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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Four Hundred and Sixtieth Meeting of the Nutrition Society was held in the Barnes Lecture Theatre, Royal Society of Medicine, 1 Wimpole Street, London on Tuesday, 6 December 1988, when the following papers were read:

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Who drops out of slimming groups? By JOYCE HUGHES* and CATHERINE GEISSLER, *Department of Food and Nutritional Sciences, King's College, Campden Hill Road, London W8 7AH* and ELIZABETH EVANS, *Slimming Magazine Clubs, 9 Kendrick Mews, London SW7 3HG*

Ashwell (1977) reported high drop-out rates as a feature of commercial weight loss groups. The present study was undertaken to elucidate reasons why members starting with at least 19 kg excess weight dropped out of such groups before achieving their desirable weight-for-height. The progress of 224 (♂ 13, ♀ 211) such members (study group) joining eight slimming groups throughout the country in an 8-week period was followed for 3 months. Information on sex, age, height, weight and weekly weight loss was abstracted from relevant group records. Of the sample 45% (*n* 102; ♂ 5, ♀ 97) had dropped out (drop-outs) within the study period (average attendance 4·7 (SD 3·9) weeks).

For comparison the same information was collected on a further group of seventy-five members (♂ 2, ♀ 73) who had successfully lost all their excess weight (completers) having started in the same excess weight range.

	Study group (*n* 224)		Drop-outs (*n* 102)		Completers (*n* 75)	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	38·4	12·0	39·2	11·7	38·0	11·5
Starting excess wt (kg)	32·1	11·9	31·4	11·1	25·3***	7·4
Starting body mass index (kg/m^2)	33·8	4·6	33·6	4·4	31·2***	2·7
Period attending group (weeks)	—	—	4·7	3·9	42·4***	20·0
Rate of wt loss (kg/week)	—	—	0·6	0·8	0·7	0·3

Significantly different from drop-outs: ***$P<0·001$.

There was no significant difference in age between the three groups. The starting excess weight and body mass index were significantly higher in drop-outs than completers, suggesting that the heavier members are more likely to drop-out. Mean rate of weight loss between the drop-outs and the completers was not significantly different; however, the range of rate of weight change within the drop-outs was much greater than in the completers (drop-outs +1·80 to −4·30, completers −0·27 to −1·46 kg/week).

To determine other easily identifiable characteristics to differentiate between potentially successful slimmers and drop-outs, questionnaires seeking additional information on behavioural and other approaches to slimming were sent to all drop-outs and completers. Responses from thirty-two drop-outs (RDO) and thirty-two completers (RC) were obtained.

Drop-outs reported more frequent dieting before joining this group (RDO 62·5%, RC 50·0% dieted >3 times) and less previous success in losing all their excess weight (RDO 18·8%, RC 22·6%). Less diet compliance in terms of less food weighing and more modification of suggested menus was found in drop-outs and they reported less integration of the diet into the family's eating habits, which suggests that they were less motivated to follow the diet and did not see the diet as a long-term eating pattern.

Fewer drop-outs found group support helpful (RDO 44%, RC 84%) and some even found it unhelpful (RDO 16%, RC 0%). This may be due to the fact that they did not attend the group sufficiently long to benefit from this support.

Ashwell, M. (1977). *Recent Advances in Obesity Research*, 11th ed., pp. 266–277 [G. Bray, editor]. London: Newman.

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How comparable are the results of a food frequency questionnaire to a 24 h diet record?

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The food frequency questionnaire (FFQ) has many advantages over other methods of dietary assessment in epidemiological studies of groups of individuals. Here we compare the nutrient intake scores derived from an FFQ with intakes derived from a 24 h diet record.

Subjects were chosen from men and women who had completed a 24 h diet record in a larger study of diet in three English towns (Cade *et al.* 1988). The FFQ was developed from the three towns study diet records. The sixty-five food items included provided up to 90% of the intake of proximate nutrients, fibre, calcium and vitamins A and C. Subjects were also asked about changes in food intake over the last 3 years. Nutrient intake scores were calculated from the FFQ and the distributions of each score were compared with the distribution of intake from the diet record. Six hundred and seventy-nine subjects were sent the FFQ and a reply-paid return envelope. Four hundred and thirty-eight questionnaires were returned completed.

Nutrient	Spearman *r**	Nutrient	Spearman *r**
Energy	0·36	Vitamin A	0·15†
Protein	0·22	β-Carotene	0·22
Fat	0·36	Retinol	0·14††
Carbohydrate	0·30	Vitamin C	0·24
Total fibre	0·26	Calcium	0·21

*Two tailed test, all P values <0·001 except for †P= 0·002 and ††P= 0·004.

The Table shows the Spearman rank order correlations between the nutrient intakes assessed by the two methods. All associations were statistically significant. Associations were weakest for vitamin A. The data have been modelled to calculate the distribution of correlations using different estimates of changes over time, within-person variation and measurement errors. These estimates were compared with the correlations found in our study. The same quintile allocation between the two methods varied between 23% for vitamin A and 27% for energy intake. Only between 1 and 5% of subjects were grossly misclassified. Taking account of changes in dietary intake or serving sizes of milk, bread and potatoes made little difference to the correlations. Generally associations between the methods were strongest among younger women and older men and for those people from Stoke-on-Trent.

The FFQ was able to categorize individuals by nutrient intakes in a similar way to a 24 h diet record.

Cade, J. E., Barker, D. J. P., Margetts, B. M. & Morris, J. A. (1988). *British Medical Journal* **296**, 1359–1362.

An improved method for measuring whole body fat and protein changes. By P. R. MURGATROYD and W. A. COWARD, *Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL*

Measurements of body density by underwater weighing, and total body water (TBW) by deuterium dilution provide the most analytically precise estimates of whole body fat. The accuracy of fat estimates is, however, limited by the assumptions of constant lean tissue density for the density method and constant lean body hydration for the TBW method (Siri, 1961). When body-weight is gained or lost the composition of lean tissue changes along with any change in fat mass (Kinney *et al.* 1968). The assumptions on which the density and TBW methods depend are therefore invalid and can lead to substantial systematic errors in fat change estimates. Using the data of Kinney *et al.* (1968), Burkinshaw (1985) has shown, for example, that a true fat loss of 0·60 kg would be interpreted by densitometry as a loss of 1·82 kg and by TBW as a gain of 0·21 kg.

If changes in skeletal minerals can be assumed to be negligible during a period of weight change, three variable components (protein, water and fat) remain and three measurements (weight, density and TBW) can be combined to derive an expression for the accurate estimation of fat change (Δf) without further assumptions. A full derivation of the expression yields:

$$\Delta f\ (kg) = 2{\cdot}741\ \Delta V - 0{\cdot}7148\ \Delta TBW - 2{\cdot}046\ \Delta Wt$$

where ΔV is the change in body volume (litres), ΔTBW is the change in TBW (kg) and ΔWt is the change in body-weight (kg). The constants are computed from the traditional density values for fat (0·900), protein (1·340) and water (0·993). ΔV is calculated as:

$$\Delta V = \text{Initial (weight/density)} - \text{Final (weight/density)}.$$

An expression for protein change (Δp) can be derived by substituting $\Delta f = \Delta Wt - \Delta TBW - \Delta p$ in the expression for Δf. Thus:

$$\Delta p\ (kg) = 3{\cdot}046\ \Delta Wt - 0{\cdot}2852\ \Delta TBW - 2{\cdot}741\ \Delta V$$

The precision of the method is dependent only on the quality of the weight, density and TBW estimates. Taking the precision of weight measurement as 0·01 kg, density measurement as 0·0023 kg/l and TBW measurement as 0·8 kg, analysis of error propagation shows that the precisions of fat and protein change estimates are 1·02 kg and 0·70 kg respectively.

The appropriate initial assumptions enable this method to represent the composition changes in Burkinshaw's (1985) example correctly. The method ensures that composition changes can be measured without the bias inherent in methods based on density or TBW alone.

Burkinshaw, L. (1985). *Progress in Medical Radiation Physics* **2**, 113–137.
Kinney, J. M., Long, C. L., Gump, F. E. & Duke, J. H. (1968). *Annals of Surgery* **168**, 459–474.
Siri, W. E. (1961). In *Techniques for Measuring Body Composition*, pp. 223–244 [J. Brozcek and A. Heuschel, editors]. Washington DC: National Academy of Sciences/National Research Council.

Body mass index in a sample of Filipino adults in relation to broad occupational categories. By V. A. CORPUS, *College of Human Ecology, University of the Philippines at Los Banos, Philippines* and E. HOINVILLE and E. A. DOWLER, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT*

In 1987, a survey of 1053 households in the central and southern municipalities of the island province of Palawan was undertaken as part of a project to produce a training programme on nutritional issues for senior agricultural planners. One objective of this survey was to investigate the use of anthropometric measurements on adults and children to measure the impact of the first phase (1980–86) of a large, integrated area development project. The data on children are reported elsewhere (Corpus *et al.* 1987). We report here the data on adults.

The survey was household based, equal numbers being randomly selected within barangays (villages) over a wide range of ecological and socio-economic conditions. Wherever possible, weight, height and age measurements were obtained on all household members, as well as information on household social, demographic and production characteristics. Co-operation was good, and ages known reasonably accurately. Males (*n* 790) and females (*n* 908) aged 18 years and over were measured. Their mean body mass index (weight/height^2; BMI) was 21·1 (SD 2·7) and 20·9 (SD 3·2) kg/m^2 respectively. There were no significant differences between municipalities; that is, no geographical variation. Men aged 61 years and over had a significantly lower BMI than those aged between 18 and 30 years, and between 31 and 60 years (ANOVA, $P<0·05$).

Main income source of household	Men *n*	BMI (kg/m^2) Mean	SD	Main income source of household	Women *n*	BMI (kg/m^2) Mean	SD
Commercial fishing	22	22·5	2·7	Commercial fishing	16	25·8	6·2†
Regular wages	99	22·0*	3·2	Irrigated farming	67	21·5	3·1
Big businessmen	43	21·8	3·1	Big businessmen	63	22·0	4·1
Irrigated farming	58	21·5	3·4	Regular wages	143	21·6*	3·8
Casual labour	70	21·3	2·6	Subsistence fishing	89	21·3	3·3
Subsistence fishing	87	21·3	2·5	Rainfed farming	140	21·3	3·7
Petty traders	44	21·1	2·5	Casual labour	91	21·3	4·3
Rainfed farming	119	21·0	2·4	Petty traders	67	20·2	2·8
Rainfed upland-farming/ shifting cultivation	232	20·2*	2·2	Rainfed upland-farming/ shifting cultivation	229	19·9*	3·1

†This group was excluded from the statistical analysis.
Significant difference between groups (ANOVA): *$P<0·05$.

Data on sixteen men and thirty-three women were excluded. The remainder (all ages) were ranked according to the main production base of the household. Numbers in some of the groups thus obtained were small; nonetheless there was a clear trend in the data on men and women. Significant differences were found between adult BMI of members of households where the income base was a regular wage, and those where livelihood was gained from agriculture whose basis is somewhat precarious (rainfed upland farmers and those practising shifting cultivation). This ranking in both men and women paralleled that obtained using anthropometric indicators of child nutritional status (Corpus *et al.* 1987). The evidence suggests adult BMI measurements can contribute to the derivation of indicators for monitoring the impact of development projects.

The survey was supported by the Food and Agriculture Organization of the United Nations. E.A.D. is partly funded by the Overseas Development Administration.

Corpus, V. A., Eusebio, J. S., Bongga, D. & Mendoza-Sumalde, Z. (1987). *Nutrition Resurvey of Selected Areas Covered by the Palawan Integrated Area.* Development project report to the Food and Agriculture Organization (unpublished).

Physique and basal metabolic rate of Gurkha soldiers serving in Britain. By S. J. ULIJASZEK, *Department of Biological Anthropology, University of Cambridge, Downing Street, Cambridge CB2 3DZ* and S. S. STRICKLAND, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT*

Proposals for the estimation of energy requirements assume that basal metabolic rate (BMR) can be reliably predicted from linear regression equations of BMR on weight, in different age groups and populations (Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU), 1985). These equations have been shown to overpredict BMR in Indian subjects at a low plane of nutrition (McNeill *et al.* 1987*a*), but not in well-nourished Indians living in Britain (Henry *et al.* 1987). In the present study, BMR was measured in seventeen well-nourished, ethnically homogeneous Gurkha soldiers stationed in Britain, and in seventeen British European controls of similar occupational background, matched by body-weight (<±1·0 kg). Individuals were between the ages of 18 and 30 years, with one exception, a Gurkha aged 33 years. BMR was measured after an 11 h fast, between 04.30 and 06.30 hours, using an Oxylog portable oxygen consumption meter modified for low flow rates (McNeill *et al.* 1987*b*). Anthropometry was carried out using methods described in Weiner & Lourie (1981).

	Gurkha (*n* 17)		British (*n* 17)		Paired
	Mean	SD	Mean	SD	*t* test
Age (years)	25·1	3·0	22·9	3·1	
Wt (kg)	67·1	4·6	66·8	4·6	
Ht (m)	1·668	0·038	1·731	0·063	**
BMI (kg/m²)	24·1	1·4	22·3	1·7	***
Σ4SF (mm)	38·3	10·8	31·8	8·0	*
BMR (MJ/d)	7·05	0·93	7·29	1·25	
BMR (kJ/kg per d)	105·1	12·2	109·8	21·4	

BMI, body mass index; Σ4SF, sum of four skinfold thicknesses: biceps + triceps + subscapular + suprailiac.

*P<0·05, **P<0·01, ***P<0·001.

Gurkhas were shorter (t=3·75, P<0·01), with greater body mass index (t=4·01, P<0·001) and sum of four skinfold thicknesses (t=2·13, P<0·05) than their matched controls. However, there was no difference in BMR between the two groups. Observed BMR values were 1·2 (SD 11·7)% lower and 2·8 (SD 18·7)% greater than those predicted from body-weight using the FAO/WHO/UNU (1985) regression equation for adult males aged 18–30 years, for the Gurkhas and British respectively.

The results of this study support the claim that ethnic differences probably have no effect on BMR in well-nourished populations. For a larger sample of Gurkhas (n 20), BMR (kJ/kg per d) was positively correlated with the number of months since their leaving Nepal (log–log plot, r 0·495, P<0·05).

Food and Agriculture Organization/World Health Organization/United Nations University (1985). *Technical Report Series* no. 724. Geneva: WHO.

Henry, C. J. K., Piggott, S. & Emery, B. (1987). *Human Nutrition: Clinical Nutrition* **41C**, 397–402.

McNeill, G., Cox, M. D. & Rivers, J. P. W. (1987*a*). *American Journal of Clinical Nutrition* **45**, 1415–1419.

McNeill, G., Rivers, J. P. W., Payne, P. R., Britto, J. J. de & Abel, R. (1987*b*). *Human Nutrition: Clinical Nutrition* **41C**, 473–483.

Weiner, J. S. & Lourie, J. A. (1981). *Practical Human Biology*. Academic Press: London.

Interactions between the intensity of exercise and the residual effect on metabolic rate. By GAIL R. GOLDBERG, P. R. MURGATROYD, H. L. DAVIES and A. M. PRENTICE, *Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

The putative phenomenon of excess post-exercise oxygen consumption (EPOC) has been the subject of a number of recent studies which have generated conflicting data (for example, Bielinski *et al.* 1985; Maehlum *et al.* 1986; Pacy *et al.* 1987). In particular, the potential usefulness of a residual exercise-induced thermogenesis as an adjunct to slimming regimens remains controversial. The existence of a threshold above which EPOC becomes significant may explain the discrepant results achieved so far. This was tested in the present study using graded levels of moderate exercise.

Ten healthy adults (six male, four female) were each studied by 36 h indirect whole-body calorimetry on five separate occasions. EPOC was assessed during sleep (23.30–08.00 hours) and during measurement of basal metabolic rate (08.00–09.00 hours) following a day containing four × 30 min periods of bicycle ergometry at the following workloads: men A = 4 × 0, B = 4 × 25, C = 4 × 50, D = 4 × 75, E = 4 × 100 Watts; women A = 4 × 0, B = 4 × 25, C = 2 × 25 plus 2 × 50, D = 4 × 50, E = 2 × 50 plus 2 × 75 Watts. Order of testing was randomized. The highest exercise level was equivalent to 62·2 (SD 6·8)% and 62·7 (SD 2·2)% maximum O_2 consumption ($\dot{V}_{O_2max}$) for the men and women respectively. On the day of exercise, subjects were given a normal diet and maintained close to energy balance (mean difference 0·19 (SD 0·45) MJ/d).

Exercise level	*n*	Sleep (% of mean) Mean	Sleep (% of mean) SD	Basal metabolism (% of mean) Mean	Basal metabolism (% of mean) SD
A	10	97·6	4·0	98·6	3·5
B	10	99·0	1·4	99·4	2·9
C	10	99·8	3·3	99·4	3·7
D	10	100·8*	2·0	100·1	2·8
E	10	103·4**	3·2	102·5*	2·2

Significantly different from exercise level A (paired *t* test): **P*<0·05, ***P*<0·01.

The Table lists the average energy expenditures after normalizing the data by expressing each subject's individual values as a percentage of their mean across all exercise levels. A dose-response relation between exercise intensity and EPOC was apparent at all levels. Analysis of variance confirmed significant linear trends for both sleep ($F_{1,38}$ = 13·03, P<0·001) and basal metabolic rate ($F_{1,38}$ = 5·26, P<0·05).

We conclude that EPOC is induced even at low levels of exercise intensity. Failure of some previous studies to detect it can be ascribed to inadequate study design and imprecision in the metabolic measurements. The magnitude of the effect is small, amounting to only 5·8% during sleep and 3·9% in the morning. This represents an average increase in this group of subjects of 235 (range 185–307) kJ/d following a day containing 2 h exercise at over 60% $\dot{V}_{O_2max}$.

Bielinski, R., Schutz, Y. & Jequier, E. (1985). *American Journal of Clinical Nutrition* **42**, 69–82.

Maehlum, S., Grandmontagne, M., Newsholme, E. A. & Sejersted, O. M. (1986). *Metabolism* **35**, 425–429.

Pacy, P. J., Webster, J. D., Isaacs, G., Hunter, S. & Garrow, J. S. (1987). *Proceedings of the Nutrition Society* **46**, 4A.

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Preschool children: an investigation of β-cell status, nutrient intake and growth. By SHELAGH M. HAMPTON, JANE B. MORGAN, LORNA E. SHANNON, SUSAN J. MCCLUSKEY and V. MARKS, *Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH*

Studies in adults have shown that serum C-peptide levels correlate with body mass index (Hampton, 1983). Measurement of C-peptide in urine and serum has been useful in the evaluation of several clinical states, e.g. diabetes mellitus, insulinomas and factitious hypoglycaemia (Marks, 1981). The aim of the present preliminary study was to examine urinary C-peptide levels in preschool children and investigate whether a relation exists between these levels and growth rate. In addition, detailed nutritional information was collected concurrently.

Early morning and 24 h urine collections were obtained on ten preschool children (mean age 28 (SD 14) months). Three children were using nappies overnight and four children were in nappies all the time during the study. A method was devised and validated for recovering urine from nappies. Body-weight, height and skinfold thickness (four sites) were measured. Information was obtained from welfare clinic records of the children's growth rates in the first year of life. During the period of urinary collection a 7 d weighed inventory record was made by the child's mother.

C-peptide levels in urine samples were measured using an established radioimmunoassay (Hampton, 1983). Early morning and 24 h C-peptide levels were correlated with growth and dietary factors (see Table).

	24 h C-peptide level (nmol/l per 24 h)		Early morning C-peptide level (nmol/l per sample)	
	r	*P*	*r*	*P*
Age (months)	0·725	<0·05	0·476	NS
Wt (kg)	0·501	NS	0·563	NS
Dietary intake:				
Energy (kJ/d)	0·206	NS	0·566	NS
Total carbohydrate (g/d)	0·131	NS	0·567	NS
Sugar (g/d)	0·326	NS	0·758	<0·05
Protein (g/d)	–0·058	NS	0·051	NS
Poskitt's Index* (%)	0·089	NS	0·668	<0·05
Growth velocity:				
(g/d over first 3 months of life)	–0·349	NS	0·745	<0·05
(kg/year)	0·420	NS	0·286	NS

NS, not significant.

*(Actual weight of child (kg)/50th centile weight at age when height on 50th centile) × 100 (Poskitt & Cole, 1978).

Early morning C-peptide level was a more sensitive indicator of basal β-cell activity than a 24 h urine sample since a 12 h fast had occurred in most of the children before the collection. No correlation was found except with age in the 24 h urine sample. Significant positive correlations were found between early morning C-peptide concentrations, sugar intake, Poskitt's Index and growth velocity.

The results of the study indicate a relation between sugar intake, growth velocity and β-cell activity at this young age; further studies with larger groups will need to be conducted to understand further the implications of these results.

Hampton, S. M. (1983). The C-peptide of proinsulin: its diagnostic use and a possible physiological role. PhD Thesis, University of Surrey.

Marks, V. (1981). *Hypoglycaemia*. London: Blackwell Scientific Publications.

Poskitt, E. M. E. & Cole, T. J. (1978). *British Medical Journal* **1**, 603–605.

Circulating and secretory antibodies to specific food proteins in adults. By JANE B. MORGAN, SHELAGH M. HAMPTON, MARY R. SMITH, RACHEL MORRIS, JULIE LOVEGROVE and V. MARKS, *Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH*

The aim of the present study was to investigate the relation between specific antibody titres and three food proteins, gliadin, ovalbumin and β-lactoglobulin in adults.

Twenty adult subjects (sixteen female, four male), aged between 20 and 64 years, were recruited into the study. A 7 d weighed food inventory record was undertaken by each subject and on the 1st day of that week a 10 ml fasting blood sample was taken. In addition the subjects were directed to provide a 5 ml saliva sample. Subjects completed a questionnaire regarding their history of food intolerance and family history of hypersensitivity.

One subject reported a history of food intolerance, and a further seven subjects reported various types of hypersensitivity. Specific IgG in serum and IgA in saliva to gliadin, ovalbumin and β-lactoglobulin were measured by the ELISA technique. Total IgG was measured by the nephelometric technique. The Table gives the results for antibody levels in saliva and serum against each food protein.

Antibody level	Gliadin			Ovalbumin			β-Lactoglobulin		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Serum IgG (μg/ml)	288	438	5–1444	645	716	0–2240	244	295	40–1160
Saliva IgA (percentage of standard)	29	39	0–146	56	122	0–364	—	—	—

Mean (SD) protein intake (g/d) for wheat protein was 15 (4), for egg protein 3 (3) and for milk protein 20 (8) in the twenty subjects studied.

Anti-ovalbumin IgG in serum was detectable in eighteen samples. Sixteen of twenty subjects had detectable levels of anti-ovalbumin immunoglobulin A in their saliva. All twenty subjects had detectable serum anti-gliadin IgG titres, and nineteen subjects had detectable levels of salivary gliadin IgA. Anti-β-lactoglobulin IgG in serum was detectable in all twenty subjects. Mean (SD) total serum IgG was 14·2 (4·3) mg/ml.

A significant correlation was observed between anti-ovalbumin IgG in serum and daily dietary intake of egg protein (r 0·46, $P<0·05$). No other significant correlations were observed. No relation was found between circulating levels of specific IgG and salivary IgA to any of the individual food proteins.

The factors that determine the levels of circulating and secretory antibodies to food proteins are complex. From this study it is apparent that protein intake does not have a primary role in determining levels of specific antibodies at this stage in life. We suggest that the results from this study demonstrate that saliva cannot be used as a non-invasive alternative to serum in the detection of specific food protein antibodies in adults.

The authors thank the Clinical Biochemistry Department at St Luke's Hospital, Guildford, Surrey for technical assistance. This study was funded in part by Cow and Gate/Nutrica Research and by the Nutritional Consultative Panel.

Patterns of food and nutrient intake in the chronically unemployed consuming high and low levels of table sugar. By M. J. GIBNEY and PAULINE LEE, *Division of Nutritional Sciences, Department of Clinical Medicine, Trinity College Medical School, St James's Hospital, Dublin 8, Irish Republic*

When nutrient intakes are expressed as a percentage of energy, individuals whose fat intake is low also have high intakes of sugar (Gibney *et al.* 1987). Whilst low intakes of fat are considered desirable, high intakes of sugar are frequently criticized on the grounds that high intakes of table sugar dilute micronutrient intake. This possibility was examined as part of a study of fifty randomly selected families living in a suburb of Dublin where 70% of households do not have an earned income. Forty-two adult men (mean age 34·2 (SD 9·6) years) and fifty-five adult women (mean age 32·6 (SD 11·1) years) were interviewed to establish diet history of a typical week in the prevailing month.

	Men		Women		Men		Women	
					(Quartile of table sugar intake)			
	Mean	SD	Mean	SD	Lower	Upper	Lower	Upper
(*n*) . . .	(42)		(55)		(10)	(10)	(15)	(12)
Intake:								
Energy (MJ/d)	13·1	3·0	8·5	2·8	11·7	15·0	7·2	11·6
Protein (g/d)	100	25	64	18	91	100	64	69
Fat (g/d)	128	33	81	24	127	123	81	83
Carbohydrate (g/d)	409	128	275	131	312	552	191	460
Starch (g/d)	200	62	122	48	198	190	120	105
Fibre (g/d)	21·5	6·4	13·9	5·0	19·6	21·1	13·8	14·1
Iron (mg/d)	13·5	3·2	8·1	2·3	13·1	12·8	7·5	8·7
Zinc (mg/d)	12·9	3·6	8·3	2·5	12·2	12·6	8·4	8·6
Vitamin C (mg/d)	60	21	45	18	57	60	43	49
Retinol (μg/d)	1507	1299	1070	1032	1484	1339	1050	1317
Table sugar (g/d)	95	99	73	100	0	242	0	231
Body mass index (kg/m^2)	24·5	3·6	23·2	4·3	25·8	23·9	23·1	23·5

Mean energy intakes for both sexes agree well with previous studies of Irish subjects (Gibney *et al.* 1987). The intakes of fibre, iron and vitamin C were, however, considerably less than has been previously recorded. Fat provided on average 37·5% of energy intake and total non-lactose sugar provided 18·7% of energy for men and 21·3% of energy for women. When the subjects were divided into upper and lower quartiles of table sugar intake, there was, with the exception of energy and carbohydrate intakes, no difference in nutrient intakes between high and low table sugar consumers. Indeed, almost all of the differential in energy intakes between the upper and lower quartiles of table sugar intake (men 3·3 MJ, women 4·4 MJ) can be explained by the differential in table sugar energy (3·9 and 3·7 MJ).

Thus high table sugar intakes do not appear to lead to lower intakes of micronutrients. Table sugar appears to supplement rather than dilute energy intakes.

This study was funded by the Combat Poverty Agency.

Gibney, M. J., Moloney, M. & Shelly, E. (1987). *Proceedings of the Nutrition Society* **46**, 14A.

Effect of 48 h starvation on the physiological response to food ingestion in normal weight women. By I. W. GALLEN, I. A. MACDONALD and P. I. MANSELL, *Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH*

We recently reported that underfeeding for 7 d was associated with a greater rise in blood glucose concentration but had very little effect on other responses to the ingestion of a test meal (Mansell & Macdonald, 1988). The aim of the present study was to examine the effects of acute starvation on the responses to a similar meal.

Eight normal weight, healthy women (age 18–46 years), taking no other medication except the contraceptive pill, volunteered to take part in the study which was approved by the Medical School Ethical Committee. Each subject was studied on two occasions (both in the follicular phase of the menstrual cycle), resting supine in a temperature-controlled room (30°) and wearing shorts and a t-shirt only. On one occasion, the subjects had fasted for 6 h (control) whilst on the other occasion they had starved for 48 h but consumed water *ad lib.* and 80 mmol sodium/d. For 30 min before and 80 min after ingestion of the test meal (30 kJ/kg body-weight; Fortisip Plus, Cow and Gate) metabolic rate (MR), respiratory exchange ratio (RER), forearm blood flow (FBF), and arterialized venous (using air at 55°) and deep forearm venous blood oxygen and glucose levels were measured.

Baseline variables before and after 48 h starvation

State		MR (kJ/min)	RER	FBF (ml/min per l)	Arterialized blood glucose (mmol/l)	Forearm uptake of Glucose (μmol/min per l)	Forearm uptake of O_2 (ml/min per l)
Control	Mean	3·82	0·77	53	4·38	13·7	2·6
	SEM	0·09	0·01	4	0·13	3·7	0·3
Starved	Mean	4·06	0·74*	94**	3·37*	1·3*	2·4
	SEM	0·11	0·01	14	0·11	1·1	0·3

*$P<0·05$, **$P<0·01$.

The effects of starvation on baseline measurements are shown in the Table. For the first 40 min after ingestion of the test meal, MR rose to similar levels in the control (4·24 (SEM 0·08) kJ/min) and starved (4·30 (SEM 0·12) kJ/min) states. However, in the subsequent 40 min, MR rose further in the control (to 4·36 (SEM 0·05) kJ/min) but did not change in the starved state (4·30 (SEM 0·12) kJ/min, $P<0·01$ compared with control). Arterialized venous glucose rose more in starvation ($P<0·05$) but the absolute levels were similar to those in the control state. After the test meal, the increases in forearm uptake of glucose and of O_2 were similar on the two occasions, but whereas FBF increased in the control, it did not change in the starved state.

Thus, 48 h starvation has similar effects to 7 d underfeeding on the blood glucose response to a test meal. Whereas underfeeding has little effect on other responses to the meal, starvation is associated with a reduction in some of the responses.

This study was supported by a Project Grant from the Wellcome Trust.

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Effect of food intake on orthostatic tolerance and finger tremor in healthy young subjects.

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The maintenance of blood pressure (BP) during standing (orthostatic tolerance) involves changes in heart rate (HR) and vascular resistance which are mediated, in part, through activation of the sympathetic nervous system, which also increases finger tremor. We recently reported that food ingestion reduced supine BP in healthy elderly women but did not significantly change finger tremor or orthostatic tolerance (Birmingham *et al.* 1988). We report here the effects of food ingestion in young subjects.

Twelve healthy subjects (age 20–22 years, six female) were studied (with Ethical Committee approval) in a temperature-controlled laboratory (25°) after an overnight fast on two occasions. Subjects rested supine on a tilt table (with a foot plate) for 30 min before HR, BP and finger tremor (right hand) were measured. The table was then moved to 25°, 45° and 65° head-up tilt, each angle being maintained for 5 min, before the subjects finally stood unsupported for 9 min. Measurements of HR, BP and tremor were made at each angle and when upright. Subjects then consumed a standard breakfast (2·4 MJ; 55% of energy as carbohydrate) on one occasion, or remained fasted on the other occasion (in balanced, random order), and remained seated for 30 min before the measurement procedures were repeated.

During tilting to 65° before food ingestion, systolic BP fell by a mean of 4 mm Hg ($P<0·05$, ANOVA), diastolic BP increased by 20 mm Hg ($P<0·001$) and HR increased by 25 beats/min. Moving to the upright position had no further effect. After food ingestion, supine systolic BP rose slightly (2 mm Hg), diastolic BP fell by 12 mm Hg ($P<0·01$) and HR rose by 7 beats/min ($P<0·01$), but the BP and HR responses to tilt were similar to those before food. By contrast when no food was consumed supine BP was unaltered, but HR was 5 beats/min lower than during the first measurements on that day ($P<0·01$). Rest tremor was unaffected by tilting but was increased after food ingestion ($P<0·01$).

Food ingestion has similar effects on diastolic BP and HR in these young subjects to those seen previously in the elderly (Birmingham *et al.* 1988). The young did display slightly different changes in systolic BP and tremor, but these are of little functional importance.

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Blood antioxidants, cholesterol and colorectal cancer in Ireland: preliminary results. By KATHRYN O'SULLIVAN and P. M. MATHIAS, *Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8* and A. TOBIN and C. O'MORAIN, *Adelaide and Meath Hospitals, Dublin, Irish Republic*

A number of recent studies have suggested a relation between the intake and biochemical status of cholesterol and blood antioxidants, such as selenium, vitamin A, vitamin C and vitamin E, and the prevalence of several forms of cancer (Mannes *et al.* 1985; Salonen *et al.* 1985; Watson & Leonard, 1986; Jacobsen & Thelle, 1987; Kromhout, 1987). To date no similar studies have been carried out in Ireland. Here colorectal cancer is the single most common invasive cancer, accounting for one in seven of all cancers reported. Adenomatous polyps (AP) of the large bowel are well-established precursor lesions for most cases of colorectal cancer. They occur in 20–50% of the population over 50 years of age, and 5% become frankly malignant within 7–15 years. The aim of the present study was to investigate the relation between the above nutrients and colorectal cancer, with a particular interest in identifying the nutritional profiles of patients with the precursor lesion, AP.

Male and female patients undergoing full colonoscopy in the Meath and Adelaide Hospitals were divided into three groups depending on the results of the colonoscopy: those with normal clear colons (controls), those with adenomatous polyps (polyp group), those with colonic neoplasms (cancer group). The groups were age matched, in their 5th–8th decade of life. Fasting blood samples were collected from each patient. The Table shows the results of analyses to date for total cholesterol, retinol, α-tocopherol and Se in plasma.

		Cholesterol (mmol/l)		Retinol (μmol/l)		α-Tocopherol (μmol/l)		Se (μmol/l)	
Group	*n*	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	66	5·6**	1·4	1·52	0·85	15·2†	6·1	0·78*	0·36
Polyp	48	5·8**	1·3	1·38	0·65	11·5	7·7	0·69	0·22
Cancer	35	4·6	1·7	1·21	0·76	11·3	7·0	0·65	0·27

Significantly different (Student's *t* test) from cancer group: **P*<0·025, ***P*<0·005.
Significantly different (Student's *t* test) from polyp and cancer group: †*P*<0·005.

These preliminary results show a lower mean cholesterol level in colon cancer, in agreement with the work of Jacobsen & Thelle (1987). Similarly, mean values for plasma α-tocopherol and Se were lower in the cancer group, as found in other studies (Willet *et al.* 1983; Salonen *et al.* 1985), although for α-tocopherol this may have been related to the significantly higher cholesterol in the controls. Of particular note is that the mean α-tocopherol level in the polyp group was significantly lower than that of the controls, with the two groups having similar cholesterol values.

The final number in each of these study groups will be 100. Further analyses will include other blood antioxidants such as plasma ceruloplasmin and plasma β-carotene, and the enzymes glutathione peroxidase and superoxide dismutase, as well as comprehensive information on dietary intake from each of the patients.

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No effect of β-carotene on the incidence of bladder cancer in *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine treated rats. By M. S. PEDRICK, J. A. TURTON* and R. M. HICKS†, *Bland-Sutton Institute of Pathology, Middlesex Hospital Medical School, London W1P 7PN*

Matthews-Roth (1985) reported thirty-three epidemiological studies of which twenty-nine showed a reduced cancer incidence with increased dietary carotenoid intake (chiefly β-carotene, BC). Information was also given on six prophylactic clinical trials of BC currently in progress, including one study with 22 000 US physicians (Hennekens & Eberlein, 1985). However, experimental evidence supporting the anti-cancer activity of BC is small (Peto *et al.* 1981). Previously we showed no effect on the response of mice to *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine (BBN) when BC was given after carcinogen treatment (Hicks *et al.* 1984). We now report the activity of dietary BC pretreatment on BBN-induced bladder cancer in vitamin-A-deficient and vitamin-A-normal rats.

Weanling female F344 rats (Harlan Olac; *n* 285) were randomized into four groups (Table). Group B received a basic vitamin-A-deficient diet (SDS) with added vitamin A (Pedrick *et al.* 1987). Group A received the group B diet until day 46 when 3 mmol BC (Roche)/kg were added. Groups C and D were given the basic diet to day 46 at which time plasma retinol was about 10% of control values. Group C was then given 3 mmol BC/kg; group D received a low level of vitamin A palmitate (VAP, Roche) in the drinking water to maintain a healthy but vitamin-A-deficient condition (Pedrick *et al.* 1988). Groups A–D were each divided into two at day 102; groups A–D were dosed with 635 mg BBN/rat in 5 weekly portions and groups E–H received BBN vehicle. Rats were killed 42 weeks later. Bladders were weighed and total tumour volume calculated before histological examination. Cancer incidence was analysed using the Fisher exact test.

Group	Diet	Dietary supplement from day 46	No. of rats	Histology of urothelium (no. of rats (%))		
				Normal	Hyperplasia	Carcinoma
A	Basic + vitamin A	BC	26	0	16(61)	10(39)
B	Basic + vitamin A	—	26	0	16(61)	10(39)
C	Basic	BC	28	0	20(71)	8(29)
D	Basic	VAP	33	0	16(48)	17(52)

BC was absorbed, colouring body fat yellow–orange and liver orange–red. Plasma BC (mg/l) at 42 weeks was: group A 2·8 (SD 1·47); group C 1·69 (SD 1·65). There were no differences in relative bladder weight or tumour volume in groups A–D. Histological findings (Table) showed no statistical evidence that dietary BC reduced the number of carcinomas in BBN-treated F344 rats. This confirms our previous findings with BC in BBN-treated B6D2F1 mice. The results also show that pretreatment with dietary BC does not prevent carcinogenesis after subsequent exposure to carcinogen.

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The effect of increasing concentrations of dietary β-aminopropionitrile on collagen cross-linkage in streptozotocin-diabetic rats. By SANA M. JANAKAT, P. C. BATES, D. J. MILLWARD, Z. A. YAHYA and J. P. W. RIVERS, *Nutrition Research Unit, London School of Hygiene and Tropical Medicine, 4 St Pancras Way, London NW1 2PE*

Collagen solubility is decreased in both human and animal diabetics, with evidence for increased intra- and intermolecular cross-linkage in type 1 collagen (Chang *et al.* 1980) and these changes are thought to contribute to several diabetic complications. β-Aminopropionitrile (BAPN) ingestion causes bone and connective tissue softening due to its inhibitory effect on lysyl oxidase and consequent collagen cross-linkage formation (Millar *et al.* 1965). It is possible, therefore, that treatment with BAPN could inhibit the increases in collagen solubility and prevent the related side-effects of diabetes. We report here preliminary studies of the effect of increasing concentrations of dietary BAPN on collagen cross-linkage in normal and diabetic rats, designed to determine the extent of the changes in collagen solubility in the diabetic rat, and to identify a concentration of BAPN which will bring the solubility of collagen to a normal level with least side-effects.

Sprague–Dawley male rats (*n* 48) average weight 120 g, were divided into eight groups. Diabetes was induced in four groups by streptozotocin (65 mg/kg body-weight, intravenously). Both control and diabetic groups were given a purified diet based on casein (200 g/kg) to which BAPN was added at concentrations of 0, 1, 2 and 4 g/kg. Collagen solubility was investigated in granulation tissue collagen deposited during a 13 d period on polyester fabric implanted subcutaneously in the hind-limbs of all rats. Measurements were made of food intake and body-weights over the 13 d period, after which time rats were killed, organs weighed, blood samples taken to measure glucose, and the polyester fabric implants excised and weighed. Collagen solubility was calculated from the proportion of hydroxyproline in the supernatent fraction and pellet from a 0·5 M-acetic acid extract.

Collagen solubility (%)

BAPN (g/kg diet) . . .	0		1		2		4	
Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	48·1	3·3	60·1	3·0	70·7	6·1	77·1	1·8
Diabetic	32·9	3·4	36·2	5·9	62·4	3·0	65·2	3·8

Collagen solubility was reduced from 48 to 33% in the diabetic rats. Administration of BAPN did not affect food intake or body-weight, but did affect collagen solubility. As indicated in the Table, a concentration between 1 and 2 g/kg would appear to reduce solubility of collagen from diabetic animals to normal levels. BAPN at 1·5 g/kg is being investigated in long-term experiments examining whether the diabetes-related pathophysiology resulting from increased collagen solubility can be inhibited without inducing any BAPN-related side effects.

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The influence of heat on the antimicrobial effect of fermented Ghanaian maize dough. By PATIENCE MENSAH, *Noguchi Memorial Institute for Medical Research, Legon, Ghana* and A. TOMKINS, B. DRASAR and T. J. HARRISON, *Clinical Nutrition Unit, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT*

The use of fermentation in the preparation of starch-rich foods such as maize, millet and sorghum is traditional among many African communities. This results in the production of a range of metabolites and a decrease in pH. Although the advantages of fermentation as a means of preventing food spoilage are recognized for dairy products such as yoghurt, the potential value of cereal fermentation has received little attention. In a previous study (Mensah *et al.* 1988) we showed a striking inhibition of growth of a limited range of strains of *Shigella flexneri* following inoculation into fermented maize dough. The present study examines the survival of *S. flexneri* following inoculation into unfermented, freshly prepared maize dough and fermented maize dough, before and after cooking.

Fermented porridge was prepared in the traditional way (Mensah *et al.* 1988) by soaking dry maize (pH 6·2) in water. A pH of 3·6 was achieved by 72 h. Strains of *S. flexneri* identified from patients with dysentery were inoculated onto nutrient agar and, after overnight growth, were suspended in phosphate-buffered saline in order to give approximately 10^8 colony-forming units. The bacterial suspension, (1 ml) was mixed into each of the porridge samples which were then kept at +20°. At 4, 8, 24 and 48 h after inoculation, portions of porridge were diluted and cultured on MacConkey, xylose lysine deoxycholate and nutrient agars for the quantitative detection of *S. flexneri*.

Proportion of samples of maize porridge with detectable S. flexneri

Period after inoculation (h)	Unfermented maize dough		Fermented maize dough	
	Cooked	Uncooked	Cooked	Uncooked
4	20/20	20/20	17/20	13/20
8	20/20	20/20	16/20	13/20
24	20/20	18/20	16/20	12/20
48	20/20	18/20	16/20	3/20

The results indicate that there is an inhibitory effect on the survival of a high proportion of strains of *S. flexneri* but this effect is reduced by cooking. The molecular basis of this response and the difference in responses between bacterial strains require elucidation. Nevertheless, fermentation as a household food strategy may have value as a diarrhoeal disease control strategy in less developed countries.

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Manipulation of rat caecal metabolism by including Avoparcin and pectin in the diet.

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Oral administration of some but not all therapeutic antibiotics alters the pattern of faecal volatile fatty acids (VFA) in healthy man (Høverstad *et al.* 1986). The present study investigated the effects of Avoparcin, a growth-promoting antibiotic, on caecal fermentation pattern in rats given no dietary non-starch polysaccharides and in rats in which caecal fermentation was stimulated by dietary inclusion of pectin.

Four groups of six male Wistar rats (initial weight 100 g) were offered daily 15 g semi-purified diet containing 0 or 100 g apple pectin/kg (added at the expense of maize starch and sucrose) each with and without 20 mg Avoparcin/kg. After 21 d, the rats were killed and caecums removed.

						Significance of dietary effects		
Pectin (g/kg diet) . . .	0	0	100	100	SE of	Avo-		Inter-
Avoparcin (mg/kg diet) . . .	0	20	0	20	Mean	parcin	Pectin	action
Growth rate (g/21 d)	142	151	145	140	3·7	NS	NS	NS
Caecal mass (g)	2·5	3·4	9·1	9·1	0·56	NS	***	NS
Caecal pH	6·6	6·4	5·4	6·0	0·19	NS	***	NS
Total caecal VFA (mmol/kg caecal contents)	123	78	136	82	15·8	**	NS	NS
Proportions of individual VFA (mmol/mol):								
Acetate	592	553	689	656	25·4	NS	***	NS
Propionate	274	380	240	284	22·0	**	**	NS
Isobutyrate	14	10	2	9	2·5	NS	*	*
Butyrate	81	29	55	26	9·5	**	NS	NS
Isovalerate	19	27	14	25	3·6	*	NS	NS
Valerate	18	tr	nd	nd	0·8	***	***	***

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

Dietary treatment had no effect on rat growth rate but pectin inclusion increased caecal mass by three times and reduced caecal pH. Total VFA concentration was significantly reduced by Avoparcin but unaffected by pectin. Proportions of individual VFA were substantially altered by diet, with pectin associated with increased acetate, reduced propionate and isovalerate and the elimination of detectable valerate. Avoparcin treatment increased propionate and isovalerate but reduced butyrate molar proportion.

Work is needed to determine the physiological significance of these changes and whether they are due to alterations in large-bowel flora or their metabolic activities.

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The intestinal microflora is required for the stimulation of intestinal epithelial cell proliferation by dietary fibre. By R. A. GOODLAD[1], B. RATCLIFFE[2], J. P. FORDHAM[3] and N. A. WRIGHT[1], [1]*Royal Postgraduate Medical School, Cancer Research Campaign Cell Proliferation Unit, Department of Histopathology, Hammersmith Hospital, Du Cane Road, London W12 0HS,* [2]*Polytechnic of North London, Holloway Road, London N7 8DB* and [3]*AFRC Institute of Food Research, Shinfield, Reading RG2 9AT*

Refeeding starved rats with a fibre-free elemental diet supplemented with dietary fibre can markedly stimulate intestinal epithelial cell proliferation throughout the gastrointestinal tract when compared with rats given the elemental diet either alone or with inert bulk (Goodlad *et al.* 1987). The present study was designed to investigate the role of hind-gut fermentation and short-chain fatty acid release in this process.

Three groups of ten germ-free (GF) Hooded Lister rats and three groups of ten similar conventional (CV) rats, were starved for 3 d and then refed for 2 d on an elemental diet (Flexical), Flexical plus kaolin (300 g/kg), or Flexical plus a fibre mixture (300 g/kg) (1 part Ispaghula gel to 9 parts Trifyba). Intestinal cell production was measured by counting the rate of entry of vincristine-arrested metaphases in microdissected crypts (Goodlad & Wright, 1982).

Crypt cell production rate (cells/crypt per h)

	Conventional						Germ-free					
	Flexical		Flexical+ kaolin		Flexical+ fibre		Flexical		Flexical+ kaolin		Flexical+ fibre	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Intestinal position sampled												
25% of small intestine	11·37	2·50	14·78	1·43	17·71*	1·67	13·03	2·58	11·74	2·21	15·94	1·53
90% of small intestine	11·86	1·89	11·00	1·95	21·60**	2·12	12·38	1·88	5·89	2·27	9·11	2·61
50% of colon	2·17	1·46	2·38	1·50	13·94***	1·60	3·67	1·45	3·04	1·75	3·12	2·02

Significantly greater than respective Flexical diet: $*P<0·05$, $**P<0·01$, $***P<0·001$.

Whilst fibre significantly increased cell proliferation in the CV rats it had no effect in the GF rats; it can therefore be concluded that it is the products of hind-gut fermentation (short-chain fatty acids?), not fibre *per se*, that stimulate intestinal epithelial cell proliferation.

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Methylmalonic acid in the rumen of cobalt-deficient sheep and its effect on plasma methylmalonic acid. By D. A. RICE*, F. P. M. O'HARTE, W. J. BLANCHFLOWER and D. G. KENNEDY, *Veterinary Research Laboratories, Stoney Road, Stormont, Belfast BT4 3SD*

Certain rumen micro-organisms require the vitamin-B_{12}-dependent enzyme methylmalonyl CoA mutase (*EC* 5.4.992) for propionate production and utilization (Allen *et al.* 1964). We examined the level of methylmalonic acid (MMA) in the rumen of cobalt-deficient lambs. Deficiency was induced in lambs fed on a barley-based diet (Co 0·007 mg/kg) for 14 weeks.

MMA (nmol/g dry matter (DM) in the rumen and plasma (μmol/l) of Co-deficient lambs (n 3))

Period on diet (weeks) . . .		0	2	4	6	8	10	12	14
Rumen MMA	Mean	1·1	212·5	544·1	79·0	212·5	172·2	255·4	153·1
	SEM	1·1	81·7	251·9	62·1	81·4	93·8	21·1	16·1
Plasma MMA	Mean	0·7	1·2	2·7	6·0	8·6	7·1	9·6	61·4
	SEM	0·2	0·3	0·2	2·6	2·9	1·6	2·0	54·8

Lambs fed on a Co-adequate diet (Co 1·0 mg/kg) maintained a relatively low level of rumen MMA throughout (12·5 (SEM 4·9) nmol/g DM). However, the lambs given the Co-deficient diet showed a rise in rumen MMA levels within 2 weeks which was sustained during the remainder of the experiment. These lambs also showed a steady rise in plasma MMA levels, which exceeded normal levels (5·0 μmol/l) after 6 weeks. The rate of increase in plasma MMA lagged behind rises in rumen MMA concentration suggesting that plasma MMA was not influenced by absorption of rumen-derived MMA. To further substantiate this, oral MMA (1·5 mmol) was given to two Co-deficient and two control lambs. Plasma and rumen MMA were monitored for up to 24 h after dosing. Despite raising mean rumen MMA levels to 22·0 and 12·6 μmol/g DM in deficient and control animals respectively, no increases in plasma MMA levels were detected in either group. It appears that rumen MMA is not absorbed into the systemic circulation and therefore does not affect plasma MMA concentrations.

This work agrees with that of McDonald & Suttle (1986) who suggested that the fermentative activity of cultured rumen microbes was affected by the use of Co-deficient substrates. It is evident from these findings that propionate utilization by rumen microbes is affected at an early stage in the development of Co-deficiency. This suggests that a diagnosis of Co-deficient feed could be based on rumen MMA values, before clinical deficiency occurs in the animal.

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Methylmalonic acid as an indicator of vitamin B_{12} deficiency in lambs fed on a cereal-based diet. By F. P. M. O'HARTE, W. J. BLANCHFLOWER and D. A. RICE*, *Veterinary Research Laboratories, Stoney Road, Stormont, Belfast BT4 3SD*

There is a need for reliable clinical markers of vitamin B_{12}/cobalt deficiency in ruminants. Raised (>5·0 μmol/l) plasma methylmalonic acid (MMA) permits diagnosis of Co-deficiency in grazing sheep (Rice *et al.* 1987). Since Lough & Calder (1976) suggested that elevated urinary ethylmalonic acid (EMA) and MMA occurred in barley-fed sheep, the use of MMA as a marker in barley-fed sheep was in doubt.

Co-deficiency was induced in eight, 10-week-old Suffolk × lambs fed *ad lib.* for 14 weeks on a diet containing (g/kg) whole barley (970), urea (14), minerals and vitamins (0·007 mg Co/kg). A parallel control group of four lambs received the same diet with added Co (1·0 mg/kg). Plasma vitamin B_{12} was measured using the Becton Dickinson radioassay kit and plasma MMA determined using capillary gas chromatography (McMurray *et al.* 1986).

In control lambs mean plasma vitamin B_{12} concentrations were above 1400 pmol/l and mean plasma MMA below 5·0 μmol/l throughout. In the deficient lambs plasma vitamin B_{12} dropped below 200 pmol/l after 5 weeks and plasma MMA rose above 5·0 μmol/l after 7 weeks.

Sequential changes in plasma vitamin B_{12} (pmol/l) and MMA (μmol/l) concentrations in sheep (n 8) fed on a Co-deficient whole barley diet

Period on diet (weeks) . . .		0	1	3	5	7	9	11	14
Vitamin B_{12}	Mean	2320	950	371	152	165	137	93	57
	SEM	74	145	61	19	23	14	11	13
MMA	Mean	0·6	0·4	0·9	2·7	6·9	11·1	12·6	200·5
	SEM	0·1	0·1	0·1	0·5	1·7	3·5	4·8	133·6

Plasma vitamin B_{12} and MMA were inversely related. The rate and magnitude of the increase in plasma MMA was similar to that observed with sheep grazing Co-deficient pastures (Rice *et al.* 1987). The low levels of plasma MMA and EMA resulting from giving a concentrate diet did not interfere with test results. It is suggested that plasma MMA is a useful indicator of vitamin B_{12} deficiency in concentrate-fed sheep and that this feeding regimen provides a useful model system for studying Co-deficiency in sheep.

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Rumen propionate production rate and absorption of fermentation end-products into the portal vein of forage and forage–concentrate fed cattle. By C. J. SEAL, A. SARKER and D. S. PARKER, *AFRC Link Research Group, Department of Agricultural Biochemistry and Nutrition, University of Newcastle, Newcastle upon Tyne NE1 7RU*

The efficiency of utilization of nutrients for growth in ruminants may be dependent on the rate and pattern of absorption from the gastrointestinal tract. The extent of metabolism by the mucosa of the rumen wall of end-products of fermentation in the rumen was investigated.

Four Friesian steers (live weight 116–137 kg) were allocated to a cross-over design and given either a forage (grass pellets) or a forage–concentrate (50:50 grass:flaked maize pellets) hourly at levels of 24 g dry matter (DM)/kg live weight (forage diet) and 19 g DM/kg live weight (forage–concentrate diet). Diets and experimental design were the same as those used in a parallel study investigating metabolism across the liver (Fitch *et al.* 1989).

Chronic indwelling catheters were established in the portal and mesenteric veins and a carotid artery. Blood and rumen samples were obtained hourly during the last 6 h of a 12 h continuous intra-ruminal infusion of [2–^{14}C]propionate (0·125 μCi/ml per min). Portal blood flow was measured by a dye-dilution method using *para*-amino hippuric acid infused into the mesenteric vein.

	n	Forage	Forage–concentrate	SEM	Significance of effect of diet (ANOVA)
Molar % VFA:	4				
Acetate		74·9	64·7	0·30	$P<0·001$
Propionate		16·2	22·3	0·56	$P<0·01$
Isobutyrate		0·4	0·9	0·09	$P<0·01$
Butyrate		7·6	8·8	0·99	NS
Isovalerate		0·4	1·3	0·06	$P<0·01$
Valerate		0·6	2·1	0·24	$P<0·05$
Propionate production rate (mmol/min)	4	2·95	3·30	0·301	NS
Portal absorption rate (mmol/min):	3				
Acetate		4·49	3·77	0·589	NS
Propionate		0·88	0·99	0·179	NS
Butyrate		0·21	0·17	0·102	NS
3-Hydroxybutyrate		0·58	0·65	0·053	NS
Portal blood flow (l/min)	3	4·96	4·62	0·107	NS

VFA, volatile fatty acids; NS, not significant.

The molar percentage of acetate in rumen fluid was significantly lower on the forage–concentrate diet and that of propionate significantly higher. There was, however, no significant difference in the rates of propionate production in the rumen between the two dietary treatments.

Portal absorption rates reflected the pattern of fermentation in the rumen with a lower uptake of acetate and an increase in propionate absorption on the forage–concentrate diet. Net absorption of 3-hydroxybutyrate resulted from the metabolism of butyrate by the mucosal tissue.

In this experiment the rate of absorption of propionate into portal blood and therefore the supply of propionate to the liver was only 0·3 of the measured production rate in the rumen on both diets. These results underline the influence of metabolism within the rumen and the mucosa on the supply of fermentation end-products to the tissues of the animal.

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Improved absorption rates of water and electrolytes from a hypotonic solution compared with two isotonic oral rehydration solutions in the intact human jejunum. By J. B. LEIPER and R. J. MAUGHAN, *Department of Environmental and Occupational Medicine, University Medical School, Foresterhill, Aberdeen AB9 2ZD*

Oral rehydration therapy (ORT) has proved to be an efficacious and cost effective method for treatment of the majority of patients suffering from diarrhoea-associated dehydration. There is, however, some debate as to the optimum formulation of the solutions used. Moderately hypotonic oral rehydration solutions (ORS) have been shown to promote faster water uptake, but reduced or similar electrolyte absorption compared with the commonly used isotonic ORS (Wapnir & Lifshitz, 1985; Hunt *et al.* 1988).

We have measured net water and solute transport from three solutions using a steady-state perfusion technique in the normal human jejunum (n 9). A triple lumen perfusion set incorporating a 150 mm mixing segment and a 300 mm test segment was positioned with the perfusion port just distal to the ligament of Trietz. The three perfusion solutions used were: solution A (Dioralyte Improved®), a hypotonic ORS with a mean (SD) osmolality of 235 (5) mosmol/kg; and solutions B (Dioralyte®) and C (Rehidrat®), two isotonic ORS with osmolalities of 302 (8) and 330 (7) mosmol/kg respectively.

Following passage through the mixing segment, concentrations in the perfusates for solutions A, B and C were: sodium (mmol/l) 69, 47 and 62; chloride (mmol/l) 88, 61 and 68; glucose (mmol/l) 68, 143 and 65 respectively. Solution A perfusates remained hypotonic (242 (17) mosmol/kg), while those of solutions B (278 (13) mosmol/kg) and C (297 (11) mosmol/kg) remained essentially isotonic. The absorption results were not normally distributed; statistical analysis was therefore performed using Friedman's two-way analysis of variance followed by Wilcoxon's matched-pairs signed rank test when the overall product effect appeared significant.

In the test segment, median net water absorption (ml/cm per h) from solution A (8·2) was faster ($P<0·05$) than from solutions B (6·1) or C (4·1); water uptake from solution B was faster ($P<0·05$) than from solution C. Absorption of sodium (μmol/cm per h) from solution A (532) was faster ($P<0·05$) than from solutions B (35) and C (84). Chloride absorption (μmol/cm per h) from solution A (553) was faster ($P<0·05$) than from solutions B (249) and C (108). There was no difference in the rates of absorption of sodium or chloride between solutions B and C. Glucose absorption (μmol/cm per h) from solution A (664) was slower ($P<0·05$) than from solution B (1281), but faster ($P<0·05$) than from solution C (411).

These results demonstrate that ORS formulations with an initial glucose content of 90 mmol/l, a sodium:chloride:glucose ratio of 1:1:1·5 and an osmolality of approximately 235 mosmol/kg promote greater water and electrolyte absorption than from currently used isotonic ORS. The faster water absorption from solution B compared with solution C may be due to the higher glucose, the slightly lower osmolality or the neutral pH of solution B. This study suggests that the replacement of isotonic ORS with suitably formulated hypotonic ORS may improve ORT.

This study was approved by the Local Ethical Committee and supported by Rorer Health Care (UK) Ltd.

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The effects of dietary ligands on zinc uptake by brush-border-membrane vesicles. By A. J. TURNBULL[1], P. BLAKEBOROUGH[2] and R. P. H. THOMPSON[1], [1]*Rayne Institute, St Thomas's Hospital, London* and [2]*AFRC Institute of Food Research, Shinfield, Reading*

The bioavailability of zinc is dependent on the concentration of several ligands in the intestinal lumen. Folic acid may inhibit Zn absorption in man—an interaction of particular importance in pregnant women and sickle cell disease patients who are given folate supplements and whose Zn nutrition is marginal (Simmer *et al.* 1987). Citrate and phytate may also impair Zn bioavailability (Menard & Cousins, 1985; O'Dell & Savage, 1960). The present study examines the effects of these ligands on the uptake of Zn by the enterocyte in vitro.

Brush-border-membrane vesicles were prepared from the intestinal mucosa of weanling piglets (*n* 3) using a technique of precipitation with magnesium (Blakeborough & Salter, 1987) and incubated for 1 min in 20 mM-Tris/0·15 M-NaCl, pH 7·5, containing 50 μg membrane protein, varying concentrations of ligand, 5 μM-Zn and carrier-free ^{65}Zn. Uptake of Zn was measured using a rapid filtration assay. At this concentration of Zn, uptake by the vesicles constitutes transport rather than non-specific binding (Blakeborough & Salter, 1987).

		Control (5 μM-Zn)	Folic acid				Phytic acid		Citric acid		
Ligand (μmol/l) . . .			0·05	0·5	5·0	50	250	500	100	500	1000
^{65}Zn uptake (nmol/mg protein)	Mean	10·15	9·81	9·09***	8·36***	9·58	3·54***	3·32***	4·17***	5·05***	4·42***
	SEM	0·21	0·24	0·24	0·25	0·24	0·15	0·16	0·23	0·023	0·23

Significantly different from control value: ***$P<0·001$.
n 18.

Phytate and citrate had potent inhibitory effects on Zn uptake at all concentrations tested, confirming previous reports of their adverse effects on Zn bioavailability. In comparison, folate only slightly inhibited uptake over a wide range of concentrations and no dose-response relation was found. Brush-border-membranes provide a useful model for studying the effects of dietary factors on the transport of Zn across the lumen–mucosa interface.

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The effect of diets adequate and deficient in calcium on the activities of plasma membrane ATPases in the hearts and diaphragms of normotensive and spontaneously hypertensive rats. By PETER BLAKEBOROUGH, CAROLINE SMALES, SHEILA G. NEVILLE and BRIAN A. ROLLS, *Human Nutrition Department, AFRC Institute of Food Research, Reading Laboratory, Shinfield, Reading RG2 9AT*

The present study investigated possible associations of hypertension with defects in calcium transport in the heart and skeletal muscle tissue (diaphragm). Groups of ten spontaneously hypertensive (SHR) and normotensive control (WKY) rats were maintained from 8 weeks of age on semi-synthetic diets containing (mg/g) 6 Ca, 4 sodium (adequate in Ca, +Ca) or 0·6 Ca, 4 Na (deficient in Ca, −Ca). From 12 weeks of age systolic blood pressures were measured by tail-cuff sphygmomanometry. At 18 weeks the rats were killed and homogenates were prepared from the hearts and diaphragms.

Blood pressures were higher in SHR compared with WKY rats and, for SHR rats only, in Ca-deficient compared with Ca-replete animals. Activities of Na^+,K^+-ATPase (*EC* 3.6.1.37), (Ca^{2+}-ATPase (*EC* 3.6.1.38) and Mg^{2+}-ATPase (*EC* 3.6.1.38) in the heart were similar, irrespective of strain of rat or diet. In the diaphragm Ca^{2+}-ATPase activity was significantly reduced when comparing SHR with WKY rats, but changing the dietary Ca did not affect the activity. Activities of Na^+,K^+-ATPase and Mg^{2+}-ATPase were unaffected by strain of rat or diet.

Strain of rat and type of diet . . .	WKY +Ca		WKY −Ca		SHR +Ca		SHR −Ca	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Blood pressure (mm Hg)	132	4	138^{NS}	4	180***	3	191*	3
Specific activity of enzymes (μmol/h per mg protein):								
Heart:								
Na^+,K^+-ATPase	0·067	0·009	0·053^{NS}	0·008	0·094^{NS}	0·018	0·093^{NS}	0·01
Ca^{2+}-ATPase	0·89	0·28	0·87^{NS}	0·4	1·22^{NS}	0·1	0·72^{NS}	0·32
Mg^{2+}-ATPase	2·34	0·25	2·79^{NS}	0·36	2·96^{NS}	0·4	2·21^{NS}	0·3
Diaphragm:								
Na^+,K^+-ATPase	0·047	0·005	0·054^{NS}	0·01	0·066^{NS}	0·012	0·061^{NS}	0·014
Ca^{2+}-ATPase	1·07	0·18	1·11^{NS}	0·2	0·47**	0·12	0·31^{NS}	0·05
Mg^{2+}-ATPase	3·72	0·83	3·11^{NS}	0·15	3·37^{NS}	0·44	2·94^{NS}	0·2

Results are means, with their standard errors, of at least four experiments. NS, not significant.

Significantly different: *P<0·05, **P<0·025, ***P<0·001. Values in the SHR +Ca column compare results between WKY +Ca and SHR +Ca. Values in the WKY −Ca and SHR −Ca columns compare results between WKY +Ca and WKY −Ca, and SHR +Ca and SHR −Ca respectively.

The results indicate that active Ca transport (catalysed by Ca^{2+}-ATPase) may be reduced in the muscle tissue of the diaphragm of SHR, compared with WKY rats. If the smooth muscle of arterioles behaves in the same fashion, Ca would build up the muscle cells, leading to increased contraction and hence hypertension would develop. Intestinal Na^+,K^+-ATPase and Ca^{2+}-ATPase are reduced in activity in SHR compared with WKY rats, and these activities are modified by altering Ca in the diets (Blakeborough *et al.* 1988). Alterations in activities of plasma membrane ATPases in SHR rats seem therefore to be tissue-specific, and it is probable that alterations in the diet only affect enzymes in the intestine.

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Effect of thyrotropin-releasing hormone and an analogue, RX77368, on thermogenesis and weight gain in genetically obese Zucker rats. By H. D. McCarthy and J. A. Carnie, *Department of Biochemistry & Applied Molecular Biology, UMIST, Manchester M60 1QD*

Impaired hypothalamic activation of the sympathetic innervation to brown adipose tissue (BAT) contributes to the obesity of the Zucker rat (Trayhurn, 1986). Griffiths *et al.* (1988) have shown that peripheral administration of TRH and its analogues increases metabolic rate in rats and appears to act centrally to stimulate BAT thermogenesis.

Intracerebroventricular (icv) administration of TRH (5–10 μg) into anaesthetized lean Zucker rats resulted in an increase in BAT temperature (1·25 (SEM 0·088)°, delay 1·0 (SEM 0·44) min; *n* 6) and a smaller increase in rectal temperature (0·88 (SEM 0·17)°, delay 6·25 (SEM 0·625) min). In a small number of hypophysectomized, Sprague–Dawley rats (*n* 3), icv administration of TRH (10 μg) caused a similar rise in BAT temperature (1·2 (SEM 0·16)°, delay 2·0 (SEM 0·66) min) and a smaller rise in rectal temperature (0·7 (SEM 0·08)°, delay 10·0 (SEM 1·0) min). Therefore, the central effect of TRH on BAT thermogenesis is not mediated through the pituitary–thyroid axis.

Intravenous administration (500 μg) of the stable TRH analogue, RX77368 (pGlu-His-(3,3 dimethyl-) ProNH_2), in lean Zucker rats caused a similar rise in BAT temperature (1·03 (SEM 0·1)°; *n* 4) indicating traversal of the blood–brain barrier.

Two groups (*n* 5 per group) of weight-matched obese male Zucker rats (starting weight 350 (SEM 14·7) g) were injected twice daily with RX77368 for 18 d (1 mg/kg subcutaneously), or sodium chloride (9 g/l). Food intake and body-weight were monitored and at the end of the experiment the thermogenic activity of interscapular BAT was assessed by measuring the GDP binding to isolated mitochondria.

Control rats gained an average 45·8 (SEM 3·93) g whereas treated rats gained 24·6 (SEM 3·75) g ($P<0·01$). Food intake was not significantly altered, but mitochondrial GDP binding was increased by 116% ($P<0·001$).

In separate animals, binding of [^{3}H]TRH to brain membrane preparations indicated no difference in the density or affinities of TRH receptors between lean and obese rats.

These results show that TRH-stimulated neurones to BAT are functional in obese rats, and a stable analogue can reduce weight gain by stimulating thermogenesis, demonstrating its potential as a thermogenic anti-obesity compound.

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Central effects of interleukin-1β and tumour necrosis factor-α on brown adipose tissue thermogenesis in genetically obese rats and mice. By J. A. CARNIE and J. A. JOHNSTON, *Department of Biochemistry, UMIST, Manchester M60 1QD* and N. J. BUSBRIDGE, M. J. DASCOMBE and N. J. ROTHWELL, *Department of Physiological Sciences, University of Manchester, Manchester M13 9PT*

Impaired diet-induced thermogenesis is fundamental to the development of many genetic obesities in rodents, including the obese (*ob/ob*) mouse and the fatty (*fa/fa*) Zucker rat. Both forms of obesity are normalized by adrenalectomy and have been ascribed to some impairment in corticotrophin-releasing factor (CRF). Obese Zucker rats also show markedly diminished responses to central injection of interleukin-1β (IL-1β) (Dascombe *et al.* 1988), a cytokine which is considered to act as an endogenous pyrogen. We have now compared the effects of IL-1β in genetically obese rats (*fa/fa*) and mice (*ob/ob*) with those of another pyrogenic peptide, tumour necrosis factor-α (TNF-α).

All injections were delivered centrally into the third ventricle of the brain of conscious animals via indwelling guide cannulae which had previously been implanted under halothane anaesthesia. Animals (adult, lean or genetically obese Zucker rats or *ob/ob* mice) were killed 60 min after treatment and brown adipose tissue (BAT) activity was assessed from in vitro mitochondrial purine nucleotide (GDP) binding.

Injection of vehicle (9 g sodium chloride/l) did not significantly affect colonic temperature in any animals. In lean mice, injection of murine recombinant IL-1β (5 ng) elicited a 0·9° rise in temperature and a significant (88%, $P<0·001$) increase in GDP binding (n 6–8). However, in obese mice the same dose of IL-1β had no effect on either body temperature or BAT GDP binding. Obese rats also exhibited impaired BAT (9% increase in GDP binding, not significant) and temperature responses (0·5° rise) compared with their lean littermates (81% increase in GDP binding, 1·9° rise in temperature, $P<0·001$).

In contrast, recombinant TNF-α (human, 2·5 μg for rats; murine, 0·7 μg for mice) caused significant ($P<0·001$) pyrogenic (0·9° rise) effects and increases (63%) in BAT activity in lean animals, which were also apparent in obese mutants (increase in GDP binding: mice 58%, rats 38%).

These results indicate that central activation of fever and thermogenesis by IL-1β, but not TNF-α, is impaired in genetically obese rats and mice. These responses may reflect the dependence of IL-1β on CRF for its thermogenic effects (Rothwell, 1988).

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Effects of a β_2-adrenoreceptor agonist clenbuterol on body-weight and muscle protein in endotoxin-treated rats. By J. J. CHOO[1,3], J. ARNOLD[1], M. A. HORAN[2], R. A. LITTLE[1] and N. J. ROTHWELL[3], [1]*North Western Injury Research Centre,* [2]*Department of Geriatric Medicine* and [3]*Department of Physiological Sciences, University of Manchester, Manchester M13 9PT*

It has been demonstrated that certain β_2 adrenoreceptor agonists such as clenbuterol stimulate muscle growth (Rothwell & Stock, 1986). These effects indicate potential uses for such compounds for animal production and also in clinical conditions associated with muscle wasting. We have investigated the effects of clenbuterol on body-weight and skeletal muscle in rats treated with endotoxin in order to mimic sepsis.

In a preliminary study, infusion of *Escherichia coli* endotoxin (0127B8, 0·5 mg/d) by osmotic mini-pump (Alzet, subcut) in young rats (male Wistar) induced fever and halted growth for 2 d, but thereafter animals recovered. Clenbuterol added to the diet (4 mg/kg) allowed animals to recover more quickly, but body-weight and muscle mass did not differ significantly between groups on day 5, although the RNA content of gastrocnemius muscle was elevated (29%) by clenbuterol treatment.

In a second experiment, rats were given two injections of endotoxin (0127B8; 1·0 then 0·5 mg/kg) 2 d apart. Fever and hypophagia were observed in endotoxin-treated rats only over the first 24 h and were unaffected by clenbuterol.

Animals were killed on the 3rd day. Endotoxin treatment significantly ($P<0·05$) reduced gastrocnemius muscle mass (9%), protein (13%) and RNA content (14%) but did not affect the ratio of RNA:protein in the muscle. Clenbuterol completely reversed the effects of endotoxin on muscle mass and protein content, and stimulated ($P<0·01$) the ratio of RNA:protein content by 19%. This latter effect might indicate an increase in protein synthesis.

These results imply that clenbuterol can modify weight gain and muscle growth in endotoxin-treated animals. This effect is most apparent during the recovery phase.

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