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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

A meeting of the Nutrition Society was held at the University of York on Wednesday, Thursday and Friday, 4–6 July 1990, when the following papers were presented:

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The effect of the oestrous cycle in rats on fat and protein selection. By H. G. ANANTHARAMAN-BARR and J. DÉCOMBAZ, *Nestlé Research Centre, Nestec Ltd, Verschez-les-Blanc, 1000 Lausanne 26, Switzerland*

Food intake is reduced just before ovulation (pro-oestrus) in the female of a number of species. In a recent study (Anantharaman-Barr *et al.* 1990) it was found that in rats the oestrous cycle had little effect on carbohydrate (sucrose) intake. The hypothesis that serotoninergic mechanisms play a role in the effect of oestrogen on appetite was therefore not supported. In the present work, we examined the effects of the oestrous cycle on fat and protein selection.

Eight Wistar rats (initial weight 200 g) were offered a dual choice of milled chow (metabolizable energy (ME) 13·4 kJ/g) and beef fat (lard) for at least four consecutive cycles. Subsequently, they were offered a choice between chow and protein (granulated casein). Adaptation for 1 week preceded each period. The stages of the oestrous cycle were identified by daily vaginal smears. Mean ME intakes at each stage were compared with those at pro-oestrus.

	ME intake (kJ/d)									
	Di-oestrus		Pro-oestrus		Oestrus		Metoestrus		Cycle mean	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE (n 32)
Choice period 1: (mean body-wt 281 g)										
Chow	109	8·8	88	7·4	116**	8·5	114**	6·0	107	4·2
Lard	228*	10·5	203	8·1	228	9·2	218	11·3	219	5·0
Choice period 2: (mean body-wt 316 g)										
Chow	127	7·3	106	8·1	119	2·8	133*	4·9	122	3·5
Casein	123	5·1	109	8·7	126*	5·5	125*	4·9	121	3·2

Significantly different from pro-oestrus (paired t test): *$P<0·05$, **$P<0·01$.

ME intake was reduced at pro-oestrus to 89% of the mean intake over the cycle ($P<0·01$), during both the period of lard and casein choice. The provision of lard resulted in hyperphagia, with fat contributing two-thirds of total ME intake. The subsequent replacement of lard by casein resulted in a reduction in energy intake, with protein (from chow and casein) contributing more than half of ME. The reduction in energy intake at pro-oestrus occurred at the expense of both chow and the macronutrient offered, since there was no interaction between the stage of the oestrous cycle and food choice either in the lard period ($P=0·15$) or in the casein period ($P=0·47$).

Those results, taken together with those of the previous study, suggest that anorexia associated with high oestrogen levels in rats at pro-oestrus is not the result of an aversion to any of the three major macronutrients.

Anantharaman-Barr, H. G., Décombaz, J., Decarli, B. & Anantharaman, K. (1990). *Proceedings of the Nutrition Society* **49**, 223A.

Stable isotope studies of albumin synthesis during normal pregnancy. By O. S. OLUFEMI, T. LIND and P. G. WHITTAKER, *University Department of Obstetrics and Gynaecology, Princess Mary Maternity Hospital, Newcastle, Tyne and Wear NE2 3BD* and D. HALLIDAY, *Nutrition Research Group, MRC Clinical Research Centre, Harrow, Middlesex*

Our serial studies of sixty-nine normal women have shown that maternal plasma protein concentration decreases during pregnancy, largely due to a 22% decrease in albumin concentration. However, after allowing for the 53% increase in plasma volume, the total amount of circulating albumin increases by about 19%, suggesting a change in metabolism. We have developed a new protocol (with ethical approval) for determination of albumin synthesis in five non-pregnant subjects, fasted overnight and during the test. We used a primed, constant infusion (12 h) of [^{15}N]glycine together with priming of the urea pool, attaining a steady state in urinary urea ^{15}N enrichment by 8 h. We measured enrichment of [^{15}N]arginine in albumin together with ^{15}N enrichment in urinary urea (by isotope ratio–mass spectrometry (IR–MS)) to compute albumin fractional synthetic rate (FSR). A simultaneous primed infusion (8 h) of [^{13}C]leucine in the same subjects (measuring [^{13}C]leucine in albumin by IR–MS and [^{13}C]keto-isocaproate by gas chromatography–MS) and thereby determining albumin synthesis independently, allowed us to validate the latter method. The protocol was also performed in five pregnant subjects (of similar age and non-pregnant weight) at 34 weeks gestation. Intravascular albumin mass was measured with Evans Blue dilution.

	Non-pregnant		Pregnant	
	Mean	SE	Mean	SE
Intravascular albumin (g)	103·9	9·9	123·0*	14·4
FSR (%/d)				
[^{15}N]glycine	6·12	0·91	7·26	1·99
[^{13}C]leucine	6·04	1·05	7·64	2·03
Intravascular rate of synthesis (g/d)				
[^{15}N]glycine	6·38	1·33	8·79*	2·04
[^{13}C]leucine	6·27	1·29	9·49*	3·18

* Pregnant significantly different from non-pregnant: $P<0·05$

The two estimates of FSR agree with each other despite the different assumptions inherent in both methods and showed that the intravascular rate of synthesis was higher at the third trimester of pregnancy, thus supporting the hypothesis of an alteration in albumin metabolism during pregnancy.

This work was supported by Birthright.

Asymmetric enhancement of cAMP in human placental tissues after treatment with bPTH(1–34). By J. M. A. WILLIAMS[1], K. R. PAGE[1], D. R. ABRAMOVICH[2], C. G. DACKE[3] and T. M. MAYHEW[1] (Introduced by P. AGGETT), [1]*School of Biomedical Sciences* and [2]*Department of Obstetrics and Gynaecology, University of Aberdeen, Aberdeen* and [3]*School of Pharmacy and Biomedical Sciences, Portsmouth Polytechnic, Portsmouth*

Fetal requirements for calcium and phosphate rise during the last stages of pregnancy. Little is known about the processes that control the supply of these minerals from mother to fetus. The dually perfused placental lobule technique is being developed to study the effects of peptides such as calcitonin, parathyroid hormone (PTH) and fetal PTH-related peptide on the movements of Ca^{2+} and phosphate from the maternal to the fetal circulations of the human term placenta. PTH has been shown to effect movements of phosphate and increase cAMP accumulation in placental fragments in vitro.

Using normal human term placentae, fetal perfusion was attained by cannulating a vein and artery supplying a lobule, while maternal perfusate was provided by inserting glass cannulae into the intervillous space of the same lobule. A second lobule in each placenta was similarly perfused and served as a control. Throughout all experiments the perfusate was a Krebs Ringer solution (KR) containing 2·4 mM Ca^{2+} and dextran sulphate (molecular weight 60 000) (20 g/l), gassed with oxygen–carbon dioxide (95:5, v/v). The whole apparatus was maintained at 37°.

Each experiment lasted 45 min and consisted of a 30 min equilibrium period followed by a 15 min experimental period during which normal KR was replaced with KR containing 10^{-4}M 3-isobutyl-1-methyl xanthine (IBMX) and bovine serum albumin (5 g/l), with the introduction of synthetic bPTH(1–34) (0·14 mg/l) to the fetal perfusate of group A (*n* 5) and the maternal perfusate of group B (*n* 5). No hormone was introduced to perfusates of control lobules. At 45 min, tissue samples were removed from each lobule and microwaved for 40 s and then homogenized in ice-cold phosphate buffer containing 10^{-4}M IBMX. All homogenates were assayed for cAMP (protein binding assay; Amersham) and normalized with total protein.

	Tissue cAMP (pmol/mg protein)					
	Control lobule		Experimental lobule		Increase relative to control (%)	
Group	Mean	SE	Mean	SE	Mean	SE
A	394	137	399	74	26·5	77·4
B	289	36	461	59	62·0*	17·0

* Significantly different from control (paired *t* test): $P<0·005$.

After exposure for 15 min to bPTH(1–34) in the maternal circulation (group B) there was a significant increase in cAMP production. After the same period of exposure to the hormone in the fetal circulation (group A) there was a non-significant increase in cAMP production. The results provide some evidence for a role of maternal PTH in controlling transfer of mineral nutrients across the placental barrier. However, further investigations using the natural hormone hPTH(1–84) will be required in order to rule out the possibility that the smaller bPTH(1–34) molecule crosses the synctiotrophoblast by extracellular pathways.

This work was supported by Action Research.

Interactions between feed protein, carbohydrate and fat on the lactational performance of rats. By N. C. FRIGGENS[1], D. E. F. HAY[2] and J. D. OLDHAM[1], [1]*Edinburgh School of Agriculture, West Mains Road, Edinburgh* and [2]*Animal Unit, Western General Hospital, Edinburgh*

Description of animal feeds solely in terms of protein and energy may be inadequate for lactation. Variation in the proportions of carbohydrate and fat as well as protein have resulted in differences in lactational performance (Nelson & Evans, 1947). This experiment investigated potential interactions between feed protein, carbohydrate and fat on lactational performance in rats.

Sprague–Dawley rats (parity 3, *n* 49) with standardized litters of thirteen pups, were allocated on day 2 of lactation to one of eight feeding treatments (*n* 5) or to an initial cull (*n* 7). The feeds, consisting of casein, groundnut oil, starch and sugar (2:1, w/w), and a mineral–vitamin supplement were offered *ad lib.* until day 14 of lactation, on which dams and litters were culled. Feed intake, maternal and litter weights were measured daily; carcasses were analysed for protein (Kjeldahl), fat (ether extract), ash and gross energy (GE).

Effect of feed composition on lactational performance

Feed no. . . .	2	4	6	8	1	3	5	7	SED
Feed composition (g/kg OM)*									
CP	300	300	300	300	150	150	150	150	
Fat	100	250	400	550	100	250	400	550	
Carbohydrate	600	450	300	150	750	600	450	300	
GE (kJ/g)	21·0	24·5	28·0	31·5	19·9	23·3	26·8	30·3	
Feed intake (g DM/12 d)	492	439	404	342	386	322	36	64	38·9
Litter gains (g/12 d) of:									
CP	52·6	51·6	47·8	46·0	30·3	26·0	8·9	10·2	3·5
Fat	34·0	49·9	56·4	49·7	22·5	21·2	4·1	6·9	5·6
Maternal gains (g/12 d) of:†									
CP	−0·9	+2·4	−1·8	−3·3	−8·3	−8·2	−19·6	−14·7	2·6
Fat	−24·3	−25·9	−18·9	−9·1	−22·7	−25·1	−26·5	−21·7	4·9

OM, organic matter; CP, crude protein (nitrogen ×6·25); GE, gross energy; DM, dry matter.
* Calculation based on measured composition of ingredients. Ash content of all feeds 100 g/kg DM.
† Covariate adjusted for initial live weight.

Dietary protein concentration affected ($P<0·001$) all variables except maternal fat gain with poor performance on low-protein feeds. Dietary protein and fat contents interacted significantly ($P<0·05$) for all variates except for maternal fat gain. Within the high-protein feeds, changes in the carbohydrate/fat content of the feeds affected ($P<0·05$) fat gains but not protein gains despite a decrease in protein intake. Increasing fat: carbohydrate ratio of the low-protein feeds caused a depression in lactation, especially at higher levels. This cannot be explained as the result of a deficit or an excess of any single nutrient.

The results show that, for lactational support, protein and energy contents alone are not a sufficient description of feed properties; form, as well as content, of dietary energy requires description.

N.F. gratefully acknowledges MAFF support.

Nelson, M. M. & Evans, H. M. (1947). *Archives in Biochemistry* **12**, 229–239.

Critical nutrient proportions for lactational success in rats. By N. C. FRIGGENS[1], D. E. F. HAY[2] and J. D. OLDHAM[1], [1]*Edinburgh School of Agriculture, West Mains Road, Edinburgh* and [2]*Animal Unit, Western General Hospital, Edinburgh*

A strong interaction between the effects of feed protein, carbohydrate and fat content on lactational performance has been shown (Friggens *et al.* 1991). The experiment reported here was designed to examine the severity of that interaction whilst eliminating the possible confound between nutrient:nutrient and nutrient:energy interactions.

Lactating rats (parity 3) with a standard litter size of twelve pups were allocated to one of three feeds (n 5) or to a control cull (n 5). The methodology was as described by Friggens *et al.* (1991).

Table 1. *Feed composition (proportions of organic matter; OM)*

Feed no. . . .	1		2		3	
	kJ/MJ	g/kg	kJ/MJ	g/kg	kJ/MJ	g/kg
Protein (N × 6·38)	178	204	178	212	254	300
Fat	569	389	644	470	569	400
Carbohydrate	253	407	177	312	177	300
Gross energy (kJ/g)		27·0		28·8		28·0

Between diets of fairly close nutrient composition, sharp changes in cumulative intake (g/12 d) and use of body reserves confirm that the interaction between components of the feed on lactation can be extremely severe. At constant protein:energy ratio, a decrease in carbohydrate content of 100 g/kg (feed 1 *v.* 2) caused mean litter growth to halve.

Table 2. *The effects of feed composition on lactational performance*

	Feed no. (Group means)				Feed 2		
	1	2	3	SED	Rat 19	Rat 2	Rat 21
Feed intake (g OM/12 d)	320	140	384	59	221	35	182
Litter gains (g OM/12 d) of:							
CP	39·7	21·6	51·8	5·2	27·7	15·2	27·6
Fat	42·0	16·4	48·8	7·8	21·3	18·8	21·8
Maternal gains (g OM/12 d) of:*							
CP	−6·8	−13·5	+0·7	2·4	−2·9	−20·6	−16·4
Fat	−29·9	−31·9	−20·1	4·1	−36·9	−25·3	−25·7

Effects of treatment $P<0·01$.

* Maternal gains covariate adjusted for initial live weight.

Individual dams given feed 2 showed a huge variability in their ability to eat this food and, consequently to lactate. Rat no. 19 was the most successful, while rat no. 2 was the poorest. This shows the importance of maternal body reserves for lactation but leaves open the question of the origins of this variation between rats.

N.F. gratefully acknowledges MAFF support.

Friggens, N. C., Hay, D. E. F. & Oldham, J. D. (1991). *Proceedings of the Nutrition Society* **50**, 5A.

Effects of long-term feeding of high-fat diets containing triolein or medium-chain triacylglycerols to lactating rats on the disposal of a meal containing the ^{14}C-labelled lipid. By PAULO F. A. SOUZA and DERMOT H. WILLIAMSON, *Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE*

Administration of an oral load of [1-^{14}C]triolein to lactating rats previously given chow results in decreased production of $^{14}CO_2$ and increased [^{14}C]lipid accumulation in mammary gland, compared with virgin rats (Oller do Nascimento & Williamson, 1986). Thus in lactation dietary fat is spared for milk lipid. In the present work we address the question, does feeding high-fat diets (long- or medium-chain fatty acid) to lactating rats lead to greater conservation of fat?

Lactating Wistar rats (eight to ten pups) were fed *ad lib.* for 8–10 d on chow (g/kg: 520 carbohydrate, 210 protein, 40 fat) or chow in which the fat content was increased to 200 g/kg by incorporation of triolein or medium-chain triacylglycerols (MCT) (C_8 and C_{10}) and reconstituted as pellets. The rats were then starved overnight and refed 5 g of the high-fat diet containing 0·3 μCi of either [1-^{14}C]triolein or [1-^{14}C]octanoate. Measurements of $^{14}CO_2$ production, tissue [^{14}C]lipid accumulation and absorption of [^{14}C]lipid were as described by Oller do Nascimento & Williamson (1986).

Long-term diet	Refed	*n*	$^{14}CO_2$ production (% absorbed dose [^{14}C]lipid/h) Mean	SE	[^{14}C]lipid accumulation (% absorbed dose [^{14}C]lipid/g per 5 h) Mammary gland Mean	SE	WAT Mean	SE
Chow	[1-^{14}C]Triolein	5	7·57	0·61	3·23	0·34	0·042	0·005
	[1-^{14}C]Octanoate	5	12·9	0·28	0·24	0·03	0·010	0·002
Triolein	[1-^{14}C]Triolein	6	6·46	0·80	2·33	0·39	0·088	0·034
MCT	[1-^{14}C]Octanoate	4	11·8	0·66	0·34	0·10	0·022	0·007

The conversion of [1-^{14}C]triolein or [1-^{14}C]octanoate to $^{14}CO_2$ and their accumulation as [^{14}C]lipid in mammary gland or white adipose tissue (WAT) was not significantly altered by long-term feeding of diets high in triolein or MCT, suggesting that there is no adaptation to increase exogenous fat uptake into the mammary gland on high-fat diets. [1-^{14}C]Octanoate was oxidized to $^{14}CO_2$ nearly 100% faster than [1-^{14}C]triolein, and the accumulation of [^{14}C]lipid in mammary gland was only 10% of that with the [1-^{14}C]triolein meal. These findings, together with the inhibition of mammary gland lipogenesis by a diet high in MCT (Souza & Williamson, 1990), would in part explain the impaired litter-weight gain on this diet.

Oller do Nascimento, C. M. & Williamson, D. H. (1986). *Biochemical Journal* **239**, 233–236.
Souza, P. F. A. & Williamson, D. H. (1990). *Proceedings of the Nutrition Society* **49**, 3A.

How is the energy budget balanced in well-nourished lactating women? By G. R. GOLDBERG, H. L. DAVIES, A. M. PRENTICE, W. A. COWARD, M. SAWYER, J. ASHFORD, P. R. MURGATROYD and A. E. BLACK, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

The mechanisms which might operate to meet the additional energy costs of lactation include increased food intake, utilization of body fat stores and energy-sparing adaptations such as decreased maintenance requirements or a reduction in physical activity.

We have studied ten women, mean age 29·3 (SD 3·7) years, at 36 weeks gestation and 4, 8 and 12 weeks lactation with a final measurement made in the normal non-pregnant, non-lactating (NPNL) state at 15 (SD 5) weeks post-weaning. All infants were totally breast-fed at 4 and 8 weeks and three had been introduced to supplementary feeds by 12 weeks. The average time of full weaning was 57 (range 24–100) weeks. Mean NPNL body mass index was 20·8 (SD 3·9) kg/m^2. Subjects remained housewives for the duration of the study. Basal metabolic rate (BMR) was measured under standard conditions by ventilated hood within a whole-body calorimeter. Total energy expenditure (TEE), body composition and breast milk output were assessed by doubly-labelled water. Weighed food intakes over 7 d were made during each measurement period. Results are summarized in the Table.

		Pregnancy	Lactation			NPNL
Period (weeks) . . .		36	4	8	12	
Wt (kg)	Mean	67·87	58·88	58·88	58·62	57·08
	SD	9·63	9·67	9·64	9·73	12·02
LBM (kg)	Mean	47·66	41·05	40·60	40·20	41·41
	SD	3·88	4·55	4·51	3·55	4·60
Fat (kg)	Mean	20·21	17·83	18·28	18·42	15·67
	SD	7·08	6·49	6·74	7·98	7·74
BMR (kJ/d)	Mean	7275	5887	5846	5640	5858
	SD	545	467	493	277	672
TEE (kJ/d)	Mean	10 334	8830	9087	8949	9775
	SD	845	1924	951	1430	1612
TEE−BMR (kJ/d)	Mean	3059	2943	3242	3309	3917
	SD	814	1738	671	1373	1117
Intake (kJ/d)	Mean	9842	10 782	11 163	10 697	9422
	SD	849	1389	997	885	1293
Breast milk (kJ/d)*	Mean	—	1971	1954	1946	—
	SD	—	440	425	434	—

LBM, lean body mass.

* Assumes a gross energy content of 280 kJ/100 g.

TEE was similar at all stages of lactation, and about 800 kJ/d lower than the NPNL value. There was no evidence of an adaptive decrease in BMR during lactation and the reduction in TEE occurred in the residual component (TEE−BMR) representing physical activity and thermogenesis. The difference was almost certainly due to changes in voluntary activity since decreases in thermogenesis, if any, are likely to be small. Food intake was similar throughout lactation and averaged 1458 kJ/d more than that during the NPNL state. Milk output averaged 700 g/d. There was no evidence of fat mobilization at peak lactation (weeks 4–12).

The extra energy demands for milk synthesis were met primarily by the increased food intake (60%) and partially by a decrease in physical activity (40%).

Effect of a milk-free diet on fetal cord blood milk antibody levels: a study of normal and atopic mothers. By J. A. LOVEGROVE, S. M. HAMPTON, JANE MORGAN and V. MARKS, *Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH*

A maternal milk-free diet during pregnancy and lactation may be of benefit in the prevention of milk allergy in infants. This study involved monitoring cow's milk specific antibody levels and their transfer to infants in pregnant mothers on a milk-free diet compared with mothers on a diet containing milk. Ten non-atopic (a) and ten atopic (b) pregnant women followed a normal diet and nine pregnant women (c) (seven atopic, two non-atopic) followed a milk-free diet for 6 weeks before delivery. Subjects (c) were provided with a hypoallergenic formula (Pepti-Junior, Cow and Gate, Trowbridge, Wilts) to consume as required. A venous and cord blood sample were taken from all subjects and during dietary restriction in group (c). All subjects completed a 7 d weighed food inventory which was analysed using Compeat (Lifeline). An indirect enzyme-linked immunosorbent assay was used to determine the total IgG against β-lactoglobulin (β-LG) and α-casein (α-cas) (Lovegrove *et al.* 1989). The Table includes the levels of α-cas and β-LG IgG levels in the various subject groups plus the milk protein ingested daily. A positive correlation ($r = 0{\cdot}9$, $P<0{\cdot}001$) was found between the maternal and cord blood levels of α-cas IgG levels. A similar positive correlation was found for maternal and cord blood levels of β-LG IgG. The levels of β-LG antibodies in maternal blood (all subjects) was significantly lower than the fetal cord blood ($P<0{\cdot}01$). The levels of α-cas antibodies were, however, not significantly different. Group (c) β-LG antibody levels were significantly reduced while on the diet ($P<0{\cdot}05$). The levels of α-cas and β-LG antibodies in atopic subjects were on average 35% higher than in normal subjects.

Group . . .	a		b		c		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
β-LG IgG								
Pre-delivery serum (μg/ml)	30·2	22·4	50·8	39·5	88·7	86·4**	54·3	55·1*
Cord serum (μg/ml)	36·8	30·1	62·2	48·9	88·9	90·6	65·5	64·2*
Diet serum† (μg/ml)	—	—	—	—	74·0	69·7**	—	—
α-cas IgG								
Pre-delivery serum (μg/ml)	12·6	12·8	30·4	45·1	64·6	96·3	33·8	59·9
Cord serum (μg/ml)	14·3	21·8	25·5	40·8	46·0	64·6	28·9	45·9
Diet serum† (μg/ml)	—	—	—	—	63·2	87·0	—	—
Milk intake (g/d)	25·1	6·7	20·2	15·6	—	—	26·7	12·0

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

† Serum obtained from mothers on milk-free diet.

The study illustrates that levels of milk IgG antibodies in fetal cord blood are dependent on the maternal levels. A milk-free diet during pregnancy reduces the maternal milk antibody levels and this is reflected in the levels of antibodies present in the fetal cord blood. The results of this study have practical implications in the treatment of infants with a family history of atopic disease.

Lovegrove, J. A., Hampton, S. M., Morgan, J. & Marks, V. (1989). *Biochemical Society Transactions* **17**, 1059–1060.

Serum and cord blood levels of anti-ovalbumin and anti-gliadin IgG in atopic and non-atopic pregnant women. By R. MORRIS, S. HAMPTON, JANE MORGAN and V. MARKS, *Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH*

IgG antibodies, including those to food proteins, are transferred via the placenta from mother to infant. The present study aimed to determine the IgG levels of two food proteins, gliadin (GLI) and ovalbumin (OVA) transferred via the placenta to infants or mothers with and without allergic disease (atopic or non-atopic).

Serum samples were collected from twelve non-atopic and twelve atopic women at between 36 and 38 weeks gestation. Cord blood samples were collected at birth. Specific IgG antibodies were determined using indirect sandwich enzyme-linked immunosorbent assays (Hampton *et al.* 1990).

The mean antibody levels in maternal serum and cord blood are shown in the Table. Antibody levels of serum and cord blood in atopic mothers and non-atopic mothers were highly correlated (GLI, r 0·9; OVA, r 0·9). As found previously (Hampton *et al.* 1990), the levels of OVA specific IgG were significantly higher than the levels of anti-GLI IgG in both serum and cord blood ($P<0·01$).

Antibody levels (μg/ml) to:	Non-atopic (n 12)				Atopic (n 12)			
	Serum		Cord blood		Serum		Cord blood	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
OVA	164	240	283	398	203	170	264	289
GLI	69	52	64	58	62	45	52	66

No significant differences in antibody levels were found between atopic and non-atopic women due to the low numbers and large range of concentrations in both groups. However, of note is that the difference in the anti-OVA IgG concentrations between atopic and non-atopic mothers was not as great in cord blood as in serum.

Anti-GLI IgG antibodies in cord blood were lower than those found in maternal serum in atopic and non-atopic women. This is in contrast to the findings for anti-OVA IgG.

The present study has shown that differences exist in the levels of specific antibodies to two food proteins in blood. Placental transfer of these antibodies would appear not to be a uniform phenomenon, with differences occurring between different specific antibodies.

This work was generously funded by the Nutritional Consultative Panel.

Hampton, S. M., Morgan, J. B., Smith, M. R., Morris, R., Lovegrove, J. & Marks, V. (1990). *European Journal of Clinical Nutrition* **44**, 151–156.

Seasonality in children's growth rates in Southern Ethiopia. By F. BRANCA[1], G. PASTORE[1], T. DEMISSIE[2] and A. FERRO-LUZZI[1], [1]*Istituto Nazionale della Nutrizione, Via Ardeatina, 546, 00179 Rome, Italy* and [2]*Ethiopian Nutrition Institute, PO Box 5654, Addis Ababa, Ethiopia*

Children's growth rates slow down in periods characterized by food shortages, greater labour demand and higher disease incidence, which cyclically affect many areas in developing countries (Rowland *et al.* 1977; Brown *et al.* 1982).

Households (204) were surveyed from May 1987 to June 1988 in an area of moderate seasonality in Southern Ethiopia, in order to assess the extent and the biological impact of seasonal stress. Data was collected on six separate occasions coinciding with key seasonal events in two overlapping agricultural cycles: the pre-harvest period, when food stocks are lowest (May in the first year and June in the second); the early harvest period (July); the main harvest (October); the coffee harvest, when cereal stocks are full and cash available (December); and the late post-harvest season (March) when starchy vegetables become the main staple food and the workload is maximal. Body-weight and stature were measured on each occasion, but stature measurements in May and October were discarded as unreliable. Household food stocks and consumption, workloads, income and expenditure were also assessed at each round by questionnaire.

The weight-for-height and the height-for-age of children below the age of 14 years corresponded in December (the best time of the year) respectively to the 22nd and to the 10th centile of the NCHS reference. The average yearly growth rates were slower than those of healthy US children, with mean z-scores for weight and height velocities of −0·88 and −1·36 (calculated from Baumgartner *et al.* 1986). A marked seasonality was detected in growth velocity with 70–83% of the yearly weight gain and 71–78% of the yearly height gain attained during the second semester of the year (July–December).

Seasonal fluctuations in growth rates have also been observed in urban areas of developing countries and in developed countries. It is unclear whether the origin of the phenomenon should be sought in the agricultural production cycle, in disease patterns or in other as yet incompletely understood physiological mechanisms.

	Weight velocity								Height velocity							
Age (years) . . .	0–4·99				5–13·99				0–4·99				5–13·99			
Sex (*n*) . . .	♂(82)		♀(74)		♂(169)		♀(141)		♂(22)		♀(18)		♂(118)		♀(90)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Yearly	−1·01	0·13	1·00	0·13	−0·94	0·05	−0·65	0·06	−1·88	0·23	−1·55	0·25	−1·46	0·12	−1·07	0·12
Jul–Dec	−0·81	0·21	−0·63	0·23	−0·42	0·09	−0·11	0·10	−0·51	0·44	−0·23	0·36	−0·14	0·19	0·27	0·19
Dec–Jun	−1·43	0·17	−1·51	0·19	−1·39	0·06	−1·18	0·09	−3·26	0·30	−2·90	0·38	−2·68	0·15	−2·35	0·18
P	<0·05[a]		<0·01[a]		<0·001[a]		<0·001[a]		<0·001[b]		<0·005[b]		<0·001[a]		<0·001[a]	

[a] By paired *t* test.
[b] By Wilcoxon matched pairs signed-rank test.

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Growth of stunted children given nutritional supplementation and psychosocial stimulation. By S. P. WALKER, S. M. GRANTHAM-MCGREGOR, C. A. POWELL and D. T. SIMEON, *Tropical Metabolism Research Unit, University of the West Indies, Kingston, Jamaica* and J. H. HIMES, *Division of Human Development and Nutrition, University of Minnesota, Minneapolis, Minnesota, USA*

Stunting or low height-for-age, is the most common form of malnutrition globally (Keller & Fillmore, 1983). We report here, the first study of the effects on growth, of nutritional supplementation targeted to stunted children.

Stunted children (*n* 129) aged 9–24 months, were recruited by house-to-house survey of several poor areas of Kingston, Jamaica, and randomly assigned to four groups: control, nutritional supplementation (1 kg whole milk powder/week providing 3142 kJ (750 kcal) and 20 g protein daily), stimulation (Grantham-McGregor *et al.* 1987) and both interventions. All groups were visited weekly. The children were measured on enrolment and 6 and 12 months later. Two children were lost to follow up.

On enrolment the children were moderately to severely stunted (height-for-age expressed in z-scores, mean −2·9 (SD 0·6)) and mildly wasted (weight-for-height −1·0 (SD 0·7)). Multiple regression analysis was used to determine the increases in the anthropometric measurements attributable to supplementation (Table). The partial regression coefficient, *B*, represents the difference between supplemented and non-supplemented groups, controlling for age, sex, initial status, stimulation group, initial dietary intakes and several socio-economic variables. Preliminary analysis indicated a modest increase in food intake.

					Circumference (mm)				Skinfolds (mm)			
	Length (mm)		Weight (kg)		Head		Arm		Triceps		Subscapular	
Interval (months)	*B*	SE	*B*	SE	*B*	SE	*B*	SE	*B*	SE	*B*	SE
0–6	10	2***	0·35	0·11**	3	1**	2	1*	0·6	0·2**	0·1	0·2
6–12	−4	2	0·01	0·08	1	1	0	1	0·2	0·2	−0·1	0·1

*$P<0·05$, **$P<0·01$, ***$P<0·001$.

There was no effect of stimulation and, in contrast to a Colombian study (Mora *et al.* 1981), no interaction between the interventions. Supplemented children had significantly increased growth, in the first 6 months only, in all measures except subscapular skinfold. The effect on weight-for-height approached significance ($P=0·10$). Supplementation therefore resulted not only in increased linear growth but also increased body fat, as measured by triceps skinfolds.

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The effects of nutritional supplementation on the development of stunted children. By S. M. GRANTHAM-MCGREGOR, C. A. POWELL, S. P. WALKER and P. FLETCHER, *Tropical Metabolism Research Unit, University of the West Indies, Kingston, Jamaica* and J. H. HIMES, *Division of Human Development and Nutrition, University of Minnesota, Minneapolis, USA*

Low height-for-age (stunting) is extremely prevalent in young children in developing countries. Stunted children usually have poor levels of development. It is uncertain whether this is due to socio-economic disadvantages, low food intake, or both, or to an interaction between the two.

We have examined the effects of 12 months of nutritional supplementation with or without stimulation on the development of stunted children. One hundred and twenty-nine stunted children aged 9–24 months, from poor neighbourhoods in Kingston, Jamaica, were randomly assigned to four treatment groups: control, nutritional supplementation, stimulation or supplementation plus stimulation. A fifth group of thirty-two non-stunted children, matched for age and sex, was also studied. All children were visited weekly. The supplement comprised 1 kg full-cream milk powder/week and the stimulation comprised structured play sessions with the mothers and children. All children had developmental assessments on the Griffiths Test on enrolment and 6–12 months later.

On enrolment, the mean developmental quotient (DQ) of the non-stunted group was significantly higher than that of the stunted groups. During the 12 months, the DQ of both the control group and the non-stunted group declined. Multiple regression analyses of the final DQ and Griffiths subscale quotients were calculated with initial age, DQ and supplementation and stimulation status as independent variables and controlling for sex, birth weight and social background.

	Mean developmental quotients on the Griffiths Test									
	Control (*n* 33)		Supplemented (*n* 32)		Stimulated (*n* 30)		Supplemented and stimulated (*n* 32)		Non-stunted (*n* 32)	
Test session	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Enrolment	96·9	9·6	97·5	8·5	100·2	9·9	97·7	7·4	106·3	9·9
6 Months	92·0	7·4	94·3	7·9	99·7	6·9	98·6	10·8	103·5	9·9
12 Months	90·2	5·8	95·2	12·1	99·2	8·2	99·9	11·8	103·1	10·1
Change 1–12 months	−6·7		−2·3		−1·0		+2·2		−3·2	

Stimulation had a significant impact on DQ, and the locomotor, hand and eye, and performance subscales; supplementation benefited DQ and locomotor subscale. The group receiving both treatments made the greatest gains, but there was no interaction between stimulation and supplementation. The findings suggest that nutritional supplementation has a small benefit on stunted children's development, and that part of the association between stunting and poor development can reasonably be attributed to low food intake. Both interventions are required to make a substantial improvement to the development of stunted children.

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Total energy expenditure during childhood and adolescence. By P. S. W. DAVIES[1], M. B. E. LIVINGSTONE[2], A. M. PRENTICE[1], W. A. COWARD[1], S. E. JAGGER[1], C. STEWART[2], J. J. STRAIN[2] and R. G. WHITEHEAD, [1]*MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ* and [2]*Biomedical Sciences Research Centre, University of Ulster at Coleraine, Coleraine BT52 1SA*

There are virtually no data on energy expenditure during childhood. We report 177 measurements of total energy expenditure (TEE) assessed by the doubly-labelled water technique in randomly selected children and adolescents aged 3–18 years from Cambridge and Belfast. The Cambridge multi-point approach (Coward, 1988) was used in the calculation of energy expenditure. Basal metabolic rate (BMR) used in the calculation of the TEE:BMR ratio was predicted from body-weight (Schofield *et al.* 1985). Results are shown in the Table.

Age (years) . . .		3	5	7	9	12	15	18
				Boys				
n		13	12	10	14	8	12	12
Wt (kg)	Mean	15·5	18·9	24·6	29·5	39·7	60·1	71·6
TEE (kJ)	Mean	5410	6880	8150	8950	10480	13470	15050
	SD	660	750	1640	1210	1470	3030	2880
TEE (kJ/kg)	Mean	351	366	333	309	246	225	201
	SD	48	42	38	51	41	32	18
RDA (kJ/kg)		414	385	347	301	236	195	185
TEE-BMR		1·52	1·76	1·82	1·84	1·76	1·85	2·01
				Girls				
n		18	16	15	15	10	11	11
Wt (kg)	Mean	14·8	18·5	26·0	29·1	49·3	58·0	62·4
TEE (kJ)	Mean	4760	6180	8170	7569	10530	10120	11090
	SD	570	1030	1470	1270	1890	1650	1870
TEE (kJ/kg)	Mean	325	333	320	261	218	177	170
	SD	43	44	74	44	33	25	32
RDA (kJ/kg)		397	368	318	259	195	169	165
TEE-BMR		1·44	1·73	1·92	1·65	1·75	1·68	1·88

At 3 years of age, in both sexes, the mean TEE was lower than current recommended daily allowances (RDA) (Food and Agriculture Organization/World Health Organization/United Nations University, 1985). This is consistent with our previous findings in younger children (Prentice *et al.* 1988). At 5, 7 and 9 years of age there was close agreement between our observations and recommendations. Beyond 9 years of age the mean TEE were greater than recommendations. At most ages the TEE:BMR ratio was surprisingly high, corresponding to a moderately active to very active lifestyle.

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Comparison of simultaneous measures of energy intake and expenditure in children and adolescents. By M. B. E. LIVINGSTONE[1], P. S. W. DAVIES[2], A. M. PRENTICE[2], W. A. COWARD[2], A. E. BLACK[2], J. J. STRAIN[1] and P. G. MCKENNA[1], [1]*Biomedical Sciences Research Centre, University of Ulster at Coleraine, Cromore Road, Coleraine BT52 1SA* and [2]*MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

We have previously demonstrated under-reporting of energy intake (EI) by weighed dietary records (WDR) in randomly selected adults when cross-validated against estimates of total energy expenditure (TEE) by the doubly-labelled water method (DLW) (Livingstone *et al.* 1990). In the present study, 7 d WDR and diet history (DH, obtained by open-ended interview) were compared with concurrent estimates of TEE by DLW in seventy-eight subjects from Belfast aged 3, 5, 7, 9, 12, 15 and 18 years. WDR were not obtained on the 3- and 5-year-olds.

Age (years) . . .		3	5	7	9	12	15	18
n		8	12	12	12	12	12	10
TEE (MJ/d)	Mean	5·26	6·09	7·62	8·78	10·54	11·71	13·50
	SD	0·68	1·26	1·38	1·26	1·10	2·77	4·11
DH (MJ/d)	Mean	5·91*	6·55	8·48*	9·28*	11·96*	11·62	12·83
	SD	0·55	0·71	1·62	1·19	2·02	3·04	3·38
DH:TEE	Mean	1·14	1·11	1·13	1·06	1·14	1·01	0·98
	SD	0·19	0·19	0·21	0·09	0·17	0·21	0·21
WDR (MJ/d)	Mean	—	—	8·19	8·45	9·36**	9·08**	9·28**
	SD			2·16	1·36	1·54	2·92	3·00
WDR:TEE	Mean	—	—	1·08	0·97	0·89	0·78	0·73
	SD			0·25	0·15	0·12	0·18	0·25

Significantly different from TEE value (paired *t* test): *$P<0·05$, **$P<0·01$.

Mean EI by WDR was significantly lower ($P<0·001$, paired *t* test) than TEE (8·85 (SD 2·25) *v.* 10·33 (SD 3·04) MJ/d) for the whole WDR group (*n* 58). The younger children (7 and 9 years) appeared to provide reliable records, but in the adolescents (*n* 36) EI was substantially underestimated, particularly by girls ($P<0·001$). The bias was >20% in thirteen adolescents and reached 50% in five subjects. In contrast mean EI by DH was slightly but significantly higher than TEE (9·62 (SD 3·14) *v.* 9·15 (SD 3·35) MJ/d), $P<0·05$ for the group (*n* 78). No age-specific trend was apparent. The large standard deviations for the DH:TEE and WDR:TEE reflect high levels of imprecision in individual measurements.

The results suggest that WDR is prone to the same under-recording of food intake in adolescents as has been reported for adults. In this study DH was more reliable at the level of group comparisons, but the significant problems with precision at an individual level remain.

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Distribution of energy intake in relation to calculated basal metabolic rate and adiposity in a national cohort study. By FIONA B. KEY[1], ALISON A. PAUL[1], T. J. COLE[1] and M. E. J. WADSWORTH[2], [1]*MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ* and [2]*MRC National Survey of Health and Development, 66–72 Gower Street, London WC1E 6EA*

Members of the 1946 National Birth Cohort were followed up at 36 years of age. A 7 d dietary record of food intake described in household measures was obtained from 983 men and 1034 women. Average portion weights were obtained from other studies of adults and mean daily energy intakes were calculated using food composition tables (Braddon *et al.* 1988).

Mean body-weight and height were 76·6 (SD 11·2) kg and 1·76 (SD 0·06) m for men and 61·7 (SD 10·8) kg and 1·62 (0·06) m for women. Basal metabolic rate (BMR) (MJ/d) was calculated from weight (kg) and height (m) (Schofield *et al.* 1985, for 30–60 years):

Male BMR = 0·048 weight − 0·011 height + 3·670
Female BMR = 0·034 weight + 0·006 height + 3·530.

Adiposity was expressed as body mass index (BMI: wt (kg)/ht^2 (m^2)). Both BMR and BMI are functions of body-weight, and were found to be highly correlated (r 0·87 for the men, r 0·90 for the women). Men and women were divided into five equal groups by quintile for energy intake, and the mean BMR and BMI for each fifth obtained.

Male			Female		
Fifths of energy intake (MJ/d)	BMI (wt/ht^2)	BMR (MJ/d)	Fifths of energy intake (MJ/d)	BMI (wt/ht^2)	BMR (MJ/d)
<8·1	25·0	7·35	<5·3	25·3	5·80
8·1– 9·4	24·8	7·37	5·3–6·5	23·3	5·65
9·4–10·5	24·4	7·31	6·5–7·4	22·7	5·59
10·5–11·9	24·2	7·30	7·4–8·5	22·4	5·57
>11·9	24·1	7·32	>8·5	22·0	5·58
SE for each group	0·2	0·04		0·3	0·03

Since BMR is a major component of energy expenditure, BMR is usually assumed to be positively related to energy intake. In fact, this correlation was negligible among men (r −0·05, not significant) and strongly negative (r −0·21, P<0·001) among women. In both sexes, energy intake was more negatively correlated with BMI than with BMR (r −0·13 for men, −0·30 for women, both P<0·001). The lack of a relationship between BMR and energy intake in men suggests that although the energy intake required for maintenance is raised in larger individuals, there is a compensating reduction in energy requirement for activity. For women the same effect operates, but in addition there is evidence (Prentice *et al.* 1985) that obese women, with high BMIs and high BMRs, tend to under-record their energy intake.

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24 h Energy expenditure and basal metabolic rate in female twins. By G. MCNEILL, J. LOVE, J. S. SMITH and D. C. MORRISON, *Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB*

Family studies of resting metabolic rate have indicated that there may be a contribution of genetic background to the variance in energy expenditure between individuals. We report here measurements of 24 h energy expenditure (24 h EE) and basal metabolic rate (BMR) in monozygotic (MZ) and dizygotic (DZ) female twin pairs which were designed to assess the possible contribution of genetic factors to variance in energy expenditure in UK women.

The women studied were seven MZ and nine DZ twin pairs, with age ranging from 16 to 63 years and body mass index (BMI) from 18·5 to 32·6 kg/m^2. There were no significant differences in age, weight or height between the two groups. 24 h EE was measured by whole body indirect calorimetry with a controlled energy intake and physical activity regime. Sleeping metabolic rate (SMR) was defined as the energy expenditure between 00·00 and 06·00 hours, excluding any periods in which significant movement of the subject was detected by an ultrasound transmitter/receiver. BMR was measured by ventilated hood indirect calorimetry on two occasions. Fat-free mass (FFM) was assessed from estimates of body fat content from underwater weighing (except in one DZ pair where skinfold thickness was used), and zygosity was determined by DNA fingerprinting. Within-pair correlations for MZ and DZ pairs (r_{MZ} and r_{DZ}) were determined by intra-class correlation.

The Table shows the mean, SD and intra-class correlation coefficients for the MZ and DZ pairs.

		MZ pairs		DZ pairs*			
		Mean	SD	Mean	SD	r_{MZ}	r_{DZ}
24 h EE:	(kJ/d)	7602	829	8184	994	0·79	0·62
	(kJ/kg per d)	135·9	16·3	133·3	14·5	0·84	0·51
	(kJ/kg FFM per d)	195·4	10·8	202·5	20·7	0·54	0·53
SMR:	(kJ/d)	4975	572	5036	466	0·77	0·69
	(kJ/kg FFM per d)	127·7	5·5	124·6	8·4	0·62	0·35
BMR:	(kJ/d)	4947	578	5148	657	0·74	0·68
	(kJ/kg FFM per d)	127·5	13·4	126·7	13·4	0·37	0·28

* *n* 7 pairs for 24 h EE and SMR measurements; *n* 9 pairs for BMR measurements.

For each variable r_{MZ} was higher than r_{DZ}, indicating a genetic contribution to the variance in these results. However, when expressed per kg FFM, there was no significant negative correlation between 24 h EE, SMR or BMR and the % fat (r 0·56, $P<0·01$; r 0·24, $P>0·05$ and r 0·13, $P>0·05$, respectively), suggesting that inter-individual differences in energy expenditure are not the largest determinants of the current level of fatness in these women.

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Does genetic variation influence energy expenditure in cystic fibrosis? By A. O'RAWE[1], J. A. DODGE[1], A. O. REDMOND[2] and I. MCINTOSH[1], [1]*Department of Child Health, The Queen's University of Belfast,* [2]*The Royal Belfast Hospital for Sick Children* and D. J. H. BROCK, *Human Genetics Unit, University of Edinburgh*

The basis for the increase in energy requirement in cystic fibrosis (CF) remains unclear. CF is now established as a heterogeneous condition with a majority of patients showing the ΔF508 mutation. The gene product has properties consistent with membrane association and nucleotide binding (Riordan *et al.* 1989). We speculate that the deletion of a phenylalanine residue may influence cellular bioenergetics by providing abnormal ATP binding domains and thus blocking the movement of high-energy phosphate bonds within the cell.

In the course of a study of basal metabolic rate (BMR) in CF, using indirect calorimetry under standardized conditions, we examined the possibility that BMR is correlated with the ΔF508 mutation. Samples were obtained from thirty-eight patients, representing approximately equal numbers of high and low metabolic rates, and genomic DNA analysis was carried out. On the basis of the results, patients were divided into three groups: (1) homozygous for the deletion (*n* 12), (2) heterozygeous for the deletion (*n* 18) and (3) without the deletion (*n* 8).

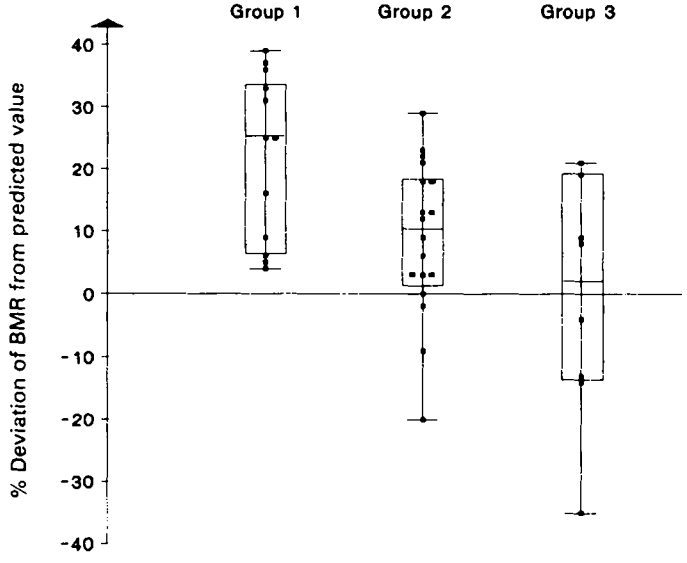

Fig. Summary of the distribution for groups 1, 2 and 3, representing the interquartile range within the box with the median marked as a line.

Group 1 had a median BMR of 25% above predicted (95% confidence limits 6–13%), group 2 median BMR of +10% (3–18%) and group 3 median BMR of +2% (−14 to +19%). Using the Mann–Whitney U test the median values for groups 1 and 3 showed significant difference (P = 0·012). There was no significant correlation between pulmonary function score and BMR and no significant difference in body mass percentile between the groups.

These findings would be consistent with the speculation that the ΔF508 allele of the CF gene product, by preventing proper binding of ATP, may provide a cellular basis for heightened energy expenditure in CF.

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Increased thermic effect of a standard meal in chronically energy-deficient human subjects. By L. S. PIERS, M. J. SOARES and P. S. SHETTY, *Nutrition Research Centre, Department of Physiology, St John's Medical College, Bangalore, India*

Twenty-nine healthy, male volunteers aged between 18 and 30 years were studied. Nine were classified into a chronically energy-deficient (CED) group, based on socio-economic class (Kuppuswamy, 1966), energy intakes (<8 MJ/d) and criteria laid down by the IDECG expert committee. The control subjects were further subdivided into two groups of ten subjects each, on the basis of their body mass index (BMI, kg/cm^2), into (a) normal weight (NW) (BMI > 20) and (b) underweight (UW) (BMI < 18). All subjects underwent a complete clinical assessment before recruitment. Anthropometric characteristics, including fat free mass (FFM) from skinfolds using the equation of Durnin & Womersley (1974) were obtained.

The thermic effect of a standard liquid meal (TEM) (g/kg: 100 protein, 150 fat, 750 carbohydrate; energy 2·5 MJ; volume 350 ml) was measured using a ventilated hood system over a period of 6 h after ingestion of the meal. All data were tested by independent *t* tests at the 5% significance level. The results in the Table indicate that the CED subjects had lower body-weights, heights, per cent fat and FFM compared with either of the control groups. BMR was lower in absolute terms but appeared to be higher when expressed per kg FFM. TEM responses in the CED subjects were significantly higher when expressed either in absolute terms, per kg body-weight or per kg FFM, or even as a percentage of the energy density of the meal. Total energy output (TEO = (BMR + TEM)/5 h) per kg FFM was also significantly higher in the CED group when compared with either control group.

	NW (*n* 10)		UW (*n* 10)		CED (*n* 9)	
	Mean	SEM	Mean	SEM	Mean	SEM
Height (m)	1·77	0·02	1·75	0·03	1·65†‡	0·02
Wt (kg)	67·3	1·7	50·8*	1·5	43·3†‡	1·1
FFM (kg)	55·3	0·9	44·4*	1·5	38·9†‡	0·9
BMR (kJ/h)	283·8	9·5	232·1*	6·1	222·3†	8·5
BMR/kg FFM (kJ/h)	5·2	0·2	5·3	0·2	5·8	0·3
TEM (kJ/5 h)	174·8	12·9	182·9	12·8	217·2†‡	9·4
TEM/kg FFM (kJ/5 h)	3·2	0·3	4·2*	0·3	5·6†‡	0·3
TEM (as % of energy in meal)	7·0	0·5	7·3	0·5	8·8†‡	0·4
TEO (kJ/5 h)	1520·0	42·6	1343·2*	22·9	1328·8†	44·8
TEO/kg FFM (kJ/5 h)	28·8	0·8	30·5	0·7	34·3†‡	1·4

NW *v.* UW, **P*<0·05; NW *v.* CED, †*P*<0·05; UW *v.* CED, ‡*P*<0·05.

The higher TEM in CED subjects may possibly be the result of an increased protein synthesis as well as an alteration in the composition of the FFM *per se*.

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Family food availability and anthropometric status in Gilan, Iran. By F. RABIEE and C. GEISSLER, *Department of Food and Nutritional Sciences, King's College, University of London, Campden Hill Road, London W8 7AH*

Data on food availability (from estimates of food purchases and consumption supplied by the individual with principal responsibility in each family) and anthropometry of 148 families comprising 935 individuals, and the individual intakes (24 h recall) of women, and children under 5 years were collected over three seasons as part of a larger study in rural Gilan, Iran. Rice and bread are the main staples, providing 46% and 20% of energy and 31% and 26% of protein respectively. The other main contributor of protein is legumes (16%). The mean annual family availability of vegetables and fruit is high (290 and 240 g/d per capita). Animal produce in the diet is very low: meat, fish, eggs and dairy foods together provide only 8% of the total energy. The daily consumption of oil, fat and butter is 17 g/capita, providing 6% of the total energy. The daily consumption of sugar is 43 g/capita, 7% of the energy. This diet provides on average 10·2 MJ (2440 kcal) energy and 65 g protein/d per person. The diet is bulky as 74% of the energy is derived from carbohydrates while fat provides only 15% of the energy. Fibre intake is 29·8 g/d per capita. This diet is therefore similar to the National Advisory Committee on Nutrition Education (1983) recommendations, although even lower in fat.

The overall family food availability meets recommended dietary intakes (RDI) for energy and protein by 102% and 135% respectively (World Health Organization/Food and Agriculture Organization, 1973). Despite marked seasonal variations in the availability of certain food items, energy and protein are adequate in all seasons. However, in the whole study population 55% are short (<95% height for age) and 30% thin (<90% weight for height). (Bray, 1975, 1976; National Center for Health Statistics, 1976). Socio-economic differences in patterns of food consumption are small because of current food policies and rationing (Rabiee & Geissler, 1990*a*). The prevalence of thinness is similar in all socio-economic groups whereas shortness is more prevalent in low income groups.

These data show that poor nutritional status, as defined by anthropometric reference standards, is prevalent despite adequacy of family food availability. This is due only in part to unequal intra-family food distribution, with low intakes in young children (Rabiee & Geissler, 1990*b*), and to the problem of growth standards in delayed adolescence (Geissler & Miller, 1983), as a high proportion of adults are also short (73%) and thin (16%).

These data suggest that the energy requirement of this population is higher than RDI due to environmental stresses such as diarrhoea and parasite infestation, or that growth is limited by specific nutrients.

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Diet quality, environment and nutritional status in Gilan, Iran. By F. RABIEE and C. GEISSLER, *Department of Food and Nutritional Sciences, King's College, University of London, Campden Hill Road, London W8 7AH*

In 1982, as part of a larger study, data were collected over three seasons in Gilan, Iran, on family food availability, individual intakes of women and children less than 5 years old, clinical deficiency signs, haemoglobin levels, and parasite infestation. Selected nutrients were compared with recommended dietary intakes (RDI) (Food and Agriculture Organization/World Health Organization, 1974).

The family diet, although adequate in energy (102% RDI) and more than adequate in protein, vitamin C and folate (135%, 128%, 131% RDI respectively) was marginally adequate in vitamin A (retinol equivalents) and iron (95%, 94% RDI) but deficient in riboflavin and calcium (55%, 70% RDI). Fat provided only 15% of energy, the low energy density being associated with deficient energy intakes and growth in young children (Rabiee & Geissler, 1990).

Despite marginal adequacy in the family diet, individual intakes of Fe were very low and of vitamin A were low even in comparison to energy intakes (children under 5 years: 48%, 62% cf 70%; women 45%, 92% cf 104% RDI). This indicates unequal distribution of food both in quantity and quality among family members.

Despite low intakes, no specific clinical signs of vitamin A deficiency were observed, however 21% of the study population had signs of riboflavin deficiency (angular stomatitis, cheilosis or both).

The prevalence of haemoglobin levels below WHO standards (World Health Organization, 1972) was 17% in the whole population, and even higher in children less than 2·5 years (24%) and 5–9 years (25%), and in lactating (27%) and pregnant (60%) women. The Fe intakes of individuals with and without anaemia were not different, suggesting that factors determining absorption and loss are more important than total intake of Fe (World Health Organization, 1972; Hallberg *et al.* 1983).

One-third of the population reported at least one single or multiple parasite infestation in the year of survey. Half (48%) of the subjects whose faeces were examined (n 89) were infested with one or more species of parasite: this may be an underestimate. Giardia (25%), ascaris (10%) and hookworm (6%) were the most prevalent. Haemoglobin levels were significantly lower in subjects with, than without parasites (115 *v.* 121 g/l, $P<0·001$). Therefore the low haemoglobin levels in this population could partially be explained by parasite infestation.

In conclusion, the low quality of the diet in respect to riboflavin, Fe and fat, rather than overall quantity, and poor environmental hygiene are determinants of the anaemia, riboflavin deficiency and poor growth that are prevalent in this population.

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Knowledge, diet, and stock decisions of small independent retailers with regard to 'healthy' foods. By B. J. TIGHE, A. WISE and P. COOPER, *Robert Gordon's Institute of Technology, Queen's Road, Aberdeen AB9 2PG*

Manufacturers have recently introduced modified food products that are considered 'healthy' because they are low in fat, sugar and salt, and high in fibre. Small independent retailers have been found to stock fewer of these items than supermarkets (Welsh Consumer Council, 1989). The present project was designed to find whether the retailer's knowledge about nutrition, or his or her individual diet is related to their decisions about which foods to stock. Shops in Aberdeen were selected from enumeration districts from the 1981 census to give a wide range of areas representing the whole city. A total of thirty-six shops were approached, three refused to participate and two were excluded because they were under the same management as shops that had been surveyed. Retailers were interviewed and asked questions about which were the 'healthiest' foods within small groups of two to four foods: wholemeal, brown and white bread; full-cream, semi-skimmed and skimmed milk; soft drinks and low-calorie/diet drinks; butter, margarine, polyunsaturated margarine and low-fat spread; tinned fruit in syrup or natural juice. They were also asked if they knew of modified versions of the following: baked beans, cheese, ketchup, jam, instant whips, crisps and flavoured yoghurts. A knowledge score was calculated by summating the correct choices made to the questions. The retailers were asked if they consumed these foods and whether they stocked them, or had ever stocked them. Scores were calculated from the total number of the 'healthier' items eaten or stocked. All scores were converted to percentages. The mean scores (ranges) were: knowledge 58% (19–88); diet 19% (0–63); and stock 65% (27–100). No significant differences were found among the six areas of Aberdeen surveyed. The Spearman's correlation coefficients were: diet and knowledge 0·41 ($P<0·05$); diet and stock 0·27; knowledge and stock 0·45 ($P<0·02$). Out of the fourteen healthier foods surveyed, 71% of the shops had stocked one or more of these foods at one time but no longer stocked them at the time of the survey. In eighteen shops this was due to low demand, in one due to bad suppliers and in eight due to other reasons. For individual foods and retailers, the number of times that a food was not consumed and not stocked (A), consumed and not stocked (B), consumed and stocked (C), or not consumed and stocked (D) were counted. For the standard and 'healthy' foods the number of foods (n 837) in each category respectively were A 2% and 31%; B 1% and 2%; C 46% and 18%; D 51% and 49% ($P<0·001$ by Chi square test). Since retailers consumed few foods they did not stock and fewer 'healthy' foods that they did stock, it would appear that their stock was mainly demand-led, so perhaps nutrition education directed at retailers would have little effect unless the customers are also educated.

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Individuality of portion weights of fat spreads. By CATHERINE H. KELLET and A. WISE, *Robert Gordon's Institute of Technology, Queen's Road, Aberdeen AB9 2PG*

A previous study showed that individuals vary widely in the weight of fat-spread they use on a slice of bread, and identified some factors that influenced the mean weight (Wise *et al.* 1990). The subjects had been asked to evaluate the spreadability of the products and were not required to taste them, thus it may be argued that the situation was unnatural. In this study, a sample of 100 people (fifty male and fifty female) was chosen by randomly approaching diners in several canteens (hospital staff (*n* 24) and visitors (*n* 24), college (*n* 28), staff in a department store (*n* 12), staff in telecommunications (*n* 12). They were asked to spread medium-sliced white bread with either four unknown spreads or two named spreads at different temperatures. They tasted these and were asked about their preference. Unknown to the subjects, the pot of spread had been preweighed and was later reweighed, and the method of spreading was observed with particular reference to the number of times they loaded the knife (dips). The spreads used were a sunflower oil-derived margarine (Flora, van den Berg) and a reduced-fat spread containing 40% fat (Gold, St Ivel). They were kept either chilled (7–9°) or at room temperature (17–22°). Subjects were asked questions which were later used to try to identify factors that might influence their 'spreading' behaviour.

			Spread		Temperature		Spread	
	Males	Females	Known	Unknown	Chilled	Room	Gold	Flora
Wt (g)	5·63	5·25*	5·21	5·67*	5·32	5·56**	5·64	5·24***
Dips	2·52	2·73	2·93	2·31**	2·86	2·39***	2·85	2·40***
Wt/dip (g)	3·31	2·66***	2·67	3·30***	2·73	3·25***	2·95	3·03

Significance was assessed by analysis of variance: *$P<0·05$, **$P<0·01$, ***$P<0·001$.

The ranges of weights (2·5–13·3 g, mean 5·4 (SD 1·8) g) and dips (1–16, mean 2·6 (SD 1·9)) were both very large and individual differences were significant ($P<0·001$). There was no correlation between weights and dips (r 0·05), and the weight/dip also ranged widely (0·4–12·1 g, mean 3·0 (SD 1·9) g). No relationships were found between weight, dips, or weight/dip and the following factors: age, social class, household composition, type of bread and spread used at home, slice thickness, whether they had recently changed the type of spread, who influenced their choice of spread, and reason for choice of spread. Although some factors influencing spreading behaviour have been identified, the major question remains: what determines the individual's choice of portion weight and number of dips?

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How much does the public know about fat intake and heart disease? By JANET CADE and JANICE TATE, *Community Medicine, South Academic Block, Southampton General Hospital, Southampton SO9 4XY*

The report of the Committee on Medical Aspects of Food Policy (COMA) (Department of Health and Social Security, 1984) has made certain recommendations for changes in dietary fat intake in an attempt to reduce the incidence of cardiovascular disease. These recommendations are being used as the basis for health promotion advice being presented by health professionals and the media. A recent study of the dietary knowledge of primary health care workers in Oxfordshire (Francis *et al.* 1989) showed some important gaps in knowledge, despite their role in providing dietary advice.

The present study was carried out to determine whether members of the general public have the minimum knowledge required to reduce their dietary risk of heart disease in accordance with the COMA recommendations for fat intake.

A systematic sample of 400 people was taken from the electoral registers for the Itchen and Test constituencies of Southampton. A questionnaire, covering letter and reply-paid envelope were sent to each individual in January 1989. The questionnaire was designed to assess knowledge on three levels: (1) selecting the food from a list of four which was 'best' for the heart, (2) knowledge of the type (saturated or polyunsaturated) and level of fat in common foods, (3) knowledge of the relationship between the different types of fat in food and heart disease.

Of the 400 questionnaires sent the final response rate was 68% (255). Overall knowledge levels were high, with twenty-one out of twenty-six questions being answered correctly by at least 70% of subjects. Five of the questions were answered correctly by only a minority of subjects.

Thirty-eight per cent of subjects thought that oily fish contained mainly saturated fat. Only 16% of subjects knew that sunflower margarine contained the same amount of fat as butter. Only 28% of subjects knew that sunflower margarine contained more fat than low-fat spreads. Only 14% of subjects disagreed with the statement that 'dietary cholesterol is the main determinant of plasma cholesterol'. Only 34% of subjects disagreed with the statement 'it is more important to cut down on cholesterol than saturated fat in the diet if we want to reduce our risk of heart disease'. The media seemed to be the main source of the information. There were no large differences in knowledge between subjects with a history of heart disease in themselves or a relative despite receiving more information from doctors compared with those without a positive history.

Differences in knowledge according to age, sex and social class were also examined. The younger group knew more than the older group. The overall knowledge score for men and women was similar. Social classes I and II knew more about the amount and type of fat in food and the relationship of fat to heart disease than did social classes III, IV and V.

In view of the high overall knowledge levels in this study, we suggest that future health education information should concentrate on clarifying the areas of misunderstanding which have been identified.

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General practitioners' knowledge, attitudes and practice regarding overweight and obesity. By JANET CADE and SHAUN O'CONNELL, *Community Medicine, South Academic Block, Southampton General Hospital, Southampton SO9 4XY*

The health risks of being overweight or obese are considerable (Royal College of Physicians, 1983). A national survey in 1981 showed that 33% of men and 24% of women were overweight (Body mass index (BMI) >25 kg/m^2) with BMI >30 in 6% of men and 8% of women (Office of Population Censuses and Surveys, 1984). However, another study of men and women selected from general practice found that only 16% of men and 22% of women had been given advice from their doctor to lose weight (Wallace *et al.* 1988).

The present study was designed to assess the knowledge, attitudes and practices of general practitioners with regard to overweight and obesity. A questionnaire was sent to a sample of general practitioners who were randomly selected from Family Practitioner Committee lists, 200 from the Portsmouth area and 200 from the Norwich area. Non-responders were sent a reminder 3 weeks after the initial mailing and 4 weeks after that doctors were telephoned to try to increase the response rate. The questionnaire included questions about doctors' attitudes to overweight; how competent they thought they were to treat it; and knowledge of the prevalence of obesity and current dietary recommendations. The response rate was 75% (299), 18% were female doctors and 82% were male.

The current prevalence of BMI >25 (27%) and BMI >30 (3%) amongst doctors was lower than that of the general population. However, 40% had BMI >25 and 12% had BMI >30 some time in their lives. Many doctors (61%) had tried to lose weight at some time and nearly half of those (48%) had found slimming easy or not very difficult.

Most (98%) of the doctors thought that it was part of their role to counsel the overweight on the associated health risks. They also felt that this counselling was worthwhile despite not finding this easy or professionally rewarding. Sixty-nine per cent thought that general practitioners should be role models and maintain normal weight. Compared with the media and the family, doctors thought that they had the least effect in persuading the overweight to lose weight. Doctors were asked to rank how prepared they were to counsel on various health promotion topics and also to rank the success of this counselling. Overall they believed that they were most prepared to counsel on stopping smoking and weight reduction and had least success in achieving permanent weight loss and reducing alcohol consumption. Most (91%) doctors weighed their patients, but only 24% calculated BMI.

The most popular methods used to educate patients about obesity and overweight were 1:1 counselling and giving out diet sheets and leaflets on healthy eating. Advice given by doctors always or often was to eat less in general (78%); specifically to eat fewer calories (75%); to exercise (77%); or to attend a slimmers' group (54%).

Only a third of doctors knew the current prevalence of overweight and even fewer knew currently recommended intakes of fat and fibre. Doctors felt that their knowledge about diet, excess weight gain and its management came mostly from experience and journals and least from textbooks, medical school and postgraduate courses.

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The effect of energy supplementation on noradrenaline-induced thermogenesis, thermic effect of a meal and basal metabolic rate in chronically energy-deficient human subjects. By M. VAZ, R. N. KULKARNI, L. S. PIERS, M. J. SOARES, A. V. KURPAD and P. S. SHETTY, *Nutrition Research Centre, Department of Physiology, St John's Medical College, Bangalore, India*

Seven chronically energy-deficient (CED) adult males underwent a period of controlled supplementary feeding (~ 3·35 MJ/d) over a period of 12 weeks. Body composition estimated from skinfolds (Durnin & Womersley, 1974) and densitometry, basal metabolic rate (BMR), thermogenic response to noradrenaline (NA) and thermic effect of a meal (TEM) were assessed before and after the 12-week period of energy supplementation. Post-supplementation there were significant increases in body-weight (mean 43·5 (SD 2) *v.* 45·3 (SD 3) kg), body mass index (16·3 (SD 0·94) *v.* 17·0 (SD 1·08) kg/m^2) and fat free mass (FFM) (38·7 (SD 2·2) *v.* 39·6 (SD 2·4) kg), all $P<0·01$. Per cent body fat was higher but not significant (11·1 (SD 1·7) *v.* 12·7 (SD 3·1)). BMR was significantly higher post-supplementation (4·8 (SD 0·3) *v.* 5·9 (SD 0·7) MJ/d; $P<0·01$) even when corrected for body-weight differences (110·5 (SD 6·6) *v.* 129·8 (SD 14) kJ/kg per d; $P<0·05$) or FFM differences (124·3 (SD 6·2) *v.* 148·5 (SD 13·9) kJ/kg per d; $P<0·01$).

In five of the original seven subjects thermogenic responses to NA (0·15 μg/kg FFM per min × 60 min) was assessed on three separate occasions; before (pre) and after (post) the period of 12-week supplementation, as well as 12 weeks following the cessation of supplementation (post + 12 weeks). Anthropometric indices (post + 12 weeks) were significantly lower than the values at post-supplementation and were not significantly different from the pre-supplementation values. NA stimulated oxygen consumption (V_{O_2}) during the three phases of the study, i.e. pre, post, and post + 12 weeks showed no significant differences when expressed in absolute terms (176·2 (SD 11·0) *v.* 176·1 (SD 15·3) *v.* 173·1 (SD 10·1) ml/min; not significant) even when corrected for FFM differences (4·5 (SD 0·2) *v.* 4·3 (SD 0·3) *v.* 4·4 (SD 0·1); not significant).

The TEM was measured in five of the original group over 6 h. The thermic response to the standard meal (g/kg: 100 protein, 150 fat, 750 carbohydrate, 2·5 MJ) lasted 5 h in all subjects. Post-supplementation values (181·0 (SD 3·0) kJ/5 h) were significantly lower than pre-supplementation values (215·0 (SD 34·9) kJ/5 h; $P<0·01$) by 15·8%. Similar results were obtained when expressed per unit body-weight (post 4·1 (SD 0·9), pre 5·0 (SD 0·9) kJ/kg per 5 h; $P<0·01$) and per unit FFM (post 4·7 (SD 1·0), pre 5·6 (SD 1·0) kJ/kg per 5 h; $P<0·01$). Total energy output (TEO) over 5 h (i.e. BMR + TEM) was not different between the two periods (post 1310 (SD 39), pre 1283 (SD 60) kJ/5 h). The changes in TEM before and after supplementation have to be interpreted in the light of our observations that the TEM responses to an identical standard meal are higher in CED subjects even when corrected for body-weight and FFM differences (Piers *et al.* 1991).

These studies were supported by the Nestlé Foundation, Switzerland, and the United Nations University, Tokyo. The indirect calorimeter facility was made possible by the Wellcome Trust, UK.

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Aerobic capacity and post-exercise recovery characteristics of chronically energy-deficient labourers. By R. N. KULKARNI, A. V. KURPAD and P. S. SHETTY, *Nutrition Research Centre, Department of Physiology, St John's Medical College, Bangalore, India*

The power output for work of a short duration in ergonometric terms is reflected by the maximal oxygen uptake (V_{O_2max}), which in turn is influenced by the nutritional status of the individual. The present study was undertaken with the objective of comparing the aerobic capacity of well-nourished healthy normal controls and chronically energy-deficient (CED) subjects.

Eight healthy male CED subjects, as defined by the IDECG expert committee, were investigated and compared with seventeen age-matched, well-nourished individuals. The CED subjects belonged to social class IV (Kuppuswamy scale) and were on habitual energy intakes of <8 MJ/d. Following a thorough clinical examination, nutritional assessment was made by anthropometry, i.e. body-weight (kg), height (m) and skinfolds (mm); fat free mass (FFM) was estimated from the sum of four skinfolds using the equation of Durnin & Womersley (1974). V_{O_2max} was estimated in all twenty-five subjects using the McDonough–Bruce protocol when in the whole body indirect calorimeter. Oxygen consumption was monitored on a 1 min mode both during exercise and continuously for 60 min after the exercise when the subjects rested quietly without moving.

The CED subjects were shorter than well-nourished controls (1·617 *v.* 1·751 m, $P<0·001$) and had lower body-weights (43·8 *v.* 58·6 kg, $P<0·001$) and FFM (39·4 *v.* 50·7 kg, $P<0·01$). They had a lower V_{O_2max} (1·75 *v.* 2·5 l/min, $P<0·001$) but the differences were not substantially different when corrected for body-weight or FFM differences. The post-exercise recovery O_2 consumption pattern showed a biexponential decline. The rate constants (k_f) during the recovery process, in the initial rapid phase, were significantly higher in the CED subjects compared with controls (0·74 *v.* 0·51/s, $P<0·005$) with the resultant half-recovery times significantly shorter in the CED group (58·8 *v.* 82·8 s, $P<0·005$). At the beginning of the second slower phase of decline the controls had a higher mean O_2 consumption over pre-exercise baselines (1·1 *v.* 5·0 ml, $P<0·02$) with the decline in O_2 consumption of the controls, showing a slope of 0·07/s and a mean half-recovery time of 10 min. In the CED subjects there was no evidence of the second slow phase of decline. The controls therefore had a significantly higher O_2 debt as shown by the area under the curve (147·3 *v.* 249·1 ml/kg FFM, $P<0·005$). Although it has been demonstrated hitherto that V_{O_2max} of undernourished subjects is low, the reduction in this aerobic capacity is largely due to reduction in body-weight and FFM (Spurr, 1988). The present study also demonstrates that the rapidity of return to pre-exercise baseline O_2 consumption in the CED subjects and the larger O_2 debt in the controls underlines a possible interesting mechanism during the recovery phase leading to some degree of energy saving in energy-deficient individuals.

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Potentiation as a possible mechanism to explain differences in dose response *v.* single dose infusion of noradrenaline while estimating thermogenic responses in human subjects. By A. V. KURPAD, R. N. KULKARNI, M. VAZ and P. S. SHETTY, *Nutrition Research Centre, Department of Physiology, St John's Medical College, Bangalore, India*

Alterations in the thermogenic component of total energy expenditure have been invoked as possible responses to reduce energy output in chronically energy-deficient (CED) individuals. We assessed the thermogenic responses to noradrenaline (NA) infusions in healthy but CED labourers (*n* 10) on habitual mean energy intakes of about 8 MJ/d and with a body mass index (BMI) of 18 kg/m^2 and other criteria as laid down by the IDECG expert committee. These responses were compared with healthy control subjects who were sub-divided into two groups: underweight subjects with a BMI of 18 kg/m^2 (comparable to the labourers, *n* 10) and normal weight subjects with a BMI of 18 kg/m^2 (*n* 7). Nutritional status was assessed by anthropometry, including skinfold thickness from which fat free mass (FFM) was calculated using the equations of Durnin & Womersley (1974). Oxygen consumption was measured by indirect calorimetry using the ventilated hood method. NA infusions were administered initially as a 30 min dose response protocol based on FFM (i.e. 0·05, 0·1, 0·15 μg/kg FFM per min) with rest periods of 60 min between each dose. Thermogenic response was assessed as the mean increment over the baseline O_2 consumption. For the initial two doses (i.e. 0·05 and 0·1 μg/kg FFM per min) significantly lower responses were seen in the CED individuals, while the highest dose produced comparable responses in all three groups.

In order to test if the order of infusion had any effect on this pattern of response, the highest dose (0·15 μg) was infused separately, in a separate single dose protocol in ten CED subjects and compared with both underweight controls (*n* 10) and normal weight controls (*n* 6). The responses to this 60 min infusion of a single dose of NA at 0·15 μg/kg FFM per min showed that the CED labourers had significantly lower responses (60%). An analysis of the response to the highest dose of NA (0·15 μg) in both protocols showed that it was much greater in the first protocol (i.e. dose response). In order to demonstrate that the response to NA depends on prior infusions of the same agonist, we undertook a third protocol to administer the highest dose of NA (i.e. 0·15 μg/kg FFM per min) for 60 min three times in succession to the same subject with rest periods of 60 min on the same day; a protocol almost identical to the dose response protocol, in a group of eight subjects. There was a significantly greater response to NA during the third identical infusion (24·16 (SD 5·21) *v.* 8·29 (SD 6·24) for the first infusion) in all subjects investigated.

The results of this study may possibly explain why NA-induced thermogenic responses have shown differences between obese and lean controls when single doses were administered (Jung *et al.* 1979), while dose response studies (Katzeff *et al.* 1986) have been unable to demonstrate such differences.

This study was supported by the Nestlé Foundation, Switzerland. The indirect calorimetry facility was made possible by the Wellcome Trust, UK.

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The effect of a graded infusion of adrenaline on metabolic rate, forearm electromyographic activity and oxygen consumption. By I. W. GALLEN, I. A. MACDONALD, K. C. F. FONE and D. G. MAGGS, *Department of Physiology and Pharmacology, University of Nottingham Medical School, Clifton Boulevard, Nottingham NG7 2UH*

The present study examines the proposition that the thermogenic effect of adrenaline is mediated in part by skeletal muscle.

Ten healthy post-absorptive subjects (five male) were studied twice, resting supine, wearing light clothing in a room at 28°. Baseline measurements were made for 30 min followed by infusion of adrenaline (A) at rates corresponding to 12·5, 25, and 50 ng/kg per min, each for 20 min, or saline (9 g sodium chloride/l; C) in random order. Metabolic rate (MR) and respiratory exchange ratio (RER) were measured continuously. Measurements of forearm blood flow (FBF) and arterialized and effluent venous blood oxygen content (from which forearm O_2 uptake was calculated) were made every 10 min. Electromyographic (EMG) activity was recorded from three silver wire suction electrodes over flexor carpi ulnaris and later transformed using fast Fourier analysis.

Baseline MR (C 5·37 (SE 0·33), A 5·26 (SE 0·30) kJ/min) and RER (C 0·82 (SE 0·01), A 0·85 (SE 0·02)) were similar. During A infusion, MR rose above baseline by +0·19 ($P<0·001$ ANOVA), +0·51 ($P<0·001$) and +0·77 kJ/min ($P<0·001$) at 12·5, 25 and 50 ng/kg per min respectively but fell by −0·25 ($P<0·01$) in C. RER rose to 0·89 ($P<0·01$) on starting A, but then fell to baseline values. Baseline FBF was similar on both occasions (C 42 (SE 4·5), A 43 (SE 4·0) ml/l per min) and rose significantly more during A infusion (by +63·2 (SE 20) ml/l per min ($P<0·01$) than in the C infusion (+17·5 (SE 6·4) ml/l per min $P<0·01$). Baseline forearm O_2 uptake was similar (C 1·70 (SE 0·4), A 2·1 (SE 0·3) ml/l per min), and did not change with either infusion. However, this method of measurement is insensitive to alteration in distribution of FBF between skin and muscle. Baseline EMG activity was similar before C and A but increased transiently (between 8 and 24 Hz) on starting A infusion, quickly returning to baseline.

Although all rates of A infusion increased whole body MR, no increase was seen in estimated forearm muscle O_2 consumption, with only a transient increase in EMG activity. Thus, these results do not support the proposal that skeletal muscle is a major site for adrenaline-induced thermogenesis in man.

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The effect on the doubly- and triply-labelled water methods of water hydrogen incorporation into body fat in growing pigs. By PAUL HAGGARTY, BRIAN A. MCGAW, MALCOLM F. FULLER and SUSANNAH L. CHRISTIE, *Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB* and WILLIAM W. WONG, *USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Texas Children's Hospital, USA*

A basic assumption of the doubly-labelled water (DLW) and triply-labelled water (TLW) methods for measuring water flux (r_{H_2O}), CO_2 production (r_{CO_2}), and fractionated water loss (X), is that the hydrogen of body water only leaves the body as water. Any loss of isotopes in other products will introduce an error into these techniques. The body fat represents the largest potential sink for water H. ^2H sequestration into the carcass fatty acids was investigated in eight pigs labelled with ^2H_2O for 21 d. Values of r_{CO_2} were measured simultaneously in respiration chambers to allow the errors on isotopically derived r_{CO_2} to be estimated. The fat content of the diet (16·3 g/kg), level of intake and stage of maturity were all designed to give the widest possible range of sequestration effects. Four animals were restricted to their estimated maintenance requirement and four were allowed to feed *ad lib.*, giving a range of weight gain from 100 to 650 g/d.

Animal no. . . .	1	2	3	4	5	6	7	8
Average wt gain (g/d)	75	97	100	141	230	431	631	671
Carcass fatty acid synthesized (g/d)	8·3	12·5	10·3	9·8	36·8	100·3	171·0	170·1
Percent error on r_{H_2O} (%)	+0·34	+0·69	+0·36	+0·30	+1·20	+2·00	+2·66	+2·37
Percent error on r_{CO_2} (%)	−1·15	−1·73	−1·11	−1·20	−2·63	−5·06	−7·83	−7·33
Error on evaporative water loss (X)	0·03	0·06	0·03	0·02	0·10	0·16	0·21	0·19

This was reflected in the estimated error on r_{H_2O} (+0·42% in the restricted group and +2·52% in the fast-growing animals) and on r_{CO_2} (−1·30 and −7·58% respectively). The error on the calculation of fractionated water loss (X) using TLW was +0·04 units in the restricted group and +0·20 units in the fast-growing animals. Extrapolation of these values to zero weight gain indicated that there would be very little turnover of the carcass fatty acids and no significant error on either r_{H_2O}, r_{CO_2} or X in weight-stable animals. However, the errors on the isotopically derived variables of r_{H_2O}, r_{CO_2} and X can be significant during rapid growth. It would therefore be prudent to consider the likely extent of water H sequestration when interpreting isotope flux results from DLW and TLW studies when there is growth or export of tissue, particularly when this involves fatty acid synthesis.

Evaluation of dual photon absorptiometry for the assessment of body composition. By N. J. FULLER[1], A. LASKEY[2], K. F. SZAZ[2], S. A. JEBB[1] and M. ELIA[1], [1]*MRC Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL* and [2]*Department of Nuclear Medicine, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ*

Although dual photon X-ray absorptiometry (DPX) is intended primarily for the determination of total body bone mineral content, it can also provide estimates of the proportion of fat, and fat free mass within the human body (by reference to a phantom-derived calibration equation). The present study was undertaken to evaluate both the reproducibility of DPX, and its potential (relative to other simple techniques) for predicting densitometric estimates of body composition.

Percentage body fat (% fat) was estimated by means of densitometry (the 'reference' method), DPX (performed in duplicate), body mass index (BMI), skinfold thicknesses (SFT), whole body impedance (WBI), and near infra-red interactance (NIRI). The equations relevant to these methods are described elsewhere (Fuller & Elia, 1989; Elia *et al.* 1990). The group of subjects studied comprised twelve healthy female (ranges of age 21–59 years, weight 48·3–67·8 kg, height 1·56–1·78 m), and sixteen healthy male (ranges of age 18–55 years, weight 59·4–90·0 kg, height 1·64–1·88 m) volunteers. The coefficient of variation for duplicate measurements of % fat was established for DPX. The bias and 95% limits of agreement were calculated for each method against densitometry (as described by Bland & Altman, 1986).

The coefficient of variation for duplicate measurements of % fat by DPX was found to be 2·66%, which is equivalent to 0·56% fat as body-weight. Estimates of % fat obtained by DPX showed closer agreement, in general, than those obtained by the other methods, when compared with densitometry (Table). In addition, there was no apparent influence of the magnitude of the estimate on the proximity of agreement (results not shown).

Table. *The bias and 95% limits of agreement (2SD) between densitometric estimates of % body fat and those obtained by other methods*

	% fat as body-weight					
	Female (n = 12)		Male (n = 16)		All subjects (n = 28)	
Method	Bias	2SD	Bias	2SD	Bias	2SD
DPX	0·09	4·46	1·01	4·42	0·61	4·46
BMI	1·67	9·75	−1·34	7·87	−0·05	9·07
SFT	−0·31	6·46	−0·94	3·66	−0·67	4·98
WBI	2·97	7·87	2·39	7·95	2·64	7·79
NIRI	−2·12	7·26	0·98	6·71	0·35	10·07

It is concluded, firstly, that DPX is a reproducible method for the determination of body composition; and secondly, that DPX agrees more closely with densitometric estimates of % fat than do the other simple methods, apart from SFT, examined in this study.

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The use of 24 h urine nitrogen to detect bias in the reported habitual food intake of individuals assessed from weighed dietary records. By S. BINGHAM, A. WELCH, A. CASSIDY and S. RUNSWICK, *Medical Research Council, Dunn Clinical Nutrition Centre, Cambridge CB2 1QL* and C. GILL, *MRC Biostatistics Unit, Cambridge CB2 2BW* and K. T. KHAW, *Clinical Gerontology Unit, Addenbrooke's Hospital, Cambridge CB2 2QQ*

All methods of determining the habitual diet of free living individuals rely on information supplied by the subjects themselves, and so the validity of results is uncertain. In individuals in energy and nitrogen balance, urine N as assessed from 8 d of complete 24 h urine collections is an objective measure of protein intake, with the expected ratio of urine N:dietary N of 81 (SE 5)% (Bingham & Cummings, 1985). We have therefore used this to validate estimates of habitual diet from weighed records kept by seventy-nine free living women aged 50–65 years over the course of 1 year. The weighed records were obtained using the PETRA (Portable Electronic Tape Recorded Automatic) dietary scales (Cherlyn Electronics, Cambridge).

At each of the four seasons 4 d of PETRA records were obtained, together with body-weight and two 24 h urine collections validated for their completeness using PABAcheck (Bingham & Cummings, 1983). The average N intake from the 16 d of records was then compared with the average N excretion in the complete 24 h urine collections. Thirty-four individuals excreted more than 91% of their reported dietary N intake in urine, and were classified as 'under-reporters'. Of these eight lost from 2 to 4 kg body-weight over the year, but seven gained 2–4 kg. No changes occurred in the other nineteen subjects.

The Table compares daily dietary intake results and body-weight for the thirty-four under-reporters and forty-five other subjects.

		MJ	Protein (g)	Fat (g)	Alcohol (g)	Sugars (g)	Starch (g)	NSP (g)	Wt (kg)
Under-reporters	Mean	7·01	65	65	9	92	103	14	75
(*n* 34)	SD	1·22	11	15	10	29	25	6	13
Others (*n* 45)	Mean	8·70	73	86	9	127	112	15	62
	SD	1·24	11	14	16	32	24	5	8
P		<0·001	<0·001	<0·001	NS	<0·001	NS	NS	<0·001

NS, not significant.

The under-reporters were significantly heavier than the other subjects and significant differences were evident between average reported intakes of energy, protein, fat and sugars from records obtained from under-reporters and those subjects whose records appeared to be valid from the 24 h urine N. There were no differences in alcohol, non-starch polysaccharide (NSP) and starch consumption. Under-reporting by a proportion of individuals within a group leads to bias in overall average intakes for some, but not all, nutrients and an artefactual extension of the range of individual values. Under-reporting is more likely to occur in overweight individuals but is not detectable by conventional methods of dietary assessment.

The dietary studies were partially supported by the Cancer Research Campaign.

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Chronic undernutrition in very-low-birth-weight infants who develop bronchopulmonary dysplasia. By D. C. WILSON[1], B. G. MCCLURE[2], H. L. HALLIDAY[2], M. MCCREID[1] and J. A. DODGE[2], [1]*Neonatal Unit, Royal Maternity Hospital, Belfast* and [2]*Department of Child Health, The Queen's University of Belfast, Grosvenor Road, Belfast BT12 6BJ*

The aim of the present study was to discover if undernutrition is a problem in sick, small, preterm infants who develop bronchopulmonary dysplasia (BPD). A retrospective study of all very-low-birth-weight (VLBW) infants managed in the Royal Maternity Hospital nursery for the first month of life, between 1 January 1987 and 31 December 1988, was performed. Energy intake on days 7, 14, 28 and 56, and outcome data were obtained from ninety-seven infants.

We compared two groups: infants who developed BPD, and gestational age-matched controls who required mechanical ventilation for at least the first three postnatal days but did not develop BPD.

	n		Gestational age (d)	Birth wt (g)	Energy intake (kJ/kg per d)			
					Day 7	Day 14	Day 28	Day 56
No BPD	22	Mean	27·4	1015	323	368	388	456
		SD	1·7	170	95·0	61·1	81·2	63·6
BPD	22	Mean	26·9	839	279	322	357	365
		SD	1·7	229	72·8	60·7	54·0	66·1
Significance			NS	P=0·05	NS	P=0·05	NS	P=0·0001

NS, not significant.

An energy intake of 500 kJ/kg per d has been recommended for premature infants to achieve satisfactory growth (American Academy of Pediatrics, 1985). We were unable to meet this guideline due to the need for fluid restriction, dextrose intolerance, frequent periods of lipid-free alimentation, and prolonged time to establish full enteral feeding. At discharge, 72% of infants with BPD had a weight less than the 3rd centile and 55% had a head circumference less than the 3rd centile, compared with 45% and 18% respectively in infants who did not develop BPD.

Limited energy intake is a major problem present in both the acute period of respiratory illness when BPD is developing, and also in the later period when infants with BPD remain ventilator-dependent. We presume this reduced energy intake to contribute to poor growth compounded by both the low energy reserves in VLBW infants, and also by increased energy expenditure due to clinical distress.

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Fruit consumption and lung function. By B. D. COX, D. E. WALTERS, SHARON E. ERZINCLIOGLU and MARGARET J. WHICHELOW, *Department of Community Medicine, University of Cambridge School of Clinical Medicine, Fenner's, Gresham Road, Cambridge CB1 2ES*

Previous studies on the Health and Lifestyle Survey (Cox *et al.* 1987) data showed that amongst correlations with other health measures, fresh fruit consumption was associated with good lung function (Whichelow & Treasure, 1990). More detailed investigation of the relationships between forced expiratory volume in 1 s (FEV_1) and fruit and vegetable intake have now been carried out in non-smokers and current regular cigarette smokers, with no history or evidence of respiratory disease. The closest association with lung function was with the consumption of fresh fruit in winter at least once a week and/or drinking fruit juice more often than 'rarely'. The factors in the non-orthogonal analyses of variance were smoking habit, socio-economic group (non-manual and manual) and fruit/juice consumption. Within the 10 year cohorts, corrections for age and height were made using covariance analysis. These analyses yielded estimated mean FEV_1 values for each factor/level, corrected for all the other factors. The mean FEV_1 values calculated both as the 'observed' and as the more usually presented 'proportion of the predicted' for persons of a given height and age were consistently higher for respondents with high fruit/juice consumption than those with low consumption (see Table). An association between FEV_1 and fruit in summer, and salads and vegetables was found, but was much less close.

Table. *Comparison of two metrics of ventilatory function illustrating the association of consumption of fruit/juice with lung function standardizing for age (within decades), social class and smoking habit*

	Proportion of predicted FEV_1				Observed FEV_1 (ml)				
Fruit/juice consumption . . . Low		High	Difference	%	Low	High	Difference	%	*n*
Age (years)									
				Men					
20–29	0·976	0·993	0·017	1·73	3898	3968	70	1·78	378
30–39	0·958	1·001	0·043*	4·39	3640	3793	153*	4·12	294
40–49	0·927	0·945	0·018	1·92	3228	3295	67	2·05	188
50–59	0·888	0·902	0·014	1·56	2800	2856	56	1·98	139
60–69	0·890	0·912	0·022	2·44	2482	2519	37	1·48	113
				Women					
20–29	0·937	0·979	0·042*	4·28	2755	2855	100	3·56	476
30–39	0·946	0·956	0·010	1·05	2645	2666	21	0·79	475
40–49	0·942	0·967	0·025	2·62	2418	2485	67	2·73	330
50–59	0·893	0·963	0·070**	7·54	2024	2187	163**	7·74	272
60–69	0·856	0·951	0·095*	10·52	1628	1802	174*	10·15	194

Difference between low and high fruit/juice consumption: $*P<0·05$, $**P<0·01$.

Cox, B. D., Blaxter, M., Buckle, A. L. J., Fenner, N. P., Golding, J. F., Gore, M., Huppert, F. A., Nixon, J., Ross, M., Stark, J., Wadsworth, M. E. J. & Whichelow, M. J. (1987). *The Health and Lifestyle Survey*. London: Health and Promotion Research Trust.

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Food frequency *v.* weighed intake data in Scottish men. By C. BOLTON-SMITH, *Cardiovascular Epidemiology Unit, Ninewells Hospital and Medical School, Dundee DD1 9SY* and A. C. MILNE, *Human Nutrition Unit, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB*

A food frequency questionnaire (FFQ) developed and validated against weighed intakes (WI) in a Welsh population (Yarnell *et al.* 1983), was used (largely unchanged) in the Scottish Heart Health Study (SHHS) (Smith *et al.* 1989) in 10 359 men and women aged 40–59 years. A validation of this FFQ in the Scottish population was undertaken in forty Aberdeenshire men, mean age 38 (range 22–57) years, who had, 1 year earlier, completed 14 d of WI. Thirty-seven men satisfactorily completed the FFQ. Analysis was by computer program using standard food tables (Paul & Southgate, 1978).

	Nutrient intake/d					% Energy/nutrient density (ND)†					FFQ *v.* WI (% difference in mean % energy and ND values)	
	WI			FFQ		WI			FFQ			
	Mean	SE	*r*	Mean	SE	Mean	SE	*r*	Mean	SE	Scotland	Wales‡
Energy (MJ)	10·83	0·35	0·32*	8·64	0·47						−20·2	−27·3
Fat (g)	101	4·2	0·46**	76·9	5·9	35·2	1·0	0·71***	32·9	1·1	−6·5	+11·8
Protein (g)	96·3	3·9	0·31*	87·0	4·5	14·9	0·4	0·33*	17·1	0·5	+14·8	−5·0
CHO (g)	317	13·0	0·32*	254	15	45·8	1·0	0·60***	46·1	1·1	+0·7	−19·8
Starch (g)	199	8·0	0·42**	167	12	28·9	0·7	0·45**	30·0	1·1	+3·8	−24·8
Sugar (g)	117	7·0	0·18	87	4·0	16·9	0·8	0·40**	16·2	0·7	−4·1	−4·8
Alcohol (g)	13·6	2·7	0·64***	10·7	1·6	3·6	0·7	0·68***	4·0	0·6	+11·1	+48·0
Fibre (g)	27·8	1·7	0·35*	26·1	1·6	11·1	0·8	0·55***	12·9	0·6	+16·2	+23·5
Vit C (mg)	89·6	9·6	0·44**	71·5	4·0	35·1	3·6	0·53***	36·5	2·3	+4·0	−15·9
Retinol (μg)	697	129	0·32*	689	72	261	45	0·43**	339	33	+29·9	
β-Caro (μg)	2394	266	0·28*	3120	374	977	142	0·28*	1603	209	+64·1	
Vit E (mg)	6·7	0·4	0·27	10·2	1·5	2·64	0·1	0·44**	5·0	0·6	+89·4	

CHO, carbohydrate; Vit C, vitamin C; Vit E, vitamin E; β-Caro, β-carotene.

r, Pearson correlation coefficients on appropriately transformed (ln, $\sqrt{}$) data: $^*P<0{\cdot}05$, $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$.

† % Energy, inclusive of alcohol; ND, nutrient density, amount/4·18 MJ (1000 kcal) for fibre and vitamins.

‡ Calculated from Yarnell *et al.* (1983).

Correlations between FFQ and WI data were improved by adjustment for energy intake, as found by Willett *et al.* (1985).

From these results, the FFQ correlated with WI data better in Scotland than it did in Wales, in spite of the lower numbers (37 *v.* 119) and the longer time interval between assessments (1 year *v.* 3–6 weeks). This may be due partly to the high percentage of non-manual workers (84%) in the Scottish sample, to the age difference, and to the 14 *v.* 7 d of WI which may have provided a better estimate of long-term intake. Different dietary habits and attitudes to food may also account for the varied differences between the FFQ and WI data for the two regions.

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Nutrient intakes from current, ex- and never smokers: results from the Scottish Heart Health Study. By C. BOLTON-SMITH, M. WOODWARD, C. A. BROWN, W. C. S. SMITH and H. TUNSTALL-PEDOE, *Cardiovascular Epidemiology Unit, Ninewells Hospital and Medical School, Dundee DD1 9SY*

Smoking may increase coronary risk by enhancing pro-thrombotic factors, by increasing free radical production, and by acting in conjunction with other aspects of unhealthy life-style, such as lack of exercise and a poor diet. While the diet of male smokers has been investigated (Fehily *et al.* 1984), other studies have been smaller and no distinction made between never and ex-smokers (Fulton *et al.* 1988; Whichelow *et al.* 1988). Food frequency questionnaire data (Yarnell *et al.* 1983) collected in the Scottish Heart Health Study (Smith *et al.* 1989), were analysed in order to determine whether nutrient intakes do differ between current (C) (*n* 1994 men and 2135 women), never (N) (*n* 1083 men and 2117 women) and ex- (X) (*n* 1301 men and 1062 women) cigarette smokers.

Group . . .	Men						Women					
	N		X		C		N		X		C	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total energy (MJ)	9·28	2·32	9·55	2·39	10·54	2·69	7·30	1·83	7·22	1·85	7·60	2·18
Total fat (% En)	35·4	5·6	34·8	5·7	34·3	6·1	39·5	5·9	38·7	6·3	39·9	6·2
SFA (% En)	15·6	3·3	15·2	3·4	15·2	3·5	17·8	3·7	17·3	3·9	18·2	3·8
PUFA (% En)	4·8	2·1	4·7	1·9	4·1	1·6	5·0	2·2	5·0	2·2	4·4	1·9
Protein (% En)	15·5	2·1	15·4	2·2	14·5	2·2	17·4	2·7	17·5	2·8	16·8	2·8
Starch (% En)	28·4	5·7	28·0	5·9	26·3	6·2	25·5	5·3	25·2	5·4	24·0	5·7
Sugar (% En)	15·6	4·5	15·9	5·1	17·7	6·0	15·7	4·3	15·4	4·4	16·4	6·6
Alcohol (% En)	5·0	5·7	6·0	6·2	7·1	7·0	1·9	2·9	3·1	3·6	3·0	3·8
Fibre (g ND)	10·4	3·5	10·0	3·5	7·9	2·5	11·8	4·0	12·2	4·2	9·8	3·7
Vit C (mg ND)	26·5	11·9	25·1	12·1	20·1	9·1	33·3	17·1	34·2	18·5	26·7	15·1
Retinol (μg ND)	319	167	309	158	324	174	397	209	399	214	411	238
β-Caro (μg ND)	1577	1117	1473	1012	1249	919	2069	1471	2186	1524	1836	1485
Vit E (mg ND)	3·7	2·8	3·5	2·4	2·7	1·8	4·1	2·9	4·2	3·0	3·3	2·4
PUFA:SFA	0·27	0·33	0·27	0·32	0·23	0·30	0·26	0·27	0·27	0·30	0·22	0·26

% En, energy inclusive of alcohol; ND, nutrient density (amount/4·18 MJ); SFA, saturated fat; PUFA, polyunsaturated fat.

Data ln or transformed, and standardized for age and social class before analysis of variance. For both sexes, differences between the groups were significant ($P<0·01$) for all the nutrients except for retinol ($P>0·02$). The raw data are presented in the Table.

Vit C, vitamin C; β-Caro, β-carotene; Vit E, vitamin E.

Clear differences in diet, other than the higher energy intakes, were evident between the C and N/X groups. For most nutrients, %En and NDs were similar for the N and X groups. Increased health awareness or reversion to pre-smoking taste preferences may account for these similarities. Although these effects were independent of age and social class, cross-sectional data can only imply that a change in diet occurs as a result of a change in smoking habit.

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In vivo measurement of changes in body composition: validation against 12 d continuous whole-body calorimetry. By SUSAN A. JEBB, W. A. COWARD, P. R. MURGATROYD, GAIL R. GOLDBERG and A. M. PRENTICE, *MRC Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL*

The accuracy of in vivo body composition techniques for the measurement of absolute body composition can only be confirmed by cadaver analysis, however changes in body composition may be independently assessed by energy balance studies. Such techniques have a high degree of accuracy relative to that of the body composition methodology. In the present study measurements were made of body density, total body water, total body potassium, skinfold thicknesses and whole-body resistance/impedance. Changes in body fat estimated by these techniques, and using a combinaton of volume (density) and water measurements in a three-compartmental model (Siri, 1961; Murgatroyd & Coward, 1989) were compared with the change calculated from energy and nitrogen balances.

Six male subjects (mean age 31 years, weight 72·74 kg, height 1·79 m, body mass index (BMI) 22·6 kg/m^2) were either over- or underfed for 12 d, whilst confined to a 30 m^2 whole-body calorimeter. Substrate oxidation was calculated using the constants of Elia & Livesey (1988). Overfeeding diets provided 50% in excess of the subjects' energy requirements; underfeeding diets contained 3·5 MJ/d. All urine and faecal samples were collected individually and analysed for energy and N. Samples of duplicate diets were also analysed.

Mean weight changes were 2·90 (SE 0·97) kg during overfeeding and −3·47 (SE 0·69) kg during underfeeding. The change in body fat mass measured by each of the methods was compared with energy and N balances using the method of Bland & Altman (1986). The results are shown in the Table.

Method	Bias* (kg)	SD (kg)
Density	−0·28	0·95
Total body water	0·33	1·48
Total body K	−1·01	2·15
Siri (1961) (eqn 21)	0·04	0·89
Murgatroyd & Coward (1989)	0·00	0·75
Skinfolds (Durnin & Womersley, 1974)	−0·59	0·58
Resistance (Valhalla Scientific Inc.)	−0·01	1·41
Impedance (Holtain Ltd)	0·65	2·97
BMI formula (Black *et al.* 1983)	−0·36	0·36

SD, standard deviation of the differences between methods.

* Bias is the mean difference between methods (energy balance − alternative method).

In comparison with energy and N balances a three-compartmental model showed the least bias and the greatest precision. The smallest change in body fat mass which can be accurately measured by such a method is 1·5 kg (2 × SD of the differences). Of the simple prediction techniques the body mass index formula and skinfold thicknesses appear to be more precise than the equations based on resistance or impedance.

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Does consumption of alcohol with a meal promote fat storage? By B. J. SONKO, G. R. GOLDBERG, P. R. MURGATROYD, W. A. COWARD and A. M. PRENTICE, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

Social eating often encourages the consumption of alcohol in conjunction with excess energy. The present study simulated a typical 'business lunch' to test whether the obligatory oxidation of alcohol suppresses fat oxidation and thereby promotes fat storage.

Five healthy male volunteers received three different meals on separate occasions. Meal A was the control diet, for meal B 38 g ethanol replaced 50% of the carbohydrate (CHO) isoenergetically, and meal C was the control diet with 38 g ethanol added. Post-prandial substrate oxidation was monitored for 20·5 h by whole-body indirect calorimetry, assays of breath alcohol and mass-spectrometric analysis of $^{13}CO_2$ in breath. Test meals contained natural maize oil enriched with [1-^{13}C]palmitic acid in order to assess exogenous fat oxidation. A second meal (0·33 × maintenance energy expenditure (1·4 × basal metabolic rate, MEE)) with the same nutrient composition as meal A was provided to all subjects 7 h after the test meal.

Protocol . . .		A		B		C	
Energy content (kJ) . . .		0·5 × MEE		0·5 × MEE		0·5 × MEE + 0·115 × MEE alcohol	
Fat:CHO:protein:ethanol§ . . .		40:46:14:0		40:23:14:23		34:36:12:18	
	Period of experiment (h)	Mean	SD	Mean	SD	Mean	SD
Exogenous [^{13}C]fat oxidation (g)	0–6	2·75	0·84	1·56	2·10	1·06**	1·13
	6–20·5	5·42	1·74	8·21*	2·64	5·71	2·02
	0–20·5	8·16	2·51	9·77	4·52	6·78	3·04
Total fat oxidation (g)	0–6	15·96	2·79	17·57	5·11	15·05	4·23
	6–20·5	33·30	7·28	45·78**	7·02	33·64	10·68
	0–20·5	49·26	8·41	63·35†	10·30	48·68	13·45
Total CHO oxidation (g)	0–6	89·90	7·79	50·46***	4·02	62·93***	7·40
	6–20·5	166·42	19·53	153·74	14·36	181·65‡	18·07
	0–20·5	251·32	26·79	204·20**	18·14	244·58‡	25·28

Significantly different from protocol A (paired *t* test): *$P<0·05$, †$P<0·02$, **$P<0·01$, ***$P<0·001$.
Significantly different from protocol B (paired *t* test): ‡$P<0·01$.
§ Expressed as % energy.

The alcohol load was completely oxidized within 6 h, providing 30% of total energy over this period. Between 0 and 6 h there was a small and marginally significant suppression in the oxidation of exogenous fat ingested with the alcohol, but there was no suppression of endogenous or total fat oxidation at any time. In contrast, post-prandial CHO oxidation (0–6 h) was suppressed on protocol C and further suppressed by the combination of the alcohol load and a reduction in CHO intake on protocol B. Following the second meal fat oxidation was substantially increased on protocol B, but not on protocol C suggesting that it was stimulated by glycogen depletion secondary to the reduced CHO intake in the test meal.

In summary, there is no evidence that alcohol consumption promotes short-term fat storage and when it replaces CHO it may actually stimulate oxidation. In this study the experimental design resulted in depleted liver glycogen stores before the business lunch. A different response might occur when hepatic glycogen is replete.

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The effect of previous feeding patterns on the oxidation and tissue [^{14}C]lipid accumulation after a meal containing [1-^{14}C]triolein in the rat. By ALISON E. TEDSTONE, VERA ILIC and DERMOT H. WILLIAMSON, *Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE*

The *de novo* synthesis of lipid in liver and white adipose tissue (WAT) in response to a meal of chow (after 12 h starvation) is higher in rats adapted to meal-feeding than in rats fed *ad lib.* (Pallardo & Williamson, 1989). This suggests that meal-feeding alters adipose tissue metabolism to promote fat deposition. To test this possibility we have examined the effects of meal-feeding on the disposal of a high fat (200 g/kg) diet containing [1-^{14}C]triolein.

Male Wistar rats were either allowed free (*ad lib.* group) or restricted (09.00–12.00 hours, meal-fed group) access to a chow diet (g/kg: 520 carbohydrate, 210 protein and 40 fat; residue non-digestible material) for 12 d. At the time of the experiment the food intake of the meal-fed group was 85% of that of the *ad lib.*-fed rats. The rats were starved for 21 h and then given 5 g chow in which 0·75 g [1-^{14}C]triolein (15% by wt, 0·3 μCi) had been incorporated and the mixture pelleted. Measurements of expired $^{14}CO_2$ (over 5 h), the accumulation of [^{14}C]lipid in tissues and the amount of [1-^{14}C]triolein absorbed were as described by Oller do Nascimento & Williamson (1986).

Feeding pattern		$^{14}CO_2$ production†	[^{14}C]lipid accumulation‡		
			Liver	WAT	Brown-adipose tissue
Ad lib. (*n* 5):	Mean	47·8	0·73	0·32	15·7
	SE	3·3	0·13	0·04	6·9
Meal-fed (*n* 4):	Mean	27·2*	1·33*	5·96***	22
	SE	6·4	0·14	0·96	5·3

Significantly different from *ad lib.* group: *P<0·05, ***P<0·001.
† % of absorbed [^{14}C]lipid/5 h.
‡ % of absorbed [^{14}C]lipid/g tissue per 5 h.

There was a 48% decrease in $^{14}CO_2$ production in meal-fed rats and this was accompanied by increased accumulation of [^{14}C]lipid in WAT (about twenty-fold) and liver (about two-fold). However, there was no significant difference in carcass weights or fat content (*ad lib.*: 6·9 (SE 1·2)%, *n* 5; meal-fed: 6·9 (SE 0·25)%, *n* 4).

These findings suggest that meal-feeding results in greater conservation of dietary lipid than *ad lib.* feeding, at least during the absorptive phase, but this does not necessarily lead to increased adiposity.

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The effect of ageing on glutamine metabolism in skeletal muscle of the rat. By M. PARRY-BILLINGS, B. LEIGHTON, G. D. DIMITRIADIS, J. BOND and E. A. NEWSHOLME (Introduced by D. H. WILLIAMSON), *Cellular Nutrition Research Group, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU*

Glutamine is taken up from plasma and utilized at a high rate by cells of the immune system and is essential for the normal function of these cells (Szondy & Newsholme, 1989). A major source of plasma glutamine is skeletal muscle: glutamine is synthesized in and released from muscle and the release process appears to be most important in control. In the elderly, muscle mass may decrease by up to 50% (Tzankoff & Norris, 1977) and there is an impairment of immune function, with infectious diseases representing major causes of morbidity and mortality (Yoshikawa, 1984). We have, therefore, investigated the effect of ageing on the metabolism of glutamine by skeletal muscle.

Soleus muscles were prepared and incubated from male Wistar rats aged 7, 13 and 85 weeks and from male Sprague-Dawley rats aged 5 and 13 weeks. The concentration of glutamine in muscle and the rate of glutamine release from muscle was decreased in older rats. However, the plasma concentration of glutamine was not affected by the ageing process.

		Glutamine concentration				Soleus, rate of glutamine release (nmol/min per g)	
		Gastrocnemius (μmol/g)		Plasma (μM)			
Strain	Age (weeks)	Mean	SE	Mean	SE	Mean	SE
Wistar	7	3·65	0·11	895	31	42·6	1·5
	13	2·95*	0·10	951	31	29·0**	1·2
	85	2·47**	0·10	832	42	32·7**	2·4
Sprague-Dawley	5	nd		1017	74	47·5	3·1
	13	nd		907	52	36·3*	1·5

nd, not determined.
*$P<0·01$, **$P<0·005$, $n<7$ for all observations.

It is suggested that the lower rate of glutamine release from skeletal muscle in addition to a reduced muscle mass in old age may decrease the supply of glutamine to cells of the immune system and thus result in a failure to provide optimal conditions for the control of the response of these cells during illness and injury. Indeed, the elderly may be particularly at risk from failure of the immune system when there is a need to respond to an immune challenge (Young *et al.* 1982).

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Lysine metabolism in young turkeys fed on diets containing different levels of lysine. By C. LINDA SAUNDERSON and PAUL E. WAIBEL*, *AFRC Institute for Grassland and Animal Production, Poultry Department, Roslin, Midlothian EH25 9PS*

Lysine is an essential amino acid required in the diet of animals. Studies using rats have suggested that the oxidation of lysine is significant only after the requirement level in the diet has been met and that oxidation continues to increase as the lysine content of the diet is raised above the requirement (Brooks *et al.* 1972).

To find out whether this phenomenon was also shown by poultry, we examined lysine metabolism in young turkeys given diets varying in lysine content. Turkey poults obtained at 1 d old were reared on a maize–soya type starter feed. At 2–3 weeks of age, six groups of six poults of similar body-weights were chosen, placed in individual cages and given one of six diets (A–F) in a Latin square design. Diets A–E consisted of a lysine-deficient basal ration containing maize, sesame meal, maize gluten meal and vegetable oil to which was added free lysine in varying amounts to give lysine contents of 7·5, 10·3, 13·7, 17·1 and 25·6 g/kg feed respectively. Diet F, the starter feed, contained 16·7 g lysine/kg feed. Each diet was given to the poults for 4–5 d before the experiment. The poults were then given [U-^{14}C]lysine (10 μCi/kg body-weight) by tube into the crop and placed in a sealed metabolism chamber. $^{14}CO_2$ exhaled was collected for 4 h and then the poult was removed from the chamber and killed by cervical dislocation. Body tissues were rapidly removed, weighed and frozen. Excreta were collected and dried. Radioactivity in CO_2, tissues and in aqueous extracts of dried excreta was measured.

Growth of the poults on diets A–E showed a typical dose-response relationship between lysine content and body-weight gain. Radioactivities (mean values) in exhaled CO_2 and in tissues are shown in the Table.

		Radioactivity (% of dose of [U-^{14}C]lysine given)					
Diet	*n*	$^{14}CO_2$	Excreta	Liver	Breast muscle	Heart	Duodenum
A	5	3·54	1·58	1·35	2·68	0·13	2·05
B	6	8·67	4·91	2·12	4·06	0·25	2·88
C	6	4·02	1·07	3·36	6·87	0·33	3·67
D	6	3·23	1·01	3·40	11·18	0·42	2·52
E	6	7·19	1·05	2·97	8·59	0·37	2·83
F	6	6·71	4·48	2·05	4·60	0·22	1·57

The results show no simple relationship between $^{14}CO_2$ or excreta ^{14}C and lysine level in the diet. There was, however, an increase in the ^{14}C retained in the liver, breast muscle and heart tissues as the lysine in the diet was increased to 17·1 g/kg (diet D).

These results suggest the $^{14}CO_2$ produced from [U-^{14}C]lysine is not an accurate index of the efficiency with which lysine is used for tissue growth or an index of the requirement of the growing poult for lysine in the diet as had been suggested for the rat (Brookes *et al.* 1972). However, as the recoveries of ^{14}C ranged from 11 to 22%, the results have to be interpreted with some caution.

We are grateful to Mr. J. MacKinlay for excellent technical assistance in these experiments.

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The effect of dietary amino acid imbalance on respiration in chick liver. By N. S. JESSOP, G. ALLAN, G. WALKER and J. P. F. D'MELLO, *Edinburgh School of Agriculture, University of Edinburgh, West Mains Road, Edinburgh EH9 3JG*

Diets containing an imbalance of amino acids have been shown to reduce feed intake and also to cause perturbations in amino acid metabolism within the liver, the most notable being an increase in the rate of protein synthesis (Harper *et al.* 1970). Uptake of certain amino acids is linked to Na^+,K^+-ATPase activity and therefore the use of imbalanced diets may result in changes in the activity of this enzyme in liver tissue. The purpose of this study was to explore this issue.

Sixteen chicks (6-d-old) were randomly allocated to one of four groups and for 14 d were given diets, *ad lib.*, which varied in their amino acid content. The crude protein (CP, nitrogen ×6·25) content of two diets, one of which was supplemented with pure tryptophan, was increased from 230 g/kg dry matter (DM) by addition of gelatin at the expense of glucose. All diets were deficient in tryptophan. At the end of this time birds were killed and liver snips were rapidly prepared and placed in minimal essential medium. Tissue oxygen consumption was measured, polarographically at 37°, for 15 min in this buffer at which time ouabain, an inhibitor of Na^+,K^+-ATPase, was added to give a final concentration of 10^{-4}M and respiration was measured for a further 15 min. The results are shown in the Table.

CP in diet (g/kg DM)	Tryptophan content (g/kg DM)	DM intake (g/chick per d)		Tissue respiration rates (nmol O_2/min per mg protein)							
				Total		Ouabain insensitive		Ouabain sensitive		% inhibition	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
230	1·32	21·89	1·56	2·34	0·09	1·88	0·06	0·46	0·09	0·20	0·01
230	1·54	29·34	1·73	1·83	0·28	1·49	0·20	0·34	0·19	0·18	0·03
330	1·22	14·64	1·74	2·12	0·14	1·55	0·13	0·58	0·09	0·27	0·04
330	1·44	16·60	1·69	1·73	0·11	1·30	0·11	0·43	0·07	0·25	0·02
Main effects:											
Protein		**		—		—		—		*	
Tryptophan		*		*		*		—		—	

*P<0·05, **P<0·01.

The results show that at both levels of dietary CP, total and ouabain-insensitive respiration were lower when tryptophan was added. This may reflect a reduction in the energetic cost of protein synthesis but it also may reflect changes in the cost of uric acid synthesis since, as tryptophan becomes less limiting, so a greater proportion of the amino acid intake may be utilized for protein synthesis by extra-hepatic tissue. As a proportion of total respiration, ouabain-sensitive respiration was higher in the livers of birds fed on diets containing 330 g CP/kg DM. On these diets a greater proportion of the amino acid intake would be deaminated and hence the energetic cost of transporting these extra amino acids into hepatocytes would result in a higher proportion of cellular energy being consumed by Na^+,K^+-ATPase activity.

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The influence of glycine and cysteine supplementation on the metabolic response to *Escherichia coli* endotoxin in rats fed on diets of marginal protein adequacy. By R. F. GRIMBLE, R. HAILWOOD, A. A. JACKSON and C. PERSAUD, *Department of Human Nutrition, Southampton University Medical School, Southampton SO9 3TU*

The metabolic response to inflammatory agents is characterized by stimulation of the immune system and enhanced turnover of tissue protein and trace elements. These changes may increase the demand for specific amino acids. In particular, glycine and cysteine, which occur in high concentrations in acute-phase proteins, and glutathione (Grimble, 1989). We investigated the effect of supplementation of a marginally adequate protein diet (100 g casein/kg) with isonitrogenous amounts of glycine (Gly; 5 g/kg), L-cysteine (Cys; 8 g/kg), an equimolar mixture of Gly and Cys (2·5 g Gly and 4 g Cys/kg) or alanine (Ala; 6 g/kg) on the response of male Wistar rats to *Escherichia coli* endotoxin (E; 1 mg/kg intraperitoneally Sigma TCA extract strain 0127:B8) or sterile non-pyrogenic saline (9 g sodium chloride/l, S). Diets were given for 1 week before injection. Growth rates, from an initial weight 149 (SE 1) g, of 3·3, 3·1, 6·1 and 6·2 g/d were observed for the Ala, Gly, Gly/Cys and Cys groups respectively. Animals were killed 24 h after injection. Saline-injected animals were pair-fed to intakes of groups given E during this period. Liver was assayed for reduced glutathione (GSH), protein and zinc; whole blood for haemoglobin (Hb) and GSH, and spleen for protein and Zn.

Diet . . .	Ala		Gly		Gly/Cys		Cys	
Injected . . .	E	S	E	S	E	S	E	S
Blood GSH/Hb (mg/g)	4·8	4·5	4·4**	3·2	4·0	4·3	4·0**	2·5
Liver								
GSH (mg/g)	18·0**	10·8	12·8**	6·1	26·9**	10·6	36·7**	20·4
Total protein (g)	1·21**	0·96	0·96	1·02	1·50	1·34	1·58**	1·05
Total Zn (μg)	361	276	267	287	389**	279	501**	271
Spleen								
Total protein (mg)	99	85	80	81	130	117	135**	94
Total Zn (μg)	16·3	12·9	11·9	11·1	23·5*	16·4	20·8**	15·0

n 5.
Significantly different from saline group (Student's *t* test): $*P<0·05$, $**P<0·01$.

Cys supplementation resulted in the most comprehensive response to endotoxin. Liver and blood GSH concentrations became elevated. Increases in total Zn and protein contents of liver and spleen also occurred. The Ala group showed a limited response of an increase in liver GSH and protein. The results illustrate the importance of Cys in the metabolic response of visceral organs to endotoxin.

Grimble, R. F. (1989). *European Journal of Clinical Nutrition* **43**, 217–230.

Glycine and cysteine supplementation influences weight gain and liver glutathione, protein and zinc of rats fed on diets of marginally inadequate protein content. By R. F. GRIMBLE, A. A. JACKSON and C. PERSAUD, *Human Nutrition Department, Southampton University Medical School, Southampton SO9 3TU*

Glycine (Gly) and cysteine (Cys) are metabolically interrelated and may be conditionally essential in situations when protein intake is marginally inadequate. Gly and Cys make up a large proportion of glutathione and liver proteins such as metallothionein, the major zinc-binding protein. We examined the effect of Gly and Cys on weight gain, and liver protein, zinc and glutathione contents in male Wistar rats (148 (SE 1) g) fed for 7 d on diets with protein contents which are thought to be marginally inadequate. Diets contained casein (80 or 100 g/kg) and Gly (5 g/kg) or Cys (4 or 8 g/kg), or 2·5 g Gly plus 4 g Cys/kg. Rats fed on 80 and 100 g casein/kg diets supplemented with alanine (Ala; 6 g/kg) acted as controls for the effects of supplementation. The study included rats fed for 7 d on adequate diets containing casein (200 g/kg) and Cys (8 g/kg). Dietary additions of Gly (5 g/kg), Cys (8 g/kg), Gly plus Cys (2·5 plus 4 g/kg) or Ala (6 g/kg) were isonitrogenous.

Amino acid supplement	Dietary protein	Wt gain (g/g protein)		Tibialis wt (mg)		Liver protein (g/liver)		Liver Zn (μg/liver)		Liver GSH (mg/g)	
(g/kg)	(g/kg)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Ala (6)	80	0·69	0·22	372	12	1·15	0·06	215	12	5·42	0·20
	100	1·61	0·10	412	9	1·16	0·05	244	17	8·42	0·36
Gly (5)	80	1·15*	0·11	408*	12	1·12	0·10	189**	10	3·82**	0·10
	100	1·78	0·14	453*	16	0·85**	0·05	222	25	8·14	0·29
Cys (4)	80	2·40**	0·13	402*	8	1·36	0·06	271	64	19·8**	2·60
	100	2·43**	0·13	427	15	1·02	0·06	269	14	26·7**	3·00
Gly/Cys (2·5/4)	80	2·39**	0·12	435**	10	1·55**	0·14	190	47	17·9**	3·90
	100	2·82**	0·05	453*	16	1·75**	0·09	323*	40	21·4**	0·57
Cys (8)	80	1·95**	0·21	397	8	1·29	0·16	188	18	32·6**	1·62
	100	2·98**	0·10	463**	10	1·77**	0·05	327**	31	29·1**	0·80
	200	1·59††	0·07	464	10	1·41††	0·08	387	36	23·9††	0·88

n 5.

Significantly different from corresponding Ala-supplemented group (Student's *t* test): **P*<0·05, ***P*<0·01.

Significantly different from 100 g casein/kg + Cys (8 g/kg) group: ††*P*<0·01.

Relative to Ala, Gly increased weight gain and tibialis mass on diets containing casein at 80 g/kg but not 100 g/kg. Cys (4 and 8 g/kg) increased weight gain and tibialis weight at both levels of dietary protein. Weight gain was less when Cys was included with 200 g of casein/kg than when a marginally inadequate (80 or 100 g/kg) level was fed.

Liver protein and Zn were only increased by Cys (8 g/kg) at the higher level of dietary protein (100 g/kg). Cys also increased liver reduced glutathione (GSH) at all levels of supplementation and dietary protein. Paradoxically, the amounts of hepatic protein, Zn and GSH were the same or larger in the group receiving the 100 g casein/kg diet than in those fed on the 200 g casein/kg diet supplemented with Cys.

While Gly *per se* had no stimulatory effect on liver GSH, protein or Zn, results from the Gly/Cys group indicate that in rats consuming 100 g casein/kg diet, Gly increases the effect of Cys (4 g/kg) on Zn and protein to that achieved by Cys (8 g/kg). These results suggest that the relative dietary amounts of Gly and Cys may have important implications for hepatic amino acid and protein metabolism, being able to transform a situation of marginal protein inadequacy to one of adequacy.

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The influence of the dietary protein intake on the response of whole body protein synthesis and degradation to feeding. By G. M. PRICE and D. J. MILLWARD, *Nutrition Research Unit, London School of Hygiene and Tropical Medicine, 4 St Pancras Way, London NW1 2PE* and P. J. H. PACY and D. HALLIDAY, *Nutrition Research Group, Clinical Research Centre, Harrow, Middlesex HA1 3UJ*

In adults overall nitrogen balance is achieved through sufficient gain of body protein from food to balance the post-absorptive losses. The magnitude of the diurnal gains and losses increases with increasing protein intake (Price *et al.* 1990) and consequently raises protein needs according to the requirements model of Millward & Rivers (1988). We report here on the mechanisms by which these variable gains and losses are achieved, with measurements of the direction and magnitude of the changes in the rates of protein synthesis and degradation during the transition from fasting to feeding with increasing amounts of dietary protein. We also report the overall rate of whole body protein turnover, as indicated by the mean of the fed and fasted rates of protein degradation in healthy adult subjects.

Each subject (four male, one female) underwent a 2-week adaptation period to isoenergetic, weight-maintaining diets at three protein levels (0·36, 0·75 and 1·5 g protein/kg per d). On the 14th day amino acid kinetics were measured by a primed, continuous 8 h infusion of [1-^{13}C]leucine covering the last 4 h of a 12 h overnight fast and the first 4 h of feeding (hourly meals: 1/12 daily intake). Leucine flux was calculated from the ^{13}C-enrichment of plasma α-ketoisocaproate and leucine oxidation from total ^{13}C excretion in expired air, using [^{13}C]bicarbonate recovery rates of 76% for fasted and 90% for fed states (Wenham *et al.* 1991).

Protein intake (g/kg per d) . . .	0·35		0·75		1·50	
	Mean	SD	Mean	SD	Mean	SD
Leucine turnover rate (mean fed/fasting appearance rate) (μmol leucine/kg per h)	103·4	11·8	105·7	9·4	106·0	5·8
Change (%) in response to feeding						
Synthesis	−6·6*	3·5	3·4	5·0	16·9*	7·3
Degradation	−19·9*	6·5	−26·6*	6·0	−41·4*	7·3
Oxidation	18·0	39·0	49·5	42·9	76·5*	13·1

*$P<0·05$ compared with no change.

As indicated in the Table the overall rate of whole body protein turnover, calculated as the mean of the fed and fasted rates of leucine appearance, did not change with increasing protein intake. Feeding induced leucine gain through an inhibition of proteolysis (leucine appearance) coupled with smaller changes in protein synthesis (leucine disappearance) which varied from a 6·6% inhibition at the lowest intake to a 16·9% increase at the highest intake. These results show that the mechanism of protein deposition during feeding varies according to the level of protein intake. The increasing diurnal gain with increasing protein intake occurs as a result of progressive inhibition of proteolysis and a change from inhibition to stimulation of protein synthesis by feeding.

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Assessment of protein metabolism in man with ^{15}N-labelled soya bean. By M. A. MCNURLAN, A. EL-KHOURY and E. MILNE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* and E. B. FERN, *Nestec Ltd, Lausanne, Switzerland*

Rates of protein metabolism in man often rely on assessing the kinetic behaviour of a single labelled amino acid. The use of ^{15}N-labelled amino acids, particularly glycine, has become widespread under conditions where continuous intravenous infusion of label is too invasive or too restraining. However, when [^{15}N]glycine is used, indices of protein metabolism must be inferred from the partitioning of this single amino acid between incorporation into protein and oxidation. We have sought to obtain more representative rates of whole-body protein metabolism with a uniformly labelled protein from soya bean.

Protein synthesis and degradation (mg N/kg body-weight per 9 h) have been assessed in six healthy volunteers during overnight fasting and during 9 h of feeding with [^{15}N]glycine and [^{15}N]soya bean. Separate rates were calculated from the excretion of label in urinary urea and ammonia, and the average of these two rates (end-product average) (Fern *et al.* 1981) is shown in the Table.

	[^{15}N]Glycine				[^{15}N]Soya bean			
	Fasting		Feeding		Fasting		Feeding	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Synthesis	158	8	240†††	8	328***	15	447***†††	15
Degradation	190	10	176	8	361***	16	380***†	14

Glycine *v.* soya bean (paired *t* test): ***$P<0{\cdot}001$.
Fasting *v.* feeding (paired *t* test): †$0{\cdot}01<P<0{\cdot}05$, †††$P<0{\cdot}001$.

Rates of protein synthesis and degradation from [^{15}N]soya bean were significantly higher than rates determined with [^{15}N]glycine in both fasted and fed states. However, both tracers showed a similar stimulation of protein synthesis with feeding (40–50%) with little or no change in protein degradation. Thus the conclusions from [^{15}N]soya bean data are comparable to those from [^{15}N]glycine, suggesting that [^{15}N]soya bean may be a useful tracer for studies on whole-body protein metabolism where a simple end-product method is appropriate. Moreover, [^{15}N]soya bean may be preferable in some situations where [^{15}N]glycine is not appropriate; for example, in young growing children who may have a specific glycine requirement (Jackson *et al.* 1981).

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Jackson, A. A., Shaw, J. C. L., Barber, A. & Golden, M. H. N. (1981). *Pediatric Research* **15**, 1454–1461.

Bicarbonate recovery: feeding *v.* time. By D. WENHAM, P. J. PACY and D. HALLIDAY, *Nutrition Research Group, Clinical Research Centre, Harrow HA1 3UJ* and G. M. PRICE and D. J. MILLWARD, *Nutrition Research Unit, London School of Hygiene and Tropical Medicine, 4 St Pancras Way, London NW1 2PE*

The use of primed continuous infusion of L-[1-^{13}C]leucine to trace whole body protein metabolism is well established. The two primary measurements with this approach are flux and oxidation, with synthesis derived from their difference. Calculation of oxidation of any ^{13}C-labelled substrate requires a correction factor to account for retention of ^{13}C label within the body bicarbonate (HCO^{-}_{3}) pool. In a variety of physiological and pathological states the correction factor most frequently used has been 0·81 (Allsop *et al.* 1978). The validity of this figure has been questioned with the demonstration of higher recoveries of HCO^{-}_{3} during feeding (84%) compared with fasting (72%). In both studies (Garlick *et al.* 1987; Hoerr *et al.* 1989) feeding immediately followed fasting, raising the possibility that the difference in recovery reflected duration of infusion rather than an actual influence of food. The present studies were designed to differentiate between these factors.

Five healthy subjects (four male, one female, age 38 (SD 14) years, body mass index 23 SD 3) kg/m^2) were studied twice, both during a primed continuous infusion of $NaH^{13}CO_3$ (2 μmol/kg per h). In study A the subject remained post-absorptive for the first 4 h while during the latter 4 h they were fed hourly. The food supplied 5/12 of daily energy of a weight-maintaining, high-protein (1·5 g/kg per d) with 2/12 supplied initially. In study B the subject was given an identical diet for 4 h only. Total carbon dioxide production was measured continuously by indirect calorimetry, and expired breath samples were collected every 20 min for $^{13}CO_2$ enrichment measurement by isotope ratio mass spectrometry. The influence of food only on $^{13}CO_2$ enrichment was established and final enrichment values corrected accordingly. The results (% recovery of administered dose) are given in the Table.

	Study A		Study B
Subject	0–4 h	4–8 h	0–4 h
1	78	94	91
2	76	89	90
3	82	93	92
4	76	86	91
5	69	88	87
Mean (SD)	76 (5)	90 (3)	90 (2)

These results demonstrate significantly higher recovery of HCO^{-}_{3} as a result of feeding rather than reflecting duration of its infusion. This study confirms that it is inappropriate to use a universal correction factor to calculate oxidation and subsequently synthesis in studies involving the influence of feeding on protein metabolism.

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Protein conservation in the obese Zucker rat during fasting. By N. G. WIJESINGHE, D. J. MILLWARD and J. P. W. RIVERS, *Nutrition Research Unit, London School of Hygiene and Tropical Medicine, St Pancras Hospital, 4 St Pancras Way, London NW1 2PE*

Studies in obese subjects are interpreted as indicating that adaptive protein conservation occurs in fasting. However, the occurrence of specific protein conservation during fasting was questioned by Dugdale & Payne (1977). They suggested that the propensity to store or mobilize body protein during feeding and fasting, defined as the p-ratio, i.e. protein energy stored (derived)/total energy stored (mobilized), was genetically determined for individuals, the same during feeding and fasting but lower in obese compared with lean individuals. Evidence for this came from analysis of published human studies and animal studies in fasted normal and cafeteria-fed obese rats (Henry *et al.* 1988). We have extended these studies, measuring for the first time, the sequential changes in fasting nitrogen excretion and metabolic rate at thermoneutrality in Fa/− (lean) and fa/fa (obese) Zucker rats to determine both the magnitude and constancy of the p-ratio in the two phenotypes.

Measurements were made in two groups of six adult male Zucker rats. The thermoneutral temperature was determined from initial measurements of the metabolic rate and found to be between 24–32° for the obese and 28–33° for the lean animals. Subsequently both groups of rats were fasted and the metabolic rate measured at thermoneutrality using a closed circuit indirect calorimeter for 20 h/d during fasting for 5 d. Simultaneous measurements of urinary N excretion were made on urine collected in the metabolic chambers. Blood was collected at 5 d for measurement of selected hormones and metabolites.

	p-ratio (energy from protein/total energy)									
Day of fast . . .	1		2		3		4		5	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lean (Fa/−)	0·184	0·026	0·170	0·026	0·164	0·034	0·158	0·031	0·157	0·034
Obese (fa/fa)	0·159	0·025	0·116	0·019*	0·102*	0·019	0·094*	0·017	0·084*	0·011

*$P<0·05$ compared with day 2.

During the 5 d fast the mean metabolic rates and fasting urinary N losses in the Fa/− rats fell by 19·6 (SD 7·6)% and 31·8 (SD 6·3)% respectively, values which were only marginally significantly different ($P=0·0726$, paired t test), and the calculated p-ratio did not significantly change (see Table). In contrast, in the fa/fa rats metabolic rates and fasting urinary N losses fell by 18·5 (SD 8·2)% and 57·3 (SD 4·5)% $P=0·0002$) respectively, with a significant fall in the p-ratio ($P=<0·0001$). This response was accompanied by a greater fall in triiodothyronine and more pronounced increase in β-hydroxybutyrate than observed in the Fa/− animals. The disproportionate fall in N excretion compared with fuel mobilized and the consequent progressive fall in the p-ratio in the fa/fa animals to a value which was half that in the lean animals indicates adaptive protein conservation in the obese rat not observed in the lean.

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Effects of food restriction on sodium and potassium balances in pregnant rats. By M. P. VAQUERO and M. P. NAVARRO, *Instituto de Nutrición y Bromatología (CSIC), Facultad de Farmacia, Ciudad Universitaria, 28040 Madrid, Spain*

Water balance is increased in pregnancy, mainly in extracellular fluid. Therefore important changes also occur to maintain the electrolyte equilibrium. In pregnant rats, sodium and potassium balances have been reported to increase as a result of the increased food intake, with no variation in faecal Na and high urinary K levels (Churchill *et al.* 1980). However, progesterone antagonizes the urinary K loss response to aldosterone (Williams *et al.* 1977). It is suggested that inadequate dietary regimens intended to limit maternal body-weight may affect intrauterine growth.

Three groups of female Wistar rats were used: two groups, pregnant (P1) and non-pregnant (NP), were fed *ad lib.* and another pregnant group (P2) was fed on the same diet restricted to the level of NP. Body-weight and food intake as well as faecal and urinary excretions of Na and K were monitored. On the 21st day laparotomies were performed and conceptus and individual fetuses collected.

Urinary excretion of Na and K during days 18–21 and fetus Na and K contents at day 21 of pregnancy

	Urine (*n* 8)				Fetus (*n* 16)					
	Na (mg/d)		K (mg/d)		Weight (g)		Na (mg)		K (mg)	
Group	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
P1	2·1	0·1	52·5	3·4	3·8	0·1	6·6	0·2	7·5	0·3
P2	0·9*	0·2	39·4*	3·1	3·2*	0·1	5·6*	0·2	6·5*	0·3

* $P<0·05$.

Food intake, and urinary Na and K excretions, were higher ($P<0·05$) in P1 than in NP. As a result of the increased intake, more Na and K were absorbed in P1 than in NP. However, in the case of Na, higher absorption efficiency ($P<0·05$) was also observed after the 14th day. In the third week, urinary Na significantly decreased ($P<0·01$), whilst urinary K was increased ($P<0·01$). Consequently, Na balance (mg/d) was twice that of the previous 2 weeks, and K balance was reduced ($P<0·05$) in the same period. On the whole, in P1, Na balance was higher ($P<0·05$) than that in NP controls and K balance remained at the NP level.

The retention of Na and K in the body was maintained in the P2 group by reducing both Na and K urinary outputs. No significant differences were found on the placenta electrolyte levels. Nevertheless, fetuses belonging to P2 dams were smaller and had lower total Na and K contents.

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The effect of inhibiting the bio-availability of additional vitamin B_6 on nitrogen utilization in the pig. By J. N. SWART, H. P. SPANGENBERG and H. C. BARNARD, *Department of Animal Science, University of the Orange Free State, Bloemfontein, South Africa*

Vitamin B_6 is widely distributed and has various functions in the body of the pig. Pyridoxal-5-phosphate (PLP), the active form of vitamin B_6, serves as a co-enzyme in several metabolic reactions involved in amino acid metabolism, where it is attached to an E-amino group of the apoenzyme via a Schiff base linkage (Driskell, 1984). As an essential vitamin in the pig, an inhibition of the bio-availability of PLP or a deficiency in the diet of vitamin B_6 could cause a detrimental effect of amino acid metabolism and impair nitrogen utilization. The addition of vitamin B_6 to the diet, on the other hand, could have a stimulating effect on N utilization and growth. The present study was designed to assess the effect on vitamin B_6 deficiency or supplementation on N utilization in pigs.

Twelve pigs, four per group, were used in an N balance study. These animals were part of a growth study where three diets were given, namely a control diet, a diet supplemented with vitamin B_6 and a diet containing a vitamin B_6 antagonist. The control diet consisted of normal substances for typical pig growth according to Agricultural Research Council (1981) standards. Vitamin B_6 (50 mg/kg diet) was added to give the supplemented diet, while 10 mg isoniazid (a vitamin B_6 antagonist)/kg live weight per pig per d was added to the control diet to form the deficient diet.

Diet . . .	Control	Vitamin B_6 supplemented	Vitamin B_6 deficient	SD	Statistical significance: *P*
Live weight	71·65	73·25	74·65	5·09	
Vitamin B_6 intake (mg/d)	35·10[a]	159·14[b]	34·74[a]	20·44	0·001
Vitamin B_6 in urine (mg/d)	1·17[a,b]	2·42[b]	0·58[a]	0·65	0·009
N intake (g/d)	73·22	65·66	72·47	10·89	
N retention (g/d)	27·68	26·66	37·61	8·70	
N retention (% of N intake)	38·95[a]	39·05[a]	52·42[b]	8·70	0·05

[a,b] Values with different superscript letters were significantly different.

The results indicate that vitamin B_6 supplementation had no beneficial effect on N retention above a normal vitamin B_6 dose. Where the bio-availability of vitamin B_6 and therefore amino acid metabolism was inhibited over a prolonged period, the pig surprisingly showed an increased ($P<0·05$) N retention. These results, along with a prominent improvement in growth rate and feed conversion efficiency to the end of the growth period, indicate a tendency of pigs to compensate for a deficiency in vitamin B_6 and consequent impaired N utilization.

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Intrinsic labelling of peas, chicken meat, eggs, goat's milk and human milk with ^{67}Zn. By T. E. FOX, S. J. FAIRWEATHER-TAIT, J. EAGLES and S. G. WHARF, *AFRC Institute of Food Research, Colney Lane, Norwich NR4 7UA*

Pea seeds, germinated in washed silica sand and watered in modified Hoagland and Arnon nutrient solution (Weaver, 1985) were transferred to black plastic buckets, whitened on the outside, so that the aerial shoots protruded through holes in the lids. The buckets were filled with the nutrient solution and were well-aerated with a laboratory pump. The solution was changed every 10 d and the ^{67}Zn added at a concentration of 0·056 μg/ml, at 8 weeks. The peas were harvested after 12 weeks and freeze-dried.

Ross I, broiler cockerels, weighing 2·025 kg, were fed on a diet of grower pellets and water *ad lib.* for 27 d. On day 23 the cockerels were given an intravenous injection of 1 ml of 1·84 mg ^{67}Zn into the branchial vein in alternate wings for 5 d. After 1 week they were killed and the breast and leg meat cooked in a microwave oven and freeze-dried. Human milk was labelled by giving an oral dose of 2·4 mg ^{67}Zn in 330 ml Coca-Cola to a lactating woman, 3·5 months post-partum, after an overnight fast. Milk was collected after 4 h and at 4-hourly intervals for 24 h. The samples were freeze-dried, ashed and fractions taken for total Zn measurement by atomic absorption spectroscopy, and Zn 64/67 ratio measurement by thermal ionization mass spectrometry. The results are shown in the Table.

Food sample	Total Zn (μg/g DM)	Zn 64/67 ratio		Total ^{67}Zn (μ g/g DM)
		R	%RSD	
Peas	46·5	2·62	0·6	6·5
Human milk	7·1	6·87	0·9	0·2
Chicken meat	45·7	3·05	0·1	5·4
Eggs	30·4	2·17	0·7	1·4
Goat's milk	39·9	3·21	0·2	0·9

Eggs were collected from a hen, previously injected with a 3 ml solution containing 2·43 mg ^{67}Zn, over 16 d. The eggs were cooked in a microwave oven as scrambled egg and freeze-dried. The highest incorporation of the isotope was 6 d after the injection with a Zn 64/67 ratio of 1·14 with enrichment decreasing to a Zn 64/67 ratio of 2·45 at day 16.

Labelled goat's milk was prepared by injecting a Cameroon pygmy goat with a solution containing 9·89 mg ^{67}Zn into the jugular vein. The goat was milked twice per day for 4 d and the samples freeze-dried separately. On analysis the results showing highest incorporation were 4–16 h after injection with a Zn 64/67 ratio of 2·62, declining over the next 88 h to a Zn 64/67 ratio of 7·28.

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Weaver, C. M. (1985). *Critical Reviews in Food Science and Nutrition* **23** (1), 75–101.

Apparent zinc absorption by rats from foods labelled intrinsically and extrinsically with ^{67}Zn. By S. J. FAIRWEATHER-TAIT, T. E. FOX, S. G. WHARF and J. EAGLES, *AFRC Institute of Food Research, Colney Lane, Norwich NR4 7UA* and H. M. CREWS and R. MASSEY, *MAFF Food Science Laboratory, Colney Lane, Norwich NR4 7UA*

A range of foods (peas, chicken meat, eggs, goat's milk, human milk) enriched with the stable isotope ^{67}Zn were prepared by means of intrinsic and extrinsic labelling procedures. Single meals containing chromium oxide as a faecal marker were fed to rats after an overnight fast. The faeces were collected until the disappearance of the marker, and were dried, homogenized and ashed. Portions of ash were analysed for Zn by atomic absorption spectrometry and ^{67}Zn by thermal ionization mass spectrometry. The degree of enrichment with ^{67}Zn was measured, and from this apparent absorption of the ^{67}Zn dose calculated (Eagles *et al.* 1989). The results are shown in the Table.

	Apparent absorption of ^{67}Zn (% of dose)					
	Intrinsic label			Extrinsic label		
Food	Mean	*n*	SEM	Mean	*n*	SEM
Peas	43·4	15	1·3	25·6	15	4·4
Chicken meat	61·7	15	2·2	57·0	14	2·6
Eggs	81·5	15	2·3	74·5	15	2·6
Goat's milk	53·5	16	1·5	65·9	15	2·2
Human milk	84·0	10	2·2	66·3	10	1·7

There were significant differences in the absorption of the extrinsic and intrinsic labels which differed in magnitude between the foods tested. Extrinsic ^{67}Zn was less well absorbed in peas, chicken meat, eggs and human milk than intrinsic ^{67}Zn, but in goat's milk the extrinsic ^{67}Zn was better absorbed than the intrinsic ^{67}Zn. The greatest disparity between intrinsic and extrinsic labels was found in peas, and the best agreement was in chicken meat, closely followed by eggs.

The results demonstrate that extrinsically added stable Zn isotopes do not fully exchange with endogenous Zn in many foods. The difference between the foods probably relates to the nature and digestibility of the food, particularly the Zn-binding constituents, and the intestinal conditions to which the food is exposed. There is clearly a need for caution when using extrinsic labels for Zn bio-availability studies.

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Zinc nutritive status and immunocompetence in anorexia nervosa. By A. MARCOS, P. VARELA and M. P. NAVARRO, *Instituto de Nutrición y Bromatología (CSIC), Facultad de Farmacia, Ciudad Universitaria, 28040 Madrid, Spain* and G. MORANDE, *Hospital de la Cruz Roja de Madrid, Spain*

Zinc metabolism has been suggested to be a potential factor in the aetiology of anorexia nervosa. Moreover, Zn is known to be the micronutrient best characterized with respect to its ability to influence immune functions, which may be altered under conditions of malnutrition. The aim of the present work was to find the relationship between Zn nutritive status and immunocompetence in anorexia nervosa.

Measurements of anthropometric indices, Zn in serum, urine and hair (determined by atomic absorption spectrophotometry) and cell-mediated immunity, evaluated by T-cell subset count (CD4 and CD8 evaluated by flow cytometry) and T-cell function throughout delayed dermal hypersensitivity (Multitest IMC), were made in patients (n 7) suffering from anorexia nervosa and in healthy control subjects (n 8). All the measurements, except the anthropometric ones, were tested in a blind study.

Zn nutritive status and cell-mediated immunity

	Control (n 7)		Anorexia nervosa (n 8)	
	Mean	SEM	Mean	SEM
Body mass index (kg/m^2)	20·4	0·40	14·67*	0·30
Serum Zn (μg/ml)	0·87	0·07	1·94*	0·12
Hair Zn (μg/g)	154	11·15	285*	53·75
Urine Zn (μg/24 h)	244	25	769*	99
CD4 (%)	45·20	1·86	43·16	5·89
CD8 (%)	26·46	2·87	38·27*	3·41
CD4:CD8	1·87	0·14	1·11*	0·08
No. of positive responses to seven antigens	4·78	1·48	2·90*	1·52
Score (mm)	14·42	4·46	6·30*	3·99

* $P \leq 0{\cdot}05$.

As expected, the anorectic patients had the lowest weight and body mass index. However, Zn levels in serum, hair and urine were much higher in the patients than in controls (123, 85 and 215% respectively). CD4 % remained unchanged in anorectic patients but CD8 was raised, leading to a lower CD4:CD8 ratio. Cell-mediated immunity was impaired in the patients since both the score (sum of induration for positive responses) and the number of positive responses (induration of 2 mm or greater) to the seven antigens were significantly much lower (56 and 39% respectively).

We conclude that, in spite of the low Zn levels usually seen with Zn-deficient diets, the high Zn levels in this study may be the consequence of a process of catabolism in patients with anorexia nervosa. The exact causes of the immune dysfunction cannot be ascertained from these studies but may be related to restricted intake of other nutrients.

Influence of tea on the availability of copper. By M. P. VAQUERO*, M. VELDHUIZEN and C. J. A. VAN DEN HAMER, *Department of Radiochemistry, Interfaculty Reactor Institute, 2629 JB Delft* and G. SCHAAFSMA, *TNO-CIVO Toxicology and Nutrition Institute, 3700 AJ Zeist, The Netherlands*

Several studies indicate that tea inhibits iron availability (Disler *et al.* 1975; Rossander *et al.* 1979). However, the possible implications of tea on copper availability have not been recognized. In a study with rats, a concentrated tea was shown to increase the liver Cu content (Greger & Lyle, 1988).

We have investigated the availability of Cu from a breakfast meal containing: white bread, margarine, jam, cheese, and the beverages tea, tea/milk, or water and water/milk as controls. The tea extract was made by brewing for 5 min a 4 g bag (English black tea) in 500 ml demineralised water after boiling. An in vitro method including dialysis was used. The dialysability of Cu (percentage of Cu dialysed) was significantly increased by tea. Milk added to the tea did not modify the dialysability of Cu.

A second study was carried out with rats using the breakfasts containing water and tea. The samples were spiked with ^{64}Cu and given orally to the animals (eight per group) in a single dose. Whole body retention of ^{64}Cu was measured over a 4 d period after the administration. In order to determine the true absorption of ^{64}Cu a control group of rats received similar trace amounts of ^{64}Cu intraperitoneally (i.p.). On the 4th day the rats were killed and blood and livers collected to measure ^{64}Cu activity.

Effect of tea on whole body retention of ^{64}Cu and liver ^{64}Cu 4 d after the administration ^{64}Cu (% of dose)

	Water		Tea		i.p.	
	Mean	SE	Mean	SE	Mean	SE
Whole body ^{64}Cu	28·9[a]	3·8	32·8[a]	3·2	51·2[b]	1·6
Liver ^{64}Cu	3·9[a]	0·3	5·0[b]	0·4	8·7[c]	0·6

[a,b,c] Mean values in a horizontal row with different superscript letters were significantly different: $P<0·05$.

The results in vivo indicate a tendency to higher absorption of ^{64}Cu with tea. Moreover, liver ^{64}Cu was higher in the tea group than in the water group of rats.

These results suggest that tea favours the solubilization and absorption of Cu, with concomitant storage of the metal in the liver.

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The effect of non-starch polysaccharide on urea kinetics in normal adult males consuming a low-protein diet. By M. LANGRAN[1,2], B. J. MORAN[2], JANE MURPHY[1], S. A. WOOTTON[1] and A. A. JACKSON[1], *Departments of* [1]*Human Nutrition* and [2]*Surgery, University of Southampton, Bassett Crescent East, Bassett, Southampton SO9 3TU*

One of the mechanisms whereby the human body responds to a low protein intake is an increase in the hydrolysis of urea (Langran *et al.* 1990). The ammonia thus produced may be retained by the body and is a potential additional source of nitrogen for the metabolic pool. The hydrolysis of urea is thought to be carried out largely by the resident colonic microflora (Gibson *et al.* 1972). One of the possible mechanisms which may control the rate of urea hydrolysis is the availability of substrate for such microflora. We hypothesized that an increase in the energy substrate available to the flora of an individual would alter urea kinetics by means of increased bacterial hydrolysis when dietary N was limited. A pilot study was devised in order to evaluate this.

Five normal adult males (age 22–43 years) were studied on two separate occasions whilst receiving one of two low-protein diets: 'fibre-free' providing 78 mg N and 124 kJ metabolizable energy/kg per d, and 'fibre-enriched' providing 76 mg N, 132 kJ, 207 mg banana fibre and 621 mg starch/kg per d. Each study was carried out over a 5 d period and 24 h urines and stools were collected throughout. During the final 18 h, urea kinetics were measured using oral [$^{15}N^{15}N$]urea and three-hourly collections of urine (Jackson *et al.* 1984). The ingested fibre exerted an effect, with the mean dry stool weight increasing from 15 (SE 1·2) g/d on the fibre-free diet to 41 (SE 14) g/d on the fibre-enriched diet ($P<0·05$, Wilcoxon Rank Sum). However, there were no significant alterations in the values obtained for urea kinetics between the two diets.

	mg N/kg per d							
Diet	P	Eu	T	S	Eu/P	T/P	S/P	P/I
Fibre-free (*n* 5)								
Mean	198	66	132	108	0·35	0·65	0·53	2·56
SE	27	10	21	17	0·06	0·06	0·04	0·25
Fibre-enriched (*n* 5)								
Mean	176	58	118	100	0·35	0·65	0·55	2·33
SE	14	6	20	18	0·07	0·07	0·06	0·20

P, total urea production; Eu, urinary excretion; T, urea hydrolysis; S, urea available to other synthetic processes; I, dietary N intake.

These results demonstrate that the addition of non-starch polysaccharide from bananas had no effect on the urea kinetics of normal adult males when N intake was marginal. We have shown that urea kinetics are significantly different between a marginal and an adequate N intake (Langran *et al.* 1990). Therefore, it would appear that the level of dietary N exerts a greater effect on urea hydrolysis than the availability of exogenous substrates to the microflora.

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The effect of non-starch polysaccharides on the magnitude and composition of faecal energy in normal healthy adults. By JANE MURPHY, S. A. WOOTTON, M. LANGRAN and A. A. JACKSON, *Department of Human Nutrition, Southampton University, Southampton SO9 3TU*

The reduction in apparent digestibility of energy, lipid and nitrogen following consumption of a raised intake of non-starch polysaccharides (NSP) (Southgate & Durnin, 1970) may be attributable to a greater faecal microbial mass (Stephen & Cummings, 1980*a*). Although the nitrogenous faecal loss is thought to be largely bacterial, the extent to which bacteria contribute to the increased faecal energy and lipid losses on high-NSP diets remains unclear. The aim of the present study was to compare the energy, lipid and N contents of the stool and the contribution made by the faecal microflora to these components in normal healthy adults following consumption of both an NSP-free and NSP-enriched diet.

Six healthy adult males (aged 22–43 years) consumed on separate occasions (1) a diet totally free of NSP, providing 78 mg N, 486 mg lipid and 124 kJ/kg body-weight per d, and (2) the same ingredients at 50% of the intake, made isoenergetic by the addition of bananas to provide 703 mg NSP and 621 mg resistant starch/kg body-weight per d. Stools were collected for the final 3 d of the 5 d study period between carmine markers. Faecal bacteria were isolated (Stephen & Cummings, 1980*b*) and the freeze-dried stools analysed for energy (bomb calorimetry), N (Kjeldahl) and lipid (Gompertz & Sammons, 1963). Bacterial energy, lipid and N were estimated from analysis of pooled samples of bacteria on each diet. The results (median values) are summarized in the Table.

	SDW (g/d)	FE (kJ/d)	FL (g/d)	FN (g/d)	BM (g/d)	BE (kJ/d)	BL (g/d)	BN (g/d)
NSP-free	16	326	1·5	0·5	3·5	70	0·5	0·1
NSP-enriched	41**	898**	2·5*	0·8**	13·0**	237**	0·5	0·3**

SDW, stool dry weight; FE, faecal energy; FL, faecal lipid; FN, faecal N; BM, bacterial mass; BE, bacterial energy; BL, bacterial lipid, BN, bacterial N.

Significantly different from NSP-free diet (Wilcoxon Rank Sum): *$P<0·05$, **$P<0·01$.

The addition of bananas to an NSP-free diet resulted in greater energy, lipid and N losses within the stool. The relative increase in dry mass, energy and N from bacteria was greater than the relative increase in stool dry weight and total energy and N within the stool. Approximately 38% of the difference in faecal dry weight, 29% of the difference in faecal energy and 66% of the difference in faecal N could be attributed to increases in bacterial mass. Bacterial lipid remained unchanged despite a difference in both bacterial mass and faecal lipid. These results suggest that the greater bacterial mass could account in part for the decrease in apparent digestibility of energy and N associated with the consumption of NSP and resistant starch from bananas.

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Influence of soluble non-starch polysaccharides and food intake on mucosal cell turnover and some gastrointestinal peptides in the rat. By I. T. JOHNSON and JENNIFER M. GEE, *AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA*

Some soluble forms of dietary fibre have been shown to stimulate cell turnover in rat small intestine, an effect which is associated with changes in enzyme and carrier activity and elevated plasma levels of enteroglucagon (Johnson *et al.* 1984, 1988). Small intestinal cell proliferation is also known to be influenced by nutrients in the gut lumen, particularly lipids (Jenkins & Thompson, 1989). It is therefore essential to consider the effect of dietary intake in studies of this type. We have investigated some gastrointestinal effects of dietary restriction *v. ad lib.* feeding in groups of growing rats fed on a range of diets containing non-starch polysaccharides.

Three groups of rats were fed *ad lib.* on a fibre-free control semi-synthetic diet (FF), or similar diets supplemented with guar gum (GG) or gum arabic (GA). Three other groups were pair-fed to the GG-fed rats and received the control diet, or similar diets supplemented with starch or cellulose. During the 28 d feeding period the GG-fed rats consumed approximately 15% less food than the other *ad lib.*-fed groups. All rats were killed at the end of this period and samples of distal ileal mucosa were obtained for the measurement of cell proliferation by the metaphase arrest technique. Venous blood was also taken for determination of the gastrointestinal peptides enteroglucagon, gastrin and neurotensin by radio-immunoassays.

The consumption of GG led to a statistically significant increase in small intestinal length. As shown in the Table, crypt cell production and plasma enteroglucagon were also higher than observed in both the *ad lib.*-fed and pair-fed control groups. However, in the GA-fed groups only plasma enteroglucagon was higher. In contrast, gastrin was reduced in all groups compared with the animals fed on the FF diet *ad lib.*, and neurotensin was significantly lower in every group except that given GG.

Mucosal cell proliferation and gastrointestinal peptides

	Ad lib.-fed						Pair-fed					
	FF		GG		GA		FF		C		S	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
CCPR†	8^{a}	2	31^{b}	10	9^{a}	2	10^{a}	2	10^{a}	2	7^{a}	2
GLI‡	120^{a}	28	369^{b}	30	296^{b}	43	57^{a}	14	65^{a}	8	107^{a}	16
Gastrin‡	383^{a}	22	327^{b}	16	288^{b}	14	314^{b}	20	297^{b}	19	283^{b}	19
Neurotensin‡	27^{a}	7	21^{ab}	2	14^{b}	1	14^{b}	2	15^{b}	2	15^{b}	4

FF, fibre-free; GG, guar gum; GA, gum arabic; C, cellulose; S, starch; CCPR, crypt cell production rate; GLI, total glucagon-like immunoreactivity.

^{a,b} Values not sharing a common superscript differed significantly ($P<0{\cdot}05$, ANOVA and least significant difference).

† Divisions/crypt per h; ‡ picogram/ml.

Both GG and GA are readily fermented by colonic bacteria but only GG is significantly viscous under intestinal conditions. These results suggest that high luminal viscosity is the major factor contributing to increased mucosal cell proliferation, rather than increased levels of enteroglucagon or the products of carbohydrate fermentation. The effect is most probably due to delayed nutrient absorption leading to an increase in luminal nutrient concentration in distal regions of the gut lumen.

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The effect of low, medium and high molecular weight grades of guar gum on postprandial blood glucose and plasma insulin in non-insulin-dependent diabetics. By S. J. GATENBY[1]*, P. R. ELLIS[1], L. M. MORGAN[2], F. M. DAWOUD[1] and P. A. JUDD[2], [1]*Department of Food and Nutritional Sciences, King's College London, Campden Hill Road, Kensington, London W8 7AH* and [2]*Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH*

Many studies have reported that guar gum attenuates postprandial glycaemia and insulinaemia in diabetic patients. However, it is still not known which types of guar gum are most effective. A study in seventeen healthy subjects (Dawoud, 1989) has shown that low molecular weight (MW) guar-gum flours are as effective as a high MW type in reducing postprandial insulinaemia. We have therefore investigated the effects of low (M60), medium (M90) and high (M150) MW guar gum on postprandial blood glucose and insulin in ten non-insulin-dependent diabetic patients.

After overnight fasts, all the patients consumed four breakfast meals (each containing 75 g available carbohydrate) in random order on separate occasions. The meals consisted of either control bread, or guar-gum breads containing guar-gum samples (7·6 g/meal) of different MW, with jam, butter and mineral water to a total meal weight of 350 g. Venous blood samples were taken preprandially and at 15, 30, 45, 60, 90, 120 and 180 min postprandially and were analysed for glucose and insulin.

All the meals containing guar gum significantly reduced postprandial glucose or insulin, or both, at the postprandial times shown in the Table, indicating that the beneficial effects of guar gum on carbohydrate tolerance are not diminished by using guar gum of low MW. As wheat breads containing low MW guar gum are more palatable than those containing high MW grades (Dawoud, 1989), such products are worthy of further investigation as therapeutic aids in the management of diabetes mellitus.

Mean changes in blood glucose (mmol/l) and plasma insulin (mU/l) from fasting values

		Postprandial time (min)							
Meal type	*n*	15	30	45	60	90	120	150	180
Glucose									
Control	14	0·15	2·44	4·89	5·88	6·95	7·00	5·67	4·73
M60 Guar gum	14	0·66	2·47	3·34**	4·27**	5·08***	5·36***	3·66**	3·81**
M90 Guar gum	14	0·55	2·24	3·87	4·69	5·52**	5·31***	4·33**	3·41*
M150 Guar gum	13	0·13	1·31	3·38*	4·13*	5·17*	4·72**	4·43	3·41
Insulin									
Control	10	2·8	20·0	38·3	43·3	55·3	60·8	52·2	23·3
M60 Guar gum	10	7·0	22·7	28·4	30·7*	45·5	23·7	32·1	22·4
M90 Guar gum	10	3·9	18·2	24·2	30·3*	33·4*	31·9	28·0	24·0
M150 Guar gum	9	4·7	23·6	23·8	28·2**	39·6	48·4	38·9	43·0

*$P<0·05$, **$P<0·01$, ***$P<0·001$.

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The effect of guar wheatflakes on postprandial blood glucose, plasma insulin and C-peptide levels in healthy subjects. By R. M. FAIRCHILD, *School of Home Economics, University of Wales College Cardiff, Cardiff CF1 3AS* and P. R. ELLIS, *Department of Food and Nutrition Sciences, King's College London, London W8 7AH* and S. LUZIO, A. BYRNE and M. A. MIR, *University Hospital of Wales, Cardiff CF4 4XW*

We have previously reported that guar gum is more effective in reducing postprandial insulinaemia, in healthy subjects, when incorporated into food rather than a pre-meal drink (Fairchild *et al.* 1990). Therefore we have investigated the effects of a new guar wheatflake on carbohydrate tolerance in healthy subjects.

Ten fasting subjects consumed control and guar wheatflake products (plus semi-skimmed milk and orange juice) for breakfast on separate occasions in random order. Each meal provided 1734 kJ, 77 g carbohydrate, 15 g protein and 5 g fat. The guar wheatflake meal provided 6·4 g guar gum (M150, Meyhall UK, Ltd). Venous blood samples were taken preprandially and 15, 30, 45, 60, 90, 120, 150, 210 and 240 min after commencing each breakfast, and analysed for blood glucose, plasma insulin and C-peptide.

The guar wheatflake meal significantly reduced the postprandial glucose (15 and 60 min), insulin (15, 60, 90 and 120 min) and C-peptide (15 and 90 min) compared with the control meal (Table).

Mean change in blood glucose (mmol/l), plasma insulin (mU/l) and C-peptide (pmol/l) from fasting values

Postprandial time (min) . . .	15	30	45	60	90	120	150	210	240
Glucose:									
Control	1·3	2·5	2·2	1·1	0·9	0·6	0·7	0·4	0·3
Guar gum	0·4**	1·9	1·6	0·5*	0·0	0·3	0·6	0·4	0·5
SEM	0·2	0·4	0·1	0·4	0·4	0·4	0·3	0·2	0·2
Insulin:									
Control	32·0	71·0	57·0	38·0	34·0	24·0	33·0	11·0	8·0
Guar gum	8·0**	63·0	46·0	20·0*	10·0**	14·0*	13·0	3·0	12·0
SEM	6·0	13·0	10·0	6·0	6·0	3·0	15·0	8·0	9·0
C-peptide:									
Control	0·6	1·0	1·1	1·0	0·8	0·8	0·7	0·4	0·3
Guar gum	0·1**	1·0	1·1	0·9	0·5**	0·6	0·5	0·4	0·3
SEM	0·1	0·1	0·1	0·2	0·02	0·1	0·1	0·1	0·1

Guar gum meal significantly lower than control: *$P<0·05$, **$P<0·001$.

The improvements in carbohydrate tolerance, in healthy subjects, resulting from the incorporation of guar gum into a wheatflake breakfast cereal indicates that this product is worthy of further investigation as a therapeutic aid in the treatment of diabetes mellitus.

We are grateful to Mr. Peter Fletcher of Newtime Foods Ltd for producing the wheatflakes, Dr. Hans Englyst for the carbohydrate analysis and Mrs. Sue Thomas for technical assistance. The study was funded by the British Diabetic Association.

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Effect of zinc supplementation on intestinal permeability and morbidity among Bangladeshi children with persistent diarrhoea syndrome. By S. K. ROY and R. HAIDER, *International Centre for Diarrhoeal Disease Research, Bangladesh* and A. TOMKINS and R. H. BEHRENS, *London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT*

Previous studies in animal models have suggested that zinc supplementation in experimental deficiency improves absorption of water and electrolytes, intestinal mucosal recovery and enhances growth (Roy & Tomkins, 1985; Roy *et al.* 1986). A double-blind randomized clinical trial with zinc acetate (4·2 mg/kg per d) against placebo was conducted over 2 weeks in a diarrhoea-treatment centre in Bangladesh, among 190 children aged 3–24 months presenting with diarrhoea lasting for more than 14 d. A standardized minced-chicken-based diet and appropriate rehydration fluid were administered to both groups. Changes in intestinal mucosal permeability were assessed by using an oral dose of 5 g lactulose and 1 g mannitol in 20 ml water on admission (day 1), after the 1st week (day 8) and at the end of the 2nd week (day 15). Reduction in lactulose excretion was significantly greater (46·0% *v.* 21·4%, $P<0·04$) in the Zn-supplemented group compared with the placebo group over the 1st week. Improvement in mannitol excretion was similar in both groups. Of 190 patients, twenty-one dropped out and twenty-eight continued with diarrhoea for longer than 15 d. When the results from the remaining 141 children were analysed, duration of recovery was not significantly different between the two groups. However, among the malnourished children (weight/age ≤70%, *n* 64), Zn-supplementation led to 25% reduction in duration (6·1 *v.* 8·1 d, $P<0·03$).

		Day 1		Day 8		
	n	Geometric mean	SD	Geometric mean	SD	Statistical significance
% Lactulose excretion/5 h						
Zn	38	0·45	3·38	0·21	4·16	$P<0·001$
Placebo	29	0·25	3·04	0·24	3·71	$P=0·8$
% Mannitol excretion/5 h						
Zn	78	1·21	3·2	2·0	2·5	$P<0·0001$
Placebo	81	1·22	2·9	2·2	2·63	$P<0·001$
L:M ratio						
Zn	78	0·39	4·0	0·13	4·1	$P<0·001$
Placebo	75	0·33	3·9	0·13	3·4	$P<0·001$

	Placebo			Zn			
Recovery (d)	*n*	Mean	SD	*n*	Mean	SD	
All patients	69	7·0	3·8	72	6·4	3·7	$P=0·4$
Weight/age ≤70% (NCHS)	30	8·1	3·7	34	6·1	3·5	$P<0·03$

The study suggests that Zn supplementation to children with persistent diarrhoea may enhance the intestinal mucosal recovery and reduce duration of diarrhoea among the malnourished.

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Energy metabolism of pigs of high genetic potential: effects of protein and energy intake.

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Recent reports (Campbell & Taverner, 1988; McCracken & Rao, 1989) suggest that (a) the accepted values for the energy costs of maintenance and of fat and protein deposition in the growing pig (Agricultural Research Council, 1981) may not be appropriate for pigs of high genetic potential, and (b) the slope of the relationship between protein deposition and energy intake changes with increasing genetic potential for lean growth.

Two studies were conducted to further investigate these aspects. In Expt 1, two replicates of five littermate, entire male, pedigree pigs were used in a 5 × 5 Latin-square changeover study of the effects of energy intake on nitrogen retention (NR) and energy retention (ER). Five feeding levels (80, 100, 120, 140 and 160 g/kg body-weight $(W)^{0.63}$) were used during five consecutive metabolism studies, each of 11 d duration, excreta being collected during the last 7 d. Heat production was measured for 1 d during each balance period and two pigs were slaughtered at 90 kg to check the accuracy of the NR and ER measurements. In Expt 2, five replicates of five littermates were randomized within litters to one of five treatments: (A) *ad lib.*, (B) 90% *ad lib.*, (C) 80% *ad lib.*, (D) 90% *ad lib.*, (E) 80% *ad lib*. Pigs on treatments A, B and C received the same diet (252 g (N×6·25; CP)/kg dry matter). The crude protein diets given on D and E provided CP intakes equivalent to A. Pigs were slaughtered at 90 kg and analysed for CP, fat and ash.

In Expt 1 the mean metabolizable energy (ME) intakes ranged from 0·85 to 1·9 MJ/d per kg $W^{0.75}$. For ER there was a significant linear relationship (R^2, 0·97) of the form,

$$\text{ER (MJ/d per kg } W^{0.75}) = 0{\cdot}734 \text{ (SE } 0{\cdot}0227) \text{ ME} - 0{\cdot}447.$$

The NR response to ME intake was linear to the highest level of ME intake achieved, and the slope was 4·6 (SE 0·50) g CP/MJ ME.

In Expt 2 the mean ME intakes (MJ/d per kg $W^{0.75}$) for groups A–E respectively were 1·35, 1·23, 1·12, 1·22, 1·11 and the ER values were 0·47, 0·40, 0·36, 0·42, 0·32. Protein retention values (g/d) were respectively 181, 158, 149, 156, 137. Using the values for protein and fat retention to calculate ER, heat production (ME−ER) was 23, 22, 17, 16 and 21% higher for A–E respectively than that calculated from the energy costs of maintenance and deposition accepted by the Agricultural Research Council (1981).

These results confirm that, in pigs of high genetic potential, (a) appetite limits protein deposition, (b) the slope of the protein/energy relationship is steeper than in pigs of poorer genetic potential, (c) heat production is considerably higher than would be predicted from the values accepted by the Agricultural Research Council (1981).

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Effects of feeding level and sex on nitrogen retention and serum insulin-like growth factor-1 in growing pigs. By J. A. TAYLOR, D. N. SALTER, W. H. CLOSE, G. H. LASWAI and A. HUDSON, *AFRC Institute for Grassland and Animal Production, Shinfield, Reading, Berks RG2 9AQ*

Many anabolic processes within the body are stimulated by insulin-like growth factor-1 (IGF-1). In entire animals, release of IGF-1 has been shown to be regulated by nutritional status (e.g. starvation/refeeding) and in reproductive tissues by gonadotropins and steroid hormones (Phillips *et al.* 1990). To investigate this further the relation between serum IGF-1 and nitrogen retention has been studied in entire and castrated pigs at different planes of nutrition and stages of growth.

In a 2 × 2 factorial experiment twelve castrated and twelve entire male pigs, of about 20 kg body-weight, were given either 2·25 (low) or 3·4 (high) × maintenance energy requirements, on a scale based on live weight. N and energy balances (including calorimetry) were measured over 7 d periods when the pigs reached approximately 30, 60 and 90 kg. Fasting blood samples were taken by venepuncture after each balance period and serum was analysed for IGF-1 and insulin.

	Live wt (kg)	Low	High	Castrate	Entire	SED
IGF-1 (ng/ml)	30	254	393***	245	402***	30·5
	60	261	387**	236	412***	39·3
	90	361	399 NS	221	539***	50·3
N retention (g/d)	30	21·2	31·0***	24·6	27·6***	0·97
	60	23·2	38·2***	27·6	33·8***	1·39
	90	25·7	33·2***	25·4	33·5***	1·76

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

Both serum IGF-1 and N retention were higher in pigs fed on the high compared with the low maintenance energy level, although differences in IGF-1 concentrations were not significant at 90 kg. Entire males had higher IGF-1 levels and N retention than castrates and these differences increased with age ($P<0·05$, SED 57·4 and 1·95 respectively). Serum insulin levels were similar for pigs on all treatments (mean 20·05 (SE 10·81) mU/l). IGF-1 was highly correlated with N retention (r 0·64, $P<0·001$, n 64).

These results indicate that high N retention, and hence rapid growth, are associated with high levels of IGF-1. This is consistent with the findings of Fletcher *et al.* (1990) for entire pigs given isoenergetic diets containing three levels of protein. The results also indicate that castration is marked by a lowering effect on IGF-1 in association with lower rates of N retention. This suggests a stimulatory effect of sex hormones on IGF-1 release and N retention.

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Immune function tests, iron metabolism and susceptibility to infection in primary undernutrition and refeeding. By JOHN MURRAY and ANNE MURRAY (Introduced by ERICA WHEELER), *Department of Medicine, University of Minnesota, Minneapolis, MN, USA*

Current dogma equates impaired immune function tests (IFTS) during undernutrition with increased susceptibility to infections. Since we have not found more infections in famine victims before refeeding, we related IFTS and serum ferritin to occurrence in infections before and after 2 weeks of refeeding in 146 Africans with >25% loss of weight. Before refeeding 2·1% had infections, 8·9% had raised C-reactive protein (CRP), 88·4% were anergic, mean serum ferritin was 779 (SD 68) ng/ml and IFTS were moderately impaired. With refeeding, weight increased 3·28 (SD 0·74) kg, 22·4% had infections, 31·5% had raised CRP, 62·3% were anergic, serum ferritin fell by 396 (SD 78·4) ng/ml and IFTS were improving. Those with infections gained more weight (3·59 (SD 0·42) kg) than those without (3·13 (SD 0·34) ($P<0·001$)) and had a greater fall of serum ferritin by 470 (SD 73) *v.* 385 (SD 59) ng/ml ($P<0·001$). Impaired IFTS did not predict increased risk of infections in undernutrition or refeeding. High iron stores before refeeding were not associated with increased infections but rapid mobilization of Fe from stores and rapid weight gain were.

Effects of vitamin C on sorbitol in eye lens of guinea-pigs made diabetic with streptozotocin. By C. J. BATES and P. H. EVANS, *Medical Research Council, Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

Recent studies have suggested that vitamin C status may influence cataractogenesis. Ascorbic acid supplements lowered sorbitol levels in erythrocytes of diabetic and non-diabetic humans, and countered the cataractogenic effect of galactose in rats (Vinson *et al.* 1989). The present study has used marginally vitamin C-deficient guinea-pigs, made mildly diabetic with streptozotocin, to investigate this new function of vitamin C.

Weanling male Dunkin-Hartley guinea-pigs, weighing 252 (SE 4) g, were divided into four matched groups, two of which received a purified diet containing ascorbic acid at 5 g/kg (Hi-C), and two received the same diet with ascorbic acid at 0·1 g/kg (Lo-C). Two days before, and again 2 weeks after starting these diets, one of each of the two diet groups received two intraperitoneal doses of streptozotocin (Boehringer, UK) at 150 mg/kg body-weight (+Stz), while the other two groups received saline (9 g sodium chloride/l) as controls (−Stz). All groups were maintained on the diets for 4 months; only one animal died prematurely. Adrenals, eye lenses and aqueous humour samples were collected. Reduced ascorbate was measured by high performance liquid chromatography with electrochemical detection; glucose and sorbitol were measured by enzyme-linked assays on a Cobas Bio centrifugal analyser.

	Lo-C (−Stz) (*n* 9)		Hi-C (−Stz) (*n* 9)		Lo-C (+Stz) (*n* 8)		Hi-C (+Stz) (*n* 9)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Final body-wt (g)	859	32	936	36	767	40	737	37
Adrenal vitamin C (μmol/g)	1·04***	0·09	11·41	0·86	1·02***	0·11	11·45***	0·74
Lens vitamin C (μmol/g)	0·19***	0·013	0·47	0·22	0·19***	0·013	0·45	0·030
Lens glucose (μmol/g)	0·42	0·08	0·34	0·05	0·62	0·08	0·81	0·05
Aqueous humour glucose (μmol/g)	4·70	0·28	4·75	0·33	5·39	0·34	6·48	0·91
Lens sorbitol (μmol/g)	1·04	0·09	1·08	0·11	1·75*	0·27	1·04	0·14

Significant differences between Lo-C and Hi-C groups by Student's *t* test and 2-way ANOVA: *$P<0·05$, ***$P<0·001$.

The results (Table) show approximately elevenfold differences in adrenal ascorbate, and 2·5-fold differences in eye lens ascorbate, attributable to dietary vitamin C. Lens and aqueous humour glucose exhibited a significant change to higher levels in the +Stz groups, but no consistent effect of vitamin C. Lens sorbitol, however, was significantly raised only in the +Stz group which received the low intake of vitamin C (Lo-C).

We suggest that the combination of low tissue vitamin C and a mild streptozotocin diabetogenic insult can significantly raise lens sorbitol levels in guinea-pigs, whereas high tissue vitamin C can counter this effect. Thus vitamin C may have anti-cataractogenic properties in vivo.

Mr. T. D. Cowen provided expert animal husbandry.

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Vitamin C in stimulated and unstimulated leucocytes in culture. By C. J. SCHORAH, D. J. ROYLE and S. W. EVANS, *Department of Chemical Pathology and Immunology, University of Leeds, Leeds LS2 9JT*

Increasingly, the functioning of white cells is being studied in vitro. In blood these cells contain high concentrations of vitamin C (Schorah *et al.* 1986), but they are often cultured for long periods in the absence of the vitamin, unless researchers are investigating the role of ascorbic acid when incubations are usually brief and undertaken on freshly isolated cells (Anderson & Lukey, 1987). Could an absence of vitamin C from cultures modify intracellular concentrations and affect white cell function? As a preliminary investigation we have examined uptake of vitamin C by both unstimulated and stimulated cells maintained in culture in the absence of vitamin C for periods greater than 5 d.

Lymphocytes were isolated from EDTA-anticoagulated blood using Lymphoprep. These cells and a macrophage line (U937) were maintained in RPMI 1640 with 10% fetal calf serum, glutamine (0·01 mmol/l), penicillin (200 IU/ml) and streptomycin (200 μg/ml) for at least 4 d. The cells were divided and half were stimulated with lipopolysaccharide (1 μg/ml) (macrophages) or concanavalin A (10 μg/ml) (lymphocytes), and after 24 h and a further 4 h the medium was made 57 μM and 1 mM for ascorbic acid and glutathione respectively. Cells were harvested at intervals after ascorbate addition for counting, protein measurement and estimation of total vitamin C by the 2,4-dinitrophenylhydrazine technique.

		Cell vitamin C* (nmol/10^8 cells)							
Time† (h) . . .		0		4		6		24	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lymphocytes	Unstimulated	20·5	5·3	39·2	7·3	46·0	2·3	22·7	0·2
	Stimulated	15·9	10·8	90·9	14·2	109·6	9·1	47·2	0·8
Macrophages	Unstimulated	0·0	0·0	129·5	10·2	144·9	8·5	48·8	4·8
	Stimulated	0·0	0·0	202·2	11·3	229·5	2·3	106·8	26·7

* Mean concentration for two separate experiments.
† After addition of vitamin C to medium.

The results show that even after extended periods without vitamin C the cells retain the ability to concentrate it. The highest levels were found after stimulation, when the concentration in cell water could be ninetyfold greater than the medium level. Decreases by 24 h could be due to falling medium levels of vitamin C and, in the lymphocytes, decreased cell viability (60% after 6 d in culture). These results could indicate the importance of including vitamin C in cultures of these cells, especially when investigating conditions such as rheumatoid or inflammatory injury and atherosclerosis, where redox mechanisms in these cells have been implicated in the disease process.

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Plasma ascorbate concentrations in human malaria. By J. KNOWLES and D. I. THURNHAM, *MRC Dunn Nutrition Centre, Milton Road, Cambridge CB4 1XJ* and A. V. S. HILL, *Institute of Molecular Medicine, John Radcliffe Hospital, Oxford* and M. GREENWOOD, *MRC Laboratories, Fajara, The Gambia* and C. M. TANG, *Royal Victoria Hospital, Banjul, The Gambia*

It would appear that plasma and erythrocyte ascorbate is increased in *Plasmodium vinkei*-infected mice, which may be a response to oxidant stress caused by the parasite and is not derived from increased liver synthesis (Stocker *et al.* 1986). To determine whether a similar response takes place in man, we measured plasma ascorbate concentrations in Gambian children (aged 1–5 years) with *P. falciparum* malaria or other diseases, and healthy, student-nurse controls (aged 20–28 years). Ascorbate was stabilized using metaphosphoric acid within 15 min of collection and measured after storage at −40° by high performance liquid chromatography (Bates & Cowen, 1988). In general median ascorbate concentrations tended to be lower for males than females but this was not significant and values for the sexes are combined.

	Malaria			Other diseases		Control
Ascorbate (μmol/l)	Cerebral (*n* 31)	Severe (*n* 24)	Mild (*n* 21)	Severe (*n* 45)	Mild (*n* 18)	subjects (*n* 44)
Median	13·8[ab]*	21·5[a]	11·6[ab]	11·1[b]	17·8[a]	14·3[ab]
Range	0–83·8	0·3–106·8	3·2–64·6	0–88·6	3·9–82·5	3·2–53·9
%<11·4	39	33	43	56	28	41

[a,b] Values with different superscript letters were significantly different (Kruskall Wallis followed by Scheffe test): $P<0·05$.

Plasma ascorbate values are shown for the different groups divided by disease severity. Highest ascorbate values were in fact obtained in the children infected with malaria but median values were not significantly higher than those in any group except children with other severe diseases. Several children with severe malaria received transfusions of fresh control blood but these cases did not explain the few high results. Furthermore, although the malaria groups contained the highest values, proportions of concentrations ±11·4 μmol/l were no different from the control group. The main cause of the low ascorbate values in the severely ill children without malaria was malnutrition: there were ten cases with mixed marasmus–kwashiorkor in whom the median ascorbate value was 5·6 μmol/l (range 0–14·6). In conclusion, there is little evidence for any change in plasma ascorbate associated with malaria or any other disease except those accompanied by malnutrition.

The above results refer to reduced ascorbate. We also incubated plasma with dithiothreitol to measure total ascorbate and, by difference, oxidized ascorbate (DHA; Okamura, 1980) but on analysis DHA results were mostly negative. Following stabilization, a variable time period up to 4 h elapsed when the samples were kept between +4 and −10°. Subsequent experiments suggest that total ascorbate falls and reduced form increases during this period but this does not affect the conclusion above.

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The effect of vitamin E deficiency on immune function in Wistar rats. By L. PURKINS, J. KELLEHER and R. V. HEATLEY, *Department of Medicine, St James's University Hospital, Leeds LS9 7TF*

Vitamin E deficiency in animals has been reported to diminish a number of immune functions, particularly those which require intact T cell functioning (Tanaka *et al.* 1979; Gabriel *et al.* 1984). The present study was designed to consider the effect of vitamin E deficiency for 6 months on immune function in Wistar rats.

Twenty-four male weanling Wistar rats with median body-weight 52 g (range 35–58 g) were randomly allocated to receive either a commercial vitamin E-deficient diet or a comparable diet containing 100 mg vitamin E/kg (Dyets Inc, USA). After 6 months on the appropriate diet the animals were killed and various measurements of immune function were made.

The vitamin E-deficient animals had a significantly lower ($P<0{\cdot}002$) final body-weight than the supplemented animals (545 g, range 475–610 g compared with 658 g, range 550–740 g). Plasma vitamin E levels were undetectable in the deficient animals, while supplemented animals had a median level of 14·8 mg/l (range 25·1–12·3 mg/l). Total white cell counts were similar in both groups, however, differential white cell counts indicated that there was a significant increase in both the percentage and absolute number of circulating neutrophils in the vitamin E-deficient group. Phenotypic analysis of T cell subsets in peripheral blood using monoclonal antibodies demonstrated that vitamin E deficiency significantly decreased the percentage of T helper cells and increased T suppressor cells, resulting in a considerable decrease in the T helper:T suppressor cell ratio ($P<0{\cdot}002$). Vitamin E deficiency caused a significant decrease in the number of T lymphocytes in the spleen ($P<0{\cdot}02$); this decrease was a reflection of a significant decrease of the T helper cells ($P<0{\cdot}002$). Mitogenic stimulation of both splenocytes and peripheral blood lymphocytes with concanavalin A was unaffected by vitamin E deficiency.

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Tissue concentrations of vitamin E, selenium and thiobarbituric acid reactive substances in ruminant calves depleted of vitamin E, or selenium, or both. By D. M. WALSH, S. KENNEDY and D. G. KENNEDY, *Veterinary Research Laboratories, Stormont, Belfast BT4 3SD*

Vitamin E (E) and selenium deficiencies are important factors in the development of nutritional degenerative myopathy (NDM) in ruminant cattle (McMurray & McEldowney, 1977). The purpose of the present study was to investigate (1) if lesions of NDM occurred in calves depleted of E but not Se and vice versa; (2) if there was evidence of lipid peroxidation in deficient calves, and (3) if E and Se were mutually protective.

Sixteen calves, approximately 14 weeks of age, were randomly divided into four groups of equal numbers. All groups were fed on a basal diet (Kennedy *et al.* 1987) which contained <3 μg α-tocopherol (α-TP)/g and <0·02 μg Se/g. The diet of each group was supplemented as follows (μg/g): 200 α-TP and 0·2 Se (group 1); 0·2 Se (group 2); 200 α-TP (group 3); no supplement (group 4). The animals were slaughtered after 147 d and samples of heart and three skeletal muscles were collected for α-TP and Se analysis. Peroxidizability was determined by measuring thiobarbituric acid reactive substances (TBARS) after incubation of homogenates with 0·25 mM-ascorbic acid.

		Heart		M. gluteobiceps		M. supraspinatus		M masseter	
Group	Analyte*	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	α-TP	7·9	0·8	3·2	2·2	5·0	0·6	8·1	1·2
	TBARS	1·43	0·61	1·3	0·22	1·52	0·67	1·16	0·49
	Se	0·23	0·03	0·12	0·01	0·15	0·01	0·16	0·01
2	α-TP	1·3[a]	0·6	0·6[a]	0·3	0·7[a]	0·2	1·0[a]	0·3
	TBARS	7·43[b]	1·34	3·56[b]	0·97	4·51[b]	0·36	5·97[b]	1·28
	Se	0·25	0·02	0·12	0·01	0·13	0·02	0·13	0·03
3	α-TP	8·6	1·1	4·25	1·3	5·0	0·4	8·9	1·2
	TBARS	1·22	0·36	0·71	0·97	1·02	0·36	2·19	2·92
	Se	0·12[c]	0·02	0·07[c]	0·03	0·06[c]	0·01	0·08[c]	0·001
4	α-TP	1·2[a]	0·5	0·5[a]	0·2	0·8[a]	0·2	1·0[a]	0·3
	TBARS	6·58[b]	0·49	4·11[b]	0·73	3·78[b]	0·9	6·48[b]	1·45
	Se	0·15[c]	0·03	0·07[c]	0·01	0·07[c]	0·01	0·09[c]	0·02

* α-TP and Se expressed as μg/g; TBARS as nmol malonaldehyde/mg protein.
[a] Groups 2 and 4 significantly different from groups 1 and 3 $P<0·001$.
[b] Groups 2 and 4 significantly different from groups 1 and 3 $P<0·01$.
[c] Groups 3 and 4 significantly different from groups 1 and 2 $P<0·05$.

Tissue α-TP and Se concentrations were independent of each other. Tissue peroxidizability was increased in E-depleted animals irrespective of Se status. No increase in peroxidizability was detected in calves depleted of Se but supplemented with α-TP. The presence of lesions of NDM in calves depleted of α-TP but supplemented with Se and vice versa indicates that these nutrients were not mutually protective.

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Inhibitory effect of isomeric *cis* and *trans* C-18 monoenoic fatty acids on Δ6-desaturase activity in human vascular endothelial cells. By K. W. J. WAHLE, LESLEY MILNE and C. R. A. EARL, *Lipid Metabolism Unit, Division of Biochemical Sciences, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Isomeric *cis* and *trans* monoenoic, and to a lesser extent dienoic, 18-carbon fatty acids are formed by catalytic hydrogenation of vegetable and fish oils during the manufacture of margarines and shortenings for human consumption. Isomers of longer chain-length, 20–24 carbons, also occur in hydrogenated fish oils. Small quantities of isomers are also formed by biohydrogenation in ruminants. Studies with various mixtures of isomers indicated deleterious effects of these compounds on the metabolism of essential fatty acids (EFA) in animal tissues (Holman, 1985).

Chemical synthesis of isomers made it possible to study their effect on EFA metabolism individually. *Cis* and *trans* 12–18:1 inhibited Δ5-/Δ6-desaturase activity and arachidonic acid (20:4*n*-6; ARA) incorporation into phospholipids in rabbit aorta and skeletal muscle (Wahle *et al.* 1987). The function and EFA metabolism of porcine blood platelets are also impaired in the presence of *trans* fatty acids (Peacock & Wahle, 1988, 1989).

The present studies were undertaken to determine the effect of 12–18:1 isomers on EFA metabolism in human vascular endothelial cells (VEC) in culture. Consumption of *trans* isomeric fatty acids have been implicated in the aetiology of ischaemic heart disease (IHD) in man (Thomas & Winter, 1987). Perturbation of EFA metabolism and possibly eicosanoid synthesis (PGI_2-prostacyclin) in vascular tissue by isomeric fatty acids could be a factor in the disease.

Endothelial cells from human umbilical vein were incubated with [1-^{14}C]linoleic acid (18:2*n*-6) or [1-^{14}C]dihomogammalinoleic acid (20:3*n*-6) for the determination of Δ6-/Δ5-desaturase activity respectively. Isomers were added in equimolar concentration to the respective substrates. Desaturation products were extracted and separated by argentation thin-layer chromatography (TLC).

The activity of Δ5-desaturase was tenfold greater than Δ6-desaturase in VEC, thereby emphasizing the rate-limiting nature of the latter. Both *cis* and *trans* 12–18:1 inhibited Δ6-desaturase activity 40–50% after 60 and 90 min of incubation, but Δ5-desaturase activity was not affected after a 4 h incubation. The isomers did not impair the incorporation of ARA into individual phospholipid or neutral lipid classes after a 2·5 or 5 h incubation. These findings differ from observations in rabbit aorta and skeletal muscle (Wahle *et al.* 1987) and emphasize the shortcomings of extrapolation between species. However, the reduced activity of the rate-limiting Δ6-desaturase in VEC when isomers were present in the incubation medium suggests that isomers could impair EFA metabolism and eicosanoid synthesis in VEC. The consequence of this on the aetiology of IHD can only be surmised.

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Inhibition of enzyme efflux from damaged skeletal muscle: a non-antioxidant role for α-tocopherol? By JOANNE PHOENIX*, R. H. T. EDWARDS and M. J. JACKSON, *Department of Medicine, University of Liverpool, Liverpool L69 3BX*

The role of vitamin E in the protection of isolated skeletal muscles against calcium-induced damage is not understood. Previous studies (Phoenix *et al.* 1989) have suggested that the role of α-tocopherol in the protection of skeletal muscle damage may be unrelated to its function as an antioxidant. To investigate this further a variety of structural analogues of α-tocopherol were used to study the importance of antioxidant activity and molecular structure on ability to inhibit calcium ionophore, A23187, (20 μM)-stimulated creatine kinase (CK; *EC* 2.7.3.2) release from an isolated rat soleus muscle preparation as previously described (Phoenix *et al.* 1989). Compounds were solubilized in ethanol and added throughout the 3 h incubation period at a final concentration of 0·23 mM. Control muscles were treated with an equivalent volume of ethanol. The release of CK was monitored for up to 120 min post-A23187 and the percentage inhibition of total CK efflux was calculated by comparison with control muscles. All compounds were also tested to ensure that they did not interfere in the CK assay. Antioxidant activity was assessed by the ability of compounds to inhibit the formation of thiobarbituric acid-reactive substances (TBARS) in ascorbate/Fe^{2+}-treated mouse skeletal muscle homogenates.

Compound	% Inhibition of CK efflux	% Inhibition of TBARS
Tocol	90·3	77·7
α-Tocopherol	83·1	82·4
5,7-Dimethyl tocol	82·2	58·7
γ-Tocopherol	80·8	34·5
α-Tocotrienol	71·0	91·7
Vitamin K_1 diacetate	63·2	6·8
Phytol	62·0	44·7
α-Tocopherol (10)	60·0	74·3
Phytyl ubiquinone	44·0	4·7
α-Tocopherol acetate	40·9	10·5
Trolox C	0·0	99·7

The results indicate that the action of tocopherol and related compounds to protect against Ca-induced skeletal muscle damage is not directly related to their antioxidant function, but suggest an important role for the hydrocarbon chain of the inhibitory molecule.

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Measurement and significance of hepatic menaquinones in rats fed on low-fibre diets. By M. J. SHEARER and N. KAZIM, *Department of Haematology, Guy's Hospital, London SE1 9RT* and J. C. MATHERS, *Department of Agricultural Biochemistry and Nutrition, The University, Newcastle upon Tyne NE1 7RU*

In a previous study (Mathers *et al.* 1990) rats given a low-fibre diet based on white rice developed severe vitamin K deficiency within 23 d. The dietary inclusion of black-eye beans (*Vigna unguiculata*) prevented the bleeding syndrome but it was not clear whether this was due to an increase in the dietary supply of phylloquinone (vitamin K_1) from the beans or the increased supply of fermentable substrate leading to an increased production of menaquinones (vitamin K_2) by the enteric bacterial flora. To gain further insight into the aetiology of vitamin K deficiency in these experiments, a sensitive method using high performance liquid chromatography (HPLC) has been developed to measure the hepatic concentrations of menaquinones (MKs), measurements of phylloquinone having already been made. A prerequisite of this assay was the ability to detect both major individual isoprenologues of the series ranging from MK-4 to MK-10 and the very low levels expected in those rats showing symptoms of vitamin K deficiency.

Rat livers were extracted with acetone, and neutral lipids partitioned into hexane as previously described (Shearer, 1986*a*). The lipid extract was then subjected to a multi-stage chromatographic procedure to, first, separate MKs as a class from other lipids by normal-phase adsorption chromatography (using solid phase extraction cartridges followed by semi-preparative HPLC) and then to separate and detect individual MKs by reversed-phase HPLC coupled to a dual-electrode electrochemical detector (Shearer, 1986*b*). Quantification of the MKs in livers was carried out by an external standard calibration method in which the height or areas of chromatographic peaks were compared with those given by a standard mixture of pure MKs. Procedural losses (usually 30–40%) were calculated and liver concentrations corrected by the addition of tritium-labelled phylloquinone as an internal standard at the extraction stage and measuring the radioactivity of an aliquot at the final chromatographic stage. The sensitivity for each MK was estimated at about 0·1 pmol/g liver.

This technique was used to determine MK concentrations in livers from groups of rats given an all-rice diet (one animal) or rice diets containing 112·5, 225 or 337·5 g beans/kg respectively (four animals per group). The only available liver from the all-rice group (a rat with severe vitamin K deficiency) showed small peaks of MK-5 and MK-6 only. The rats from other groups showed a wide spectrum of MKs with MK-6 and MK-10 predominating. Total MKs ranged widely from 0·7 to 40 pmol/g. Interestingly, the only two rats with prolonged prothrombin times of >60 s and 24 s showed the lowest total hepatic MK contents of 0·7 and 2·5 pmol/g respectively.

These experiments show the potential of this method for measuring low hepatic concentrations of MKs in rats including individual animals with vitamin K deficiency. Further work is needed to determine the roles of dietary phylloquinone and enteric MKs in preventing vitamin K deficiency, and also whether the rats derived their hepatic MKs by direct absorption from the large bowel or indirectly by coprophagy.

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The effect of high viscosity guar gum flour on the rate of glucose absorption and net insulin production in the portal blood of the pig. By F. G. ROBERTS[1,2], A. G. LOW[1], S. YOUNG[1], H. A. SMITH[1] and P. R. ELLIS[2], [1]*AFRC Institute for Grassland and Animal Production, Shinfield, Reading RG2 9AQ* and [2]*King's College London, Department of Food and Nutritional Science, Campden Hill Road, London W8 7AH*

It is well established that guar gum can decrease post-prandial peripheral blood glucose and plasma insulin levels. However, its effects on the rates of glucose absorption and net insulin production have not been studied directly. Accordingly, we have fitted four 30 kg pigs with permanent catheters in the mesenteric artery and hepatic portal vein, and an ultrasonic probe on the hepatic portal vein to measure blood flow rate (Transonic Systems, Ithaca, NY, USA). In addition a T-piece cannula was placed in the jejunum 2·0 m distal to the pylorus to sample digesta for 'zero shear' viscosity measurement and to relate changes in viscosity to glucose absorption (Roberts *et al.* 1990).

Semi-purified diets containing 0, 20 or 40 g/kg of a high-molecular-weight guar gum (Meyprogat 150, Meyhall Chemical (UK) Ltd) were given twice daily at a level of 40 g/kg body-weight per d. The guar gum was fully hydrated before feeding. Blood samples were taken (6 ml/vessel) at −10, 0, 10, 20, 30, 40, 50, 60, 90, 120, 150, 210 and 240 min after feeding, for glucose and insulin measurements; 10-g samples of digesta were collected at the same time for 'zero shear' viscosity measurements. Continuous blood flood measurements were made allowing quantitative absorption and production to be calculated (Rérat *et al.* 1980).

Table. *Glucose absorption (g) and insulin production (mUnits) following meals containing 0, 20 or 40 g guar gum/kg*

Time (min) . . .	0	10	20	30	40	50	60	90	120	150	180	210	240
Guar gum (g/kg diet)													
						Glucose							
0	0·7	6[a]	7[a]	9[a]	10[a]	6[a]	6[a]	24[a]	25[a]	17[a]	18[a]	23[a]	19[a]
20	1·0	3[b]	6[a]	7[b]	8[b]	7[a]	7[a]	22[a]	18[b]	19[a]	16[b]	13[b]	16[b]
40	2·0	2[c]	4[b]	5[c]	6[c]	4[b]	4[b]	12[b]	15[c]	10[b]	9[c]	12[b]	8[c]
						Insulin							
0	96	238	682[a]	1122[a]	492	692[a]	757[a]	1502[a]	1068[a]	161[a]	921[a]	767	1104[a]
20	65	236	103[b]	169[b]	216	385[b]	187[b]	646[b]	819[a]	956[b]	1264[b]	596	1098[a]
40	99	254	208[b]	150[b]	357	61[c]	227[b]	568[b]	423[b]	570[c]	432[c]	665	455[b]

[a,b,c] Different superscript letters within columns indicate significant differences between treatments: $P<0·05$.

Guar gum depressed the rates of glucose absorption and insulin secretion, but the effect of the 20 g guar gum/kg diet was closer to that of the 40 g/kg diet. The viscosity of the digesta was inversely related to glucose absorption and net insulin production. This indicates much more directly than hitherto a relationship between polysaccharide viscosity and glucose absorption.

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Effects of sucrose-feeding on in vivo rates of hepatic fatty acid, cholesterol and glycogen synthesis in rats given dietary guar gum or volatile fatty acid salts. By LAURENTINA M. R. PEDROSO, J. C. MATHERS and HEATHER J. FINLAYSON, *Department of Agricultural Biochemistry and Nutrition, The University, Newcastle upon Tyne NE1 7RU*

Fermentation in the large bowel (LB) results in the production of volatile fatty acids (VFA) which are absorbed and may influence metabolism in the gut mucosa and other tissues. For example, we have shown that feeding VFA salts, or their production by LB fermentation of guar gum, reduced the activities of hepatic lipogenic enzymes (Pedroso *et al.* 1990). We now report in vivo rates of hepatic synthesis of fatty acids, cholesterol and glycogen for these animals.

Male Wistar rats (five per diet) were offered semi-purified diets based on maize starch (M) and casein. In three diets, 350 g sucrose (S)/kg replaced M, two diets (one M and one S) each contained 100 g guar gum/kg and a further pair of 100 g VFA salts/kg. Hepatic synthesis rates in vivo were measured as ^3H incorporation into saponifiable lipid (fatty acid; Stansbie *et al.* 1976), non-saponifiable lipid (cholesterol) and glycogen (Holness *et al.* 1988) 1 h after intraperitoneal injection of ^3H_2O.

	Hepatic synthesis rates in vivo (μmol ^3H_2O incorporated/h)					
	Fatty acids		Cholesterol		Glycogen	
Diet	Per g liver	Total liver	Per g liver	Total liver	Per g liver	Total liver
M-Basal	5·3	43	0·84	6·7	5·3	42
S-Basal	9·3	80	1·25	11·7	8·2	70
M-Guar gum*	5·7	38	1·82	12·1	5·9	39
S-Guar gum	6·7	54	1·77	14·3	7·1	56
M-VFA	8·6	68	0·78	6·2	5·3	42
S-VFA	10·0	94	1·21	10·2	6·4	55
SEM (*n* 5)	1·66	14·3	0·171	1·36	0·72	5·5

* Four rats.

Synthesis rates of all three fractions were higher in sucrose-fed animals and significantly so for cholesterol ($P<0·01$) and glycogen ($P<0·01$) on a total liver basis. There was no evidence that VFA affected hepatic lipogenesis, in contrast to the stimulatory effects of medium-chain fatty acids reported recently (Souza & Williamson, 1990). Cholesterol synthesis rates were similar for basal and VFA-fed rats but were, on average, 76% greater for the guar gum groups. This increased cholesterol synthesis may be a response to reduced bile acid reabsorption.

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Effects of ageing on the caecal fermentation response to oat-feeding in rats. By J. C. MATHERS[1], JULIA KENNARD[1] and O. F. W. JAMES[2], [1]*Department of Agricultural Biochemistry and Nutrition* and [2]*Department of Geriatric Medicine, The University, Newcastle upon Tyne NE1 7RU*

It has been observed in rats (Key & Mathers, 1987), in in vitro models of large bowel (LB) fermentation (Goodlad & Mathers, 1988) and in man (Scheppach *et al.* 1988) that altering substrate supply can have profound effects on the pattern of volatile fatty acid (VFA) end-products produced in the LB, with potentially important effects on tissue metabolism (Cummings & Englyst, 1987). The in vivo studies have been confined to young animals. In the present study we have compared the responses of young and elderly rats.

Six (three male, three female) young (4 months) and six (three male, three female) elderly (27 months) Wag Rij rats obtained from EURAGE (TNO Institute for Experimental Gerontology, Rijswijk, The Netherlands) were offered 15 g/d for 17–18 d of either a semi-purified diet based on maize starch, sucrose and casein and containing 20 g cellulose/kg diet (basal) or a diet containing 500 g oat meal/kg included at the expense of starch, sucrose and casein (+Oats). Oats are rich in non-starch polysaccharides and were expected to greatly increase supply of fermentable substrate to the LB.

Age . . .	Young		Old		SE of mean	Significance of treatment effects		
Diet . . .	Basal	+Oats	Basal	+Oats	(*n* 6)	Age (A)	Diet (D)	A×D
Caecal pH	7·2	6·5	7·1	6·7	0·11	NS	***	NS
Total VFA (mmol/kg caecal contents)	86	150	82	146	10·3	NS	***	NS
	Proportions of individual VFA (mmol/mol)							
Acetate	600	495	621	631	28·2	*	NS	NS
Propionate	192	120	204	186	9·7	***	***	**
Butyrate	96	319	78	139	21·8	***	***	**
Isobutyrate	51	23	38	18	6·4	NS	**	NS
Isovalerate	34	18	34	15	1·9	NS	***	NS
Valerate	25	15	26	8	2·3	NS	***	NS
Hexanoate	3	10	nd	3	0·5	**	**	NS

nd, not detected; NS, not significant. *$P<0·05$, **$P<0·01$, ***$P<0·001$.

Age had no effect on caecal pH or total VFA concentration and, for both age groups, dietary oats reduced pH whilst increasing total VFA. However, the pattern of caecal VFA was markedly affected by age and diet and there were important age × diet interactions. Oat-feeding reduced both acetate and propionate molar proportions in the young but in the elderly had no effect on acetate and only a minor effect on propionate. Of particular interest was the more than threefold increase in butyrate proportion in young oat-fed rats compared with the more modest increase in the older animals.

A reduction in the ability of the aged LB to produce butyrate may contribute to the prevalence of LB disorders in the elderly since this VFA appears to have specific protective properties (Cummings & Englyst, 1987).

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Effects of dietary fibre (grass) level and time of day on caecal concentrations of volatile fatty acids and uric acid in the fowl. By C. J. SAVORY and ANNE I. KNOX (Introduced by J. M. MCNAB), *AFRC Institute for Grassland and Animal Production, Poultry Department, Roslin, Midlothian EH25 9PS*

Effects of dietary fibre level on caecal concentrations of volatile fatty acids (VFA) and uric acid do not appear to have been studied in the fowl. An opportunity to do so arose in an experiment where intestinal absorption was compared in immature layer-type hens (12–14 weeks old, weight 1·0–1·5 kg) preconditioned for at least 3 weeks to a layer's mash diet with 0 (basal), 100, 200 or 400 g dried grass added per kg (neutral detergent fibre 52, 89, 125 and 198 g/kg respectively). These birds had free access to food until testing, and were killed after approximately 60 min luminal perfusion (with buffer and 10 mM-sugar solutions) of either jejunum or caecum under halothane anaesthesia. One or both intact caeca could thus be removed from each bird. Since fowls typically evacuate their caeca about twice a day, near times of lights on and lights off (Clarke, 1979), composition of their contents might change with time, during microbial fermentation of sub- strate introduced in a refill. To test this, roughly equal numbers of caecal samples were analysed on each dietary treatment from each of the three times when birds were killed (10.30, 12.30 and 15.30 hours). pH and concentrations of VFA and uric acid were measured in homogenized caecal contents. ANOVA were done on data adjusted to allow for effects of analytical run, number of caeca removed, and diet/time of day interaction.

Mean wet weight of contents of one caecum (g), and pH and concentrations of VFA (μmol/g wet wt) and uric acid (mg/g wet wt) in caecal contents

	Dietary grass addition (g/kg, *n* 9 or 10)					Time of day (hours, *n* 12–14)			
	0	100	200	400	Linear grass†	10.30	12.30	15.30	Linear time†
Wt	3·8	4·2	4·5	4·6	*	4·2	4·4	4·2	
pH	7·1	7·2	7·2	7·2		7·3	7·1	7·1	
Acetic	23·0	20·4	16·7	16·5	***	18·0	19·4	19·8	
Propionic	6·0	5·7	5·5	5·9		5·6	5·9	5·8	
Butyric	3·5	5·0	4·1	4·6		3·0	3·9	6·3	***
Iso-butyric	0·22	0·27	0·25	0·27		0·31	0·23	0·22	**
Valeric	0·47	0·67	0·42	0·48		0·47	0·44	0·60	
Iso-valeric	0·38	0·47	0·47	0·52	*	0·53	0·43	0·42	**
Uric	0·37	0·38	0·52	0·60	**	0·50	0·43	0·44	

† Regressions against increasing grass or time: *$P<0·05$, **$P<0·01$, ***$P<0·001$.

Increasing grass in the diet was associated with increases in the weight of caecal contents and in concentrations of iso-valeric and uric acids, and with a decline in acetic acid which comprised 58–69% of total VFA present. Butyrate also increased, but not significantly so. These VFA responses to higher dietary fibre are like those found in the rat (Demigné & Rémésy, 1985). Here, butyrate levels increased during the day while iso-butyrate and iso-valerate declined. Values of pH were all close to neutrality, perhaps due to production of both VFA and ammonia (from uric acid) by caecal microbes. The fact that neither weight of caecal contents nor uric acid level declined with time of day supports the proposal (Gasaway *et al.* 1976) that filling of caeca in galliform birds, with liquids and fine particulate matter, is continuous.

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Continuous in vitro incubation as a model to study microbial metabolism in the hind-gut of pigs. By G. BREVES and J. DREYER, *Institute of Animal Nutrition, Federal Agricultural Research Centre, 3300 Braunschweig, Federal Republic of Germany*

The rumen simulation technique (Czerkawski & Breckenridge, 1977) was modified and applied to in vitro studies on microbial hind-gut metabolism in pigs. Caecal contents were obtained from fistulated animals, and gauze-filtered caecal fluid was used to start the in vitro system. Nylon bags containing freeze-dried particles from the caecum were introduced into the incubation vessels as a pre-digested substrate to be fermented. In each experiment equilibration for 8 d was followed by 6–8 sampling days, with volatile fatty acids (VFA) and gas production, microbial protein synthesis and digestibilities of organic matter (OM) and fibrous carbohydrates being measured.

In the first set of experiments the donor animals were adapted to a grain–soya-bean meal diet with 110 g dried sugar beet pulp/kg. Under these conditions the effects of changes in liquid and particle retention times on microbial metabolism were tested. By increasing the perfusion rate of the isotonic buffer solution the mean liquid retention time in the incubation vessel was decreased from 9·6 to 4·8 h. This resulted in significant increases in VFA and gas production and higher digestibilities of OM, cellulose and hemicellulose. Similar results were obtained by increasing the particle retention time from 24 to 48 h. In the second set of experiments incubations were performed with three diets differing in cellulose:hemicellulose ratio. Increasing the cellulose content of the diet from 44 to 109 g/kg dry matter and decreasing the hemicellulose content from 152 to 50 g/kg resulted in significantly higher digestibilities of OM, cellulose and hemicellulose. With diet 1 (109 g cellulose, 50 g hemicellulose/kg) VFA production was more than twice as high as with the other two diets. Microbial protein synthesis was not affected by diet composition.

Effect of diet composition on microbial metabolism

Cellulose content (g/kg) . . .	109		87		44	
Hemicellulose content (g/kg) . . .	50		101		152	
	Mean	SD	Mean	SD	Mean	SD
pH	6·27[a]	0·04	6·36[b]	0·06	6·42[c]	0·01
VFA production (mmol/d)	22·6[a]	1·9	8·0[b]	0·9	9·4[b]	0·4
OM digestibility (%)	43·7[a]	3·5	38·5[b]	1·8	34·0[c]	0·7
Microbial protein synthesis (mg/d)	225·0[a]	19·0	269·0[a]	44·0	262·0[a]	20·0
Efficiency of growth (mgN/gDOM)	21·8		30·0		36·7	

VFA, volatile fatty acids; OM, organic matter; DOM, digestible organic matter.
[a,b,c] Values with different superscript letters were significantly different, $P<0·05$ (Scheffé test).

In order to test the transferability of the in vitro data, fistulated pigs were used for in vivo experiments with all three diets. In these studies the hind-gut digestibilities of OM, cellulose and hemicellulose were calculated from measurements in situ. Nylon bags containing freeze-dried particles of the respective diet were placed into the caecum for 24 h. With both techniques similar relative differences between the three diets were obtained, indicating the suitability of the continuous incubation technique as a model to study microbial metabolism in the hind-gut.

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Effect of carbohydrate source and protein level on selected plasma characteristics and acetate metabolism of ovine perirenal adipose tissue in vitro. By N. D. SCOLLAN and N. S. JESSOP, *Department of Agriculture, University of Edinburgh, Edinburgh EH9 3JG*

On feeding isoenergetic diets reduced efficiencies of metabolizable energy (ME) use for growth were observed when the main dietary carbohydrate source was sugar beet feed (fermentable fibre) rather than barley (fermentable starch) (Emmans *et al.* 1989). This may reflect inefficient utilization of acetate. Scollan *et al.* (1988) reported the potential for increased interconversion between acetate and acetyl CoA when sugar beet replaced barley in the diet. MacRae *et al.* (1985) suggested that enhanced amino acid supply might ameliorate the reduced energetic efficiency observed with high-forage diets. The present study investigated the interaction between dietary protein supply and carbohydrate source on levels of plasma acetate, glucose and insulin and acetate metabolism in isolated adipocytes.

Sixteen Blackface wethers (approximately 27·7 kg) were allocated to one of four treatments based either on barley (B) or sugar beet pulp (S) at two levels of dietary protein (L and H, 115 and 201 g crude protein (nitrogen × 6·25)/kg respectively). ME intakes were similar across treatments. On day 28, after semi-continuous feeding for 3 d, blood was sampled every 4 h for 24 h. At slaughter (after 63 d), samples of perirenal adipose tissue were collected, adipocytes prepared, and the rates of [1-^{14}C]acetate incorporation into lipid and carbon dioxide assessed.

	Treatment								Main effects	
	BL		SL		BH		SH		Diet	Protein
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	(B *v.* S)	(L *v.* H)
Acetate (mM)	0·64	0·10	0·83	0·07	0·48	0·07	0·57	0·05		*
Glucose (mM)	4·39	0·16	4·42	0·07	4·36	0·10	4·08	0·07		
Insulin (μU/ml)	25·8	2·45	28·5	2·56	21·6	1·10	22·3	1·56		
Acetate to CO_2 (nmol/2 h per 10^6 cells)	294	20·6	421	40·0	310	27·0	355	44·0	*	
Acetate to lipid (nmol/2 h per 10^6 cells)	1306	101	1200	137	1632	101	1714	58		*

*$P<0·05$.

Diets based on sugar beet pulp produced higher plasma acetate concentrations, with little difference in plasma glucose and insulin. The rate of acetate incorporation into carbon dioxide was lower for barley diets ($P<0·05$) and higher levels of protein increased the rate of acetate incorporation into lipid ($P<0·05$). The results are consistent with amino acid supply facilitating acetate incorporation into lipid, but the mechanism remains unknown.

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Effect of intraruminal propionate infusion on rumen fermentation and whole body glucose turnover rate in forage-fed steers. By C. J. SEAL, C. S. ROPER and D. S. PARKER, *AFRC Link Research Group, Department of Agricultural Biochemistry and Nutrition, University of Newcastle, Newcastle upon Tyne NE1 7RU*

The importance of propionate as a precursor for glucose synthesis in the ruminant is well-established but the evidence for its effect on glucose turnover is often unclear, since dietary manipulations also influence the balance of other fermentation end-products as well as propionate. As a consequence the supply of other oxidative intermediates will change visceral glucose demand which forms a significant proportion of total glucose turnover (Weighart *et al.* 1986). The present experiment was designed to investigate the effect of varying propionate supply without affecting rumen fermentation on glucose metabolism.

Four Friesian steers (live weight 125–152 kg), each with a rumen fistula and a chronic carotid artery catheter, were fed on a grass-pellet diet (31 g nitrogen, 234 g crude fibre, 19·8 MJ gross energy/kg dry matter (DM)) hourly at 28 g DM/kg live weight per d. Each received by random allocation for 7 d a continuous intraruminal infusion of propionic acid (PA) in water, equivalent to 1 mol PA/1·44 litres per d, and a control infusion of the same volume of distilled water. Blood and rumen fluid samples were obtained during the last 6 h of a 16 h intraruminal infusion of (1) [2-^{14}C]acetate (0·25 μCi/ml per min), (2) [2-^{14}C]propionate (0·125 μCi/ml per min), and (3) a 7·5 h continuous intrajugular infusion of [6-^{3}H]glucose (1·5 μCi/ml per min).

		Propionate infusion rate (mol/d)			Significant effect of infusion
	n	0·0	1·0	SEM	(ANOVA)
Molar % VFA	4				
Acetate		71·6	65·9	2·34	$P<0·05$
Propionate		17·3	24·9	2·28	$P<0·01$
Butyrate		11·1	9·2	1·47	NS
Acetate production rate (mmol/min)	4	5·42	5·59	0·46	NS
Propionate production rate (mmol/min)	3	2·01	3·05	0·43	NS
Glucose turnover rate (mmol/min)	3	1·69	2·49	0·30	NS
Proportion of glucose from propionate	3	0·61	0·54	0·09	NS

VFA, volatile fatty acids; NS, not significant.

The pattern of fermentation within the rumen was unaffected by the infusion of PA since although the molar percentage of acetate in rumen fluid was significantly lower and that of propionate significantly higher in PA-infused animals, acetate production rate was not affected by the infusion, and propionate production rate was increased by an increment comparable to the infusion rate of exogenous PA.

Glucose turnover rate was not significantly affected by treatment but tended to be higher for PA-infused animals, suggesting that the supply of propionate *per se* may influence whole body glucose metabolism, although the proportion of glucose derived from propionate did not change.

The effect of propionate on visceral metabolism of glucose and volatile fatty acids is being further investigated.

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Digestibility of wheat middlings by the weaned pig: comparison of balance and indicator methods. By DEVINA MCCLEAN[1], K. KING[1,2] and K. J. MCCRACKEN[1,2], [1]*Department of Food and Agricultural Chemistry, The Queen's University of Belfast* and [2]*Department of Agriculture, Northern Ireland, Newforge Lane, Belfast BT9 5PX*

The young pig can utilize diets containing high levels of by-products such as wheat middlings (pollard) but the net efficiency of energy utilization is low (Pals & Ewan, 1978; McCracken *et al.* 1986). This is probably related to high levels of hind-gut fermentation. As a preliminary stage in studies to elucidate the contributions of the small and large intestine to energy utilization, an experiment was conducted to determine the effect of level of inclusion of pollard in a highly digestible diet on apparent digestibility and to assess the suitability of titanium dioxide as an indicator.

Three replicates were conducted each using eight littermate pigs weaned at 4 weeks of age and randomly allocated to one of eight diets. The basal diet contained (g/kg): dried skim milk 350, fish meal 150, starch 500, and the other diets were produced by diluting the basal diet with pollards in 100 g/kg stages to 700 g/kg. Minerals–vitamins were added to all diets (10 g/kg) and titanium dioxide was included at 1 g/kg. The pigs were given a weaner diet (McCracken & Caldwell, 1980) for 1 week followed by the experimental diet for 2 weeks, of which the balance period was the last 7 d.

Dry matter (DM) digestibility decreased linearly from 0·95 for diet 1 to 0·74 for diet 8 and organic matter digestibility decreased from 0·97 to 0·77. The mean recovery of titanium dioxide in DM was 0·97 (SEM 0·005) for the eight diets. Digestible energy:gross energy decreased from 0·96 to 0·74 respectively for diets 1 to 8. The calculated apparent digestibility coefficients for organic matter and energy in the wheat middlings were 0·69 and 0·64 by linear extrapolation of the equations $y=0{\cdot}9692-0{\cdot}0028x$ and $y=0{\cdot}9631-0{\cdot}0032x$ respectively. The digestible energy of the pollards was 12·29 MJ/kg DM.

It is concluded that the effects of inclusion of wheat middlings on apparent digestibility are linear up to inclusion at 700 g/kg, and that titanium dioxide is very suitable as an inert indicator for the determination of faecal DM excretion.

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Patterns of children's malnutrition in an area of Southern Somalia with multiethnic composition. By F. BRANCA[1,2], A. ALAS ALI[3] and T. D'ARCA[2] (Introduced by A. FERRO-LUZZI). [1]*Istituto Nazionale della Nutrizione, Via Ardeatina, 546, 00195 Rome, Italy*, [2]*Comitato Internazionale per lo Sviluppo dei Popoli, Via Marziale, 47, 00136 Rome, Italy*, and [3]*Ministry of Health, Somali Democratic Republic*

The growth performance of 694 children below 5 years of age was assessed in a rural area of Southern Somalia along the banks of the river Juba. Children were randomly selected from a censused population of 20 000 composed of three main ethnic groups: people of Bantu origin, settled in the area since the last century; Southern Somali, recently attracted to the area by working opportunities in industrial agriculture; and Northern Somali, former nomadic pastoralists resettled by the Somali government after the droughts of 1974 and 1978. A primary health care programme was implemented in the area by the Italian non-governmental organization CISP.

Anthropometric measurements included body-weight and recumbent length or standing height. Mothers were then interviewed about the composition of their household, their socio-economic level, children's feeding pattern, immunization status and recent disease episodes.

Children's mean weight-for-height was significantly larger among Bantu children (Table) than in the other groups. Conversely, height-for-age was closer to the NCHS reference among Northern Somali children than among Southern Somali and Bantu children. Among Northern Somali children the prevalence of moderate/severe stunting (9·2%) and of moderate/severe wasting and stunting (1·6%) was also lower than in any other group.

Table. *Anthropometric indicators in three ethnically different groups of children living in the same area of Southern Somalia*

	Bantu			Southern Somali			Northern Somali		
	n	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM
Height-for-age Z score	212	−1·66[a]	0·15	241	−1·20[b]	0·12	123	−0·71[c]	0·14
Wt-for-height Z score	211	−0·66[a]	0·08	240	−1·26[b]	0·07	123	−1·08[b]	0·09

[a,b,c] Means with different superscript letters are significantly different ($P<0·05$).

Multiple regression analysis showed that, among Bantu children, the use of health facilities and the absence of recent disease were better predictors of weight-for-height than socio-economic factors. Among the Northern Somali, income from both agriculture and salaried work was associated with better weight-for-height, whereas the number of siblings that a child had was negatively correlated. None of the variables, however, showed predicting value among the Southern Somali.

The higher prevalence of phenomena of longitudinal growth retardation in one group and of decreased body size in another group of children living in the same area would suggest that the growth pattern and the final body build of the different groups are the result of an interaction between genetic programming and environmental factors.

Anthropometric indices and risk of mortality allowing for socio-economic differences among Ugandan children. By V. VELLA[1,5], A. TOMKINS[1], I. CORTINOVIS[2], T. MARSHALL[3], J. NDIKU[4] and V. AYATOMIZEK[4], [1]*Centre for Human Nutrition and* [3]*Department of Medical Statistics, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT,* [2]*Department of Biostatistics, University of Milan, Italy,* [4]*Ministry of Health, Uganda* and [5]*UNICEF*

The relative risk of morbidity (Tomkins *et al.* 1989) and mortality (Briend & Zimicki, 1986) varies with nutritional states and according to the anthropometric criteria used. However, there is a need to allow for socio-economic factors as confounders in these associations. A baseline survey was carried out in April–May 1988 in the district of Mbarara, Southwest Uganda.

Weight, height and mid-upper arm circumference were measured in 4320 children age 0–60 months. Health and socio-economic variables were collected from each household. After 12 months a follow-up survey was carried out in order to assess the number of children who had died.

Nineteen socio-economic variables were analysed through a programme of Multiple Correspondence Analysis (Benzecri, 1973). From the data set, seven socio-economic groups were initially developed which were then reduced to three because of similarities between some of the groups.

Group 1 (1614 children) was the most advantaged with a higher education, and a high prevalence of government workers and professionals who were able to hire agricultural labour; radio ownership was relatively high. Group 2 (1149 children) was mainly composed of cattle keepers and producers of export crops, with primary education, not hiring labour and not working on other people's land; radio ownership was less prevalent. Group 3 (596 children) was the most disadvantaged with minimal or no education, mainly subsistence farmers working on other people's land. The proportion of children dying associated with different cut-off points of anthropometric indicators was analysed using logistic regression which gave smoothed or averaged predicted percentages (Table).

	Wt for age SD score			Height for age SD score			Wt for height SD score			Mid-upper arm circumference SD score		
Group	<−3	<−2	>−2	<−3	<−2	>−2	<−3	<−2	>−2	<12·5	12·5–13·5	>13·5
1	4·8	3·3	1·8	2·5	2·2	2·0	8·3	4·1	1·9	7·4	3·5	1·4
2	6·0	4·3	2·5	3·5	3·2	2·8	9·9	6·0	2·8	10·0	4·6	2·0
3	8·1	5·7	3·3	4·6	4·2	3·7	11·3	7·9	3·8	13·0	5·4	2·3

These findings show that malnutrition is an important risk factor for mortality in all socio-economic groups in this population.

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Malnutrition as a risk factor for mortality from infectious diseases among Ugandan children. By V. VELLA[1,3], A. TOMKINS[1], J. NDIKU[2] and V. AYATOMIZEC[2], [1]*Centre for Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT,* [2]*Ministry of Health, Uganda* and [3]*UNICEF*

Recently it has been suggested that for certain infections, malnutrition is not an important cause of death. Rather the higher risk of mortality is related to the poor socio-economic conditions in which the undernourished children live (Aaby, 1988). The present study examined the relationship between nutritional status and subsequent risk of death from specific causes. A baseline survey was carried out in South West Uganda in April–May 1988. Height, weight and mid-upper arm circumference (MUAC) were measured in 4320 children age 0–60 months. After 12 months a follow-up survey assessed the mortality of the children during the preceding year. The relative risk of mortality (compared to 1·0) from specific diseases was assessed in comparison to different cut-off points of anthropometric indicators at the start of the study.

Table. *Relative risk for cause of deaths from infectious diseases*
(95% confidence intervals in parentheses)

		Fever	Diarrhoea	ARI	Measles	Other causes
Wt/age, SD	<−3	6·5* (1·3–31)	10·0*** (3–32)	3·2 (0·7–14)	8·5** (2·2–31)	1·6 (0·2–12)
	<−2	2·4 (0·6–9)	5·6*** (2·2–14)	1·2 (0·3–4)	1·4 (0·3–6)	2·4 (0·9–6)
	>−2	1	1	1	1	1
Height/age, SD	<−3	4·2* (1·3–13)	1·4 (0·4–4)	3·3* (1·2–9)	1·4 (0·3–7)	2·3 (0·5–9)
	<−2	0·5 (0·1–5)	1 (0·3–3)	1·5 (0·5–5)	2 (0·5–6)	0·8 (0·2–2)
	>−2	1	1	1	1	1
Wt/height, SD	<−2	2·3 (0·3–18)	5·7** (2–16)	1·4 (0·2–10)	4·7 (1–21)	4·2* (1·3–14)
	>−2	1	1	1	1	1
MUAC (mm)	<125	6·2** (1·8–21)	12·3*** (4·3–33)	9·5*** (3·7–24)	4·6* (1·2–18)	2·5 (0·7–8)
	<135	0·8 (0·1–6·7)	6*** (2·1–17)	1·3 (0·3–6)	2·6 (0·6–10)	2·3 (0·8–6)
	>135	1	1	1	1	1

ARI, acute respiratory infections.
*$P<0·05$, **$P<0·01$, **$P<0·005$.

The study shows the importance of anthropometric indicators as risk factors for specific mortality. The best predictor was MUAC. Malnourished children appeared to have significantly higher risk of death from fever, measles, diarrhoea and acute respiratory infections while there was no evidence of higher risk for other causes of death. Diseases which are influenced by nutritional status have a higher fatality rate among malnourished children in this community.

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Impact of *Ascaris lumbricoides* and other intestinal helminths on intestinal permeability and growth in Bangladeshi children. By C. A. NORTHROP-CLEWES and P. G. LUNN, *Medical Research Council Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ* and E. ROUSHAM and C. G. N. MASCIE-TAYLOR, *Department of Biological Anthropology, University of Cambridge, Cambridge*

The impact of infection with *Ascaris* and other parasitic helminths on growth and intestinal permeability has been investigated in 2 to 5-year-old children living in a rural community in the Jamalpur region of Bangladesh. One hundred and twenty children were recruited into the study and their nutritional status, parasite load and intestinal permeability assessed. On study days, children were invited to attend the Save the Children Clinic in Pullakandi in groups of twenty and asked to bring a recent faecal sample with them. A portion of the stool sample was fixed in Schaudinn's fluid to allow later examination for helminth eggs and protozoan cysts. To measure intestinal permeability, each child was given a 20 ml drink which contained 4 g lactulose and 1 g mannitol, following which urine was collected for 5 h. Total urine volume was measured and a portion frozen for later analysis. Whilst at the clinic, anthropometric measurements were made and mothers were questioned about their child's health and diet. Half of the children were then treated, initially with pyrantel pamoate to allow *Ascaris* size and number to be measured, then, at 2-monthly intervals, with mebendazole. The remaining sixty children received placebos at each visit.

Following therapy for 8 months, the prevalence of *Ascaris* had fallen from 76% to 3%. Hookworm prevalence fell from 6% to 2% and *Trichuris* from 64% to 12%. At this time, intestinal permeability, expressed as the ratio of lactulose: mannitol (L/M) was significantly lower in treated children (ln L/M −1·36 (SD 0·47), geometric mean 0·26) than in untreated counterparts (ln L/M −1·04 (SD 0·52), geometric mean 0·35, $P<0·01$). However, in both groups, values were more abnormal than they had been initially (ln L/M −1·56 (SD 0·46), geometric mean 0·21, $P<0·05$ for the treated group and ln L/M −1·38 (SD 0·58), geometric mean 0·25, $P<0·01$ in untreated children). Two months after anthelmintic administration commenced, treated children were heavier (weight/age Z-scores −2·24 and −2·60, $P<0·05$) and taller (height/age Z-scores −1·82 and −3·07, $P<0·01$) than untreated counterparts. However, after 8 months, despite better intestinal permeability, this improved growth had faltered and the difference between the groups no longer reached significance.

The failure to sustain early growth improvement might be explained by a marked increase in the prevalence of the protozoal intestinal parasite, *Giardia lamblia* which occurred only in the group of children receiving anthelmintic. Before they were treated only 3% of children in this group harboured this parasite, but 8 months later stool examination revealed that 32% were infected. In the untreated group 17% of children had giardiasis initially, but 8 months later the prevalence was similar at 15%.

We conclude that although some improvement has been observed, the beneficial effects of deworming have been confounded by a concomitant increase in *Giardia lamblia* prevalence. Clearly a more sophisticated statistical analysis will be required to resolve the individual effects of each parasite.

Sire effects on growth and carcass characteristics of Friesian steers during the finishing period. By K. J. MCCRACKEN[1,2], C. A. MOORE[1], R. W. J. STEEN[1,2], E. F. UNSWORTH[1,2], D. J. KILPATRICK[1,2] and F. J. GORDON[1,2], [1]*Department of Agriculture for Northern Ireland* and [2]*The Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX*

The factors affecting growth, feed conversion efficiency (FCE) and carcass composition of cattle include breed, strain within breed, sex, stage of maturity, energy intake and diet form. Most of these factors have been quantified (Agricultural Research Council, 1980). However, the effects of energy intake remain controversial (McCracken & Unsworth, 1989) and there is little evidence from nutritional studies on the extent of variation within breeds. In a study designed primarily to examine the effects of plane of nutrition, Friesian steers (*n* 48) were selected from seven sires (*n* 11, 9, 11, 6, 6, 3, 2 respectively) and reared under standard conditions to 380 kg. They were randomized within four weight blocks to one of four feeding levels (*ad lib.*, and 90, 80, 70% of *ad lib.*) and one of three slaughter weights (500, 550, 600 kg), balancing for sires as far as possible, and individually fed on a diet of (g/kg): 700 silage, 300 concentrate. At slaughter all carcass and offal components were collected, weighed and retained for chemical analysis.

Although there were no significant differences, due to sire, in rate of daily gain up to 380 kg (0·94, 1·00, 0·99, 1·02, 0·96, 1·05, 0·96 kg respectively, SEM 0·029; *n* 6) there was a significant difference ($P<0·05$) during the finishing period. FCE was inversely related to live-weight gain (LWG) during the finishing period but the sire effect just failed to be significant. The combined internal fat depots (TIF), ie. kidney and channel fat, omental and mesenteric fat; as per cent empty body-weight (EBW) (taken to represent carcass fatness) were significantly affected ($P<0·001$) by sire and the range (7·9–10·7) was greater than that for the adjusted plane of nutrition means (8·4–10·3). There was a significant linear relationship (r 0·69) between TIF:EBW % (y) and FCE (x) of the form, $y=3·62+0·624$ (SEM 0·116)x.

Sire . . .	NI	DG	SS	HJ	PA	PB	AM	Signifi-cance,	SEM
n . . .	10	9	11	6	6	3	2	*P*	(*n* 6)
ME intake (MJ/kg $W^{0·75}$ per d)	0·72	0·72	0·73	0·75	0·73	0·73	0·73	NS	0·011
LWG (kg/d)	0·67	0·79	0·68	0·81	0·64	0·71	0·69	0·05	0·053
FCE (kg DM intake/kg gain)	9·7	8·3	9·7	8·3	10·3	8·7	8·6	0·08	0·69
TIF:EBW (%)	10·7	7·9	9·5	8·4	10·4	8·0	9·8	<0·001	0·52

ME, metabolizable energy; W, body-weight; DM, dry matter.

These results emphasize the extent of strain differences within breed and the importance of taking account of sire effects in the design of nutritional studies on cattle.

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The influence of adrenalectomy on the 'anabolic' effect of testosterone implants in rats. By C. J. H. WOODWARD[1], R. E. OAKEY[2] and E. M. WHITAKER[1], [1]*Department of Physiology* and [2]*Division of Steroid Endocrinology, Department of Chemical Pathology, University of Leeds, Leeds LS2 9NQ*

Subcutaneous implants of testosterone in silastic tubes significantly increase weight gain and lean body mass in gonadectomized rats of both sexes (Woodward *et al.* 1990). In the present study we have investigated whether the absence of the adrenal hormones alters this anabolic effect.

Male and female Wistar rats, weighing approximately 190 g and 150 g respectively, were gonadectomized (GDX) and either adrenalectomized (ADX) or sham-operated. Half of the rats were given testosterone implants (i.d. 2 mm; length 10 mm) (IMP). After 5 weeks the animals were killed and the carcasses analysed for water, fat and defatted dry matter. Fat-free mass was calculated as the sum of carcass water and defatted dry matter.

	Final body-wt (g)		Wt gain (g)		Fat-free mass (g)		Fat (g)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Males:								
GDX	315	6·8	128	5·4	255	5·5	42	2·5
GDX/IMP	333	4·6	147*	3·1	264	5·4	49*	3·4
GDX/ADX	242	9·5	54	8·8	196	7·7	28	1·5
GDX/ADX/IMP	288***	5·7	100***	5·6	232***	5·2	34	1·2
Females:								
GDX	242	5·1	96	2·6	189	3·4	36	2·8
GDX/IMP	259*	6·0	112*	5·4	202*	3·5	39	2·9
GDX/ADX	200	3·2	54	2·6	162	2·2	22	1·7
GDX/ADX/IMP	236***	6·8	91***	5·3	195***	5·3	23	1·5

n 8 rats per group.

* Significantly different from non-implanted group of the same sex and adrenal status: **P*<0·05, ****P*<0·001.

The testosterone implants increased weight gain by approximately 15% in GDX rats of both sexes (see Table). GDX/ADX rats grew more slowly than GDX animals. The implants caused larger increases in weight gain and fat-free mass when given to GDX/ADX animals than they did in GDX rats. Thus the interaction between the effects of adrenalectomy and testosterone was significant ($P<0·05$) for both sexes, in respect of both weight gain and fat-free mass.

It is concluded that adrenalectomy enhances the 'anabolic' effect of testosterone implants. It is not clear, however, whether this is caused specifically by the absence of the adrenal hormones, or whether it is a consequence of the lower growth rate caused by adrenalectomy.

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Metabolism in vivo of essential fatty acids in man using linoleic acid labelled with carbon-13 stable isotope. By A. SMITH, MOIRA THOMAS and K. W. J. WAHLE, *Lipid Metabolism Unit, Division of Biochemical Sciences, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Considering the putative importance of dietary essential fatty acids (EFA) of both the *n*-6 and *n*-3 families in preventing or ameliorating a wide variety of clinical disorders in man, surprisingly little is known about the metabolism of these lipids in vivo. Our understanding of the dietary and endocrine regulation of EFA metabolism, particularly through control of the activity of the Δ5-/Δ6-desaturase enzymes, is based largely on observations from animal studies using tissue preparations either in vivo or ex vivo (Brenner, 1981). Studies on EFA metabolism in animals in vivo generally use substrates labelled with radioactive carbon-14 or tritium (^{3}H) but their use in human studies, particularly in the young, is discouraged due to the potential (albeit small) hazard involved. Deuterium (^{2}D), a non-radioactive stable isotope, was used by El Boustani *et al.* (1989) as a marker in dihomo-γ-linolenic acid (DLA, 20:3 *n*-6) in order to study the plasma lipid incorporation and Δ5-desaturation of this EFA in man in vivo. These workers found decreased Δ5-desaturation of DLA to arachidonic acid (ARA, 20:4 *n*-6) and decreased DLA incorporation into plasma lipids in diabetics when compared with healthy subjects; impaired DLA metabolism was restored with insulin treatment. Human tissues exhibit impaired EFA metabolism at the Δ6-desaturase enzyme, which is regarded as the rate-limiting reaction in the pathway (Chapkin & Ziboh, 1984). Whether this pertains in vivo is not clear.

[1-^{13}C]Linoleic acid (LA, 18:2 *n*-6) was synthesized by electrolytic decarboxylation of unlabelled LA with chain extension using K^{13}CN and given to healthy volunteers in order to address this question. Present studies show that within 2 h, the label was incorporated into neutral lipid (NL), phospholipid (PL) and unesterified fatty acids (UEFA) of blood plasma. This suggests that some LA is absorbed directly into the blood rather than into lymph. Products of LA elongation (20:2 *n*-6) and Δ6-desaturation (18:3 *n*-6) increased with time in all plasma lipids; a steady state was achieved after 8 h in UEFA and after 12 h in NL and PL. Chain-elongation of 18:3 *n*-6 to 20:3 *n*-6 (DLA) also occurred but no label was found in ARA. This suggests that either Δ5-desaturase activity or the specific activity of substrate is low. Use of [1-^{13}C]DLA will clarify these points.

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The contractile characteristics of rat skeletal muscle following consumption of a low-protein diet. By L. B. LEVY and S. A. WOOTTON, *Department of Human Nutrition, Southampton University, Southampton SO9 3TU*

Inadequate intakes of energy and protein results in skeletal muscle wastage. The aim of the present study was to examine the influence of muscle wastage associated with the consumption of low-protein diets on the contractile characteristics of skeletal muscles selected to reflect the differing muscle fibre types: soleus (type I), extensor digitorum longus (EDL, type II_B) and tibialis anterior (TA, type $II_{A/B}$).

Muscle function was assessed *in situ* under Sagatal anaesthesia in male Wistar rats (initial weight 100·3 (SE 1·7) g) at the commencement of the study (CONTROL) and following 21 d during which the animals received a 150 g protein/kg diet (FED) or a 5 g protein/kg diet (LP) *ad lib*. Contractile characteristics, elicited via supramaximal stimulation of the sciatic nerve, were assessed during single twitches (1 Hz) and during stimulation of increasing frequencies up to 200 Hz using a microcomputer. Fatigability was assessed by Burke's Paradigm (Burke *et al.* 1973). Briefly, this is the decline in force generation over 5 min of intermittent stimulation at a frequency of 40 Hz (330 ms train:670 ms rest), the fatigue index being defined as the force generated at 2 min as a ratio of that generated at the commencement of stimulation.

Growth in the FED group resulted in a 2·5-fold increase in body-weight ($P<0·01$) compared with CONTROL, while the LP diet was associated with weight loss (29%, $P<0·01$). Muscle weight was raised in all muscles studied in the FED group (61%, $P<0·01$) while a 25% lower weight was exhibited by the muscles of animals of the LP group ($P<0·01$).

In general, growth in the FED animals was associated with a 1–2·5-fold greater force generation in absolute terms (*v.* CONTROL) but no alteration in either force–frequency relationship or fatigue index was noted. Time to peak tension in the TA muscle of the FED group was 7% faster ($P<0·01$) than that of CONTROLS.

All muscles of the LP group exhibited maintenance and in some cases enhancement of force generation despite reduced muscle mass, resulting in raised force generation per unit muscle (50–100%, $P<0·01$). Twitch time of the soleus muscle of the LP animals was slower (40%, $P<0·01$) but was unaltered in the EDL and TA. Force generation of 50 Hz was raised in the EDL (15%, $P<0·01$), lower in the soleus (7%, $P<0·05$) and unaltered in the TA. The fatigability of the muscles tended to be raised, reaching statistical significance only in the TA (30%, $P<0·01$).

Thus, in contrast to the preservation of skeletal muscle function under conditions of undernutrition without loss of muscle mass (Levy & Wootton, 1989, 1990), muscle wastage following consumption of a low-protein diet resulted in functional alterations in skeletal muscle function which would be expected to reduce the working capacity of the animal.

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Characterization of the haemodynamic responses to the ingestion of a high-fat and a high-carbohydrate meal in young subjects. By M. B. SIDERY[1], I. A. MACDONALD[1] and L. J. FULLWOOD[2], [1]*Department of Physiology and Pharmacology* and [2]*Department of Medicine, University of Nottingham Medical School, Clifton Boulevard, Nottingham NG7 2UH*

In healthy, elderly subjects, a high-carbohydrate meal has effects on heart rate and blood pressure which are not seen with a high-fat meal (Potter *et al.* 1989). The present investigation was designed to assess the cardiovascular effects of similar meals in ten healthy, young subjects (four female, mean body mass index 21·6 kg/m^2, age range 22–35 years). Each subject was studied on two occasions after an overnight fast, resting supine, before and after the ingestion of test meals (2·5 MJ) containing 71% of the energy as fat or 72% as carbohydrate (60% of the carbohydrate was starch, the remainder being sucrose) (Potter *et al.* 1989). Measurements of cardiac output (indirect Fick), calf blood flow (BF) (venous occlusion plethysmography), mesenteric BF (Duplex Ultrasound), heart rate and blood pressure (BP) (automated oscillometry), and arterialized–venous blood glucose were made during fasting and for the first 60 min postprandially.

Cardiac output rose by approximately 1 litre/min postprandially with no significant difference between the meals. Calf BF rose slightly after the carbohydrate meal, but fell within the first 15 min after the fat meal and remained low throughout the remainder of the experiment ($P<0·03$, ANOVA). Fifteen min after the carbohydrate meal, mesenteric BF had increased by 135 ml/min, and subsequently declined towards fasting levels. By contrast, after the fat meal, mesenteric BF rose progressively to a peak increase of 183 ml/min at 45 min ($P<0·05$, ANOVA). Heart rate increased by approximately 5 beats/min after each meal but there were no significant changes in BP. Blood glucose rose by 2·3 mmol/l after the carbohydrate meal and 0·5 mmol/l after the fat meal ($P<0·05$, ANOVA).

These results confirm the observations of Qamar & Read (1988), that a high-fat meal gives maximal responses of mesenteric BF at a later stage than a high-carbohydrate meal. The postprandial changes in gut blood flow do not correspond with the changes in plasma glucose levels. The postprandial changes seen in BP by Potter *et al.* (1989) would not be explained by the differences in mesenteric BF but could be influenced by the differences in limb BF.

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The effect of a low-fat diet on hormone and metabolite concentrations following a mixed meal in healthy subjects. By LINDA M. MORGAN, JACKI A. TREDGER, JACQUELINE WILKINSON, CHRISTINE M. WILLIAMS, A. ZAMPELAS and VINCENT MARKS, *Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH*

We have previously shown (Morgan *et al.* 1988) that a low-fat diet affects gastric emptying rates and postprandial gastric inhibitory polypeptide (GIP) and triacylglycerol levels following oral fat in healthy human subjects. GIP plays a major role in the stimulation of insulin secretion via the entero-insular axis. Its modification by dietary change could be relevant in determining the degree of hyperinsulinaemia which occurs following a meal, and consequent association with the pathology of various hyperinsulinaemic states in which dietary factors also play a part. The present study investigates the effect of a low-fat diet on hormone and metabolite concentrations following a mixed meal.

Plasma GIP, C-peptide, insulin, glucose and triacylglycerol levels were determined in eleven healthy non-obese subjects (mean age 22·8 (SD 6·3) years, body mass index 21·6 (SD 1·9) kg/m^2), for 3·5 h following a test meal (4184 kJ (1000 kcal), 36 protein, 53 g fat, 100 g carbohydrate) before and after a 6 week period on a low-fat diet (LFD). Paracetamol (1·5 g) was given with the meal on each occasion as an index of liquid gastric emptying (Holt *et al.* 1976). Dietary intakes of major nutrients were calculated from 10 d records of weighed food intake, for both habitual diets and the LFD. During the LFD period mean daily fat intake fell from 90 (SD 25) g to 37 (SD 15) g ($P<0·01$). Total daily energy intake showed a small but significant fall from 8800 (SD 1820) to 7650 (SD 2100) kJ (2104 (SD 434) to 1828 (SD 501) kcal) on the LFD ($P<0·01$). Total daily carbohydrate intake rose from 230 (SD 47) g to 281 (SD 70) g ($P<0·01$), and sugar intake from 102 (SD 26) g to 136 (SD 47) g ($P<0·01$). Fasting cholesterol fell from mean levels of 4·35 (SD 0·63) mmol/l to 3·97 (SD 0·51) mmol/l on the LFD ($P<0·01$). Fasting triacylglycerol rose from mean values of 0·66 (SD 0·26) mmol/l to 0·91 (SD 0·32) mmol/l ($P<0·025$). Fasting GIP, insulin, C-peptide, HDL cholesterol, apolipoproteins A-1 and B and glucose were unaltered by the LFD. Postprandial glucose tolerance was improved following the LFD (peak glucose 8·1 (SD 1·2) mmol/l with habitual diet *v.* 6·6 (SD 1·1) mmol/l on LFD, $P<0·01$). Postprandial GIP, insulin, C-peptide and triacylglycerol concentrations were similar following both test meals. There was a tendency to lower postprandial insulin secretion following the LFD between 90–210 min as evidenced by lower mean insulin and C-peptide levels over the latter part of the test. Liquid gastric emptying rates following the test meal were unaltered by the LFD. Failure of the LFD to attenuate the postprandial GIP response in this study was contrary to expectations. It is possible that the GIP responses observed were not simply the result of a restricted fat intake, but a combination of multiple dietary changes including an increase in sucrose consumption, which has been previously observed to raise postprandial GIP levels.

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Fasting and postprandial hormone and metabolite concentrations in normal and diabetic subjects taking dietary fish-oil supplements. By CHRISTINE M. WILLIAMS, F. MOORE, J. WRIGHT, A. ZAMPELAS and L. MORGAN, *Division of Nutrition and Food Science, University of Surrey, Guildford, Surrey GU2 5XH*

The hypotriacylglycerolaemic effects of fish-oil supplements are well documented in normal subjects and patients with hypertriacylglycerolaemia (Phillipson *et al.* 1985). Most studies have measured fasting concentrations; less is known about effects of fish oils on postprandial triacylglycerol concentrations and on concentrations of the lipogenic hormones such as insulin and gastric inhibitory polypeptide (GIP). Fish-oil supplements have been suggested to be of particular benefit to patients with non-insulin dependent diabetes mellitus (NIDDM) in whom hypertriacylglycerolaemia is common. However, potentially adverse effects have been reported in this group including elevated low-density-lypoprotein-cholesterol and apo-B concentrations, and raised fasting glucose concentrations (Sorisky & Robbins, 1989). Fasting total and high-density-lipoprotein(HDL)-cholesterol concentrations and fasting and postprandial insulin, GIP, triacylglycerol and glucose concentrations were determined in normal (*n* 6) and NIDDM (*n* 5) subjects, before and 6 weeks after a period of dietary fish-oil supplementation (9 capsules/d, providing 2·7 g *n*-3 fatty acids). Postprandial metabolite and hormone concentrations were determined over a 3·5 h period, following a standard mixed test meal (4184 kJ (1000 kcal), 36 g protein, 53 g fat, 100 g carbohydrate).

Difference in plasma concentration between second (post fish oil) and first (pre fish oil) test meals for 0, 60 and 180 min post meal

Time post meal (min) . . .	NIDDM (*n* 5)						Normal (*n* 6)					
	0		60		180		0		60		180	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Insulin (mU/l)	0·27	1·4	0·29	0·6	0·96	1·3	1·06	0·4	8·1	10·1	2·7	6·9
GIP (ng/l)	−181	250	−622	952	−288	445	−75	70	−226**	124	−1439	1341
Glucose (mmol/l)	0·11	2·50	−1·03	0	−0·22	2·9	−0·3	0·04	0·46	2·6	0·8	1·3
Triacylglycerol (mmol/l)	0·27	1·4	0·34	0·67	0·96	1·3	−0·72*	0·6	−0·7*	0·5	−1·2**	0·6
Total cholesterol (mmol/l)	0·33*	0·2	–	–	–	–	−0·50	0·8	–	–	–	–
HDL-cholesterol (mmol/l)	−0·02	0·2	–	–	–	–	−0·1	0·3	–	–	–	–

*$P<0·05$, **$P<0·01$.

Fish-oil supplementation did not affect insulin and glucose concentrations but lowered GIP concentrations in normal (60 min) and NIDDM (15 and 30 min) subjects (values not shown). It lowered triacylglycerol concentrations in normal but not NIDDM subjects, and increased total cholesterol concentrations in NIDDM subjects.

Failure to observe hypotriacylglycerolaemic effects of fish oils and measurement of elevated total cholesterol concentrations following fish-oil supplementation in diabetic subjects suggests fish-oil supplements may not be beneficial in NIDDM.

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The effects of peripheral and central glucocorticoids on insulin secretion in the obese Zucker rat. By M. STUBBS, *Department of Human Nutrition, University of Southampton, School of Biological and Physiological Sciences, Bassett Crescent East, Southampton SO9 3TU* and D. A. YORK, *Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, California, USA*

The obese (*fa/fa*) Zucker rat displays excessive body-weight gain, hyperphagia (Zucker & Antoniades, 1972), insulin resistance (Terretaz *et al.* 1986) and hyperinsulinaemia (Zucker & Antoniades, 1972). Adrenalectomy will normalize the body-weight gain in the obese animal (Bray & York, 1979); this is believed to result from release of hypothalamic corticotrophin releasing factor secretion from inhibition by glucocorticoids (Rohner-Jeanrenaud *et al.* 1989).

In the present study, adrenalectomy (adx) of post-weaning (9-week-old) obese Zucker rats decreased basal plasma insulin levels (530 (SE 56) *v.* 151 (SE 59) μU/ml, sham *v.* adx) and reduced the hypersecretion of insulin induced by intravenous (12·6 mg/kg) glucose load (724 (SE 134) *v.* 424 (SE 64) μU/ml, sham *v.* adx, 2 min after the start of glucose infusion). The hypersecretion of insulin in the adx animals was restored (1058 (SE 238) μU/ml, 2 min after the start of infusion) after 1 week of daily corticosterone injections (20 mg/kg body-weight). Chronic administration of dexamethasone (15 ng/d) into the third cerebral ventricle of adx Zucker rats produced a greater increase in the 2 min post-glucose plasma insulin levels compared with saline-infused controls (increases of 397 (SE 89) and 136 (SE 53) μU/ml on respective basal levels). Treatment of 7-week-old obese rats with glucocorticoid antagonist RU 486 for 1 week (30 mg/kg per d) also reduced 2 min post-glucose insulin levels (182 (SE 47) *v.* 439 (SE 85) μU/ml above basal levels, RU 486 *v.* vehicle treated). These results suggest that glucocorticoids are responsible for the excess parasympathetic activity in the *fa/fa* rats and that their site of action is in the central nervous system.

Bray, G. A. & York, D. A. (1979). *Physiological Reviews* **59**, 719–809.
Rohner-Jeanrenaud, F., Walker, C.-D., Greco-Perotto, R. & Jeanrenaud, B. (1989). *Endocrinology* **124**, 733–739.
Terretaz, J., Assimacoupoulos-Jeannet, F. & Jeanrenaud, B. (1986). *Endocrinology* **118**, 674–678.
Zucker, L. M. & Antoniades, H. N. (1972). *Endocrinology* **90**, 1320–1330.

Genetic influence on fatness and fat distribution in women. By J. LOVE and G. MCNEILL,
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Although obesity in man is known to have a strong familial tendency, the relative importance of genetic and environmental factors is not well known. A recent Canadian study (Bouchard *et al.* 1988) suggests that genetic factors may be particularly important in determining fat distribution, but that family environment has a larger effect than genetic constitution on body mass index (BMI) and body fat (%). We have investigated genetic and environmental contribution to fat and fat distribution in UK women using a classical twin study.

Estimates of body fat and fat distribution were made in nineteen healthy adult female twin pairs (nine monozygotic (MZ), ten dizygotic (DZ)) aged 16–63 years. There was no significant difference between MZ and DZ twins in age, height, weight or BMI ($P>0{\cdot}05$ for all variables). Body fat (%) and fat free mass (FFM) were estimated from underwater weighing (UWW), except in three pairs of twins in whom UWW was not possible, and body fat (%) was estimated from skinfold thickness. Regional fat distribution was assessed from the ratio of extremity to trunk skinfolds (E:T); the ratio of subcutaneous fat (sum of six skinfolds) to fat mass (S:T), and the ratio of waist to hip circumference (WHR). Zygosity was determined by DNA fingerprinting. Concordance for all variables was assessed by intraclass correlation coefficients: r_{MZ} in MZ pairs and r_{DZ} in DZ pairs. The Table shows the mean and intraclass correlation coefficients r_{MZ} and r_{DZ}.

	MZ pairs (n 9)		DZ pairs (n 10)			
Variable	Mean	SD	Mean	SD	r_{MZ}	r_{DZ}
Height (m)	1·60	0·062	1·60	0·056	0·89	0·29
Weight (kg)	58·61	8·16	63·80	12·78	0·78	0·71
BMI (kg/m^2)	23·41	2·62	25·13	5·28	0·73	0·74
Body fat (%)	31·30	9·10	34·40	9·10	0·79	0·68
FFM (kg)	39·84	5·01	41·37	3·96	0·84	0·43
E:T (mm/mm)	0·84	0·20	1·03	0·27	0·79	−0·04
S:T (mm/kg)	4·39	1·23	4·40	1·07	0·90	−0·27
WHR (mm/mm)	0·77	0·05	0·77	0·05	0·41	0·50

Intraclass correlation coefficients for height, FFM, E:T and S:T were considerably higher in MZ than DZ twins. This suggests that genetic factors are important in the determination of these variables. WHR did not show the same pattern of genetic influence. High intraclass correlations in both MZ and DZ twins were seen in weight, BMI, % fat and WHR, suggesting that the within-pair resemblances in these variables may be more related to common environment than common genes.

Bouchard, C., Perusse, L., Leblanc, C., Tremblay, A. & Theriault, G. (1988). *International Journal of Obesity* **12**, 205–215

The effect of formaldehyde-treatment of barley on starch digestion in sheep. By MARIA E. ORTEGA-CERRILLA and HEATHER J. FINLAYSON, *Department of Agricultural Biochemistry and Nutrition, The University, Newcastle upon Tyne NE1 7RU*

Increasing the quantity of glucose available for absorption from the small intestine (SI) of ruminants may improve production efficiency by reducing energy utilization for glucose synthesis. Increased flows of glucose, as starch, to the SI of both cattle (John, 1976) and sheep (Fluharty & Loerch, 1989) have been observed in response to feeding formaldehyde (HCHO)-treated maize. The present study investigated the effect of HCHO treatment of barley on the rate of starch degradation in the rumen and on the flow of starch to the SI of sheep.

Rolled untreated barley (UB) (438 g dry matter (DM)/d; 671 g starch/kg DM) or HCHO-treated (30 g HCHO/kg crude protein) barley (TB) (438 g DM/d; 622 g starch/kg DM) was given together with a compound feed (454 g DM/d; 193 g starch/kg DM) in two equal portions to four surgically modified sheep. Starch degradation from UB and TB was assessed using the dacron bag technique (Mehrez & Ørskov, 1977). Starch flow to the SI was calculated based on its concentration in a total 24 h collection of duodenal digesta and on the recovery of a flow marker (Cr_2O_3).

	Constants from fitted disappearance curves ($p = a + b - be^{-ct}$)			In vivo starch measurements		
Barley	*a*	*b*	*c*	Intake (g/d)	Flow to SI (g/d)	Disappearance before SI
UB	0·290	0·704	1·10	380·0	15·2	0·959
TB	0·272*	0·710	0·47*	360·0	17·6	0·951
SEM	0·0030	0·0142	0·131		2·70	0·0073

*$P<0·05$.

HCHO-treatment significantly reduced the rate (*c*) of starch degradation and the proportion of rapidly soluble starch (*a*). There were no significant differences in the flow of starch to the SI or the proportion of starch disappearing before reaching the SI, suggesting that the reduced degradation rate was nullified by rumen retention time. HCHO-treatment may, however, result in increased efficiency in the rumen due to the more gradual release of fermentable substrate and this may be reflected in improved productive performance without an increase in the flow of starch to the SI.

MEO-C was in receipt of an ORS award. The study was supported by Svenska Lantmännens Riksförbund, Sweden.

Fluharty, F. L. & Loerch, S. C. (1989). *Canadian Journal of Animal Science* **69**, 173–180.
John, A. (1976). Protection of starch from rumen microbial fermentation and its utilization in the lower gut of cattle. PhD Thesis, University of Illinois.
Mehrez, A. Z. & Ørskov, E. R. (1977). *Journal of Agricultural Science, Cambridge* **88**, 645–650.

The effect of different pairs of feeds offered as a choice on food selection by sheep. By X. Z. HOU, G. C. EMMANS, D. ANDERSON, A. ILLIUS and J. D. OLDHAM, *Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG*

We have shown previously that sheep can select from a choice of two feeds, differing in protein concentration, in a systematic way in relation to their maturity (Hou *et al.* 1989), and that individual sheep are willing to do work to sustain a choice (Hou *et al.* 1991). There is good evidence that growing pigs are able to make consistent systematic choices from a variety of pairs of feeds (Kyriazakis, 1989). The purpose of the present experiment was to examine the abilities of growing sheep to do the same.

Twenty-four Greyface sheep were used, twelve aged 3 months and twelve aged 17 months at the start of the experiment. Four pelleted feeds (A, B, C, D) were used, with similar energy and mineral concentrations, and crude protein (CP, nitrogen × 6·25) concentrations of 234, 180, 123 and 66 g/kg dry matter respectively. These were offered in pairs to the sheep in individual pens in an experimental design based on 3 × 3 Latin squares such that sheep within a square were of the same age and one feed (primary feed) was always present and paired with the other three feeds (secondary feeds) in a changeover fashion. Treatment periods were 28 d long; fresh food was offered once daily and *ad lib.* consumption of the two feeds on offer was recorded daily. Water was always available. Body-weight was recorded weekly.

Table. *Amounts of feed eaten by young and old sheep (g/d)*

Feed		Young				Old			
		1		2		1		2	
1	2	Mean	SE	Mean	SE	Mean	SE	Mean	SE
A	B	618	46	1142	47	904	56	1219	42
A	C	872	45	906	57	769	20	1316	29
A	D	1023	58	704	43	1268	61	742	41
B	C	791	28	886	20	1153	61	870	40
B	D	1142	54	605	40	1166	69	728	30
C	D	1363	55	364	19	1294	52	554	59

The choices made by sheep of different ages did not differ significantly, but total dry matter intake was greater ($P<0·05$) in the older sheep (1997 (SE 18) *v.* 1736 (SE 6) g/d respectively). Live-weight gain was not affected by treatment but was greater ($P<0·05$) in the younger sheep (318 (SE 7) *v.* 212 (SE 13) g/d respectively). Food selections were clearly systematic and converged around a mean of 156 (SE 1·9) g CP/kg dry matter in total food selected where the combination on offer allowed this. For the CD, AB combinations this was not possible and the overall variation in choice was greatest for this treatment.

We conclude that sheep have a certain ability (though less precise than shown with pigs) (Kyriazakis, 1989) to make systematic choices between feeds according to dietary protein density.

Hou, X. Z., Emmans, G. C., Anderson, D., Illius, A. & Oldham, J. D. (1989). *Animal Production* **48**, 649.

Hou, X. Z., Lawrence, A. B., Anderson, D., Illius, A. & Oldham, J. D. (1991). *Proceedings of the Nutrition Society* **50**, 95A.

Kyriazakis, I. (1989). Growth, feed intake and diet selection in pigs. PhD Thesis, University of Edinburgh.

Operant studies on feed selection in sheep. By X. Z. HOU, A. B. LAWRENCE, A. ILLIUS, D. ANDERSON and J. D. OLDHAM, *Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG*

Both pigs (Kyriazakis, 1989) and sheep (Hou *et al.* 1991) appear to make purposeful selections between a choice of available feeds. An operant approach to study feeding motivation has proved useful in behavioural studies with pigs (Lawrence & Illius, 1989). This paper presents the results of some exploratory studies with sheep to test their motivation to sustain a particular feed choice.

Four adult Suffolk × Greyface wethers were used. They were offered, for 10 d, free choice between a pair of pelleted feeds of similar energy and mineral concentrations, but with either 111 (LP) or 231 (HP) g crude protein (CP, nitrogen × 6·25)/kg dry matter.

At the end of this time they had established a particular pattern of choice between LP and HP; they were then offered one food (normally the one eaten in greatest amount) from an operant feeding device, with the other food (and water) available *ad lib*. The operant feeder responded to button presses and the number of presses/food reward (20 g/delivery) was varied over the range 1–30, with 1–4 d allowed for recording of intake and selection at a given ratio.

Total food dry matter intake varied little within sheep (3017 (SE 13·7); 3425 (SE 17·8); 3202 (SE 14·6); 3246 (SE 22·7) g/d for sheep 1–4 respectively). The Table shows the effect of increasing rate of presses for a reward on the percentage of total food intake eaten as the HP feed when either LP or HP was offered via the operant feeder. The results are presented separately for those occasions when LP was offered by the operant feeder (i.e. HP was freely available from a bucket) and vice versa.

Feed in operant feeder . . .		LP					HP			
Ratio for reward . . .		1	5	10	15	30	1	5	10	15
Sheep no. 1	Mean	13·0	—	20·0	35·0	68·0	16·0	—	—	—
	SE	2·3		2·0	1·8	5·3	3·3			
2	Mean	9·0	—	9·0	30·0	84·0	10·0	—	—	—
	SE	1·3		2·3	1·7	2·9	1·5			
3	Mean	48·0	69·0	70·0	84·0	—	52·0	21·0	31·0	21·0
	SE	6·3	1·3	11·5	1·4		3·5	2·8	2·8	1·7
4	Mean	29·0	25·0	38·0	52·0	—	29·0	25·0	3·0	6·0
	SE	5·3		3·7	2·8		2·9	7.5	1·2	0·7

This exploratory study suggests that there was strong motivation in these sheep to make the food selections which they made. Their reasons for making them are less clear at present but the operant approach promises to be a useful tool to explore this issue further.

Hou, X. Z., Emmans, G. C., Anderson, D., Illius, A. & Oldham, J. D. (1991). *Proceedings of the Nutrition Society* **50**, 94A.

Kyriazakis, I. (1989). Growth, feed intake and diet selection in pigs. PhD Thesis, University of Edinburgh.

Lawrence, A. B. & Illius, A. W. (1989). *Applied Animal Behaviour Science* **24**, 273–285.

A comparison of a split diet system and choice feeding on food intake and growth of broilers. By F. SHARIATMADARI and J. M. FORBES, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

Choice-feeding systems in which birds have access to high- and low-protein feeds have been proposed as allowing birds to meet their own energy and protein requirements (Emmans, 1979). In practice, however, it is difficult to modify automatic feeding equipment to deliver two feeds. A pilot study was therefore carried out to investigate the ability of broiler chickens to meet their requirements when high- and low-protein feeds were offered alternately. The day consisted of a 12 h lighting schedule.

Individually caged male broilers (five per treatment) were offered *ad lib.* access to isoenergetic diets varying in protein content (a low-protein diet containing 70 g/kg, provided by wheat and a high-protein diet containing 300 g/kg, provided by wheat, fish meal and soya bean meal) concurrently (A), LP in the 1st half of the day and HP in the 2nd half of the day (B), HP in the 1st half of the day and LP in the 2nd half of the day (C), and LP and HP given on alternate days (D), from 5 weeks to 10 weeks of age.

Treatments . . .	A	B	C	D	SED
High-protein intake (g/bird per d)	$90{\cdot}2^{a}$	$89{\cdot}3^{a}$	$70{\cdot}6^{b}$	$74{\cdot}1^{ab}$	7·62
Low-protein intake (g/bird per d)	$72{\cdot}9^{b}$	$82{\cdot}4^{ab}$	$95{\cdot}6^{a}$	$81{\cdot}0^{ab}$	8·58
Total food intake (g/bird per d)	$163{\cdot}1^{a}$	$171{\cdot}7^{a}$	$166{\cdot}2^{a}$	$151{\cdot}1^{a}$	6.90
Protein intake (g/bird per d)	$34{\cdot}2^{ab}$	$35{\cdot}2^{a}$	$30{\cdot}4^{ab}$	$29{\cdot}6^{b}$	2·16
Live-wt gain (g/bird per d)	$59{\cdot}9^{a}$	$61{\cdot}4^{a}$	$63{\cdot}8^{a}$	$58{\cdot}9^{a}$	4·66
Abdominal fat (g/bird)	$54{\cdot}2^{ab}$	$53{\cdot}9^{ab}$	$68{\cdot}8^{a}$	$37{\cdot}4^{b}$	9·25
Fat deposition (g/bird)	$391{\cdot}0^{ab}$	$330{\cdot}0^{b}$	$427{\cdot}0^{a}$	$325{\cdot}0^{b}$	36·9
Protein deposition (g/bird)	$332{\cdot}0^{a}$	$381{\cdot}0^{a}$	$352{\cdot}0^{a}$	$336{\cdot}0^{a}$	27·7

[a,b] Mean values in the same horizontal row with different superscript letters were significantly different: $P<0{\cdot}05$.

The results show that broilers on split diets can regulate their intake and grow as well as those given free choice. The significantly lower abdominal fat and lower carcass fat content shows that treatment D gives better performance.

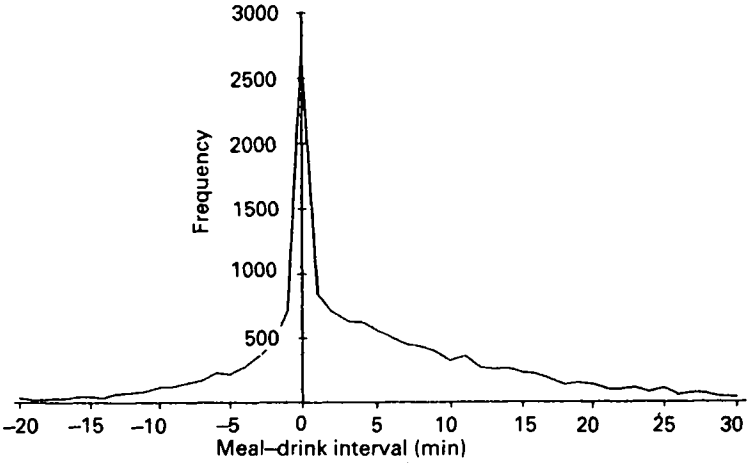
Emmans, G. C. (1979). In *Recent Advances in Animal Nutrition*, pp. 31–39 [W. Haresign and D. Lewis, editors]. London: Butterworths.

The drinking behaviour of lactating cows offered silage *ad lib*. By J. M. FORBES, C. L. JOHNSON and D. A. JACKSON, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

Animals drink water in proportion to their food dry matter intake (Silanikove, 1989) but little is known about the temporal relationship between feeding and drinking in cattle.

Drinking behaviour was monitored individually in twelve lactating Friesian–Holstein cows from October until the following April, using an automatic system in which cows were identified as they ate or drank and the weight of food and water were continuously monitored (Forbes *et al.* 1987). During this period of 196 d a total of 10 614 drinks was taken, an average of 4·5/cow per d, compared with 15·1 meals of silage per d. Visual inspection of daily records showed that most drinks were large (>5 litres) and occurred during some spontaneous meals of silage. To quantify this over the whole winter period, time intervals between the onset of drinking and the termination of the previous feeding, and between the termination of drinking and the onset of the next feeding period were calculated.

In 2672 cases (12·6% of the number of intervals recorded) drinking occurred within 1 min of the start or end of feeding. With longer intervals the frequency declined smoothly; a further 2815 drinks (13·3%) occurred between 1 and 10 min of the end of a meal while 5512 (26·0%) drinks were followed by feeding starting between 1 and 10 min later. Thus, 51·9% of all meal–drink and drink–meal intervals were within 10 min.



In a period of 40 d during December and January in a subsequent year, with twelve different cows, very similar results were obtained. Of 4018 intervals, 449 (11·2%) were less than 1 min, 451 (11·2%) were meal–drink intervals of between 1 and 10 min and 1218 (30·3%) were drink–meal intervals of between 1 and 10 min, giving a total of 52·7% of intervals of 10 min or less.

There is a clear tendency, therefore, for drinking to occur in association with some meals, but by no means all. Further analysis of the data is required (e.g. relationship between meal and drink sizes) before the relevance of meal-related drinking in cows can be elucidated.

Forbes, J. M., Jackson, D. A., Johnson, C. L., Stockill, P. & Hoyle, B. S. (1987). *Research and Development in Agriculture* **3**, 175–180.

Silanikove, N. (1989). *Appetite* **12**, 163–170.

The effect of drinking water on food-intake responses to manipulations of rumen osmolality in sheep. By J. P. BARRIO, S. T. BAPAT and J. M. FORBES, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

The osmolality of rumen fluid has been suggested as an important factor influencing voluntary food intake. However, some of the work showing the clearest effects of manipulating rumen osmolality has been performed with animals denied access to drinking water (Grovum & Bignell, 1989). As rumen fluid often rises to around 450 mosmol/kg after large meals we have infused salts to achieve this level of osmolality, with and without drinking water.

Five rumen-fistulated sheep were kept individually in pens equipped for continuous monitoring of food (a complete, pelleted diet) and water intakes. From 09.00 to 13.00 hours the following treatments were applied in random order: no infusion; infusion into the rumen of 1·0 mol sodium chloride in 1·0 litre water; infusion of 1·0 mol potassium chloride in 1·0 litres water; infusion of 1·0 litre water. Each treatment was applied on a total of three occasions with free access to drinking water and three occasions when drinking water was withheld during the 4 h treatment period.

	With access to drinking water				Without access to drinking water				
Treatment . . .	Control	NaCl	KCl	Water	Control	NaCl	KCl	Water	SED
Food intake (g/4 h):									
During infusion	860	326	680	715	347	225	82	628	238
After infusion	512	254	319	502	243	461	179	879	175
Water intake (g/4 h):									
During infusion	1211	2492	1914	739	—	—	—	—	307
After infusion	892	1229	937	527	1124	3011	4567	2316	526
Osmolality during infusion (mOsmol/kg)	291	398	381	294	278	429	456	311	55

Food intake was significantly depressed when drinking water was not available. Infusion of salts depressed food intake but significantly stimulated water intake. Infusion of water tended to increase food intake and significantly depressed water intake. During the 4 h after the end of treatment food intake was still significantly increased when water had been infused into water-deprived sheep; water intake was much higher ($P<0·001$) in previously deprived sheep.

The effects of manipulating the osmolality of rumen fluid on food intake are much greater when drinking water is withheld and its importance in the control of food intake could be overemphasized under these conditions.

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Grovum, W. L. & Bignell, W. W. (1989). *Proceedings of the Nutrition Society* **48**, 3A.

The effect of force-feeding on the relationship between body fat and food intake in broilers. By A. Y. YALDA and J. M. FORBES, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

Increasing the body fat content of White Leghorn cockerels by force-feeding approximately twice their *ad lib.* food intake prevented them from eating for about 10 d when returned to *ad lib.* conditions. Thereafter they began to eat when the body fat was decreasing toward normal (Lepkovsky & Furuta, 1971) which implied that excessive fatness inhibited food intake. However, the surgical removal of the abdominal fat pad did not affect subsequent food intake in female broiler chickens (Taylor & Forbes, 1988).

In order to determine whether excessive fatness would affect feeding in broilers, eighteen males were caged individually at 4 weeks of age and assigned to two groups. One group was fed *ad lib.* until 11 weeks of age and the other force-fed 100% of the *ad lib.* level in addition to having free access to the food from 4 to 7 weeks of age, and thereafter fed *ad lib.* until 11 weeks of age. Body-weight and tibia length measurements were carried out weekly, and food intake was recorded daily. Birds were killed at 7, 9 and 11 weeks for carcass analysis and abdominal fat weight.

Effect of force-feeding on body-weight, food intake and tibia length

		Ad lib.			Force-fed		
Age (weeks) . . .		7	9	11	7	9	11
Body-wt (g)	Mean	2095[b]	2978[a]	3215[a]	2308[a]	3082[a]	3167[a]
	SE	73·2	176·1	256·6	26·0	182·0	117·0
Total food intake (g/bird per d)	Mean	146[b]	162[a]	146[a]	191[a]	197[a]	179[a]
	SE	7·0	16·1	27·9	4·0	4·6	7·5
Tibia length (mm)	Mean	40[b]	60[b]	82[a]	49[a]	73[a]	83[a]
	SE	3·3	3·7	1·7	0·8	2·5	2·5

[a,b] Means in the same row at the same age with different superscript letters are significantly different: ($P<0·05$).

Force-feeding the broilers 100% of *ad lib.* intake did not prevent some voluntary eating. On returning to *ad lib.* feeding the voluntary food intake was sharply increased from 45 g/bird per d to 197 g/bird per d. Body-weight was increased by 10% and cessation of force-feeding did not cause losses in body-weight due to the increase of voluntary food intake. Abdominal fat pad and total lipid content of the carcass were significantly increased by the end of force-feeding, and at 9 and 11 weeks the force-fed birds still tended to have a greater abdominal fat content compared with those fed *ad lib.* While the protein and ash content were not affected significantly, the force-feeding significantly increased growth as measured by length of tibia.

Thus, following force-feeding of male broilers there is a rapid increase in voluntary intake to a level at least as great as control birds and body fatness tends to remain higher. It appears that voluntary food intake in the broiler is less sensitive to body fatness than in the layer chickens.

Lepkovsky, S. & Furuta, F. (1971). *Poultry Science* **50**, 573–577.
Taylor, C. G. & Forbes, J. M. (1988). *Proceedings of the Nutrition Society* **47**, 90A.

The effect of adding water to the diet on body fatness and food intake in broiler chickens.

By A. Y. YALDA and J. M. FORBES, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

It has been shown that adding water to the food of rats can increase food intake and growth (Keane *et al.* 1963). In cockerels of a laying strain, Lepkovsky & Furuta (1960) found that adding water to the food improved feed conversion efficiency and growth. Adding high-moisture by-products to the food improved the performance of laying hens (Thorne *et al.* 1988).

In order to study the effect of adding water to the food of modern broiler chickens, thirty-two male broilers were housed in individual cages at 4 weeks of age and divided into four groups. One group was fed on a standard grower feed (991·5 g dry matter/kg) *ad lib.* until 9 weeks of age, while the other groups were offered the same feed but with additions of 250, 500 and 750 g water/kg respectively, until 7 weeks, and thereafter fed with no added water until 9 weeks of age. Drinking water was available at all times. Body-weight and food and water intake data were obtained weekly. Birds were killed at 7 and 9 weeks for carcass analysis.

Age (weeks) . . .	7					9				
Added water (g/kg) . . .	0	250	500	750	SED	0	250	500	750	SED
Body-wt (g)	1883[a]	1863[a]	1834[a]	1839[a]	463	2834[a]	2757[a]	2519[a]	2585[a]	263
Food intake (g DM/bird per d)	140[a]	129[a]	132[a]	133[a]	10·2	167[a]	147[a]	133[a]	126[a]	25·6
Water intake (ml/bird per d)	313[a]	248[a]	315[a]	238[a]	66·8	317[a]	321[a]	290[a]	284[a]	57·0
Total water intake* (ml/bird per d)	326[a]	297[a]	399[a]	355[a]	65·7	317[a]	321[a]	290[a]	284[a]	57·0
Total carcass lipid (g/bird)	202[a]	142[ab]	134[ab]	117[b]	17·0	227[a]	177[b]	166[b]	95[c]	12·8
Abdominal fat (g/bird)	25·8[a]	16·8[b]	17·3[b]	13·0[b]	3·4	38·0[a]	23·8[b]	20·0[b]	14·8[b]	4·4
Total carcass protein (g/bird)	241[a]	280[a]	318[a]	277[a]	22·2	396[a]	395[a]	404[a]	339[a]	22·1

DM, dry matter; SED, standard error of the difference between means.

[a.b.c] Means in the same row for the same age with different superscript letters were significantly different: $P<0·05$.

* Total water intake is water in food plus water drunk *ad lib.*

Addition of water to the food at different levels did not affect protein deposition but resulted in less fat deposition, both in the abdominal fat pad and in the whole carcass, associated with the tendency for dry matter intake to be reduced. On return to the normal dry diet there was no tendency to overeat, nor to regain the same body fat content as control birds. Diet-induced changes in body fatness are not readily compensated for in broilers.

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Dietary intakes of smokers and non-smokers in the Northern Ireland population. By J. J. STRAIN, K. A. THOMPSON and M. E. BARKER, *University of Ulster, Coleraine BT52 1SA, Northern Ireland*

Dietary studies of middle-aged men drawn from the general population in South Wales (Fehily *et al.* 1984) and from around Edinburgh (Fulton *et al.* 1988) have indicated that smokers appear to have different dietary habits from non-smokers. In the present study dietary intakes of smokers were compared with those of non-smokers in a random sample, aged 16–64 years, of the Northern Ireland population.

Smoking habits were assessed by questionnaire and subjects were classified as either current regular smokers or non-smokers. Dietary intakes were measured by a 7 d weighed inventory and nutrients calculated from a data base derived from food tables (Paul & Southgate, 1978). Statistical analysis of data, which were logarithmically transformed where appropriate, was by analysis of variance controlling for age and socio-economic status.

	Men				Women			
	Smokers (*n* 111)		Non-smokers (*n* 147)		Smokers (*n* 132)		Non-smokers (*n* 200)	
Daily intake	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Energy (MJ)	10·32	2·44	10·79	2·45	6·90	1·82	7·29	1·89
Protein (g)	81**	18	87	20	58	14	61	15
Fat (g)	104	29	111	30	72	23	77	24
Starch (g)	150***	47	179	59	107***	34	118	36
Sugars (g)	97	46	103	38	65*	32	74	31
Fibre (g)	19·1***	7·5	22·7	7·6	14·7**	5·3	16·8	5·8
Alcohol (g)	24·3***	31·7	8·4	16·1	7·1***	10·8	3·1	7·9
Calcium (mg)	883*	307	998	336	633*	214	712	256
Magnesium (mg)	317	103	336	106	216*	75	238	84
Iron (mg)	12·6**	4·1	14·7	6·5	9·8	4·8	10·6	4·5
Zinc (mg)	10·2**	2·9	11·3	3·1	7·5	2·3	7·8	2·3
Vitamin D (μg)	2·3*	1·9	2·9	1·9	1·8**	1·6	2·3	1·8
Vitamin E (mg)	4·2***	1·8	5·5	4·2	3·3***	1·4	3·9	1·5
Thiamin (mg)	1·2***	0·3	1·5	0·5	1·0	0·5	1·0	0·3
Riboflavin (mg)	1·8*	0·6	2·0	0·7	1·3	0·6	1·4	0·6
Folate (μg)	176	59	174	61	115*	43	126	43

Significantly different from the respective non-smokers: *$P<0·05$, **$P<0·01$, ***$P<0·001$.

Apart from the expected large differences in alcohol consumption between smokers and non-smokers, smokers also had significantly lower starch and dietary fibre intakes. The differences in carbohydrate intakes were reflected in the food groups data with smokers consuming less cereal products and cakes and puddings than non-smokers. There were also significant differences in intakes of some of the micronutrients and these differences are reported in the Table.

These results provide further support to previous reports which have identified associations between smoking and dietary habits.

This work was supported by the Health Promotion Research Trust.

Fehily, A. M., Phillips, K. M. & Yarnell, J. W. G. (1984). *American Journal of Clinical Nutrition* **40**, 827–833.

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Paul, A. A. & Southgate, D. A. T. (1978). *McCance and Widdowson's The Composition of Foods*. London: H.M. Stationery Office.

Diet and alcohol intake: a study amongst male university students. By T. R. KIRK and C. SOULSBY, *Department of Dietetics and Nutrition, Queen Margaret College, Edinburgh EH12 8TS* and C. A. BROWN and C. SMITH, *Cardiovascular Epidemiology Unit, Ninewells Hospital and Medical School, Dundee DD1 9SY*

A self-administered dietary questionnaire, described elsewhere (Kirk *et al.* 1991), was used to examine energy and nutrient intakes in relation to drinking habits in male first year university students. In the 7 d period before completion of the questionnaire, sixty-three students (14%) consumed no alcohol (1), 182 students (40·5%) consumed between 1 and 20 units (2), 172 students (38·3%) consumed between 21 and 50 units (3) and thirty-two students (7·1%) consumed in excess of 50 units (4). There were significantly higher intakes ($P \leq 0{\cdot}001$) of energy, fat, protein, total carbohydrate and sugar in the group consuming over 50 units (4) compared with the non-drinkers (1), and a linear trend between units of alcohol consumed and intakes of each of these nutrients.

Group . . .	(1)		(2)		(3)		(4)		Statistical significance	Linear
Nutrient intake (/d)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	(1) *v.* (4)	trend
Energy:										
kJ‡	7795	1900	8443	2217	10 220	2170	12 940	3272	***	†
kcal‡	1863	454	2018	530	2442	519	3093	782	***	†
kJ§	7795	1900	8120	2188	9247	2138	10 916	3117	***	†
kcal§	1863	454	1941	523	2210	511	2609	745	***	†
Total fat (g)	75·1	24·5	78·9	27·6	88·6	27·2	105	400	***	†
Saturated fat (g)	32·6	11·3	33·4	13·1	38·1	12·9	44·8	19·0	***	†
Protein (g)	80·9	21·8	81·0	23·9	88·1	22·8	101·4	37·4	***	†
Total CHO (g)	234·4	56·4	240·8	66·0	273·3	67·8	320·0	91·8	***	†
Sugar (g)	74·7	24·6	79·4	24·1	100·6	30·1	132·3	41·4	***	†
Vitamin C (mg)	52	22	51	23	50	25	46	18	NS	NS

CHO, carbohydrate; NS, not significant.
t test: ***$P<0{\cdot}001$.
One-way ANOVA including test for linear trend: †$P<0{\cdot}05$.
‡ Including alcohol.
§ Excluding alcohol.

As alcohol consumption increased there was a trend towards adopting 'unhealthy' food habits: breakfast was less likely to be eaten, consumption of chips increased and of fresh fruit decreased, daily intake of sugar added to tea and coffee increased. (Differences between (1) and (4) were significant in each case, $P<0{\cdot}05$.)

It appeared that in young males who were heavy drinkers, alcohol energy did not displace food energy. Indeed, heavy drinking was associated with additional consumption of fat and sugar, which, if sustained, could increase the risk of obesity and coronary heart disease later in life.

The study was sponsored by the Vitamin Information Service.

Kirk, T. R., Soulsby, C., Brown, C. & Smith, C. (1991). *Proceedings of the Nutrition Society* **50**, 103A.

Smoking and diet: a study amongst male university students. By T. R. KIRK and C. SOULSBY, *Department of Dietetics and Nutrition, Queen Margaret College, Edinburgh EH12 8TS* and C. A. BROWN and C. SMITH, *Cardiovascular Epidemiology Unit, Ninewells Hospital and Medical School, Dundee DD1 9SY*

A self-administered dietary questionnaire enquiring about lifestyle and incorporating a modification of the Medical Research Council food frequency questionnaire (Yarnell *et al.* 1983) was mailed out to 750 male first-year students in self-catering accommodation at six UK universities. The response rate was 65·5%.

A small proportion of students were cigarette smokers, 6·2% smoking less than 10/d (light smokers, LS) and 8·8% over 10/d (heavy smokers, HS). Estimated daily intakes of energy and nutrients are given in the Table.

Group . . .	Non-smokers (1)		LS (*n* 28) (2)		HS (*n* 40) (3)		Statistical significance		
Nutrient intake (/d)	Mean	SD	Mean	SD	Mean	SD	1 *v.* 2	2 *v.* 3	1 *v.*3
Energy									
kJ	9268	1085	8430	2560	10806	3364	NS	**	***
kcal	2212	259	2012	611	2579	803	NS	**	***
kJ†	8698	1596	7772	2208	9570	2996	*	**	*
kcal†	2076	381	1855	527	2284	715	*	**	*
Fat (g)	83·5	29	72·8	25·4	94·6	28·4	NS	**	*
Protein (g)	85·7	24	71·7	24·0	87·7	31·0	**	*	NS
Total CHO (g)	256·4	67·8	236·7	64·4	288·5	97·4	NS	*	**
Sugar (g)	88·2	28·7	86·5	31·5	117·2	49·0	NS	**	***
Alcohol (g)	24·1	21·4	26·8	18·7	51·0	29·9	NS	***	***
Vitamin C (mg)	50	23	50	21	52	30	NS	NS	NS

CHO, carbohydrate; NS, not significant.
t test: **P*<0·05, ***P*<0·01, ****P*<0·001.
† Excluding alcohol.

A U-shaped relationship was found between amount smoked and intakes of energy, dietary fat, total carbohydrate, sugar and alcohol. This may offer an explanation for earlier reports of a U-shaped relationship between amount smoked and obesity index (Khosla & Lowe, 1971; Noppa & Bengtsson, 1980).

The research was sponsored by the Vitamin Information Service.

Khosla, T. & Lowe, C. R. (1971). *British Medical Journal* **4**, 10–13.
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Yarnell, J. W. G., Fehily, A. M., Milbank, J. E., Sweetman, P. M. & Walker, C. L. (1983). *Human Nutrition: Applied Nutrition* **37A**, 103–112.

Dietary behaviours and blood biochemical nutritional indices in Northern Ireland. By M. E. BARKER, S. I. MCCLEAN, J. J. STRAIN and K. A. THOMPSON, *Centre for Health and Social Research, University of Ulster, Coleraine BT52 1SA*

The dietary behaviours of a random sample of adults (*n* 592) aged 16–64 years from Northern Ireland were investigated as part of a cross-sectional population study of diet, lifestyle and health (Barker *et al.* 1989). Dietary assessment was by a 7 d weighed record and a non-fasting blood sample was also obtained from consenting subjects over 18 years of age (*n* 522). Food intake was described in terms of forty-one food groups. Using principal component analysis four distinct dietary patterns were generated, which were identified as a 'traditional' diet, a 'cosmopolitan' diet, a 'convenience' diet and a 'meat and two veg' diet (Barker *et al.* 1990). The relationship between these dietary patterns and blood measurements of nutritional and health status were examined by calculating partial correlations which were controlled for age and smoking habit.

The 'traditional' dietary pattern was negatively correlated with measurements of iron status viz haemoglobin (r −0·12, $P<0·05$) and serum ferritin (r −0·18, $P<0·01$) in men and transferrin saturation (r −0·12, $P<0·05$) in women. In contrast the 'meat and two veg' behaviour was positively associated with serum ferritin in men (r 0·22, $P<0·01$). Both the 'cosmopolitan' and 'convenience' diet were positively correlated with folate and vitamin B_{12} status in women. The 'cosmopolitan' behaviour was positively associated with erythrocyte folate (RCF) (r 0·11, $P<0·05$), serum folate (r 0·18, $P<0·01$) and serum vitamin B_{12} (r 0·18, $P<0·01$). Similarly the 'convenience' behaviour was positively associated with RCF (r 0·12, $P<0·05$), serum folate (r 0·15, $P<0·01$) and serum vitamin B_{12} (r 0·18, $P<0·01$).

There were also significant associations between the dietary patterns and serum lipids. In women serum total cholesterol levels were negatively associated with the 'cosmopolitan' behaviour (r −0·11, $P<0·05$). HDL-cholesterol (HDLC) levels were positively correlated with the 'cosmopolitan' (r 0·21, $P<0·001$) and the 'convenience' (r 0·20, $P<0·001$) behaviours in women. In men the 'cosmopolitan', 'convenience' and 'meat and two veg' behaviours were positively associated with HDLC levels; the respective correlation coefficients were r 0·12, $P<0·05$, r 0·16, $P<0·01$ and r 0·19, $P<0·01$.

White blood cell (WBC) and platelet levels in men showed no significant associations with dietary behaviours, whilst in women the 'traditional' and 'cosmopolitan' diets showed significant negative associations; the respective correlations were for WBC r −0·11, $P<0·05$ and r −0·15, $P<0·01$ and for platelets r −0·10, $P<0·05$ and r −0·11, $P<0·05$.

These results demonstrate that food combinations may influence blood measurements of nutritional health. However, it would appear that these interactions between diet and blood measurements may be confounded by other factors.

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Barker, M. E., McClean, S. I., Thompson, K. A. & Reid, N. G. (1990). *British Journal of Nutrition* **64**, 319–329.

Urinary plant oestrogen excretion in post-menopausal women. By A. CASSIDY and S. BINGHAM, *Medical Research Council, Dunn Clinical Nutrition Centre, Cambridge CB2 1QL* and K. SETCHELL and D. WATSON, *Clinical Mass Spectrometry Unit, Children's Hospital Medical Center, Cincinatti, Ohio 45229, USA*

Considerable interest has developed in the potential biological significance of two groups of diphenols found in plants, the lignans and isoflavones. Lignan precursors and isoflavones are found in plants and are metabolized by the colonic flora to enterodiol and enterolactone (lignans) which are absorbed and excreted in urine, together with the isoflavones, daidzein, genistein and equol. These diphenols are stereochemically very similar to oestradiol and may therefore be biologically active in humans (Setchell & Adlercreutz, 1988). However, little is known of the amount of plant oestrogens found in the habitual diets of individuals.

Detailed individual dietary information comprising weighed dietary records for 16 d, has been obtained from eighty women aged 50–65 years over four seasons of 1 year, together with eight 24 h urine collections, and four fasting blood samples. Eighty-six 24 h urine collections, shown to be complete by the PABA check technique (Bingham & Cummings, 1983) were selected from thirty-six healthy post-menopausal women not using hormonal replacement therapy. These were subsequently analysed for lignans and isoflavones using a combined gas chromatography–mass spectroscopy technique, utilizing select ion monitoring (Setchell, 1985). The urinary excretion of the plant oestrogens is shown in the Table.

	Excretion (μg/d)				
Oestrogen	Median	Mean	SD	Minimum	Maximum
Enterodiol	25·1	62·3	105·6	0·2	532·7
Enterolactone	115·3	156·1	187·2	4·0	931·4
Equol	1·05	3·0	10·1	0	61·3
Genistein	13·9	21·5	30·9	4·5	165·5
Daidzein	14·9	29·9	48·3	3·2	8·0
Total lignans	143·3	218·4	262·7	4·6	1141·6
Total isoflavones	31·4	54·4	76·1	9·7	394·1

Median excretion of total lignans was 143 μg/d, and 31 μg/d of total isoflavones. Correlation analysis on the transformed urinary values and dietary data suggested that non-starch polysaccharide consumption was related to enterodiol excretion (r 0·32, $P<0·05$). The low median rate of excretion of isoflavones is in agreement with low average estimates from total diet analyses (Jones *et al.* 1989) but a wide range in individual excretion was evident from the present study (see Table). The effect of increased consumption of phytoestrogens on hormonal status is presently being investigated.

The dietary studies were partially supported by the Cancer Research Campaign.

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Increased urinary excretion of 5-oxoproline in vegetarians. By C. PERSAUD, P. BISHOP and A. A. JACKSON, *Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO9 3TU*

In an earlier study in which urinary 5-oxoproline (5OP) was measured the impression was gained of a difference in the rate of excretion between omnivores and vegetarians. The present study was conducted to determine whether a difference in urinary 5OP could be demonstrated between the two dietary groups.

Urine collections were made for 24 h in twelve omnivore males, twelve vegetarian males, twelve omnivore females and twelve vegetarian females. No effort was made to control the dietary intake. Urine was collected into acid and stored frozen. 5OP was isolated by short column ion exchange chromatography, converted to glutamic acid by acid hydrolysis and the glutamic acid assayed with glutamate dehydrogenase. Statistical differences were sought using Wilcoxon Rank Sum test.

	Omnivore		Vegetarian		
	Mean	SE	Mean	SE	*P*
Body mass index (kg/m^2)	22·8	0·52	21·4	0·48	NS
5OP (μmol/d)	225	28	335	30·3	<0·01
5OP (μmol/kg per d)	3·5	0·5	5·4	0·5	<0·005
5OP/creatinine (μmol/mmol)	18·7	2·5	30·3	1·8	<0·001

Both groups had a similar height, weight and body mass index. As expected creatinine excretion was significantly increased in omnivores compared with vegetarians (13·5 (SE 0·9) *v.* 11·3 (SE 0·7) mmol/d). There was a significant increase in urinary 5OP for vegetarians relative to omnivores, regardless of whether 5OP excretion was expressed per d, per unit body mass, or relative to creatinine excretion. Males were significantly taller and heavier than females and excreted significantly more oxoproline and creatinine each day. However, 5OP excretion/kg body-weight and 5OP/creatinine were not different between the sexes.

The results suggest that the urinary excretion of 5OP may be related to either metabolic mass or muscle mass. However, the results do not exclude a more complex relationship between the synthesis of creatine, the metabolic demand for glycine, and 5OP excretion. The basis of the difference between omnivores and vegetarians is less obvious. It may reflect a difference in renal function or in the handling of 5OP in the renal tubule between the two diets, but does not rule out metabolic differences associated with either the formation or utilization of 5OP within the body.

Given the important role played by 5OP in cellular function and the metabolism of amino acids, the specific basis of the observed difference in urinary excretion of 5OP between omnivores and vegetarians deserves more detailed exploration.