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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

A meeting of the Nutrition Society (Irish Group) was held at the University of Ulster at Coleraine, Coleraine, Northern Ireland on Wednesday, Thursday and Friday, 19–21 June 1991, when the following papers were read.

Attitudes to food allergy: a survey of general practitioners in Dublin. By S. SUGRUE and N. P. KENNEDY, *Division of Nutritional Sciences, Department of Clinical Medicine, Trinity College Medical School, St James's Hospital, Dublin 8, Republic of Ireland*

There has been increased media interest and public concern in relation to 'food allergy' in recent years. It is generally accepted in the medical literature that the term 'food allergy' refers only to immune-mediated food intolerance, although it has a wider meaning in lay use. General practitioners (GPs) have an important role in the management of suspected food reactions, as they are the primary care providers in the Irish system of health care.

The aims of this study were to determine firstly, the extent to which GPs consider 'food allergy' to be a problem; secondly, which foods they identify to cause 'food allergy' and thirdly, how they diagnose and manage 'food allergy'.

A sample of 100 GPs was randomly selected from those practising in the Eastern Health Board area. A postal survey was carried out by means of a self-administered questionnaire. Following a single postal reminder seventy questionnaires were returned, of which sixty-seven were suitable for analysis. Of these sixty-seven, thirty-eight (57%) felt that 'food allergy' was uncommon (affecting >1% but <10% of the population), fifteen (22%) that it was common (affecting >10% but <50%), eleven (16%) that it was rare (affecting <1%), two (3%) that it was very common (affecting >50% of the population) and one was not sure. When asked how frequently they encounter suspected 'food allergy', thirty-four (51%) of GPs indicated less than once per month, fourteen (21%) more than once per month but less than once per week and thirteen (19%) more than once per week. In the experience of the GPs, the foods most often implicated in 'food allergy' included milk (indicated by forty-nine GPs, 73% of the evaluable responses), artificial colours (fifteen, 22%), eggs (twelve, 18%), wheat (ten, 15%), fish (ten, 15%), shellfish (nine, 13%), chocolate (eight, 12%) and food additives (five, 7%). When 'food allergy' is suspected, six GPs (9%) would undertake diagnostic tests themselves, eighteen (27%) would refer to a hospital specialist and fifty-eight (86%) advise exclusion of one or more foods. None would refer to a dietitian.

These results demonstrate that GPs have a realistic perception of the prevalence of 'food allergy', when compared with the reported perceived prevalence of food intolerance elsewhere (Young *et al.* 1987), but this exceeds the expected prevalence of true 'food allergy'. 'Food allergy' was not defined in the questionnaire and the range of foods implicated by the GPs in our study indicates a tendency to include non-immunologically-mediated food intolerances within the diagnosis of 'food allergy'.

S. Sugrue is the recipient of a postgraduate scholarship from the National Dairy Council (Ireland).

Young, E., Patel, S., Stoneham, M., Rona, R. & Wilkinson, J. D. (1987). *Journal of the Royal College of Physicians* **21**, 5–11.

Dietary control in the first year of life and its associated problems in children with phenylketonuria. By S. A. MCGRATH and P. MATHIAS, *Department of Biological Sciences, College of Technology, Kevin Street, Dublin 8* and I. SAUL, G. DARLING and E. R. NAUGHTEN, *The Metabolic Unit, The Children's Hospital, Temple Street, Dublin 1, Republic of Ireland*

Phenylketonuria (PKU) is one of a group of metabolic disorders which requires a semi-synthetic diet from the first detection in infancy. The aim of this study was to determine the effect of diagnosis and the discipline of the diet on the coping ability of the parents, as manifested by growth in the infants and control of blood phenylalanine levels within a desirable level.

Nineteen subjects were selected for the study (median age 1·72 years; range 0·34–4·42 years) and divided into three groups according to parity, as this would affect the feeding experience of the parents. These were: (I) first child, first PKU (*n* 8); (II) second child, first PKU (*n* 6); (III) second child, second PKU (*n* 5). Growth was assessed retrospectively using centile records of the first year. For the same period blood phenylalanine levels, as an index of dietary control, were calculated using the mean level attained. The values related to common childhood illness, which can be a cause of excessively elevated levels, were excluded. The desirable control range being 200–400 μmol/l (Naughten *et al.* 1987). A questionnaire was designed to assess parental management of the diet. Questions related to knowledge of the disorder, pyscho-social problems, understanding of the diet and practical problems related to feeding (Acosta & Werz, 1987; Fehrenbach & Peterson, 1989). The questionnaire was coded under the following headings: understanding; ability to cope; quality of support of health care; quality of support of family and others. It was envisaged that the questionnaire score would reflect the control of phenylalanine achieved.

Results indicated that, overall, the total questionnaire score correlated significantly with phenylalanine levels using the Spearman rank test for non-ordinal data ($P<0·025$). The mean (SD) phenylalanine control levels in the three groups were: (I) 420 μmol/l (SD 148); (II) 446 μmol/l (SD 190); (III) 364 μmol/l (SD 161). There was no significant difference between the groups. As expected, children in group (III) had a higher mean questionnaire score (57) than those in the other two groups (group (I), 44; group (II), 40). Anthropometric data showed that none of the infants were undernourished (<3% weight for height) but that children in group (II) were more likely to be overweight (>90% weight for height) than the other two groups. This suggested that parental pressure and anxiety transferred to infant feeding, especially in group (II) where considering a child 'special' may predispose to overfeeding.

In conclusion, this study has demonstrated a proven need for counselling and education facilities for parents undertaking a difficult dietary regimen.

Acosta, P. B. & Werz, E. (1987). *Journal of the American Dietetic Association* **72**, 164–169.
Fehrenbach, A. M. & Peterson, L. (1989). *Journal of Consultative Clinical Psychology* **57(2)**, 237–241.
Naughten, E. R., Kiely, B., Saul, I. & Murphy, D. (1987). *European Journal of Paediatrics* **146**, A23–A24.

The relationship of human milk growth factor activity with maternal body mass index and maternal age. By G. P. SHERRY, V. J. MCKELVEY and W. S. GILMORE, *Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA* and S. MILLAR and P. LOANE, *Jubilee Maternity Hospital, Belfast City Hospital, Belfast BT9 7AB, Northern Ireland* and M. KELL and J. M. FLETCHER, *Unilever Research, Colworth Laboratory, Sharnbrook, Bedford MK44 1LQ*

This abstract was awarded the prize for the best student presentation.

The physiological significance of polypeptide growth factors and hormones present in the milk from various species has been clarified by studies on neonatal animals and on cell lines in vitro (Koldovsky, 1989). In addition, the absence of certain polypeptides in infant feeding formulas has been reported (Carpenter, 1980). In the present study we have investigated the ability of human milk samples to stimulate a rat intestinal epithelial cell line (RIE-1) that is dependent on polypeptide growth factors for proliferation.

The RIE-1 cells (Blay & Brown, 1984) were grown to confluence in microtitre plates (7×10^3 cells/well) in Dulbecco's modification of Eagle's medium (DME) containing 10% (v:v) fetal calf serum. Quiescence was induced by replacing the medium with DME containing 0·5% (v:v) fetal calf serum 48 h after plating. Milk samples were obtained from twelve healthy mothers and cellular and fatty material removed by centrifugation. These were then diluted 1:10 in phosphate-buffered saline (9 g sodium chloride/l, PBS) and 166 μl added to each well of the quiescent RIE-1 cultures.

Proliferation was estimated by the incorporation of [^{3}H]-thymidine (1μCi/well) added to replicate cultures at 0, 8 and 16 h after milk sample addition. Results were expressed as the cumulative stimulation index (CSI) of the three labelling periods. The CSI is the tritiated thymidine incorporation (in cpm) induced by the milk sample, divided by the value obtained in the control experiment where the milk sample was replaced by PBS.

The CSI values of ten mothers ranged from 25–60 and their body mass indices (BMI) ranged from 23·5–30·5. Further, an inverse relationship was demonstrated between BMI and CSI (r 0·89). Milk samples from two additional subjects with higher BMI values of 33·2 and 36·4 were not consistent with this inverse relationship.

Samples from day 4 of lactation showed an inverse relationship between maternal age and milk CSI (r 0·76). Mothers (n 5) in the age range 21–28 years had a mean milk CSI of 70·2 (SD 20·9) as compared to mothers (n 5) in the 29–40 age range, who showed a mean CSI of 34 (SD 19·6).

It is clear that human milk can stimulate significant growth of RIE-1 cells. There were large differences in activity between individuals and the results suggest that mitotic activity may be related to age and BMI of lactating mothers.

Further work on larger cross-sections of human milk samples is required to validate the functional significance of these findings for growth of the small intestine of the neonate.

RIE-1 cells were a generous gift from K. Brown of the Institute of Animal Physiology, Cambridge.

Blay, J. & Brown, K. D. (1984). *Cell Biology International Reports* **8**, 551–560.
Carpenter, G. (1980). *Science* **210**, 198–199.
Koldovsky, G. (1989). *Journal of Nutrition* **119**, 1543–1551.

Snack energy and nutrient intakes of Northern Ireland adolescents. By P. J. ROBSON and J. J. STRAIN, *Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA* and G. W. CRAN[1], J. M. SAVAGE[2], E. D. PRIMROSE[2] and C. A. G. BOREHAM[3], [1]*Department of Statistics and Operational Research,* [2]*Nuffield Department of Child Health* and [3]*Physical Education Centre, The Queen's University, Belfast BT12 6BJ, Northern Ireland*

It is often suggested that the snacks consumed by adolescents consist of high-energy foods which contribute little else in the way of nutrients to the daily diet (Greenwood & Richardson, 1979). However, few attempts have been made to evaluate the current snacking patterns of adolescents in the UK. In the present study, the snacking habits of 1015 randomly-selected adolescents attending sixteen representative post-primary schools (251 boys aged 12; 258 girls aged 12; 252 boys aged 15; 254 girls aged 15) in Northern Ireland were assessed.

Habitual food intake data were collected by the dietary history method obtained in open-ended interview. Children did eat defined meals at breakfast, lunch and dinner. Snacks were, therefore, defined as all foods and drinks which were consumed between meals.

Total mean (SD) daily intakes of energy and selected nutrients by the 12-year-old boys, 12-year-old girls, 15-year-old boys and 15-year-old girls respectively were: energy (MJ/d), 11·6 (3·3), 9·6 (2·7), 13·5 (3·6), 9·5 (2·7); fat (g/d), 123 (39·0), 101 (33·0), 142 (42·9), 101 (34·2); sugars (g/d), 152 (61·7), 128 (49·4), 171 (61·8), 125 (50·9).

Snack intake of energy and selected nutrients expressed as percentages of total daily intakes

	12 year olds				15 year olds			
	Boys		Girls		Boys		Girls	
Nutrient	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Energy	30·8	9·4	29·9	7·9	31·0	8·8	32·8	8·6
Protein	21·5	8·5	19·8	7·8	21·3	8·0	21·8	8·0
Fat	29·6	10·9	28·9	9·3	29·5	9·6	32·4	9·9
Carbohydrate	33·7	9·4	33·1	8·5	33·3	9·6	35·0	9·2
Sugars	46·0	12·8	44·1	12·3	46·2	12·8	46·1	13·5
Iron	26·0	9·6	24·9	8·6	26·1	9·5	27·5	9·3
Calcium	33·5	12·0	31·9	12·4	32·5	12·2	33·2	12·0
Vitamin C	28·0	17·1	27·6	17·4	26·6	17·1	27·4	17·5
Thiamin	22·9	11·2	21·4	10·2	23·1	11·5	23·1	9·5
Riboflavin	25·8	12·8	23·8	13·2	25·8	13·0	25·5	12·9

More snack energy (30–34%) was derived from cakes, puddings and biscuits than from the other food groups. Confectionery (20–21%), vegetables including potato products (14–19%), dairy products (9–12%), beverages (6–7%), fruit (4–6%) and fats and oils (4–5%) also contributed an appreciable amount of the total energy from snacks. Snacks appear to contribute substantial amounts of energy and sugars to the daily diet of adolescents. Contrary to popular opinion, however, snacks also contribute important amounts of other nutrients.

This work was supported by the Northern Ireland Chest, Heart and Stroke Association.

Greenwood, C. T. & Richardson, D. P. (1979). *World Review of Nutrition and Dietetics* **33**, 1–41.

L110140 (Fluoxetine) is an antidepressant and appetite suppressant: does it have thermogenic properties? By C. M. MURPHY[1], J. F. ANDREWS[1], J. STINSON[2] and G. H. TOMKIN[2], [1]*Department of Physiology, Trinity College, Dublin 2* and [2]*Department of Diabetes and Endocrinology, Adelaide Hospital, Dublin 8, Republic of Ireland*

Fluoxetine is an effective antidepressant and a potent and selective inhibitor of serotonin uptake. Fluoxetine is chemically unrelated to tricyclic, tetracyclic and other available antidepressant agents and, while it inhibits the re-uptake of serotonin into rat brain synaptosomes, it has no appreciable effect on the re-uptake of tritiated noradrenaline or dopamine. Further studies showed Fluoxetine to have weight-reducing effects also. The weight reduction caused by Fluoxetine is believed to be mainly as a result of appetite suppression. Since other serotinergic compounds such as dexfenfluramine have thermogenic properties which result in an increase in metabolic rate (Levitsky *et al.* 1986) in addition to their appetite suppressing properties, we decided to assess whether Fluoxetine caused an increase in metabolic rate.

The study was a double-blind cross-over trial. Each arm of the trial consisted of 2 weeks treatment with a 6-week cross-over between them. Resting oxygen consumption (RMR) and oxygen consumed after an oral glucose load (diet-induced thermogenesis, DIT) were measured by open circuit calorimetry.

Subjects consisted of twenty-one post-menopausal women and nine men, all of whom had a body mass index (BMI) >30.

		Oxygen consumption ml/min								
		RMR		Total DIT		Peak DIT		Time to peak	Weight (kg)	
	Visit	Mean	SD	Mean	SD	Mean	SD	(min)	Mean	SD
					Placebo					
Placebo run in	1	277·5	58·5	1548	263	337·9	47·9	62	97·6	17·5
24 h response	2	276·5	64·9	1523	266	338·2	59·4	59	97·6	17·5
14 d response	3	272·8	53·7	1569	379	328·8	56·6	61	97·3	17·3
					Fluoxetine					
Placebo run in	1	273·5	52·4	1535	252	336·9	45·0	65	98·25	18·0
24 h response	2	268·5	46·7	1506	263	327·8	48·8	66	98·25	18·0
14 d response	3	272·6	46·6	1493	276	330·7	51·2	58	97·09*	17·8

Visit 3 *v.* visit 1 (ANOVA for paired data): *$P<0·05$.

Based on the analysis carried out, no significant thermogenic effect is evident from Fluoxetine. However, it is a potential weight-reducing agent.

Levitsky, D. A., Schuster, J. A., Stallone, D. & Strupp, B. J. (1986). *International Journal of Obesity* **10,** 169–173.

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Effects of copper deficiency and induction of streptozotocin (STZ)-diabetes on trace element mineral status in the rat. By B. M. MCDERMOTT, J. J. STRAIN and P. R. FLATT, *Human Nutrition and Diabetes Research Groups, University of Ulster at Jordanstown, Newtownabbey, Co. Antrim BT37 0QB, Northern Ireland*

Alterations of micronutrient status, including copper, have been implicated in the β-cell dysfunction and pathogenesis of diabetes. The aims of the current experiment were: (1) to evaluate the effects of STZ-diabetes on trace mineral status and, (2) to investigate the effects of Cu deficiency on the induction and acute metabolic consequences of STZ-diabetes.

Two groups (*n* 12) of male Sprague-Dawley rats were housed individually and fed diets containing adequate (4 mg/kg) or deficient (0·6 mg/kg) quantities of Cu *ad lib.* After 46 d six rats from each group were injected with STZ (65 mg/kg body-weight) to induce diabetes, and these rats were then pair-fed against control rats in the same dietary group. On day 50, the rats were sacrificed and blood and organs removed for analysis of the Cu status indices, superoxide dismutase (*EC* 1.15.1.1, CuZnSOD) cytochrome-c-oxidase (*EC* 1.9.3.1, CCO) and caeruloplasmin (*EC* 1.16.3.1, CPL) and the trace elements Cu, zinc, manganese and iron.

	Cu-adequate				Cu-deficient				Statistical effects†		
	Control		Diabetic		Control		Diabetic				
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Cu	Diabetes	Cu × diabetes
Body-wt (g)	340	10·4	293	10·2	342	8·6	292	8·8	NS	***	NS
Hepatic											
CuZnSOD (U/g protein)	373	56·0	441	70·0	163	36·0	196	28·0	***	NS	NS
Cu (μg/g)	11·5	0·77	16·0	2·3	3·7	0·58	5·3	0·47	***	*	NS
Fe (μg/g)	239	23·4	229	25·9	387	52·6	635	64·6	***	*	*
Zn (μg/g)	76·0	5·0	95·6	14·2	54·5	7·3	73·4	4·2	*	*	NS
Cardiac											
CuZnSOD (U/g protein)	84·8	7·0	80·0	13·0	51·3	14·0	49·3	6·0	**	NS	NS
CCO (U/mg protein)	2·01	0·2	3·29	1·1	0·95	0·2	0·87	0·1	**	NS	NS
Cu (μg/g)	32·8	4·3	30·3	1·8	24·1	2·8	19·7	1·6	*	NS	NS
Renal											
Mn (μg/g)	2·67	0·3	1·86	0·3	4·01	0·3	2·58	0·6	**	**	NS
Plasma											
CPL (U/l)	28·1	17·0	93·7	34·0	0·03	0·03	ND	—	**	NS	NS
Glucose (mg/l)	20·0	0·59	53·0	4·40	18·8	1·46	50·2	4·41	NS	***	NS
Fe (mg/l)	2·18	0·08	1·28	0·08	1·23	0·18	1·08	0·17	**	*	*

† Statistical significance by two-way ANOVA: $*P<0·05$; $**P<0·01$; $***P<0·001$.
NS, not significant; ND, not detectable.

These results indicate marked changes during the acute phase of diabetogenesis. Such changes appear to be exacerbated by Cu deficiency.

This work was supported by the Northern Ireland Chest, Heart and Stroke Association.

Control of manganese absorption in sucking and weanling rats. By A. L. FRAILE and A. FLYNN, *Department of Nutrition, University College, Cork, Republic of Ireland*

It has been reported that the efficiency of manganese absorption in rats decreases at about the time of weaning (Keen *et al.* 1986). This study outlines age-related changes in the control of Mn absorption in young rats. These animals are weaned at 21 d, but generally start consuming solid food at 17 d.

Two Mn solutions (3 and 3000 mg/l) were prepared with $MnSO_4$ in water and labelled with ^{54}Mn (1 μCi/ml): 0·2 ml was given by gavage to 16-, 17-, 18-, 20-, 21-, 23-, and 28-d-old rats (4–6/group), previously fasted for 18 h. Animals were killed 6 h later and stomach, small intestine (SI), and caecum–colon removed. SI was perfused with 6 ml 0·15 M-sodium chloride. ^{54}Mn in tissues was determined in a well gamma counter. ^{54}Mn absorption (% dose) was calculated as follows:

$$^{54}Mn \text{ absorbed } (\%) = 100 - (\text{stomach} + \text{SI perfusate} + \text{caecum–colon}) (\%).$$

^{54}Mn absorption (%) was very high in 16- and 17-d-old rats and was unaffected by dose. There was a considerable decrease in ^{54}Mn absorption at 6 h between 17 and 21 d of age for both dose levels with little further change up to 28 d. This decrease was more marked at the higher dose level.

Absorption of ^{54}Mn (% dose) in rats at different ages

		3 mg Mn/l			3000 mg Mn/l	
Age (d)	*n*	Mean	SEM	*n*	Mean	SEM
16	6	89·8	0·8	6	86·5	3·2
17	4	91·4	0·8	4	86·4	2·5
18	5	80·8	1·8	6	59·7	1·7
20	6	39·4	7·2	6	15·8	5·3
21	5	27·9	2·1	5	11·6	1·6
23	6	32·8	6·1	5	19·3	7·8
28	5	30·3	8·4	6	16·8	4·6

When ^{54}Mn absorption was determined at shorter assimilation times (0·5–2 h) in 21-d-old rats, a maximum of 83% of Mn was absorbed at the low dose level (3 mg/l) at 0·5 h, a maximum of 48% was absorbed at 2 h at the higher dose level (3000 mg/l) and a maximum of 68% was absorbed at 0·5 h at an intermediate dose level (300 mg/l).

These results show that there is no control of Mn absorption in 16- and 17-d-old rats. In 21-d-old rats, rapid biliary excretion of freshly-absorbed Mn appears to be the principal mechanism for controlling Mn retention. However, regulation of intestinal absorption cannot be excluded as a contributory mechanism for Mn homeostasis at higher dose levels.

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Keen, C. L., Bell, J. G. & Lönnerdal, B. (1986). *Journal of Nutrition* **116**, 395–402.

Bioavailable zinc concentrations in soya milks and cow's milk. By M. REDDY and A. FLYNN, *Department of Nutrition, University College, Cork, Republic of Ireland*

Recently, soya milks have become widely available as substitutes for cow's milk. Soya products contain phytic acid which has been shown to reduce the nutritional bioavailability of zinc and other trace elements. In this study, the bioavailability of Zn in cow's milk and soya milks is compared using the sucking rat model which we have described previously (Brennan *et al.* 1989).

Zn content of single samples of pasteurized, homogenized cow's milk (C) and five soya milks supplied in liquid form (Granose (G; Granose Foods Ltd, Newport Pagnell, Bucks), Provamel (Pr; Provamel Division, Vandemoortele (UK) Ltd, Hounslow, Middlesex), Unisoy (U; Unisoy Milk 'n' by-Products Ltd, Stockport, Cheshire), Plamil (Pl; Plamil Foods Ltd, Folkstone, Kent) and Sunrise (S; Soya Health Foods Ltd, Manchester)) were determined by atomic absorption spectrophotometry following wet ashing. All milks were extrinsically labelled with ^{65}Zn (1μCi/ml) and 0·3 ml was given by gavage to 16-d-old rats (5–6/group), previously fasted for 18 h. Animals were killed 6 h later and stomach, small intestine (SI) and caecum–colon removed. SI was perfused with 6 ml 0·15 M-sodium chloride. ^{65}Zn was determined in a well gamma counter.

Uptake (% dose) of ^{65}Zn from cow's milk and soya milks in sucking rats

		Stomach		SI perfusate		Caecum–colon		Absorbed†‡	
Milks	*n*	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Cow's milk	5	1·3	0·2	3·7	0·6	4·7	0·8	90·2	1·3
Granose	6	0·9	0·1	39·7*	11·3	31·6	10·9	27·9**	1·4
Provamel	6	0·8*	0·1	12·4	3·5	61·3**	4·9	25·5**	2·0
Unisoy	6	0·9	0·1	8·2	2·0	65·3**	1·9	25·6**	1·4
Plamil	6	1·4	0·3	13·9	4·5	51·9**	4·8	32·8**	3·6
Sunrise	5	0·7*	0·1	8·1	4·0	61·6**	2·4	29·5**	2·2

Significantly different from cow's milk: *$P<0·05$, **$P<0·01$.

† Absorbed = 100 − (stomach + SI perfusate + caecum–colon).

‡ Since the small intestine is the principal site of absorption of Zn in the rat it was assumed that the ^{65}Zn reaching the caecum–colon was largely unabsorbable, although there is some evidence that some absorption of Zn can occur in the caecum–colon (Seal & Mathers, 1989).

Zn concentrations (mg/l) in milks were 5·0(C), 5·1(G), 3·6(Pr), 3·2(U), 1·3(Pl) and 3·7(S). Zn absorption (%) from all soya milks was significantly lower than from cow's milk, but there were no significant differences between soya milks. Bioavailable Zn (mg/l), estimated from milk Zn concentration and % absorption, was 4·54(C), 1·42(G), 0.92(Pr), 0·82(U), 0·43(Pl) and 1·09(S). These results show that soya milks are much poorer sources of bioavailable Zn than cow's milk. This is probably due to chelation of Zn with phytic acid in the gastrointestinal tract.

Brennan, M. M., Flynn, A. & Morrissey, P. A. (1989). *Proceedings of the Nutrition Society* **48**, 39A.
Seal, C. J. & Mathers, J. C. (1989). *British Journal of Nutrition* **62**, 151–163.

Effect of phytate on iron and zinc absorption from a model food system in sucking rats. By K. CASHMAN, A. FLYNN and M. HARRINGTON, *Department of Nutrition, University College, Cork, Republic of Ireland*

Phytate is a strong inhibitor of zinc absorption but its effect on the absorption of other trace elements is less well understood. This study compares the effect of added phytate on absorption of Zn and iron in a model food system (infant formula) using the sucking rat model which we have previously described (Brennan *et al.* 1989).

Sodium phytate was added at concentrations of 0–8 mM to reconstituted cow's milk-based infant formula (SMA Gold, Wyeth Laboratories) containing 6·7 mg Fe/l and 5·0 mg Zn/l. Formulas were extrinsically labelled with both ^{59}Fe and ^{65}Zn, (1 μCi/ml each) and allowed 24 h for isotopic equilibrium to be attained. Wistar rats, (16 d old), fasted for 16 h, were given 0·2 ml formula by gavage. Animals were killed 6 h later and the stomach, small intestine (SI) and caecum–colon removed. SI was perfused with 6 ml 0·15 M-NaCl. ^{59}Fe and ^{65}Zn were determined in a well gamma counter. Absorption of ^{59}Fe and ^{65}Zn (% dose) were calculated as:

Absorption (%) = 100 − (stomach + SI perfusate + caecum–colon)(%). Since the small intestine is the principal site of absorption of Zn in the rat it was assumed that ^{65}Zn reaching the caecum–colon was largely unabsorbable, although there is some evidence that some absorption of Zn can occur in the caecum–colon (Seal & Mathers, 1989).

Effect of phytate on ^{59}Fe and ^{65}Zn absorption (% dose) from infant formula in 16-d-old sucking rats

		^{59}Fe		^{65}Zn	
Food	*n*	Mean	SEM	Mean	SEM
Infant formula	6	93·7	1·7	91·3	0·8
+0·8 mM-phytate	6	79·2	3·1	25·5	3·2
+1·6 mM-phytate	6	66·5	2·0	20·3	1·7
+3·2 mM-phytate	6	44·8	1·7	19·9	2·8
+8·0 mM-phytate	6	22·9	4·2	8·4	3·0

Over 90% of ^{59}Fe and ^{65}Zn was absorbed from infant formula alone. Addition of phytate to the formula reduced the absorption of ^{59}Fe and ^{65}Zn in a dose-dependent manner. This inhibitory effect was much more marked for ^{65}Zn than for ^{59}Fe.

These results show that phytate inhibits the absorption of non-haem-Fe and Zn, although its effect on Fe is much weaker than on Zn.

Brennan, M. M., Flynn, A. & Morrissey, P. A. (1989). *Proceedings of the Nutrition Society* **48**, 39A.
Seal, C. J. & Mathers, J. C. (1989). *British Journal of Nutrition* **62**, 151–163.

The effect of cereal source and processing on post-prandial glucose, insulin and GIP responses to starch in man. By R. ELLIOTT, LINDA MORGAN, JACKI TREDGER and J. WRIGHT, *School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

A range of factors, including cereal source and processing, have been shown to influence the rate and extent of starch digestion in simple starch test meals. These studies investigate the extent of the effects in different types of commercially-available cereal products. The first study investigated the possible effect of different cereal sources used to make bread. A wholemeal wheat bread (Allinson Wholemeal Bread) was compared with a 20% oat-supplemented (Waitrose Wholemeal Bread with oatmeal) and a 50% rye-supplemented wholemeal bread (Waitrose Italian Style Dark Rye Bread), selected on the basis of similar processing and composition except for cereal source. The second study investigated the effect of different processing on cereal starch from one source. Three wheat-based breakfast cereals, processed by puffing (Quaker Puffed Wheat), flaking (Force Wheat Flakes) and extrusion (Shredded Wheat, RHM Foods Ltd), were compared. Six healthy, non-obese volunteers took part in each study. On three occasions after an overnight fast, subjects consumed 50 g carbohydrate loads of either breakfast cereal or bread together with 250 ml skimmed milk. Venous blood was taken from an antecubital vein at frequent intervals before and for 180 min following the meals, and analysed for glucose, insulin and the insulin-stimulating gastrointestinal hormone GIP. In the first study, no significant differences were found in the post-prandial incremental responses (0–180 min) between the different breads for either glucose, insulin or GIP. In the second study, the incremental responses (0–180 min) for glucose and insulin were significantly lower following consumption of puffed wheat compared with extruded wheat.

	Area under the curve					
	Glucose (mmol/l per h)		Insulin (mU/l per h)		GIP (ng/l per h)	
	Mean	SEM	Mean	SEM	Mean	SEM
Puffed wheat	13·9*	0·3	47·6‡	4·5	2413	157
Shredded wheat	15·2*	0·3	73·7‡	9·3	2369	257
Flaked wheat	14·5	0·5	68·8	12·1	2086	115

Figures marked * or ‡ are significantly different ($P<0·05$) from each other.

There were no significant differences in post-prandial GIP responses between the three breakfast cereals.

We conclude that substitution of starches from different cereal sources in commercially-available bread is without effect on glucose tolerance and insulin secretion. Processing in wheat-starch breakfast cereals did influence glucose tolerance and insulin secretion but the observed effect was small. It appears that the starch processing undergone in the production of commercially-available breads and breakfast cereals is severe enough to make the starch readily digestible, regardless of cereal source and processing used, thus minimizing any differences in metabolic responses.

Effects of orally-administered amino acids on circulating gastric inhibitory polypeptide (GIP) and insulin concentrations in genetically-obese hyperglycaemic (ob/ob) mice.

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The markedly raised basal GIP concentrations of ob/ob mice have been shown to play a key role in the promotion of the hyperinsulinaemia and associated metabolic abnormalities of the obesity–diabetes syndrome (for review see Bailey & Flatt, 1988). Previous studies have examined the effects of individual sugars and fatty acids on GIP release in ob/ob mice (Kwasowski *et al.* 1985; Flatt *et al.* 1989).

In the present investigation, the effects of oral administration of eight L-amino acids (alanine, arginine, cysteine, glycine, histidine, hydroxyproline, lysine and threonine) individually or as an amino acid mixture, on plasma GIP, insulin and glucose concentrations were examined in 18-h fasted, 24–26-week-old Aston ob/ob mice. At a dose of 5·4 mmol/kg body-weight, arginine, cysteine, histidine and the amino acid mixture were equipotent in terms of increasing plasma GIP and insulin concentrations. Alanine, hydroxyproline and lysine similarly increased plasma GIP but insulin concentrations were unchanged. In contrast, threonine failed to affect either GIP or insulin concentrations. There was no correlation between either the incremental or integrated GIP and insulin responses, and none of the amino acids administered affected circulating glucose concentrations.

Integral and integrated GIP and insulin responses of 18-h fasted ob/ob mice to individual amino acids or an amino acid mixture (six mice/group)

	Incremental GIP response for 0–30 min (pmol/l)		Integrated GIP response for 0–120 min (pmol/l per h)		Incremental insulin response for 0–30 min (nmol/l)		Integrated insulin response for 0–120 min (nmol/l per h)	
Amino acid	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Alanine	181	17	103	41	1·38	0·28	1·24	0·34
Arginine	72	32	88	31	1·77	0·47	2·72	0·30
Cysteine	136	28	135	35	2·68	0·71	1·96	1·11
Glycine	44	7	72	47	1·91	0·41	1·89	0·62
Histidine	68	19	24	24	1·82	0·39	2·61	0·25
Hydroxyproline	86	38	72	18	0·70	0·17	0·75	0·28
Lysine	107	25	70	37	1·36	0·43	0·90	0·38
Threonine	99	37	55	50	0·86	0·24	1·21	0·27
Amino acid mix	127	25	190	52	2·29	0·64	1·95	0·37

The results indicate that a range of essential and non-essential neutral and basic amino acids stimulate the release of GIP in ob/ob mice. However, GIP made only a modest contribution to the stimulation of insulin secretion following administration of amino acids in the presence of basal glucose concentrations.

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The role of insulin and GIP in lipid metabolism in obese (ob/ob) mice. By J. OBEN[1], LINDA MORGAN[1], J. FLETCHER[2], P. R. FLATT[3], J. M. KNAPPER[1] and V. MARKS[1], [1]*School of Biological Sciences, University of Surrey, Guildford GU2 5XH,* [2]*Unilever Research, Colworth Laboratory, Bedford MK44 1LQ* and [3]*Diabetes Research Group, Department of Biological and Biomedical Sciences, University of Ulster, Coleraine BT52 1SA, Northern Ireland*

The genetically obese (ob/ob) mouse is hyperinsulinaemic and has high circulating levels of the gastrointestinal hormone GIP. These hormones have previously been shown to stimulate fatty acid synthesis (FAS) and lipoprotein lipase (LPL) activity in adipose tissue from lean rats. This study investigates the role of insulin and GIP in fat metabolism in adipose tissue from lean and obese mice.

Explants (0·3–0·5 mg) were prepared from adipose tissue of lean and obese (ob/ob) mice, and also from obese mice passively immunized with anti-GIP antiserum γ-globulin. The effects of insulin and GIP on FAS and LPL activity were measured. FAS was determined by measuring the incorporation of ^{14}[C]acetate into saponifiable fat. LPL activity was determined using ^{3}[H]triolein as substrate.

In lean mice, insulin (5 nM) and GIP (4 nM) significantly stimulated FAS from a basal value of 0·92 (SE 0·08) pmol acetate/mg wet wt per 2 h to 3·73 (SE 0·42) and 2·06 (SE 0·14) pmol acetate/mg wet wt per 2 h respectively ($P<0·01$) in subcutaneous adipose tissue. In obese mice, the basal rate of FAS was significantly less (0·34 (SE 0·12) pmol acetate/mg wet wt per 2 h) and neither insulin nor GIP had any stimulatory effect. LPL activity (μM free fatty acid/mg tissue per 15 min) is shown in the Table.

	Lean mice						Obese mice					
	Basal		GIP (4 nM)		Insulin (5 nM)		Basal		GIP (4 nM)		Insulin (5 nM)	
Adipose tissue	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Omental	2·9	0·3	4·0*	0·3	6·8*	0·6	18·0	0·5	24·5*	1·7	23·1*	0·9
Subcutaneous	4·2	0·3	4·0	1·0	4·3	0·6	15·5	3·1	15·9	4·2	16·0	2·6
Epididymal	4·7	0·8	5·3	0·6	9·2*	0·8	16·3	2·8	14·8	3·7	15·1	2·9

Significantly different from basal value: (*$P<0·05$)

In lean mice, insulin significantly stimulated LPL activity in epididymal and omental adipose tissue. GIP significantly stimulated LPL activity in omental adipose tissue only. In obese mice, basal LPL activity was significantly higher than in lean. However, their adipose tissue was less responsive to insulin and GIP.

In passively-immunized obese mice, both insulin and GIP stimulated FAS in a manner intermediate between the lean and obese mice (FAS at 0·1 nM GIP, 0·62 (SE 0·03), 0·19 (SE 0·03) and 0·43 (SE 0·04); at 0·6 nM insulin, 0·62 (SE 0·03), 0·19 (SE 0·01) and 0·31 (SE 0·03) for lean, obese and passively-immunized obese mice respectively. The loss of response to GIP and insulin observed in adipose tissue of obese mice possibly results from reduced sensitivity due to high circulating levels of these hormones in the obese state. This loss of response is partially reversed in obese mice passively immunized with anti-GIP. These findings suggest a possible role of GIP in the control of fat synthesis in genetically obese animals.

Effects of intraduodenal administration of fat or glucose on gastric inhibitory polypeptide concentration in portal blood of goat kids. By J. P. MCCARTHY, P. A. MARTIN, A. FAULKNER and D. J. FLINT, *Hannah Research Institute, Ayr KA6 5HL*

In simple-stomached animals gastric inhibitory polypeptide (GIP) augments glucose-stimulated insulin release (Morgan *et al.* 1988), and has been demonstrated in vitro to have direct anabolic effects on adipose tissue (Beck, 1989). GIP secretion has not been studied in ruminants, where such actions could have implications for milk production and fattening. We have therefore monitored GIP responses to intraduodenal administration of fat and glucose in goat kids. Though normally absorbed from the ruminant small intestine in relatively small amounts, these nutrients were chosen because they are the major GIP secretagogues in simple-stomached animals.

Kids were given milk only, to inhibit development of rumen function (pre-ruminants), or allowed access to hay and straw (ruminants). When 63 (SE 1) d old (live weight 13 (SE 1) kg) the animals were fasted overnight, anaesthetized and fitted with a portal vein catheter. Blood samples were taken before and for 90 min after 15 g fat (as double cream) or glucose (as 500 g/l solution) was injected directly into the duodenum.

			Plasma GIP concentration (pg/ml)									
Time (min) . . .			0	10	20	30	40	50	60	70	80	90
	n											
Pre-ruminant:												
Fat	5	Mean	330	310	356	386	386	438	574**	643**	658*	695*
		SE	57	63	62	57	56	70	84	77	101	121
Glucose	7	Mean	349	410	345	334	307	283	270*	251	267*	274
		SE	42	55	47	43	38	29	24	17	42	31
Ruminant:												
Fat	7	Mean	230	219	261	362	356	346	403*	436**	380	423*
		SE	42	57	50	65	58	73	70	60	68	75
Glucose	7	Mean	252	253	224	214	203	191	190*	174	171*	169
		SE	63	86	67	58	52	39	50	29	40	49

Significantly different from time 0 (paired *t* test): $*P<0{\cdot}05$; $**P<0{\cdot}01$.

By 90 min after fat or glucose injection, the respective increases in blood triacylglycerol and glucose concentrations were 119 (SE 37) mg/l and 3·8 (SE 0·8) mM in pre-ruminants and 67 (SE 15) mg/l and 3·5 (SE 0·7) mM in ruminants. GIP concentrations were significantly increased in response to fat, and decreased after glucose administration (see Table). Basal GIP concentrations and GIP responses to fat tended to be higher in pre-ruminants than in ruminants but these differences did not reach statistical significance.

These results indicate that fat is a GIP secretagogue in both pre-ruminant and young ruminant animals. GIP responses in adult ruminants receiving conventional feedstuffs are being investigated.

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Modification of lipoprotein lipase activity in response to gastric inhibitory polypeptide (GIP) in rats intubated with triolein. By J. OBEN[1], LINDA MORGAN[1], J. FLETCHER[2] and V. MARKS[1], [1]*School of Biological Sciences, University of Surrey, Guildford GU2 5XH and* [2]*Unilever Research, Colworth House, Sharnbrook, Bedford MK44 1LQ*

Fat synthesis and incorporation into adipose tissue of rats is stimulated by the gut hormones GIP and glucagon-like polypeptide (GLP)-1 (7-36)amide. The mechanism for this stimulation is not known but could involve enzymes concerned with adipose tissue fat deposition. This study investigated the influence of GIP, GLP-1 (7-36)amide and insulin in modifying lipoprotein lipase (LPL) activity in rats.

Twelve male rats (96–105 g) either received 1 ml of triolein by stomach intubation daily between 08.30 and 09.30 h (triolein intubated group), or 1 ml of water (control group) after an overnight fast. Both groups were allowed to feed *ad lib.* on chow after intubation. Daily food intakes and weight gains were measured. After 14 d, animals were killed and their omental, subcutaneous and epididymal adipose tissues excised. Adipose tissue explants (0·3–0·5 mg) were cultured for 2 h in the presence of GIP, GLP-1 (7-36)amide, and LPL activity was determined in the homogenates prepared from them using ^{3}[H]triolein as substrate.

During the trial period, chow intake was not significantly different for the two groups. Weight gain was, however, significantly higher in triolein-intubated rats than the control group (130·3 (SD 2·3) g *v.* 124·8 (SD 2·4) g) ($P<0·05$). LPL activity (μM FFA/mg tissue per 15 min) is shown in the Table.

	Control group			Triolein group		
Adipose tissue	Basal	GIP (4 nM)	Insulin (1 nM)	Basal	GIP (4 nM)	Insulin (1 nM)
Omental	3·37	11·54*	8·12*	5·01†	20·40*†	12·25*†
Subcutaneous	2·77	2·92	4·67*	6·31†	16·30*†	15·40*†
Epididymal	4·62	9·32*	5·43*	8·42†	22·20*†	18·23*†

† Significantly different ($P<0·05$) from equivalent control group; * significantly different ($P<0·05$) from basal level.

Basal LPL activity was significantly higher in the triolein-treated compared to the control group. LPL activity was stimulated by GIP and insulin but not GLP-1 (7-36)amide (4 nM).

Stimulation of fatty acid incorporation into adipose tissue by GIP may, like insulin, be due to increasing LPL activity. The increased LPL activity in the triolein-treated group could reflect an increased amount of LPL protein present, an adaptive measure to cope with the excess fat. GLP-1 (7-36)amide-stimulation of fatty acid incorporation must, however, be regulated via a different mechanism. The increased stimulation by GIP and insulin of LPL activity found in fat-treated rats may therefore facilitate the uptake of circulating triglycerides.

Effects of neonatal under- and overnutrition on the function of the entero–insular axis in rats. By J. M. KNAPPER, *School of Biological Sciences, University of Surrey, Guildford GU2 5XH* and P. R. FLATT, *Diabetes Research Group, Department of Biological and Biomedical Sciences, University of Ulster, Coleraine BT52 1SA*

The present study evaluated in rats the effects of litter size and neonatal nutrition on glucose homeostasis and function of entero-endocrine cells responsible for the synthesis and secretion of two insulin-releasing hormones, GIP (glucose-dependent insulinotropic polypeptide) and GLP-1 (7-36)amide (glucagon-like peptide-1 (7-36)amide).

Rats were suckled in litters of four (overfed), ten (normal) and sixteen (underfed) for 21 d post-partum prior to weaning onto normal laboratory diet *ad lib.* From day 3 onwards, body-weights of pups from the various litters were significantly different (Table).

	Body-wt of offspring (n 6)					
Days post-partum . . .	0		3		41	
Litter	Mean	SD	Mean	SD	Mean	SD
Overfed	6·5	1·2	11·4*	0·7	99·5**	1·9
Normal	6·7	0·9	10·3*	0·4	84·7**	2·3
Underfed	7·1	0·7	8·6*	0·7	77·3**	1·0

Significance of differences between groups at 3 d and 41 d post-partum (multiple ANOVA): *$P<0·05$; **$P<0·01$.

On days 17 and 42, rats from each group (3 × n 6) were fasted for 18 h prior to administration of an oral glucose load (52·8 kJ/kg body-weight). All groups showed superior glucose tolerance on day 17 compared with day 42 ($P<0·05$). This was not accompanied by changes of the plasma insulin response. Elevated fasting plasma GIP and insulin concentrations ($P<0·05$) were observed in all groups on day 17. Compared with other groups, the GIP response of overnourished rats to oral glucose was greater ($P<0·01$) on day 17. Basal GIP concentrations and the acute GIP response to glucose were similar in all groups by day 42. On day 17, small intestines of overnourished rats had higher ($P<0·05$) concentrations (pmol/g wet wt) of GIP (mean (SEM) n 6; 234 (50)) compared with normal (157 (40)) and undernourished (158 (46)) rats. These differences were no longer apparent at 42 d. In comparison, intestinal GLP-1 (7-36)amide concentrations (pmol/g wet wt) were increased ($P<0·05$) in overfed (99 (19)) and underfed (161 (30)) rats on day 17, compared with normal controls (62 (20)). By day 42, the concentration of GLP-1 (7-36)amide had increased in normal rats (139 (29)) beyond that of overfed rats (107 (16)), but the underfed rats had the highest GLP-1 (7-36)amide concentrations (203 (40); $P<0·01$).

These observations indicate that overnutrition during the neonatal period transiently augments the function of GIP-secreting cells. Changes of intestinal GLP-1 (7-36)amide concentrations observed following overnutrition and undernutrition were not normalized 21 d after weaning.

Influence of vegetable oils and α-tocopherol supplementation on tocopherol concentrations in chick tissues. By P. J. A. SHEEHY, P. A. MORRISSEY and A. FLYNN, *Department of Nutrition, University College, Cork, Republic of Ireland*

There is considerable interest in the possibility that heated and/or oxidized fats may be detrimental to health. The feeding of diets containing thermally-oxidized rapeseed oil at four levels of deterioration, but containing similar concentrations of α-tocopherol acetate (α-TA), decreased rat serum and liver α-tocopherol (α-T) levels in a graded fashion, suggesting that substances present in oxidized oils promote peroxidation in liver (Izaki *et al.* 1984). Consumption of heated sunflower oil by chicks increased TBA-reactive substances and reduced α-T in several tissues, the effects being partially offset by dietary supplementation with α-TA (Sheehy *et al.* 1990). The aim of this study was to compare the effects of heated sunflower and linseed oils on the α-T status of chick tissues.

Sunflower and linseed oils were heated at 140° for 24 h with constant aeration. Forty-eight 1-d-old chicks were randomized into six groups, and fed diets containing 80 g/kg fresh sunflower oil (FSO), fresh linseed oil (FLO), heated sunflower oil (HSO), heated linseed oil (HLO), α-TA-supplemented heated sunflower oil (HSE) or α-TA-supplemented heated linseed oil (HLE). Diets were prepared weekly and stored at 4°. Diets contained 0·05 g/kg BHT to protect lipids during storage, and 0·05 g α-T/kg (except for HSO and HLO which were devoid of α-T).

Chicks fed HSO and HSE had normal growth, but feeding HLO and HLE caused a significant growth depression. In general, concentrations of α-T in tissues of chicks fed FSO were greater than in those fed FLO. In the majority of tissues, α-T levels were significantly reduced by consumption of heated, or heated–supplemented oils. The depression in tissue α-T status was more pronounced in chicks fed HSE than in those fed HLE. Plasma α-T was a good indicator of α-T concentrations in other tissues ($r > 0{\cdot}71$, $P < 0{\cdot}01$).

	α-Tocopherol concentration*													
	Sunflower oil							Linseed oil						
	HSO		HSE		FSO		HSE/FSO	HLO		HLE		FLO		HLE/FLO	
	Mean	SE	Mean	SE	Mean	SE	Mean	Mean	SE	Mean	SE	Mean	SE	Mean	*r*
Plasma	0·80[a]	0·07	6·58[b]	0·50	19·1[c]	3·13	0·34	0·65[a]	0·03	7·84[b]	0·90	11·3[c]	1·14	0·69	—
Heart	1·47[a]	0·56	15·4[b]	1·22	41·2[c]	1·81	0·37	0·23[a]	0·15	16·4[b]	1·73	35·3[c]	1·63	0·46	0·96
Spleen	0·32[a]	0·09	9·51[b]	1·39	25·0[c]	4·26	0·38	0·21[a]	0·12	15·8[b]	2·20	31·1[c]	2·30	0·51	0·81
Lung	0·04[a]	0·02	6·09[b]	0·44	18·6[c]	1·21	0·33	2·04[a]	1·51	17·2[b]	2·70	16·1[b]	2·16	1·07	0·71
Liver	0·17[a]	0·07	7·27[b]	0·84	15·9[c]	2·34	0·46	0·19[a]	0·08	7·05[b]	0·88	13·0[c]	0·60	0·54	0·96
Thigh muscle	1·64[a]	0·47	7·71[b]	0·80	17·8[c]	1·45	0·43	1·14[a]	0·18	10·9[b]	0·84	19·8[c]	2·21	0·55	0·82
Breast muscle	1·75[a]	0·38	5·81[b]	0·16	12·5[c]	0·98	0·46	2·50[a]	0·43	7·03[b]	0·45	9·35[c]	0·62	0·75	0·92
Cerebrum	1·96[a]	0·28	3·68[b]	0·51	6·97[c]	1·60	0·53	2·07[a]	0·46	3·76[b]	0·29	5·69[b]	1·10	0·66	0·90
Cerebellum	1·30[a]	0·38	3·11[b]	0·13	4·97[c]	0·36	0·63	1·84[a]	0·41	2·19[a]	0·25	2·76[a]	0·17	0·79	0·75

[a–c] For each oil, mean values in a horizontal row with different superscript letters were significantly different: $P < 0{\cdot}05$ (Student's *t* test for unpaired data.)

* μg/ml plasma or μg/g tissue.

The results support the proposal that heated oils induce in vivo peroxidation and accelerate the turnover of α-T, and suggest that the products responsible, and their target tissues, depend on the fatty acid composition of the dietary lipid.

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Measurement of radical-trapping antioxidant parameter (TRAP) activity in plasma using chemiluminescence. By RUTH COLL, *Biomedical Research Centre, University of Ulster, Coleraine BT52 1SA, Northern Ireland* and D. I. THURNHAM, *MRC Dunn Nutrition Laboratories, Milton Road, Cambridge CB4 1XJ*

Wayner *et al.* (1987) described the measurement of TRAP activity in plasma using an oxygen electrode. The technique is, however, very time-consuming; one sample can take up to 90 min to measure. Recently, workers in Birmingham (G. Thorpe and T. P. Whitehead, personal communication) and Finland (Metsa-Ketela, 1991) have described methods to measure TRAP based on the ability of plasma antioxidants to quench radical-generated chemiluminescence. We have combined and modified these to produce the following method.

Into an LKB Wallac 1251 luminometer (Bio-Orbit Ltd) is placed a cuvette containing 400 μl phosphate-buffered saline (PBS; pH 7·4; Sigma), 200 μl 2:1 mixture of 2,2′-azo-bis-(2-amidinopropane HCl) (Polysciences Ltd, Northampton; 600 mg in 5 ml PBS) and sodium perborate (10 mg in 30 ml PBS), 200 μl sodium linoleate (Sigma, 9·45 mg in 50 ml PBS) and 200 μl luminol (17·7 mg in 5 ml dimethylsulphoxide). After 4 min is allowed to stabilize light emission, 6 nmol standard (30 μl 0·2 mmol/l 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TROLOX; Aldrich) is added, followed, after luminescence recovers, by 30 μl plasma (diluted 1:5 in PBS). Quenching times ('T') for TROLOX and plasma were calculated from their time of addition to the point when light emission had recovered to 10% of the previous luminescence. Total assay time is 15 min.

$$TRAP^{CHEM}\ \mu mol/l = \text{'T' plasma/ 'T' TROLOX} \times 2000$$

where 2000 is obtained from stoichiometric factor for vitamin E multiplied by the nmol TROLOX added per tube expressed per litre plasma (1000/6).

The TRAP activity of sixty-nine plasma samples from healthy persons using the chemiluminescence assay was significantly higher (paired *t* tests) in men, but not women, than that obtained using the oxygen electrode ($TRAP^{OX}$) method of Thurnham *et al.* (1990) (Table). Furthermore, $TRAP^{CHEM}$ but not $TRAP^{OX}$ values showed evidence of correlating with α-tocopherol concentrations when examined by sex (men r 0·278 NS, women r 0·414 P=0·014) or smoking habits. TRAP measured by both methods was significantly higher (*t* tests) in men than women (Table) but there were no differences between smokers and non-smokers.

	n	$TRAP^{OX}$ (SD)		$TRAP^{CHEM}$ (SD)		*P*
All cases	67	695	(188)	757	(199)	<0·01
Men	32	750[a]	(202)	838[b]	(192)	<0·02
Women	35	645[a]	(160)	684[b]	(179)	NS

Significant differences between sexes: [a]P<0·02; [b]P<0·001.

Stoichiometric factors for the main plasma antioxidants using the chemiluminescent assay were ascorbate 1·5, urate 1·7 and protein Sulphydryl Stoichiometric Factor 0·4 and these were the same as those obtained by the oxygen electrode method (1·5, 1·7, 0·2, respectively) except for protein. The higher TRAP activity obtained by the chemiluminescence method may be due to greater efficiency of protein to trap radicals in this system.

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Iron status, antioxidant enzymes and tumour load in male and female rats treated with 1,2-dimethylhydrazine (DMH). By H. E. BRISTOW, J. J. STRAIN and R. W. WELCH, *Human Nutrition Research Group, University of Ulster at Jordanstown, Newtownabbey, Co. Antrim BT37 0QB, Northern Ireland*

Dietary iron (Fe) has been shown to enhance tumour growth in mice treated with carcinogen (Siegers *et al.* 1988), and it has been suggested that Fe overload may increase cancer risk in humans (Stevens *et al.* 1988). This study investigated the effects of Fe on antioxidant enzymes and on DMH-induced colon tumour incidence on male and female rats.

Forty-eight weanling Sprague-Dawley rats (twenty-four female; twenty-four male) were fed a modification of the American Institute of Nutrition purified rat diet, containing either 15 mg/kg Fe (LFe) or 400 mg/kg Fe (HFe). From weeks 8–14, all rats were given a weekly subcutaneous injection of DMH (30 mg/kg body-weight) to induce colon tumours. The rats were sacrificed at week 20. On removal of the colon, all rats were found to have tumours, and tumour load was recorded.

Fe status measurements and those antioxidant enzyme activities which showed significant differences are given in the Table.

	Low iron				High iron						
	Female		Male		Female		Male				
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Fe	Sex	Fe × sex
Iron indices											
Haemoglobin (g/l)	99·8	4·6	66·6	9·8	98·0	5·9	97·9	9·8	NS	*	*
Plasma Fe (μg/dl)	183	26·9	69	15·2	207	18·1	139	15·7	*	***	NS
Liver Fe (μg/g)	390	52·6	105	17·5	1149	105	476	61·6	***	***	**
Transferrin saturation (%)	28·6	3·9	12·2	3·3	34·8	4·6	25·1	3·9	*	**	NS
Erythrocyte enzymes											
Catalase (U/mg protein)	14·7	1·78	20·9	2·52	13·4	1·2	16·7	3·59	NS	*	NS
Liver enzymes											
Catalase (U/mg protein)	30·7	1·69	40·6	3·03	36·5	3·56	49·2	1·93	*	***	NS
GST (U/mg protein)	65	6·2	125	7·5	68	7·5	125	5·8	NS	***	NS
Tumour load											
(no. per colon)	2·67	0·43	9·09	1·5	3·17	0·49	4·45	0·68	*	***	**

NS, not significant; *$P<0·05$; **$P<0·01$; ***$P<0·001$ (two-way ANOVA).

Results indicate that Fe status was significantly higher in rats fed the HFe diet compared with those fed the LFe diet, and also that Fe status was significantly higher in females compared with males. Male rats had significantly higher erythrocyte catalase (*EC* 1.11.1.6), hepatic catalase and glutathione-S-transferase (*EC* 2.5.1.18) activities. Hepatic catalase activity was also significantly higher in HFe rats. Tumour load was significantly higher in male rats, and was significantly higher in those rats fed the LFe diet. There was also a significant Fe × sex interaction.

These results suggest that low Fe status increases the tumour load of DMH-treated rats in contrast to earlier findings with mice (Siegers *et al.* 1988). The detoxifying enzyme cytochrome P-450, is known to have significantly lower activities in the intestines of Fe-deficient rats (Dhur *et al.* 1989). Thus decreased activities of this enzyme may be implicated in the increased tumour load in the Fe-deficient rats in the current study.

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Effect of high dose antioxidant vitamin supplements on serum markers of oxidant damage.

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Excessive free radical production or suboptimum free radical regulatory mechanisms, can result in the initiation of lipid peroxidation and the accumulation of lipid peroxides in blood. The antioxidant vitamins α-tocopherol and ascorbate inhibit lipid peroxidation by scavenging free radicals (Anderson & Lukey, 1987; Leibovitz *et al.* 1990). There is also good in vitro experimental evidence that ascorbate acts synergistically with α-tocopherol (Barclay *et al.* 1983).

The aim of this study was to examine the effect of high-dose dietary antioxidant supplementation, using α-tocopherol and ascorbate separately or in combination (1 g α-tocopherol and/or 1 g ascorbate per d), on plasma levels of products of lipid peroxidation. The degree of lipid peroxidation was assessed by the thiobarbituric acid test for malondialdehyde-like material (MDA-LM). Another putative measure of oxidant damage, i.e. estimates of linoleic acid isomerization (DC/LA), was also made.

Thirty-two healthy subjects were randomized into four groups of eight and received either: placebo, ascorbate, α-tocopherol or ascorbate and α-tocopherol for 28 d. Blood samples were collected on day 0 and day 29 and results are given in the Table.

Group . . .		Treatment							
		Placebo		Ascorbate		α-Tocopherol		Ascorbate + α-tocopherol	
	Day	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Ascorbate (μmol/l)	0	76	7·1	62	10·2	83	9·5	79	4·0
	29	69[a]	7·6	115[b]*	13·1	62[a]	7·8	104[b]*	9·5
α-Tocopherol (μmol/l)	0	24	1·7	27	2·4	29	2·2	28	1·5
	29	23[a]	1·7	27[a]	2·5	57[b]*	3·8	53[b]*	4·4
MDA-LM (μmol/l)	0	2·35	0·28	1·83	0·20	1·93	0·31	1·99	0·23
	29	1·81	0·23	1·53	0·09	1·64	0·11	1·65	0·19
DC/LA (×100%)	0	0·66	0·13	0·85	0·18	0·70	0·08	0·84	0·20
	29	0·81	0·04	0·93	0·18	0·81	0·11	1·10	0·20

[a,b] Means in horizontal rows with different superscripts are significantly different by the Newman Kuels test: $P<0·01$.

* Significantly different from day 0 at $P<0·01$ (paired t test).

The present study demonstrated that in these healthy young adults, antioxidant vitamin supplementation did not significantly affect the circulatory levels of lipid peroxides, as assessed by MDA-LM, or the degree of isomerization of linoleic acid (DC/LA).

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Immune responses in calves depleted of vitamin E, or selenium or both. By J. M. POLLOCK, D. M. WALSH, D. P. MACKIE, D. G. KENNEDY and S. KENNEDY, *Department of Agriculture, Veterinary Sciences Division, Stormont, Belfast BT4 3SD, Northern Ireland*

Vitamin E (vitE) and selenium have been considered to affect immune responses in cattle (Hutcheson, 1989). This study was initiated to investigate if deficiencies of vitE and Se had either independent or additive effects on immunity.

Sixteen calves were randomly allocated to four diets at approximately 16 weeks of age. A basal diet providing <3·0 μg/g α-tocopherol and <0·02 μg/g Se was fed to all calves (Kennedy *et al.* 1987). This was, or was not, supplemented with α-tocopherol (200 μg/g) and/or Se (0·2 μg/g) to give four diets: vitE−/Se−, vitE+/Se−, vitE−/Se+ and vitE+/Se+. Comparing deficient with supplemented calves, by 59 weeks of age these diets resulted in a mean plasma α-tocopherol concentration of 1·77 *v.* 12·74 μmol/l and a mean erythrocyte glutathione peroxidase activity of 71 *v.* 242 U/g Hb.

All calves were immunized with Keyhole Limpet haemocyanin (KLH; Sigma Chemical Co. Ltd, Poole, Dorset), a novel antigen, at 51 and 54 weeks of age. Serum anti-KLH antibodies were measured using an indirect ELISA. No significant differences in anti-KLH IgG levels were detected between diets.

Lymphocyte transformation test (LTT) responses to KLH, pokeweed mitogen (PWM) and concanavalin A (ConA) were measured for each calf. These tests were made in both culture medium containing autologous serum (AS) and fetal calf serum (FCS).

There were significant effects of diet on LTT responses between 50 and 59 weeks of age, although not always consistently. The AS cultures showed that supplementation with vitE, with or without Se, enhanced unstimulated responses ($P<0·05$). Also in AS cultures, supplementation with Se alone increased responses to KLH, while supplementation with vitE and Se together increased responses to PWM ($P<0·05$).

However, no effect of diet on unstimulated cultures occurred in FCS. The effect of Se-enhancement of KLH responses seen in AS was apparent only as a trend in FCS ($P<0·05$). Also in FCS, responses to PWM were increased by supplementation with vitE, with or without Se, but were decreased by Se supplementation alone ($P<0·05$). The differences between cultures in AS and FCS may be due partly to the low concentration of vitE in FCS (1·6 μmol/l). LTT responsiveness may be affected when cells are transferred to in vitro conditions different from their in vivo history.

No effects of diet on ConA responses were detected. ConA is considered to stimulate T-cells while PWM stimulates both T-cells and B-cells. The present results suggest that vitE and Se deficiencies affect only cell populations responsive to PWM. Furthermore, vitE and Se deficiencies may have both discrete and interactive effects on immune responses.

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The effects of increased vitamin E supplementation on profitable commercial broiler production. By D. G. Kennedy[1], E. A. Goodall[2], S. G. McIlroy[1], D. W. Bruce[1] and D. A. Rice[1], *Department of Agriculture,* [1]*Veterinary Sciences Division, Stormont, Belfast BT4 3SD* and [2]*Biometrics Division, Newforge Lane, Belfast BT9 5PX, Northern Ireland*

Contradictory results have been obtained in previous trials measuring the effects of vitamin E supplementation on broiler performance. Previous trials may have been hampered by insufficient randomization, numbers of birds and controls. The present study was initiated to investigate the effects of additional vitamin E supplementation on the performance and profitability of broiler production in an integrated poultry organization.

Ten matched pairs of broiler breeder flocks were fed either their normal diets or their normal diets supplemented with additional vitamin E, to contain a total concentration in feed of 180 IU/kg, throughout their lives. Progeny from these flocks were then randomly assigned to groups receiving either a normal or vitamin E-supplemented diet. Thus, four categories of broiler flocks were studied: broilers fed either a normal or supplemented diet which were the progeny of broiler breeders which were fed either a normal or supplemented diet. A total of 168 broiler flocks containing over 3 million birds which had no evidence of clinical disease were studied. The performance criteria measured included mortality, feed conversion efficiency, average weight per bird and income. In addition, data relating to the size, age, stocking density, sex, litter conditions and disease status of all flocks were collected. Random samples of feedstuffs were analysed for vitamin E concentration by HPLC for quality control purposes.

Vitamin E supplementation had no effect on the performance of broiler breeders. Similarly, the vitamin E status of broiler breeder flocks had no statistically significant effects on the subsequent performance of their progeny. However, when all broiler flocks receiving the supplemented diet, irrespective of the vitamin E status of their parents, were compared to all broiler flocks receiving the normal diet, irrespective of the vitamin E status of their parents, the feed conversion ratio was improved by 0·8% ($P<0·05$) and the average weight per bird was increased by 1·4% ($P<0·05$). Additionally, there was a significantly increased net income per 1000 birds equivalent to 8·52% of the target income set for all flocks in that organization ($P<0·05$). However, when the cost of the additional vitamin E was taken into consideration (equivalent to 5·7% of the target net income per 1000 birds), the adjusted net income per 1000 birds was increased by 2·82%. This difference was not statistically significant, although a positive cost benefit was still obtained.

This study was supported by F. Hoffmann-La Roche, Basel, Switzerland.

Elevation of serum insulin levels by the artificial pancreas and by oral carbohydrate loading stimulates cholesterol synthesis and storage in humans. By J. C. STINSON[1] (introduced by F. J. ANDREWS), D. OWENS[2], P. COLLINS[2], A. JOHNSON[2] and G. H. TOMKIN[1], [1]*Department of Diabetes and Endocrinology, Adelaide Hospital, Dublin 8* and [2]*Department of Biochemistry, Royal College of Surgeons in Ireland, Dublin 2, Republic of Ireland*

Hyperinsulinaemia is associated with atherosclerosis in diabetic and non-diabetic patients. The aim of this study was to examine the relationship between serum insulin and cholesterol synthesis.

In the first part of this study six young (18–36 years) insulin-dependent diabetics were studied using the insulin–glucose clamp method with the Biostator (artificial pancreas). All patients were normocholesterolaemic and had a normal body mass index (23·7 (SEM 0·45) kg/m^2). The patients all underwent a high insulin (intravenous infusion rate 1·2 mU/kg per min), low glucose (4 mmol/l) clamp which lasted 4 h following an overnight fast. Serum insulin levels rose by 103·2% (SEM 17·8%). Cholesterol synthesis was measured by [^{14}C]acetate incorporation into lymphocytes (a viable and accessible cell model). The rate of acetate incorporation increased over the 4 h from 1026 (SEM 120) to 1199 (SEM 64) pmol/min per mg cell protein ($P<0·05$). Lymphocyte cellular total cholesterol was also measured before and after the hyperinsulinaemia clamp. The total cell cholesterol rose significantly from 34·7 (SEM 6·12) to 56·84 (SEM 8·31) ng/mg cell protein ($P<0·05$).

To assess if these changes could be induced by more physiological stimulation we studied the effects of oral high-carbohydrate feeding in six controls, six obese non-insulin-dependent diabetics and six obese non-diabetic subjects. After an overnight fast, each patient ate a set meal of 5·65 MJ (1350 kcal; 55% carbohydrate, 25% fat, 20% protein). Serum insulin levels and [^{14}C]acetate incorporation were measured before and after 4 h.

With the obese non-diabetic subjects there was a significant rise in both the insulin, 17·5 (SEM 4·1) to 54·5 (SEM 12·4) μIU/ml ($P<0·05$) and the acetate incorporation, 1200 (SEM 131) to 1658 (SEM 249) pmol/min per mg cell protein ($P<0·05$). The obese diabetic subjects showed a smaller rise in serum insulin 14·9 (SEM 1·9) to 34·1 (SEM 8·7) μIU/ml ($P<0·05$). However, the rise in the rate of acetate incorporation, 502·7 (SEM 196·3) to 1272·2 (SEM 468) pmol/min per mg cell protein, was not significant ($P=0·09$) in this group although five out of six showed an increase. The one patient whose acetate incorporation failed to rise was the only patient not to show an increase in serum insulin. There was no change in acetate incorporation or insulin levels in the controls. Blood sugar rose only in the diabetic subjects (9·1 (SEM 2·6) to 15·4 (SEM 3·8) mmol/l).

This study suggests that insulin stimulates cholesterol synthesis both under highly artificial conditions and in a more physiological setting, and whilst hyperglycaemia potentiates this effect, it will not on its own produce this effect. We suggest that these findings may explain in part the association between hyperinsulinaemia and atherosclerosis.

Serum amino acids during insulin-dependent diabetic and normal pregnancies and their relationship to estimates of fetal weight. By O. S. OLUFEMI, P. G. WHITTAKER, P. HUMES and T. LIND, *University Department of Obstetrics and Gynaecology, Princess Mary Maternity Hospital, Newcastle upon Tyne NE2 3BD*

Serial measurments of twenty-three amino acids were made in fourteen insulin-dependent diabetic (D) and nine normal (N) mothers during pregnancy and again at 12 weeks post-partum (PP). Maternal serum was taken in the morning from diabetic subjects (2 h post-prandial) and from normal mothers (fasted). In six non-pregnant subjects this breakfast gave no significant difference in amino acid content between fasted and post-prandial samples. Significant and consistent decreases in amino acids during gestation (compared with PP values) were seen in only five amino acids, the falls having occurred during early pregnancy in both subject groups. Threonine rose during pregnancy.

Stage of gestation . . .		12 weeks		34 weeks		12 weeks PP	
Amino acid (μmol/l)	Subjects	Mean	SD	Mean	SD	Mean	SD
Threonine	N	149	51	219†††	50	146	31
	D	186	54	282†††	65	154	38
Glycine	N	258***	53	261**	56	408	121
	D	236*	35	227*	41	381	137
Tyrosine	N	44***	12	41*	8	61	18
	D	58*	11	50**	16	70	14
Ornithine	N	95***	35	87***	20	165	47
	D	77***	15	84**	29	129	41
Taurine	N	100*	58	91**	31	157	39
	D	99	40	86	25	124	71
Glutamine	N	774*	230	751**	249	1016	352
	D	521	214	495	160	505	145
Total	N	3157***	507	3265*	548	3811	649
	D	3161	570	3230	429	3441	637

Significantly higher than 12 weeks or 12 weeks PP (paired *t* test): †††$P<0{\cdot}001$.
Significantly lower than 12 weeks PP (paired *t* test): *$P<0{\cdot}05$; **$P<0{\cdot}01$; ***$P<0{\cdot}001$.

Diabetic mothers had significantly higher levels of proline, arginine, histidine and the aromatic group (phenylalanine, tyrosine and tryptophan) but surprisingly lower levels of the gluconeogenic amino acid glutamine, relative to the normal mothers during gestation and post-partum. Total amino acid values were similar in the two groups.

Stage of gestation . . .		12 weeks		34 weeks		12 weeks PP	
Amino acid (μmol/l)	Subjects	Mean	SD	Mean	SD	Mean	SD
Proline	N	86	32	86	31	138	97
	D	166*	103	185***	59	194	55
Arginine	N	88	24	114	38	83	29
	D	151*	88	136	38	129*	43
Aromatics	N	162	38	158	32	193	19
	D	244**	60	212**	40	239*	56
Histidine	N	77	19	96	30	81	25
	D	107**	26	115	35	110*	23
Glutamine	N	774	230	751	249	1016	352
	D	521*	214	495**	160	505**	145

Significant difference between N and D: *$P<0{\cdot}05$; **$P<0{\cdot}01$; ***$P<0{\cdot}001$.

Significant negative correlations ($P<0{\cdot}01$) were found at 34 weeks gestation between branch chain, non-essential or total amino acids and sonar estimates of fetal weight (but not subsequent birth weight) supporting the possibility that maternal serum amino acid levels may influence fetal growth.

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Glycine and urea metabolism during normal and diabetic pregnancies. By O. S. OLUFEMI, P. G. WHITTAKER and T. LIND, *University Department of Obstetrics and Gynaecology, Princess Mary Maternity Hospital, Newcastle upon Tyne NE2 3BD*

Glycine-N and urea kinetics were determined post-absorptively during late gestation in six normal pregnant subjects and two subjects with pregnancy complicated by insulin-dependent diabetes mellitus (IDDM), using continuous infusions of [^{15}N]glycine and [^{13}C]urea with appropriate priming. The study was repeated in the same women at 4 months post-partum. Stable isotope enrichment was measured by gas chromatography–mass spectrometry. A steady state enrichment was found for glycine and urea in both groups of subjects; glycine and urea turnover were quantified by isotope tracer dilution.

1. During normal pregnancy, the glycine-N flux was unchanged relative to the post-partum period (2·23 (SD 0·32) *v.* 2·20 (SD 0·29) mg N/kg per h; but elevated flux (2·78 (SD 0·27) mg N/kg per h) was found in mothers with diabetes relative to normal mothers which decreased post-partum (2·24 (SD 0·01) mg N/kg per h).

2. Urea turnover (as reflected in the flux measurements) was depressed during normal pregnancy (7·00 (SD 0·25) mg/kg per h) compared to post-partum values (13·42 (SD 1·62) mg/kg per h; $P<0·001$); the same phenomenon occurred in IDDM women but to a lesser extent (7·82 (SD 0·49) *v.* 10·51 (SD 2·10) mg/kg per h, pregnant and non-pregnant state respectively).

3. No relationship was found between amino acid flux and estimates of fetal weight determined at test and subsequent birth weight in normal subjects.

We conclude that, post-absorptively in both non-diabetic and diabetic pregnant subjects, the lower plasma and urinary urea seen was a consequence of decreased urea production which may be important in conserving nitrogen, and the higher glycine-N flux shown in diabetic pregnant subjects would suggest that glycine metabolism was altered in diabetic pregnancy possibly to satisfy feto–placental metabolic needs.

Insulin sensitivity in pregnancy: studies with the euglycaemic clamp technique. By K. STANLEY, C. BRUCE and R. FRASER, *Centre for Pregnancy Nutrition, University Department of Obstetrics and Gynaecology, Northern General Hospital, Sheffield S5 7AU*

Studies using the euglycaemic clamp to demonstrate insulin resistance in pregnancy have been limited (Botta, 1988; Ryan, 1988). We report a study to assess the extent of insulin resistance in the third trimester of normal pregnancy.

Euglycaemic clamps were performed in nine healthy non-pregnant women in the follicular phase of the menstrual cycle, age 30·5 (SEM 1·2) years and in nine healthy women at 34–37 weeks gestation, age 32·1 (SEM 0·7) years. Two of the pregnant women were potential diabetics; both had normal 75 g oral glucose tolerance test at 32 weeks gestation.

In vivo sensitivity to insulin was measured during an insulin infusion of 40 mU/m^2 per min following an initial bolus of 2000 mU/m^2 (Bergman, 1989). Arterialized venous blood was obtained (oxygen saturation 95–98%), and plasma glucose was clamped at 4·5 mmol/l, using a variable infusion of 10% dextrose. 'Steady state' was achieved by 75 min and maintained for a further 60 min. Mean glucose infusion rates over each 30-min period of constant glycaemia were assessed. Steady state was considered to have been achieved if the mean plasma glucose throughout each 30-min period was within 5% of the target, and if the coefficient of variation of the plasma glucose was less than 6%. In the prepregnancy studies there was less than 8% variation in the mean glucose infusion rate over the two 30-min periods (mean 3%, range 0·8%); in the third trimester studies, all but three showed less than 8% variation in mean glucose infusion rates over the two 30-min periods (mean 2%, range 0·5–12%).

The glucose infusion rates were 278 (SD 52) mg/m^2 per min in the non-pregnant state, and 139 (SD 48) mg/m^2 per min in the third trimester ($P<0·001$). This represents a 50% reduction in insulin sensitivity by the third trimester of pregnancy, although of course completeness of suppression of hepatic glucose output cannot be assessed in human pregnancy.

This reduction in sensitivity to insulin in normal pregnancy has important effects on all classes of fetal substrate provision.

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Insulin resistance and insensitivity in pre-eclampsia. By A. M. RICKETTS[1], H. M. PAYNE-ROBINSON[1], T. E. FORRESTER[1], F. BENNETT[1] and J. HALL[2], [1]*Tropical Metabolism Research Unit* and [2]*Department of Obstetrics and Gynaecology, University of the West Indies, Kingston, Jamaica*

In normal pregnant (NP) women glucose tolerance is impaired, as assessed both by intravenous glucose tolerance tests (IVGTT) (Burt, 1955) and oral glucose tolerance tests (OGTT) (Benjamin & Casper, 1967). An even greater impairment of glucose tolerance occurs in a hypertensive complication of pregnancy, pre-eclampsia (PE), when assessed by either IVGTT (Singh, 1976) or OGTT (Long *et al.* 1977). This impairment of glucose homeostasis in PE has not been fully examined.

In this study, glucose homeostasis in twelve PE patients and thirteen NP women was investigated by OGTT. Insulin receptor binding to white blood cells (WBC) (/10^7 cells per ml) and red blood cells (RBC) (/4×10^9 cells per ml) were measured on fasting blood samples. Plasma insulin and glucose were measured in fasting samples before, and at timed intervals after, a 75 g oral glucose load. The data were log transformed to allow parametric statistical analysis. The results in the fasting state are shown in the Table.

	WBC insulin bound (%)		RBC insulin bound (%)		Insulin (mU/l)		Glucose (mM)		Insulin glucose ratio	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PE	2·27*	1·27	5·60	2·01	27·8*	23·3	4·38	0·54	6·21*	4·97
n	8		8		12		11		11	
NP	4·47	2·4	10·63	9·41	9·61	8·41	4·12	0·29	2·264	1·85
n	10		10		13		13		13	

*$P<0·05$.

Plasma glucose at 30 min was significantly higher in the PE than the NP ($P<0·05$), although repeated measures analysis showed that there was no significant difference between the glucose response curves of the two groups ($P=0·6$). Insulin resistance, defined as exaggerated and delayed insulin peaks at 60 min after the glucose load, was seen in both groups. This effect was more pronounced in the PE, who displayed a higher insulin peak at 60 min which approached statistical significance ($P=0·069$).

In conclusion, the increased insulin response observed in pre-eclampsia, although not statistically significant, suggests a difference in the manner in which an oral glucose load is handled when compared to the normals. The lower insulin receptor binding suggests that the abnormality in glucose homeostasis may occur as a result of tissue insensitivity to insulin. This is supported by the observed difference in the fasting insulin:glucose ratio. The data suggest that the abnormal glucose homeostasis in pre-eclampsia is the result of a significant fasting hyperinsulinaemia and an accompanying insulin insensitivity.

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The effects of ethanol and dietary protein level on weight gain, serum insulin and glucose, and pancreas weight in rats. By J. ANNA NIKOLIĆ[1], VESNA KOKO[2], LJILJANA PETRONIJEVIĆ[3], JASMINA VARAGIĆ[2] and VERA TODOROVIĆ[2], [1]*INEP-Institute for Endocrinology, Immunology and Nutrition, 11080 Zemun,* [2]*Institute for Medical Research, 11000 Belgrade* and [3]*Institute for Diseases of the Digestive System, Clinical Centre, 11000 Belgrade, Yugoslavia*

As part of a study on the effects of chronic ethanol intake, an experiment was set up with seventy male Wistar rats, aged 2 months and initial body-weight (BW) 241 g. Four groups of fifteen rats received limited amounts of a natural diet in a 2×2 factorial scheme. The factors were adequate (26% of gross energy (GE)) or decreased (6% of GE) intake of protein (groups NP and LP) and partial replacement of carbohydrate by ethanol (15–24% of GE; groups NPA and LPA). The remaining rats (NPS) received *ad lib.* the same pelleted diet as group NP.

Group . . .	NPS	NP	NPA	LP	LPA		
Energy intake (kJ/d) . . .	618	381	393	383	388	LSD	SEM
Final BW (g)	513[a]	359[c]	426[b]	369[c]	361[c]	37	13
Pancreas wet wt (g)	1·53[a]	1·30[b]	1·33[b]	1·26[b]	0·99[c]	0·18	0·07
Relative pancreas wt (g/kg BW)	2·99[cd]	3·63[a]	3·15[bc]	3·42[ab]	2·74[d]	0·40	0·14
Serum glucose (mM)	6·03[a]	5·47[b]	5·32[b]	5·33[b]	5·16[b]	0·55	0·19
Serum insulin (mIU/l)	42·2[a]	21·0[b]	21·6[b]	24·8[b]	20·1[b]	9·3	3·3
Serum T_3 (nM)	0·79[b]	0·62[c]	0·68[bc]	0·72[bc]	1·42[a]	0·16	0·06

[a.b.c.d] Mean values in the same horizontal row with different superscript letters were significantly different (analysis of variance): $P<0·05$.

The Table shows that the mean GE intake of group NPS over 32 weeks was about 60% higher than each of the other groups. The final BW after 24 h starvation, pancreas weight, serum glucose and insulin levels were significantly higher than in the remaining groups. There were no significant differences in glucose and insulin between any of the groups with limited dietary intake. However, reduction of protein intake and replacement of starch by ethanol led to an additive decrease in relative pancreas weight. This may reflect a decreased requirement for pancreatic digestive enzymes.

Serum albumin and total protein levels were not affected by the dietary treatments but triiodothyronine (T_3) levels were higher in group LPA than in any of the other groups. This is interesting in connection with the known effect of low protein intake on T_3 (Jepson *et al.* 1988) and the controversy about the action of ethanol (Teschke *et al.* 1983). The difference in T_3 levels between groups NPS and NP may result from insulin-mediated stimulation of hepatic monodeiodination of T_4.

Thus, with limited dietary GE intake, partial replacement of carbohydrate by ethanol and/or protein did not affect basal control of glycaemia. However, 60% increased daily consumption of energy induced a degree of insulin resistance.

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The effects of feeding protected polyunsaturated fatty acids and α-tocopherol to calves depleted of α-tocopherol and selenium. By D. M. WALSH, D. G. KENNEDY, S. KENNEDY and E. GOODALL, *Department of Agriculture, Veterinary Research Laboratories, Stormont, Belfast BT4 3SD, Northern Ireland*

Nutritional myopathy (NM) frequently occurs in cattle following turnout to spring pasture. It is associated with low vitamin E and selenium intake during the inwintering period. Turnout to pasture results in rapid increases in plasma linolenic acid (LA) and α-tocopherol (α-TP) concentrations (McMurray & McEldowney, 1977). These workers hypothesized that NM was due to the toxic effects of grass LA. An experimental model that reproduced the clinical signs and pathology of spontaneous NM was devised (Rice, 1981; Kennedy *et al.* 1987). It was based on feeding linseed oil that had been protected against ruminal hydrogenation as a source of polyunsaturated fatty acids (PUFA) to ruminant calves. However, this model did not consider the possible protective effects of the relatively high α-TP content of grass. The aim of this study was to determine if NM could be induced in calves by feeding protected PUFA and α-TP simultaneously.

Five calves were maintained on a low vitamin E and Se basal diet (Kennedy *et al.* 1987) for 26 weeks. They were then fed the basal diet supplemented with 500 g protected PUFA and 0·75 g α-TP/d for 11 d. Blood samples and biopsies of the gluteal muscles were collected daily for 5 d for α-TP and LA analyses.

Time (d) . . .	0	1	2	3	4	5
Muscle α-TP (ug/g)	0·692	0·754	1·072	1·345*	1·401*	1·413*
SD	0·061	0·069	0·081	0·201	0·067	0·138
Muscle LA (% total fatty acids)	0·8984	0·9252	1·349	1·402*	2·372*	2·233*
SD	0·0587	0·0467	0·2071	0·1482	0·2071	0·4050

* Values significantly different from time 0 value (Student's *t* test for paired data): $P<0·05$.

All calves remained clinically normal. Concentrations of LA and α-TP rose simultaneously in both plasma and muscle. These results indicate that spontaneous NM is unlikely to result from differences in the rates of incorporation of these substances into muscle. Further studies are required to determine if differences in the ratio LA:α-TP in grass are important in the pathogenesis of NM.

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Is mulberry heart disease in pigs the result of uncomplicated vitamin E deficiency? By M. R. NOLAN, S. KENNEDY, W. J. BLANCHFLOWER and D. G. KENNEDY, *Department of Agriculture, Veterinary Sciences Division, Stormont, Belfast BT4 3SD, Northern Ireland*

Mulberry heart disease (MHD) is one of the most common causes of sudden death in weaned, fast-growing pigs. It is characterized by myocardial haemorrhage, necrosis, microthrombosis and fibrinoid degeneration of arterioles, hydropericardium and pulmonary oedema. Many of these lesions have been experimentally induced by feeding diets low in vitamin E and selenium (Grant, 1961; Van Vleet *et al.* 1977*a*,*b*) and MHD has come to be regarded as a vitamin E and Se deficiency disease. However, the pathology of spontaneous MHD is not identical to that of the experimentally-reproduced disease. Several studies have indicated that Se is not aetiologically important in the pathogenesis of MHD (Lindberg *et al.* 1972; Moir & Masters, 1979; Rice & Kennedy, 1989). Furthermore, pigs that have died from spontaneous MHD have lower tissue concentrations of α-tocopherol in spite of apparently adequate dietary α-tocopherol contents (Wallimann *et al.* 1984; Rice & Kennedy, 1989). The aim of this study was to investigate if MHD could be induced in pigs by feeding a diet low in vitamin E, but supplemented with Se.

Eleven 5-week-old pigs were divided into a principal group of six pigs and a control group of five pigs. The principal pigs were fed a basal low vitamin E diet (3·5 μg/g α-tocopherol) while the control group received the basal diet supplemented with 60·8 μg/g α-tocopherol for 32 weeks. The basal diet contained 0·2 μg/g Se. Blood samples were collected from all animals at weekly intervals and analysed for plasma α-tocopherol concentration. Lipid peroxidation was estimated by measuring tissue concentrations of 4-hydroxynonenal.

Plasma α-tocopherol concentrations in the principal group were significantly lower than those in the control group from day 24 onwards. At the end of the experiment (32 weeks) they were: principal 7·0 (SD 0·7) μmol/l and control 1·0 (SD 0·1) μmol/l ($P<0·001$; *t* test). At necropsy, no pathological changes were seen in any animal. Mean heart α-tocopherol concentration in the principal group (1·82 μg/g) was significantly lower than in the control group (12·64 μg/g). It was also lower than the concentration found in pigs with spontaneous MHD (mean 2·63 μg/g) (Rice & Kennedy, 1989). Concentrations of 4-hydroxynonenal were significantly higher ($P<0·002$; *t* test) in the hearts of principal pigs (1·53 (SD 0·14) μg/g) than in control pigs (0·11 (SD 0·02) μg/g).

These results indicate that although vitamin E metabolism is altered in spontaneous MHD (Kennedy & Rice, 1989), the disease is not the result of uncomplicated dietary vitamin E deficiency. Although high dietary supplementation with α-tocopherol has been shown to reduce the incidence of spontaneous MHD, further studies are required to determine other aetiopathogenetic factors and the role of α-tocopherol in this disease.

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In vitro binding of bile acids to dietary fibres. By G. QUINN, A. FLYNN and P. A. MORRISSEY, *Department of Nutrition, University College, Cork, Republic of Ireland*

There is evidence that some sources of dietary fibre alter cholesterol metabolism and reduce serum cholesterol concentrations. Although the mechanism by which dietary fibre exerts its effect on serum cholesterol is not understood, it has been suggested that adsorption of bile acids to dietary fibre leads to increased excretion in faeces, thereby increasing the demand for cholesterol for the synthesis of bile salts to maintain pool sizes (Story & Lord, 1987). In this study the adsorption of bile acids to a number of dietary fibres in vitro was investigated. Cholestyramine, which is known to bind bile acids strongly and reduce serum cholesterol, was included for comparison.

Samples of fibre from pea, apple, wheat and barley and cholestyramine were added to 4 ml of 0·1 M-phosphate buffer, pH 7·0, and allowed to hydrate at 37° for 1 h (all fibre samples were obtained from SOFALIA, Paris, France). One ml of ^{14}C-labelled (1 μCi/ml) bile acid solution (250 μM glycocholate or taurocholate (sodium salts) in 0·1 M-phosphate buffer, pH 7·0) was added to each tube and gently agitated for 2 h at 37°. After centrifugation for 30 min at 2000 *g*, supernatant ^{14}C was measured in a liquid scintillation counter and bile acid binding determined.

The fibres bound both glycocholate and taurocholate in the following order: apple>barley>wheat>>pea. The apple, barley and wheat fibres bound slightly more glycocholate than taurocholate. Cholestyramine bound much more taurocholate and glycocholate than any of the fibres. For all the fibres, the bile acid in the supernatants was totally dialysable through 10 000–12 000 molecular weight (MW) cut-off dialysis membranes, indicating that there was no binding of bile acids to high molecular weight soluble fibre fractions. When the fibre content of the assay was varied from 20–100 mg, bile acid adsorption increased in direct proportion to the fibre content.

Adsorption of glycocholate and taurocholate to fibre preparations in vitro

Fibre preparation . . .	nmol bound/g fibre			
	Glycocholate		Taurocholate	
	Mean	SEM	Mean	SEM
Barley	314	11	215	31
Wheat	300	41	229	13
Apple	351	15	308	16
Pea	83	20	101	27
Cholestyramine	2440	1	2470	1

When 100 mg of apple fibre was incubated with a range of concentrations of glycocholate or taurocholate (25–250 nmol/5 ml incubation), adsorption of both bile acids to the fibre increased linearly with increasing bile acid concentration.

These results show that apple, barley and wheat fibres and to a lesser extent pea fibre, bound both glycocholate and taurocholate. Thus, these fibres may increase faecal bile acid excretion.

Story, J. A. & Lord, S. L. (1987). *Scandinavian Journal of Gastroenterology* **129**, 174–180.

The effect of baked bean consumption on nutrient intake and serum cholesterol levels in human subjects. By JACKI TREDGER, LINDA MORGAN, AVRIL ANDERSON and J. WRIGHT, *School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

We have previously shown (Shutler *et al.* 1989) that daily consumption of a 450 g can of baked beans for 2 weeks caused a significant reduction in mean dietary fat intake and mean serum cholesterol levels in young normocholesterolaemic males. The present study investigated whether smaller quantities of beans were effective in lowering cholesterol levels in normal and hyperlipidaemic subjects.

Seventeen normocholesterolaemic males (38–60 years, total cholesterol 5·8 (SE 2·3) mmol/l) were monitored for 3 weeks and six hyperlipidaemic subjects (35–59 years, cholesterol 7·5 (SE 0·3) mmol/l) were monitored for 4 weeks on their usual diets. They then ate 225 g/d baked beans for 4 weeks. Fasting blood samples were taken at weekly intervals and dietary intakes (3 d diet records) were recorded regularly on both usual and bean-supplemented diets, and for a further 3-week follow-up period when they resumed their usual diets. Bean consumption caused significant increases in sodium intake from 2987 (SE 226) to 4300 (SE 210) mg Na/d in both groups ($P<0·02$), and a significant rise in fibre intake from 25 (SE 2) to 43 (SE 3 g/d in the normocholesterolaemic subjects ($P<0·01$). In normocholesterolaemic subjects bean consumption had no effect on circulating total cholesterol, HDL-cholesterol, triacylglycerols or apolipoproteins A1 and B. In the hypercholesterolaemic subjects cholesterol fell significantly ($P<0·05$) from the point of entry into the trial. This reduction continued throughout the basal and bean-supplemented period, and reached a nadir after 3 weeks of bean consumption. During the follow-up period the mean serum cholesterol rose to a level which was no longer significantly different from that at the point of entry to the study. Mean apolipoprotein B levels showed a similar trend to total cholesterol levels. Mean HDL-cholesterol levels were lower during the follow-up period than the basal period but were unaffected by bean consumption. These data indicate that subjects modified their diet on entry to the study so causing a reduction in mean serum cholesterol which continued throughout the basal period and for 3 weeks of the bean-supplemented period. It is possible that after 7 weeks in the study, subjects' compliance with their modified diet was reduced causing the increase in mean serum cholesterol level seen in the fourth week of bean consumption and during the follow-up period. This was consistent with a significant rise in dietary cholesterol intake ($P<0·05$) observed in these subjects during the follow-up period. It would therefore appear that daily consumption of 225 g baked beans is insufficient to have any significant effect on serum cholesterol levels.

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The effect of rice and wheat bran supplements on plasma lipids in healthy adult male volunteers. By M. A. T. FLYNN, D. D. SUGRUE and M. J. GIBNEY, *Nutriscan Ltd., The O'Reilly Institute, Trinity College Dublin, Dublin 2, Republic of Ireland*

Eighty-five healthy adult men were recruited into the preliminary phase of the study which involved a blood lipid analysis. Of these, sixty men were selected, thirty with the highest levels of blood cholesterol and thirty with the lowest levels of blood cholesterol (6·5 *v.* 5·1 mmol/l). The men in each group were randomly assigned to receive supplements (34 g) of either wheat bran providing approximately 9 g of insoluble dietary fibre, or rice bran providing approximately 12 g of dietary fibre of which 94% was insoluble and 6% soluble, in the form of three biscuits per day. Blood samples were taken at the beginning and end of the 4 week supplementation period. In the group as a whole, no significant effects of bran supplementation on serum lipids were observed. When the high- and low-cholesterol groups were considered separately, there was no significant effect of bran supplementation on blood lipids in the low cholesterol group, while the high cholesterol group showed a significant fall in blood total cholesterol (6·6 *v.* 6·3 mmol/l; $P<0·05$) with the rice bran supplement but not with the wheat bran supplement. No other effects on blood lipids were observed.

Examination of dietary intakes indicated increased fibre intakes in both groups ($P<0·001$) but also found that bran supplementation affected changes in components of the diet that are known to affect blood lipids. These changes of a reduction in percentage energy derived from saturated fat (initial intake 15·7%, final intake 14·6%, $P<0·05$) and a non-significant increase in percentage energy derived from polyunsaturated fat (initial intake 6·9%, final intake 7·4%, NS) did not, however, explain more than 24% of the observed fall in plasma cholesterol (Keys *et al.* 1965). This would seem to indicate a possible cholesterol-lowering potential for rice bran in hypercholesterolaemic men.

	Before supplementation: total plasma cholesterol (mmol/l)		After supplementation: total plasma cholesterol (mmol/l)	
	Mean	SD	Mean	SD
Rice bran group				
Lower initial blood cholesterol (*n* 12)	5·1	0·8	5·3	0·8
Higher initial blood cholesterol (*n* 15)	6·6	1·1	6·3	1·0*
Total rice bran group (*n* 27)	5·9	1·2	5·8	1·0
Wheat bran group				
Lower initial blood cholesterol (*n* 12)	5·1	0·6	5·3	0·9
Higher initial blood cholesterol (*n* 12)	6·3	0·6	6·2	0·8
Total wheat bran group (*n* 24)	5·7	0·9	5·8	1·0

* Significantly lower than presupplementation level: $P<0·05$.

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Indices of body-fat distribution in angiographically-defined coronary artery disease in man. By M. A. T. FLYNN[1], M. B. CODD[2], M. J. GIBNEY[3], E. KEELAN[1] and D. D. SUGRUE[1], *Departments of* [1]*Cardiology* and [2]*Medical Statistics and Epidemiology, Mater Hospital, Dublin 1* and [3]*Department of Clinical Medicine, Trinity College Dublin Medical School, St James' Hospital, Dublin 8, Republic of Ireland*

Anthropometric measurements descriptive of obesity, body-fat distribution and build were made in 186 males undergoing coronary arteriography. Using stepwise multiple logistic regression analysis, two indices of body-fat distribution were independently associated with coronary disease, while the widely-used indices of obesity, namely body mass index and percentage body-fat were not. A significant disease association was found for the waist:thigh circumference ratio (waist:thigh ratio) which was included as the first step in the multivariate model ($P<0{\cdot}005$). The waist:hips circumference ratio (waist:hip ratio), a widely-used body-fat distribution index, was found to have a negative association with disease ($P<0{\cdot}05$). These opposing directions of two significant indices clearly demonstrate how, at this stage, there is a strong need for caution in decisions concerning which of the numerous indices of body-fat distribution are important. None the less, this study does suggest that use of body-fat distribution indices may improve risk factor assessment of cardiac patients.

Risk factors found to be independently associated with significant disease

	Insignificant disease *n* 72*		Significant disease *n* 114*		Stepwise multiple logistic regression
	Mean	SD	Mean	SD	
Waist:thigh ratio	1·8	0·0	1·9	0·1	$P<0{\cdot}005$
Height	172·5	6·0	170·0	6·0	$P<0{\cdot}05$
History of hypertension	*n* 18 (33%)		*n* 59 (47%)		$P<0{\cdot}01$
HDL-cholesterol	1·0	0·3	0·9	0·2	$P<0{\cdot}005$
Waist:hip ratio	1·0	0·05	1·0	0·05	$P<0{\cdot}05$
Duration of cigarette smoking (years)	*n* 59		*n* 100		$P<0{\cdot}05$
	22·8	11·2	27·4	10·4	

* Except where otherwise stated.
SD, standard deviation.

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Regulation of insulin binding to sheep adipocyte membranes. By SARA WASTIE and P. J. BUTTERY, *Department of Applied Biochemistry and Food Science, University of Nottingham, Sutton Bonington LE12 5RD* and R. G. VERNON, *Hannah Research Institute, Ayr KA6 5HL*

Insulin action on sheep adipose tissue metabolism can be modulated in vitro by glucocorticoids such as dexamethasone and growth hormone. Dexamethasone inhibits glucose utilization in the presence and absence of insulin and inhibits lipogenesis at low levels of insulin, but acts synergistically to increase lipogenesis at higher insulin levels (Vernon & Finley, 1988). The presence of growth hormone in the culture medium inhibits the insulin-induced increase in fatty acid synthesis in sheep adipose tissue (Vernon & Finley, 1988). One mechanism by which these hormones may exert their efforts could be through the insulin receptor. This study was designed to investigate the effect of dexamethasone and growth hormone on insulin binding to sheep adipocyte membranes.

Finn–Dorset Horn crossbred wethers (6–9 months old) were fed on a diet of cereal (500 g/d) and hay *ad lib*. Samples of subcutaneous adipose tissue were collected aseptically, and used either immediately for the isolation of adipocyte membranes or maintained in tissue culture for 48 h at 37° as described previously (Vernon & Finley, 1988). The culture medium was supplemented with insulin (I; 100 ng/ml), dexamethasone (D; 10 nM) or growth hormone (GH; 100 ng/ml) as described in the Table. Adipocyte membranes were prepared as described previously (Watt *et al.* 1991) with slight modifications. Adipocyte membranes were incubated for 16–18 h at 4° in the presence of 0·4 ng ^{125}I-insulin (final concentration 1 ng/ml). The assay was terminated by the addition of 1 ml 0·15 M–NaCl and centrifugation. The supernatant was discarded and the radioactivity incorporated in the pellet was determined using a gamma counter. Correction was made for non-specific binding by determining the amount of ^{125}I-insulin bound in the presence of 10 μg insulin.

	^{125}I-insulin bound (pg/mg protein)							
Treatment . . .	None	I	D	GH	I+D	I+GH	D+GH	I+D+GH
Mean	36·8	24·2	53·6	41·3	27·9	22·6	52·8	35·3
(*n* 10) SEM	6·6	5·7	8·2	7·7	4·4	4·2	7·4	6·1

Analysis of variance showed that inclusion of insulin in the culture medium decreased ($P<0·01$) insulin binding to the membranes. This was not unexpected since insulin is known to down-regulate its receptors in other species and cell types. In contrast, culture with dexamethasone increased ($P<0·01$) insulin binding to adipocyte membranes. The mechanism is not certain but in view of previous studies with other cell types it is probable that increased receptor synthesis is involved. The dexamethasone-induced increase in insulin binding was prevented by the inclusion of insulin. Growth hormone in the culture medium had no effect on insulin binding. Thus some effects of dexamethasone on insulin action in sheep adipose tissue may be due to changes in insulin receptor binding. Growth hormone, however, appears to exert its effect at a post-binding level.

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Fatty acid-binding protein in brown adipose tissue of the newborn lamb. By ASIM K. DUTTA-ROY, YIMING HUANG and P. TRAYHURN, *Division of Biochemical Sciences, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The fatty acid-binding proteins (FABPs) are a family of low molecular weight (14 000–15 000 Daltons) cytosolic proteins which have been widely implicated in fatty acid metabolism (Veerkamp *et al.* 1991). These proteins play an important role in the transport of fatty acids into various tissues, as well as in their subsequent esterification, compartmentation and β-oxidation (Veerkamp *et al.* 1991). FABPs have been purified and characterized from several tissues, including the liver, heart and kidney (Veerkamp *et al.* 1991).

FABP has also been identified and characterized in white fat (of rats, pigs and humans), but it has not been reported in the other form of adipose tissue namely brown adipose tissue (BAT). BAT is the major site of non-shivering thermogenesis in mammals, and is especially prominent in the newborn (see Himms-Hagen, 1989). Fatty acids are the primary fuel for thermogenesis, and are also considered to provide an intracellular signal for the acute activation of uncoupling protein (Himms-Hagen, 1989). We have now examined perirenal BAT from newborn lambs for the presence of FABP; large quantities of active BAT occur in the perirenal region of the newborn lamb, making it a potentially valuable source of the tissue for the isolation and characterization of a protein.

Five, full-term male or female lambs (mean wt 3·1 (SE 0·4) kg) were obtained within 12 h of birth. Perirenal BAT was rapidly removed weighed (mean wt 17·8 (SE 3·9 g)), and homogenized in a 20 mM-Tris-HCl buffer (pH 7·4) containing 1 mM-EDTA, 0·1 mM-PMSF and 0·25 mM-sucrose. A cytosolic fraction was prepared by centrifugation at 110 000 *g* for 70 min, and subjected to ammonium sulphate (700 g/l) fractionation. The supernatant left after the addition of ammonium sulphate was then dialysed. The dialysate was concentrated by ultrafiltration (Amicon YM membrane, 3500 mol wt cut-off), and subjected to a combination of size exclusion and ion-exchange chromatography (Dutta-Roy *et al.* 1987). SDS-polyacrylamide gel electrophoresis of the final preparation showed a single band, mol wt ~15 000 Daltons. The purified protein and earlier fractions bound [^{14}C]oleate, with a K_d value of 1·10 (SE 0·15) μM.

These results indicate that perirenal BAT of the newborn lamb contains FABP, and the protein appears to be present at a high level (2–4 times that in rat liver). We suggest that FABP is likely to play an important role in the provision and utilization of fatty acids for thermogenesis in BAT of the newborn animal.

We are most grateful to Dr. John Robinson for providing the newborn lambs.

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The role of succinate in ovine cobalt–vitamin B_{12} deficiency. By P. B. YOUNG, D. G. KENNEDY, W. J. McCAUGHEY and W. J. BLANCHFLOWER, *Department of Agriculture, Veterinary Sciences Division, Stormont, Belfast BT4 3SD, Northern Ireland*

It has been widely accepted that the primary metabolic defect in ovine cobalt–vitamin B_{12} deficiency is impaired propionate metabolism (Marston *et al.* 1961). This occurs as a result of a decrease in the activity of methylmalonyl CoA mutase which participates in the sequence of reactions which metabolize propionate to succinate. If correct, the biochemical sequelae of inhibition of the mutase should include a reduction in gluconeogenesis and succinate availability.

Using a modification of a GLC assay for methylmalonic acid (McMurray *et al.* 1986) we have measured the sequential changes in rumen and plasma concentrations of succinate in sheep fed on a Co-deficient whole barley diet (O'Harte *et al.* 1989). Eight 5-month-old Suffolk cross lambs were randomly allocated to two equal groups and fed *ad lib.* on either a Co-deficient (4·2 μg Co/kg) or a Co-sufficient diet (1 mg Co/kg) for 14 weeks. The mean weekly voluntary food intake of the two groups did not alter during the course of the study, but was 18% lower ($P<0·005$; *t* test) in the Co-deficient animals than in controls.

In the Co-sufficient animals there was no change in either the rumen or plasma concentrations of succinate at any time during the experiments. However, in the animals fed on the Co-deficient diet, the rumen concentrations rose 100-fold within 2 d ($P<0·05$), peaking at a level 1000 times that in the control animals after 6 weeks. In addition, the plasma concentration of succinate in the deficient animals rose significantly from 8 μmol/l to 26 μmol/l after 10 weeks ($P<0·05$). This suggested that the rumen succinate was absorbed. Proof that rumen succinate was absorbed was obtained when we measured a twenty-twofold increase in the portal vein succinate concentration of a Co-deficient sheep 60 min after oral administration of 0·15 mol sodium succinate.

In the normal rumen, succinate produced by, for example, *Bacteroides succinogenes*, is rapidly metabolized to propionate by, for example, *Propionibacterium shermanii*, in reaction sequence which is the exact reverse of that which occurs in tissue. This pathway includes a vitamin B_{12}-dependent methylmalonyl CoA mutase enzyme which may also be affected by the Co deficiency. Using a GLC assay for rumen volatile fatty acids we have demonstrated that the increase in rumen succinate concentration occurs concomitantly with a decrease in rumen propionate. It therefore appears likely that succinate accumulates in the rumen due to a decrease in production of propionate.

The production and absorption of succinate in the rumen of Co-deficient sheep has major implications for the pathogenesis of this disorder. Clearly, the animals do not suffer from impaired succinate availability as would be predicted by the accepted view of Co deficiency. In the light of these findings, and our earlier study which reported decreases in the activities of both vitamin B_{12}-dependent enzymes in ovine Co deficiency (Kennedy *et al.* 1990), we are now examining some of the basic mechanisms which underlie this disorder.

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The effects of cobalt–vitamin B_{12} deficiency on methionine synthase activity and phospholipid methylation in the sheep. By D. G. KENNEDY[1], P. B. YOUNG[1], A. M. MOLLOY[2], J. M. SCOTT[2], D. G. WEIR[3], S. KENNEDY[1] and W. J. BLANCHFLOWER[1], [1]*Department of Agriculture, Veterinary Sciences Division, Stormont, Belfast BT4 3SD,* [2]*Department of Biochemistry* and [3]*Department of Clinical Medicine, Trinity College, Dublin 2, Republic of Ireland*

Methionine synthase (*EC* 2.1.1.13) is one of two mammalian vitamin B_{12}-dependent enzymes. We have previously shown that the activity of this enzyme is significantly reduced in the liver and kidney of moderately vitamin B_{12}-deficient sheep (Kennedy *et al.* 1990). Methionine synthase through S-adenosyl methionine (SAM) and S-adenosyl homocysteine (SAH), is intimately involved in the metabolic supply of methyl groups to a wide variety of acceptor molecules, including the membrane component, phosphatidyl ethanolamine (PE). It is widely accepted that the activity of the SAM-dependent transmethylation enzymes is controlled by the ratio of SAM to SAH. Low SAM:SAH ratios are associated with decreased activity of the transmethylation enzymes.

Two groups of four 5-month-old sheep each were fed a barley-based diet containing either 4·3 ng/g Co (deficient) or 1000 ng/g Co (sufficient) for 28 weeks. Tissue samples obtained at slaughter, were analysed for holo-synthase activity; SAM and SAH; phosphatidyl choline (PC) and PE. The Table shows the results obtained.

	Holo-synthase (units/g)				SAM:SAH				PC:PE			
	+Co		−Co		+Co		−Co		+Co		−Co	
Tissue	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Liver	6·4	(0·7)	0·9	(0·2)	5·4	(1·5)	2·7	(1·3)	1·5	(0·1)	1·1	(0·1)
Kidney	5·6	(0·3)	0·8	(0·1)	1·7	(0·5)	1·2	(0·5)	0·8	(0·1)	0·8	(0·02)
Brain	3·7	(0·2)	3·2	(0·3)	6·5	(1·8)	5·7	(1·0)	1·0	(0·1)	0·7	(0·03)
Cord	7·2	(0·3)	4·2	(0·3)	5·9	(0·9)	6·2	(0·9)	ND		ND	

Significant reductions in holo-methionine synthase activities were observed in liver, kidney and spinal cord, but not in brain. Despite the significant decreases in kidney and cord methionine synthase activity, a significant change in the SAM:SAH ratio was observed only in liver. The ratio of PC to PE was decreased in liver and unchanged in kidney, in line with the observed changes in the SAM:SAH ratio in these tissues. However, there were significant changes in the PC:PE ratio in the brain of vitamin B_{12}-deficient animals, despite the fact that this tissue showed changes in neither methionine synthase activity nor the SAM:SAH ratio. We propose that PC synthesis in ovine brain is heavily dependent on active transport of choline across the blood–brain barrier.

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The use of rat intestinal epithelial cells to study the growth-promoting properties of porcine, bovine and human milk. By G. P. SHERRY, *Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA, Northern Ireland* and J. M. FLETCHER, *Unilever Research, Colworth House, Sharnbrook, Bedford MK44 1LQ*

The presence of hormone-like growth factors, capable of stimulating cell replication in vitro and in vivo has been demonstrated in the mammary secretions of several species (Koldovsky, 1989). Growth-promoting activity is generally reported to be high in colostrum, and to fall as normal milk production proceeds. In the present study the ability of porcine, bovine and mid–late lactation human milk to promote in vitro cell replication has been studied. In contrast to most previous studies, this study used rat intestinal epithelial cells (RIE-1); an untransformed, non-tumorigenic, non-established cell line (Blay & Brown, 1984).

RIE-1 cells of passage 19 or less were grown to confluence in microtitre plates (7×10^3/well) using Dulbecco's modification of Eagle's medium (DME) containing fetal calf serum (FCS) (10% v/v). Quiescence was induced by replacing the medium with DME containing 0·5% (v/v) FCS. Fat and cellular materials were removed from milk samples by centrifugation. They were then diluted in phosphate buffered saline (PBS) and added to the plates (166 μl/well) 24 h before harvesting.

Proliferation was estimated by the incorporation of [^{3}H]thymidine added (1 μCi/well) either 16 or 20 h after milk addition.

Results are expressed as stimulation index (S.I.), that is, [^{3}H]thymidine incorporation induced by milk samples (cpm) relative to control PBS (cpm).

FCS added at 10% (v/v) gave a maximum S.I. of 9·3 and 14·7 after 4 and 8 h of labelling respectively. Pooled sow's milk (n 2) collected at 21 d of lactation, gave a maximum S.I. of 4·8 and 28·3 after 4 and 8 h of labelling respectively, at a dilution of 10% (v/v). Pooled cow's milk (n 6) collected in mid–late lactation gave a maximum S.I. of 7·2 after 8 h of labelling, at a dilution of 2·5% (v/v). A sample of human milk, collected at 49 d of lactation gave a maximum S.I. of 12·5 after 8 h of labelling, at a dilution of 10%. Hydrolysis of sow's milk protein with trypsin, or denaturation by heat treatment or acid precipitation completely abolished any stimulatory activity.

Use of an epithelial cell line has shown that contrary to previous reports (Shing & Klagsbrum, 1984; Cera *et al.* 1987), the growth-stimulatory activity of mammary secretions persists well after the colostral phase.

The magnitude of the stimulatory effect is dependent on the time of the label addition before cell harvesting. Abrupt withdrawal of sow's milk from the piglet weaned at 21 d of age may contribute to the decline in small intestinal mucosal growth and function observed in piglets weaned at this age.

RIE-1 cells were a generous gift from K. Brown of the Institute of Animal Physiology, Cambridge.

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Effects of variety and harvesting method on the energy value of mature peas. By R. M. WEIGHTMAN[1,2], J. C. MATHERS[1] and S. J. WILCOCKSON[2], *Departments of* [1]*Agricultural Biochemistry and Nutrition* and [2]*Agriculture, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU*

Dry peas contain appreciable quantities of non-starch polysaccharides, oligosaccharides and slowly digestible starch, which are substrates for fermentation in the large intestine. Variation in the concentration of these carbohydrate fractions may influence the digestible energy (DE) and metabolizable energy (ME) concentrations. This study was designed to measure DE and ME concentrations in peas grown in 1989. The peas comprised three varieties; Solara (S), Princess (P) and Bohatyr (B) each harvested by one of three methods; hand harvested at the desiccant application stage (HH), combine harvested after desiccant application (D) or after reaching full maturity (C).

For a 16 d balance period, sixty male Wistar rats were fed 15 g/d of ten semi-purified diets. Fifteen rats were fed a basal diet (based on starch and casein), and forty-five rats were fed nine test diets containing a 1:1 w/w mixture of the basal diet with milled peas. Gross energies of diets, peas and faeces were determined by adiabatic bomb calorimetry. Urinary energy output was calculated from urinary nitrogen excreted using the factor 22·55 kJ/g N and that portion from peas in the test diets assumed to be 0·5 of total output.

DE concentrations (MJ/kg dry matter) were calculated by two methods. Method 1 was the classical procedure, assuming no associative effects between the basal and pea components of the diets. Method 2 was based on Livesey (1989) which does not require knowledge of the GE concentrations of the test diets, but only of the peas.

	Variety			Treatment			SEM
	S	P	B	HH	D	C	(*n* 15)
Method 1:							
DE	16·0	15·9	15·8	16·1	15·8	15·8	0·06
ME	15·7	15·5	15·5	15·7	15·6	15·5	0·06
Method 2:							
DE	16·0	16·0	15·9	16·1	15·9	15·9	0·06
ME	15·7	15·7	15·6	15·8	15·6	15·6	0·06

Estimates of ME were, on average, 0·99 DE with no between treatment differences in urinary N output and hence estimated urinary energy loss. Both methods of calculation provided similar estimates of DE concentration but between treatment differences tended to be greater with Method 1 which detected a significantly lower value for conventional pea variety Bohatyr, than for semi-leafless varieties Princess and Solara. DE content of hand-harvested peas was consistently greater than that for combine-harvested peas.

These results suggest that the energy value of dry peas differs albeit slightly between varieties and may be affected by the harvesting strategy adopted.

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Conventional factorial calculation underestimates the metabolizable energy value of peas.

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Conventional calculation of the metabolizable energy (ME) content of foods is based upon the chemical determination of available carbohydrate (i.e. carbohydrate assumed to be digested in the small intestine) plus protein and lipid, and the use of energy factors for each of the components (Paul & Southgate, 1978). Energy potentially available from non-starch polysaccharides (NSP) and oligosaccharides of the raffinose family (OS) are discounted despite the growing evidence that these carbohydrates can be extensively fermented in the large bowel (LB). While discounting may be relatively unimportant for mixed diets relatively low in NSP (Southgate & Durnin, 1970) it would seriously underestimate the ME value of individual foods or food components, rich in fermented carbohydrates. A recent in vivo determination, using rats, of the ME content of peas (Weightman *et al.* 1991) produced estimates which were considerably greater than those given by Paul & Southgate (1978). The present study attempted to determine whether the conventional calculation of ME is reliable for peas which are rich in NSP and OS.

The total starch, protein and lipid contents of the peas (g/kg dry matter (DM)) used by Weightman *et al.* (1991) were determined by conventional methods, and glucose, sucrose and OS by HPLC. NSP was assumed to be organic matter not accounted for by these components. ME (MJ/kg DM) was calculated as described by Paul & Southgate (1978). ME made available by LB fermentation of NSP and OS was calculated assuming digestibilities of 0·79 and 1·00 respectively (Goodlad & Mathers, 1990) and 9·4 MJ absorbed energy/kg fermented carbohydrate (Mathers, 1991).

	Variety			Treatment			SEM*
Component	S	P	B	HH	D	C	(*n* 12)
Starch	559	518	493	488	520	562	16·7
Glucose + sucrose	31·8	46·0	32·8	35·7	36·3	38·6	1·22
Ether extract	14·3	15·3	13·9	15·1	15·0	13·4	3·04
Crude protein	183	190	186	182	189	190	4·95
Calculated ME (MJ)	14·1	14·1	13·2	12·7	13·9	14·6	0·03
Modified calculated ME (MJ)	15·2	15·2	14·8	14·6	15·2	15·4	0·12

* Four field replicates for each treatment.

S, Solara; P, Princess; B, Bohatyr; HH, Combine harvested; D, desiccant application; C, full maturity.

Conventional calculation of ME from chemical analysis was, on average, 13·8 MJ/kg DM, very similar to Paul & Southgate's (1978) value but only 0·88 of the in vivo determined value. Including the estimates of ME made available by LB fermentation removed much of the bias in calculated ME values when compared with the in vivo value, resulting in means of 15·1 and 15·6 MJ/kg DM respectively.

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Hepatic drug metabolism in chronically-diabetic streptozotocin-treated rats. By C. R. BARNETT and P. R. FLATT, *Diabetes Research Group, Department of Biological and Biomedical Sciences, University of Ulster, Coleraine BT52 1SA, Northern Ireland* and C. IOANNIDES, *Molecular Toxicology Research Group, Division of Toxicology, School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

Insulin-dependent diabetes mellitus influences the metabolism of xenobiotics by modifying their rates of oxidation. The major xenobiotic metabolizing system is the cytochrome P-450-dependent mixed-function oxidase system which comprises a number of different families with differing substrate specificities. Streptozotocin-induced diabetes mellitus has been shown to increase the levels of hepatic cytochrome P-450 proteins belonging to the P-450 I, II, III and IV families (Barnett *et al.* 1990*a*,*b*). Almost all animal studies were confined to the short-term effects of the disease, and the long-term effects of chemically-induced diabetes have been overlooked. In the present study, thirty male Wistar albino rats were divided into six groups of five animals. Three groups were rendered diabetic by the administration of a single intraperitoneal injection of streptozotocin (65 mg/kg dissolved in 0·1 M-citrate buffer, pH 4·5) while the remaining three groups received buffer alone and served as controls. At 4, 8 and 12 weeks, one group of diabetic and one group of control animals were killed, microsomal extracts prepared and mixed-function oxidase activities determined. At 4 weeks the diabetic animals displayed increased pentoxy- and ethoxyresorufin *o*-dealkylase, *p*-nitrophenol and lauric acid hydroxylase and ethylmorphine *N*-demethylase activities. However, by 8 weeks the extent of induction of *p*-nitrophenol and lauric acid hydroxylase activities were decreased, whereas the level of ethylmorphine *N*-demethylase activity was similar to that of the control animals. The activity of diabetic microsomes towards ethoxy- and pentoxyresorufin was not significantly altered from that observed at 4 weeks. By 12 weeks the elevation of *p*-nitrophenol hydroxylase, lauric acid hydroxylase and ethylmorphine *N*-demethylase were not significantly different from control levels, although the activity towards ethoxy- and pentoxyresorufin was still as significant as that observed at 4 weeks. Determination of the plasma glucose and ketone body concentrations revealed that all diabetic animals were hyperglycaemic throughout the study, however, the severity of ketosis decreased with the duration of the disease. In recent studies, animals that were rendered hyperketonaemic by manipulation of the diet but remained normoglycaemic, displayed enhanced mixed-function oxidase activities (Barnett *et al.* 1988) and increased levels of the same cytochrome P-450 proteins as observed in acute streptozotocin-induced diabetes, implicating ketosis in the diabetes-induced changes.

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The influence of metabolic events on the regulation of energy balance in cystic fibrosis. By A. O'RAWE and J. A. DODGE, *Department of Child Health, The Queen's University of Belfast, Belfast BT12 6BJ* and J. THOMSON and A. O. B. REDMOND, *The Royal Belfast Hospital for Sick Children, Belfast BT12 6BE, Northern Ireland*

The achievement and maintenance of energy balance in cystic fibrosis (CF) is central to clinical management and promotes longevity. The documented elevation in energy requirement in CF is a multifactorial trait and is subject to wider variability.

Using indirect calorimetry we measured resting metabolic rate (RMR) and respiratory quotient (RQ) 12 h post-prandial, in fifty CF patients and thirty age-matched healthy controls aged 5–28 years. There was no significant difference in body mass percentile (BMP) for the two groups, values were 97% and 103% respectively. CF patients were identified by genotype group, (ΔF508/ΔF508, homozygotes for common CF mutation; ΔF508/N, heterozygotes for common mutation and N/N, other uncharacterized mutations).

Group	Resting metabolic rate (% predicted)		Respiratory quotient	
ΔF508/ΔF508	121*	(116–126)	0·88**	(0·87–0·89)
ΔF508/N	109	(103–116)	0·86	(0·85–0·87)
N/N	104	(97–111)	0·86	(0·85–0·87)
Control	101	(98–104)	0·80	(0·79–0·82)

Values are means (95% confidence interval).
Significantly different from control values: *$P<0·005$, **$P<0·001$.

Group comparisons were made using one way analysis of variance. In addition to a significant contribution from genotype to RMR, there was a highly significant difference in RQ between patients and controls. That this was not a diet-induced response was confirmed by calculating food quotients (FQ) on thirty subjects from 7-d weighed intake data. The average FQ was 0·87 and there was no correlation betwen RQ and FQ on regression analysis. An increase in RQ suggesting altered substrate utilization appears to be a metabolic consequence of CF, irrespective of genotype. Specific CF genotypes are therefore at greater risk of nutritional compromise with high RMRs combined with a potential RQ/FQ imbalance. This has implications for strategies for nutritional supplementation.

A randomized study of starter enteral feeding regimens in a mixed intensive care unit. By C. A. McCABE[1], G. G. LAVERY[2] and B. J. ROWLANDS[1], [1]*Department of Surgery, Queen's University of Belfast, Belfast BT12 6BJ* and [2]*Regional Intensive Care Unit, Royal Victoria Hospital, Belfast BT12 6BA, Northern Ireland*

The widespread use of starter regimens for patients receiving enteral nutrition is based on the hypothesis that the sudden introduction of high osmolar loads will overwhelm the absorptive capacity of the small intestine causing diarrhoea (Silk, 1987). Patients with normal gastrointestinal function are not, however, influenced by the osmolality of enteral feeds (Keohane *et al.* 1984).

In critically-ill patients the delivery of, and nutritional efficacy of, enteral nutrition can be difficult to achieve due to the high incidence of gastrointestinal intolerance.

This study was designed to assess the use of starter regimens in intensive care patients. Twenty-two patients in a mixed intensive care unit were prospectively randomized to receive either a full regimen (group 1) or starter regimen (group 2). (Seven female; fifteen male; age 17–66 years (median 41); weight 60–83 kg (median 69) (group 1); 64–159 kg (median 82) (group 2).) Full strength regimen is the total requirement given via a full strength, full volume isoenergetic feed. Starter regimen is the requirement met over 3–4 d via feed dilution and gradual volume initiation. All feeds were delivered by continuous pump infusion. Energy requirements were assessed using a metabolic cart (Senseromedics 2900Z) in ten patients and the Harris Benedict formula, plus appropriate stress factor, was used in the remaining twelve patients. Requirements were calculated to be 7·98 (SD 0·94) MJ (1918 (SD 227) kcal) in group 1 and 8·92 (SD 2·20) MJ (2145 (SD 529) kcal in group 2.

Efficacy of feed was determined by daily nitrogen balance in twenty-one patients calculated by subtracting N output (+2 g for insensible losses) from N intake. Total urinary N was determined by pyrochemiluminescence (Antek analyser). This is the most accurate determinant of N output for critically-ill patients (Dechert *et al.* 1990). Daily negative N balance for group 1 was 2·52 (SE 0·69) g and for group 2 balance was 5·03 (SE 0·69) g. Daily N balance was significantly better in the group receiving full strength regimen ($P<0·024$). Tolerance to enteral feeds was assessed by the level of gastric residuals and the incidence of diarrhoea. No differences were detected between the groups.

This study shows the institution of enteral nutritional support at full strength and volume to intensive care patients, results in greater energy intake, less negative N balance and no greater incidence of gastrointestinal side effects. The practice of using traditional starter regimens for intensive care unit patients should be abandoned.

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Application of a newly developed diet questionnaire for use in health education. By M. G. O'DONNELL, *Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA, Northern Ireland*, M. NELSON, *Department of Food and Nutritional Sciences, King's College, London W8 7AH* and P. H. WISE, *Department of Endocrinology, Charing Cross Hospital, London W6 8RF*

A new previously-validated questionnaire-based system for dietary health education was applied in a sample of healthy subjects (age range 25–64 years) who were allocated to one of three groups for health education (O'Donnell *et al.* 1991).

Group C: Control group, no education.

Group L: Leaflet group, received a booklet pack containing practical suggestions for desired dietary changes.

Group F: Feedback group, received the new form of advice, i.e. personalized feedback messages generated from analysis of their questionnaire by the 'DIET' computer program, along with the booklet pack.

Questionnaire (QUEST A) and fasting blood samples were used to establish baseline dietary intakes and biochemical variables. Body-weight and serum fat fractions were the objective measures of change used in the study. Three months after dietary advice, subjects were assessed again by questionnaire (QUEST B), and blood sample.

Sixty-nine per cent of subjects responded (forty-eight males, sixty-one females). One way analysis of variance of the differences between QUEST A and QUEST B showed that there were no statistically significant differences between the nutrients for the three groups for all the nutrients with the exception of % energy from total sugars. Examination of intakes after dietary advice within the feedback group revealed significant change in the desired direction in the reported intakes of protein ($P<0{\cdot}02$), total sugars ($P<0{\cdot}01$), P:S ratio ($P<0{\cdot}02$), alcohol ($P<0{\cdot}04$), zinc ($P<0{\cdot}02$), vitamin B_{12} ($P<0{\cdot}05$) and vitamin D ($P<0{\cdot}02$). There were no significant differences in intake for total fat or dietary fibre.

Analysis of the biochemical data showed no statistically significant differences in biochemical variables or body-weight between the three groups or within the feedback group.

This study coincided with the publication of data showing considerable under reporting in energy intakes from weighed diet surveys (Livingstone *et al.* 1990). The questionnare in the present study was validated against 16 d of weighed record. Doubts about weighed dietary data led to re-examination of energy intakes (EI) obtained from the questionnaire and weighed records in the validation study. These were expressed as a multiple of each subject's predicted basal metabolic rate (BMR). The 1985 WHO report on energy requirements has estimated the requirement of an adult sedentary lifestyle as $1{\cdot}55 \times$ BMR (FAO/WHO, 1985). Seventy-five per cent of subjects had weighed record energy intakes less than $1{\cdot}55 \times$ BMR, while 94% of subjects reported questionnaire energy intakes less than $1{\cdot}55 \times$ BMR.

Thus there are far-reaching implications for the future planning of studies relating to personalized dietary feedback.

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Health attitudes and dietary behaviour in Northern Ireland. By M. E. BARKER, S. I. MCCLEAN and K. A. THOMPSON, *Centre for Health and Social Research, University of Ulster, Coleraine BT52 1SA, Northern Ireland*

The Health Attitude Inventory (HAI), a subject-administered questionnaire developed at the MRC Epidemiology Unit, South Wales, was used to measure the health attitudes of a representative sample of 592 subjects aged 16–64 years from Northern Ireland. The HAI utilizes a psychological model (Fishbein & Ajzen, 1975) in which behaviour is construed as a function of attitudes, which are in turn a product of beliefs and values. In the HAI, attitudes towards fat and fibre are measured through beliefs and values towards fried food and dairy produce, and wholemeal bread and vegetables, respectively. A high attitudinal score for fat indicates a perception of fat as health threatening, whilst the respective score for fibre indicates a perception of fibre as health promoting. Dietary behaviour was assessed by 7 d weighed records (Barker *et al.* 1989). Statistical analysis was carried out using Pearson correlation coefficients and analysis of variance.

Attitudinal scores for fat and fibre were greater in women than men. The sex difference was statistically significant ($P<0{\cdot}001$) for fibre. Fat score showed significant ($P<0{\cdot}05$) positive associations with marital status in men; married men tended to have greater scores. Age was also positively associated with fibre score in men ($P<0{\cdot}05$) and women ($P<0{\cdot}001$). Non-smoking women tended to have greater fibre scores ($P<0{\cdot}05$), as did women from non-manual socio-economic groups ($P<0{\cdot}05$).

There was a significant (r $-0{\cdot}14$, $P<0{\cdot}01$) inverse association between fat score and fat intake in women. Fat score was significantly ($P<0{\cdot}05$) inversely correlated with mean chip consumption in men (r $-0{\cdot}13$) and women (r $-0{\cdot}12$). Fat score was positively correlated with milk consumption in men (r $+0{\cdot}13$, $P<0{\cdot}05$). Mean yoghurt intake was positively (r $+0{\cdot}38$, $P<0{\cdot}001$ and r $+0{\cdot}48$, $P<0{\cdot}001$) related to fat score in men and women respectively. Butter consumption was negatively correlated with fat score in men (r $-0{\cdot}04$, NS) and women (r $-0{\cdot}22$, $P<0{\cdot}001$).

There were significant ($P<0{\cdot}001$) positive associations between fibre score and fibre intake in men (r $+0{\cdot}23$) and women (r $+0{\cdot}29$). Fibre score was positively associated with mean vegetable intake (excluding potatoes) in men (r $+0{\cdot}26$, $P<0{\cdot}001$) and women (r $+0{\cdot}36$, $P<0{\cdot}001$). Consumption of potatoes was also positively ($P<0{\cdot}001$) correlated with fibre score (r $+0{\cdot}39$, r $+0{\cdot}40$) in men and women respectively. Wholemeal bread intake was positively associated with fibre score in men (r $+0{\cdot}29$, $P<0{\cdot}001$) and women (r $+0{\cdot}37$, $P<0{\cdot}001$).

Whilst these correlations between attitude scores and dietary behaviour were small, it is evident that dietary behaviour is influenced by the interaction of sociocultural and diet/health attitudinal variables.

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Dietary inadequecies in a free-living aged group in Dublin. By B. MCLAUGHLIN, M. BRADLEY and J. KEVANY, *Department of Community Health, Trinity College, Dublin 2* and M. CANTON and B. CONNOLLY, *Teagasc Research Centre, Fermoy, Co. Cork, Republic of Ireland*

The percentage of the population over the age of 65 is steadily increasing with a current figure of 15% and a projected figure of 20% by the year 2030. Nutrition problems in this age group can arise as a result of physical and social problems. Reduced movement from stroke or arthritis can limit exposure to sunlight and may even result in a loss of autonomy in respect of feeding. Drug–nutrient interactions can occur especially with anticonvulsants (Grahame-Smith & Aronson, 1984). Absence of teeth or poorly fitting dentures have been associated with reduced intake of macro- and micronutrients (Morley *et al.* 1988). Reduced finances can limit the purchasing of foods.

The study population consisted of forty subjects, three males and thirty-seven females, over the age of 65 years and living in the inner Dublin area. Each had dietary intake assessed by three separate 24 h recalls carried out in the subject's own home. This included for each subject, 1 weekend day and 2 midweek days. Information on medications, limitation of movement and chewing problems were also noted.

The results were as follows (mean (SD)): energy 6·13 (1·41) MJ, 76% of US recommended daily allowance (RDA); vitamin C 47 (22) mg, 77% of RDA; vitamin D 1·3 (1·1) μg, 25% of RDA; folic acid 118 (36) μg, 65% of RDA; zinc 7·9 (3·0) mg, 64% of RDA. No subject showed any clinical manifestations of deficiency. Although this methodology necessarily has limitations, these findings may suggest: (a) that RDA levels are set too high for certain nutrients in the over 65 age group; or (b) subclinical malnutrition can exist in the over 65 age group (Garry *et al.* 1982). If the latter is true then the current policy of setting up at risk registers for the elderly may help to isolate these problems and facilitate intervention at an early stage.

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The effects of including or excluding sucrose in a weight reduction programme. By MARY MCCREERY, *The Blackrock Clinic, Co. Dublin, Republic of Ireland*

The effects of the composition of diets used in weight-reducing diets have been extensively studied under strict metabolic conditions. Few studies have examined dietary advice to ambulatory subjects other than that of Baron *et al.* (1986), who found low carbohydrate diets to be more effective than low fat, high fibre diets in weight reduction programmes. Most such programmes exclude sugar given that it is a source of energy which can be discarded through the use of sugar-substitutes with zero energy value and because sugar does not provide any of the micronutrients. The present study set out to examine the necessity for such advice.

Forty-four healthy overweight volunteers were recruited from a fitness club and consecutively assigned to either a standard (4·2 MJ, 1000 kcal) diet or the same diet with an additional allowance of 0·84 MJ, (200 kcal) as either sucrose or sucrose-containing sweets or soft drinks. Forty subjects completed the weight reduction programme, with data gathered at the start of the programme and at 2 and 10 weeks thereafter. The results are summarized in the Table for initial and final values.

Sex . . .	Male				Female			
Sucrose . . .	Included		Excluded		Included		Excluded	
No . . .	*n* 10		*n* 9		*n* 10		*n* 11	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body-wt (kg):								
Initial	92·1	8·9	98·0	11·5	79·4	10·0	76·2	8·8
10 weeks	87·0	7·9	94·4	11·4	74·7	10·2	70·7	8·8
Body mass index (kg/m^2):								
Initial	29·1	1·9	30·3	3·8	28·9	2·5	27·2	2·7
10 weeks	27·6	1·5	29·1	3·8	27·2	2·5	25·4	3·0

The decline in body-weight and body mass index was highly significant ($P<0·01$) for all groups. Analysis of variance showed no significant effect of sex or dietary treatment on the 10-week weight loss.

These data show that advice for the complete exclusion of sucrose from balanced energy deficit diets is not necessary for successful weight reduction. Because compliance with the dietary advice given cannot be independently verified, this and other comparable studies, can evaluate only the efficacy of advice and not efficacy of the treatment.

This work was carried out under contract to the Irish Sugar Company.

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Assessment of energy expenditure and patterns of physical activity in healthy children, aged 7–15 years. By M. B. E. LIVINGSTONE[1], P. S. W. DAVIES[2], A. M. PRENTICE[2], W. A. COWARD[2], J. J. STRAIN[1], C. N. STEWART[1] and C. A. MAHONEY[3], [1]*Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA, Northern Ireland,* [2]*Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL* and [3]*Physical Education Centre, Queen's University, Belfast BT12 6BJ, Northern Ireland*

It has recently been suggested that children from the UK seldom engage in physical activity of high enough intensity to promote cardiovascular health (Armstrong *et al.* 1990).

In this study total energy expenditure (TEE) was measured for thirty-six (nineteen male, seventeen female) free-living healthy children aged 7–15 years, by the doubly-labelled water method. Daytime patterns of physical activity were assessed simultaneously for 2–3 d by heart-rate (HR) monitoring. Standardized measurements of basal metabolic rate (BMR) were made by indirect calorimetry. Peak aerobic power (PV_{O_2}) was assessed on a motorized treadmill using a graded exercise protocol. A FLEX HR which defined the lower limits of physical activity was identified. The number of minutes spent at or above individual HRs equivalent to 50% PV_{O_2} and 70% PV_{O_2} were calculated as approximating to the lower levels of moderate and vigorous activity respectively.

Sex (*n*) . . .	Younger children (7–9 years)				Older children (12–15 years)			
	Boys (11)		Girls (9)		Boys (8)		Girls (8)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Weight (kg)	27·6	7·9	27·9	6·0	46·4	7·4	49·0	9·5
TEE (MJ/d)	8·79	1·76	7·52	0·84	10·8	0·75	9·77	1·71
BMR (MJ/d)	4·74	0·69	4·39	0·62	6·45	0·69	5·59	0·82
TEE:BMR	1·86	0·27	1·73	0·17	1·69	0·19	1·67	0·11
TPA (h/d)	8·9	1·1	6·5	1·8	6·1	1·6	3·3	1·1
(% daytime)	74·3	11·2	55·4	20·3	51·1	7·0	27·3	17·5
MVPA (h/d)	1·5	0·6	0·7	0·3	0·9	0·4	0·3	0·2
(% daytime)	12·7	4·6	5·4	2·1	7·2	2·8	2·1	1·3
VPA (h/d)	0·6	0·3	0·2	0·1	0·5	0·3	0·1	0·1
(% daytime)	4·8	2·1	1·3	0·2	3·8	2·5	1·1	0·7

TEE, total energy expenditure; BMR, basal metabolic rate; TPA, total physical activity (time above FLEX HR); MVPA, moderate and vigorous physical activity; VPA, vigorous physical activity.

Data were analysed by *t* tests. Boys spent approximately one-third more time in total physical activity (TPA) (time above FLEX HR) than girls (7·7 (SD 1·8) *v.* 5·3 (SD 2·0) h/d, $P<0·01$). An inverse relationship was observed between age, duration and intensity of physical activity. Younger children spent significantly more time in TPA (7·9 (SD 2·4) h/d) and moderate and vigorous physical activity (MVPA) (1·1 (SD 0·6) h/d) than older children (TPA, 4·7 (SD 2·4) h/d, $P<0·001$; MVPA, 0·6 (SD 0·4) h/d, $P<0·001$). Within each age category boys had significantly greater MVPA and vigorous physical activity (VPA) than girls.

The lower levels of physical activity by girls and the decline in activity with age in both sexes particularly of MVPA are of potential concern in relation to long-term cardiovascular health.

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