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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Four Hundred and Fifty-first Meeting of the Nutrition Society was held in the Royal Society of Medicine, 1 Wimpole Street, London on Monday and Tuesday, 23/24 May 1988, when the following papers were read:

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The effect of habitual breakfast omission on the energy cost of rest, exercise and dietary-induced thermogenesis during consumption of weight-maintenance and low-energy diets. By RICHARD MOODY, Polytechnic of the South Bank, Borough Road, London SE1 0AA

Feeding patterns may influence body-weight during consumption of normal weightmaintenance diets (Fabry, 1967) and low-energy diets (Debry *et al.* 1973; Mahler, 1972). To investigate this, regular feeders $(n \ 3, RF)$ who eat breakfast every day, and p.m. feeders $(n \ 3, PMF)$ who never consume more than a drink at this time, recorded their energy intake for 7 d (RF 9.96 (SEM 0.6), PMF 6.62 (SEM 0.39) MJ/d) and on day 8 undertook the morning programme shown in Fig. 1 (series 1) (body mass index (kg/m², BMI): RF 23.71 (SEM 0.57), PMF 24.65 (SEM 3.46)). Both groups then severely reduced their energy intake for 3 d (RF 3.01 (SEM 0.32), PMF 2.56 (SEM 0.21) MJ/d) and then repeated the programme (series 2) (BMI: RF 23.02 (SEM 0.45), PMF 24.14 (SEM 3.48)).

	←	→ X0 ←	→ XB ←	→ X0 ←	—→ XO
Time of day (hours)	06.00	07.00	08.00	09.00	10.00

Fig. 1. Morning programme. ←→→, Resting period; X, resting period, energy expenditure measured; B, liquid Complan[®] breakfast (1.68 MJ) given; O, exercising, energy expenditure measured.

Energy expenditure was measured by indirect calorimetry at rest and during a well-practised and standardized arm-pulling activity on a dual pulley system. Each set of four work rates and corresponding expenditure values were used to determine the energy cost of 20 watts work (Table).

		i	RF			PI	MF		RF+I	PMF
Series	1		2		1		·	2	1+	2
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
		Ene	rgy expend	liture (J	/s per kg	body-wt)			
Rest (all periods)	1.04	0.03	1.14*	0.04	1.20	0.04	1.25	0.04	-	-
20 watts work	2.71	0.2	3.04***	0.2	2.77	0.19	2.71	0.16		
	DIT	(% inci	ease over p	orepran	dial restin	ig metal	olic rate))		
Rest	9.8	1.61	19.8	4-31	11.5	0.44	15.8	0.62	14.23	1.54
20 watts work	24.8	7.9	30.6	7 ·1	39.2	5.2	27.2	10.9	30.46++	3.91

DIT, dietary-induced thermogenesis.

Significantly different from series 1 RF group: *P<0.05, ***P<0.001. Significantly different from at rest group: $\dagger^{\dagger}P<0.01$.

The very-low-energy diet increased the energy cost of rest and light exercise for the RF group but not for the PMF group who could be adapted to their morning starvation through increased energetic efficiency. DIT was not influenced by total daily energy intake or feeding pattern, although all subjects doubled resting DIT levels during light exercise. This well-known effect indicated an important area of energy conservation for those who (a) omit meals and (b) consume the majority of their energy in the evening with the associated symptoms of lethargy.

Debry, G., Rohr, R., Azouaou, R., Vassilitch, I. & Mottaz, G. (1973). In Regulation of Energy Balance in Man, pp. 308-309 [M. Apfelbaum]. Paris: Masson and Co.

Fabry, P. (1967). Feeding Pattern and Nutritional Adaptations. London: Butterworths.

Mahler, R. (1972). Acta Diabetologica Latina 9, 449-465.

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Measurement of arteriovenous differences across an adipose depot in man. By K. N. FRAYN, S. W. COPPACK, P. L. WHYTE, S. M. HUMPHREYS and L. L. NG, Sheikh Rashid Diabetes Unit, Radcliffe Infirmary, Oxford OX2 6HE

Our knowledge of the regulation of fat storage and mobilization in human adipose tissue in vivo is indirect, based either on whole-body experiments or on studies of isolated adipose tissue. We have sought to study human adipose tissue metabolism more directly by measurement of arteriovenous differences across a suitable depot, the subcutaneous adipose tissue of the anterior abdominal wall. The venous drainage from this tissue runs towards the groin in small vessels which merge before entering the femoral triangle where they meet the femoral vein. Muscle and adipose tissue drainage are completely separated by the superficial fascia, although skin and adipose tissue must inevitably be linked. In trial experiments the blood flow in these superficial vessels was found to be low and variable, often ceasing during a study. We have recently passed a Seldinger-type catheter through a small superficial vein towards the groin, where the vessels are larger and flow more consistent. Concentrations were compared with those in arterialized blood from a heated hand.

In nine experiments in five normal subjects fasted overnight, plasma non-esterified fatty acid (NEFA) concentrations in adipose tissue drainage were very high (up to $4\times$ arterial concentration), as were blood glycerol levels (mean (sE), μ mol/l, venous ν . arterial; NEFA, 730 (60) ν . 270 (30); glycerol, 175 (20) ν . 44 (4)). Fractional extraction of glucose was low (venous 4.94 (0.09), arterial 5.06 (0.10) mmol/l). After an oral glucose load (75 g) arterial NEFA levels declined, as did the arteriovenous difference (at 60 min after glucose, NEFA venous 120 (30) ν . arterial 60 (20) μ mol/l); the extraction of glucose increased (venous 9.17 (0.86) ν . arterial 9.60 (0.92) mmol/l). All arteriovenous differences were significant by paired t test (P < 0.05).

This technique opens up many possibilities for investigating the physiology and pathophysiology of fat storage and mobilization in man, and thus the metabolic basis for body-weight regulation.

The studies were approved by the Central Oxford Research Ethics Committee.

Influence of a 3-month programme of brisk walking on walking performance, plasma cholesterol and body fatness in middle-aged women. By A. HUDSON¹, A. E. HARDMAN¹, P. R. M. JONES² and N. G. NORGAN², ¹Department of Physical Education and Sports Science and ²Department of Human Sciences, University of Technology, Loughborough, Leicestershire LE11 3TU

There is continuing uncertainty regarding the amount and kind of exercise necessary, or the degree of physical fitness needed, to influence the risk factors associated with ischaemic heart disease (Shaper, 1987). The purpose of the present study was to examine the effects of a 3-month programme of brisk walking on walking performance, body fatness and plasma cholesterol in a group of initially sedentary women aged 30–62 years (thirty-two walkers (W), eighteen controls (C)). Maximum oxygen uptake was predicted from submaximal O_2 uptake and heart rate during stepping exercise. Walking performance was assessed as the time to walk 1 mile (1.61 km) at maximum pace. In addition, subjects completed a 1 mile treadmill walk at a pace which they had previously selected as 'brisk'. Venous blood samples were obtained after an overnight fast for the determination of plasma total cholesterol (TC) and high-density-lipoprotein cholesterol (HDL-C). Skinfold thickness was measured at biceps, triceps, subscapular, suprailiac and anterior thigh. Subjects walked briskly for 200 min per fortnight, increasing to 350 min by the end of 3 months (e.g. eight walks/fortnight for a minimum of 20 min on each occasion).

Predicted maximum oxygen uptake (\dot{V}_{O_2max}), time to walk 1 mile, heart rate during a standard treadmill walk, plasma HDL–C concentration and the ratio TC:HDL–C, before (pre) and after (post) a programme of brisk walking

		-	₂max per min)	1 mile time (min)		Treadmill heart rate (beats/min)			L–C nol∕l)	TC:H	IDL-C
Group		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
W :	Mean	27.0	29.1*	14.14	13-36*	129	125*	1·17	1.47*	5.27	3.79**
	SD	4.9	4.4	1.02	1.03	19	13	0.44	0.28	0.97	0-91
C :	Mean	28.7	27.6	14.30	14.60	130	130	1.33	1-45	4.03†	3.78
	SD	4.4	4 ⋅0	$1 \cdot 10$	1.32	19	18	0.27	0.26	0.99	0.90

Significantly different from pre group: *P < 0.05, **P < 0.01. Significantly different from W: †P < 0.05.

Body mass (mean and sD) was decreased for W (63.5 (8.7) v. 62.7 (8.3) kg, P < 0.05 paired t test) but not for C (60.2 (8.7) v. 60.3 (8.5) kg, not significant). Anterior thigh skinfold was decreased for W (28.5 (6.6) v. 27.9 (6.2) mm, P < 0.05) and increased for C (29.5 (6.7) v. 30.4 (6.1) mm, P < 0.05) but there was no significant change in the sum of skinfolds at the other four sites (W 58.1 (16.5) v. 57.1 (15.7) mm; C 59.9 (21.9) v. 61.0 (19.8) mm).

These results indicate that regular, brisk walking can improve walking performance and provoke a favourable change in the TC:HDL-C ratio.

Shaper, A. G. (1987). Current Opinion in Cardiology 2, 571-585.

Carbohydrate supplementation before or during exercise, or both, has been much studied, usually using glucose or fructose (Ahlborg & Felig, 1976; Koivisto *et al.* 1985). The present study examined the effects of chain length of carbohydrate ingested (i.e. glucose or maltodextrin) with and without the addition of guar gum.

Five healthy male subjects rode a bicycle ergometer at an exercise intensity corresponding to 65% maximum consumption oxygen $(V_{O_2 max})$ for 90 min, followed by a ride to volitional exhaustion at 75% $\dot{V}_{O_2 max}$. The subjects ingested 1 g/kg body-weight of the test substance in 400 ml water before exercise. The five solutions were placebo (P), glucose (G), glucose + guar gum (G+Gu), maltodextrin (MD), and maltodextrin + guar gum (MD+Gu). Blood samples were taken from an indwelling catheter at rest, after 15, 30, 60 and 90 min of exercise, and at the point of exhaustion. Samples were analysed for adrenaline, noradrenaline, insulin, glucagon, glucose and non-esterified fatty acids (NEFA).

There were significant differences between the treatments for adrenaline (P<0.01), insulin (P<0.05), glucose (P<0.01) and NEFA (P<0.01) according to ANOVA. Subsequent analysis using HSD tests showed that significantly higher adrenaline levels were found for P compared with MD (P<0.05), significantly lower levels of insulin for P compared with MD (P<0.05), significantly lower levels of glucose for P compared with G+Gu (P<0.05), and significantly higher levels of NEFA for P compared with G and MD (P<0.05). No significant differences were found between the carbohydrate treatments for any of the blood variables measured. Student's t test revealed that significantly longer times (s) to exhaustion were obtained for G (372 (sp 155), P<0.05), G+Gu (423 (sp 156), P<0.05), MD (250 (sp 45), P<0.05), and MD+Gu (483 (sp 167), P<0.01) compared with P (163 (sp 53)), and also for MD+Gu compared with MD (P<0.05).

These results support the use of carbohydrate supplementation in prolonged exercise, and suggest that the type of carbohydrate may influence the degree of benefit.

This study was supported by Powell & Scholefield Ltd, Liverpool.

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Koivisto, V. A., Harkonen, M., Karonen, S. L., Groop, P. H., Elovainio, R., Ferrannini, E., Sacca, L.
& De Fronzo, R. A. (1985). Journal of Applied Physiology 58, 731-737.

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Effects of food carbohydrates on large intestinal fermentation in vitro. By J. S. GOODLAD and J. C. MATHERS, Department of Agricultural Biochemistry and Nutrition, The University, Newcastle upon Tyne NE1 7RU

Dietary manipulations designed to increase the intake of non-starch polysaccharides alter markedly the volatile fatty acid (VFA) patterns in the rat caecum (Key & Mathers, 1987, 1988). The reasons for this altered bacterial metabolism are unknown but may include (i) altered substrate supply, (ii) modifications in gut flora or (iii) changes in rates of bacterial growth resulting from reduced caecal transit time (Goodlad & Mathers, 1987).

To investigate the effects of specific carbohydrates on VFA production and bacterial growth, we incubated anaerobically 0.5 g portions of selected substrates with filtered and buffered pig large-intestinal contents. Incubation proceeded at 37° for 0, 2, 4, 6, 10 and 24 h. The incorporation of ³⁵S into TCA-precipitable material was used as a measure of bacterial growth and net VFA production calculated after correction for blanks (no added substrate). The Table gives means for two incubations from each of two pigs after 24 h.

Substrate	Cellulose	Starch	Pectin	Raffinose	Pea fibre	SE of mean
Net VFA production (mmol/flask)	0.63	3.14	3.40	3.83	2.87	0.271
Proportions of individual VFA						
(mmol/mol):						
Acetate	641	474	802	527	587	40 ·7
Propionate	205	107	132	343	257	15.9
Butyrate	10	388	57	109	123	28.1
% added ³⁵ S incorporated into						
TCA-precipitable material	1	19	5	10	12	2.1

Cellulose proved to be a poor fermentation substrate with little VFA production and low ³⁵S-incorporation. Although net VFA production rates were relatively similar for the remaining four carbohydrates, there were large differences ³⁵S-incorporation suggesting very variable coupling of substrate fermentation to bacterial growth. In addition, VFA patterns were distinctly different for these carbohydrates, with pectin characterized by high acetate, raffinose by high propionate and starch by high butyrate proportions. If fermentation in vivo is similar, considerable opportunities for manipulation of largeintestinal bacterial metabolism by altering substrate supply appear possible.

J. S. G. holds a SERC CASE studentship in collaboration with Unilever Research.

Goodlad, J. S. & Mathers, J. C. (1987). Proceedings of the Nutrition Society 46, 149A. Key, F. B. & Mathers, J. C. (1987). Proceedings of the Nutrition Society 46, 11A. Key, F. B. & Mathers, J. C. (1988). Proceedings of the Nutrition Society 47, 101A.

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Effects of intraruminal infusions of sodium acetate on silage intake by dairy cows. By J. N. MBANYA, M. H. ANIL and J. M. FORBES, Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT

We have shown the dose-related effects of sodium acetate on hay intake by the lactating dairy cow (Anil *et al.* 1987). A similar study has now been performed on silage intake.

Six rumen-fistulated Friesian cows in late lactation (mean milk yield 8 kg/d) were used in a 7×7 Latin-square design with one column omitted. They were housed individually and offered grass silage (dry matter (DM) 190 g/kg; crude protein, 143 g/kg DM; modified acid-detergent fibre, 408 g/kg DM; ether extract, 44 g/kg DM; ash, 97 g/kg DM; pH 4·1) ad lib. and a daily total of 3 kg concentrates. The treatments imposed over 3-h periods from 11·00 to 14.00 hours on each experimental day were: 0 (no infusion), water, 6, 9, 12, and 15 mol sodium acetate in 4·5 litres water, and 15 mol sodium chloride in 4·5 litres water (see Table). Rumen fluid samples were collected at hourly intervals for determination of sodium, osmolality and volatile fatty acid concentrations. Silage intakes were measured during 3-h infusion and during 2-h post-infusion periods.

			Water (l)			acetate ioi)		NaC1 (mol)	
Treatments and									
amounts		0	4.5	6	9	12	15	15	SED
Silage intake (kg):									
During infusion	Mean	9.0	10-6	10-4	9-4	7.6	5.0**	7.6	1.9
Post-infusion	Mean	4.9	4.2	8∙3	7.1	5.0	3.7	4.9	1.3
Na levels (mg/ml):									
During infusion	Mean	6-5	6-2	7.5	8.5	9.2	11.9*	10.2*	1.4
Post-infusion	Mean	6.2	5.9	7.3	8.3*	8.7*	10.6*	8.6*	1.0
Osmolality (mOsmol/	l):								
During infusion	Mean	270	290	309	329	336	419*	358	44
Post-infusion	Mean	268	274	304	301	305	363*	309	34

SED, standard error of the difference.

Significantly different from water: *P<0.05, **P<0.01.

Silage intake was significantly reduced by the highest level of sodium acetate during infusions. Sodium chloride had a tendency to depress intake during infusion. Mean rumen fluid Na levels and osmolalities during infusions increased consistently with the increasing amount of salt infused and was significant, compared with the control values, for the 15 mol sodium acetate treatment, and Na levels were also significant for the 15 mol sodium chloride treatment. Mean rumen fluid Na correlated significantly with mean rumen fluid osmolality during infusion ($r \ 0.94$) and during 2 h post-infusion ($r \ 0.89$), and with silage intake during 5 h ($r \ -0.36$) and 2 h post-infusion ($r \ -0.33$) periods.

Intraruminal sodium acetate depresses silage intake of lactating dairy cows, and it appears that this is as likely to be due to osmotic effects as it is to the effects of acetate.

Anil, M. H., Mbanya, J. N., Symonds, W. H. & Forbes, J. M. (1987). Proceedings of the Nutrition Society 46, 29A. The therapeutic value of barley in the treatment of diabetes mellitus. By G. S. MAHDI and D. J. NAISMITH, Department of Food and Nutritional Sciences, King's College, London W8 7AH

Certain foods are traditionally used in some developing countries alone or in conjunction with modern medication for the management of diabetes mellitus. In Iraq barley flour is substituted for wheat flour for bread making. To investigate the alleged beneficial properties of barley, young rats (100 g), eight rats per group, were fed for 4 weeks on semi-synthetic diets in which carbohydrate was supplied in the form of (Iraqi) barley flour, maize starch or sucrose, the protein contents being equalized (at 200 g/kg) using casein. Although rates of growth were not significantly different, the rats fed on the barley diet consumed 10% more energy. However, faecal weight was increased threefold, and the digestibilities of energy and protein were reduced by 12% (P<0.001) and 9% (P < 0.001) respectively, when compared with the groups consuming starch or sucrose. In a second experiment adult rats (ten animals per group) were made diabetic by injection with streptozotocin (70 mg/kg). After 8 weeks on the experimental diets, the mean (SE) non-fasting plasma glucose concentration (mmol/l) in the rats fed on barley $(32 \cdot 2 (1 \cdot 3))$ was significantly lower than that in rats fed on either maize starch $(42 \cdot 2 (3 \cdot 0))$. P < 0.005) or sucrose (41.3 (4.6), P = 0.03). These differences were reflected in the values for mean daily water consumption (ml): barley (238 (9.5)), starch (298 (24), P < 0.05), sucrose (388 (34), P < 0.001).

The protective effect of barley might be due to properties of its constituent starches, or to its remarkably high content of chromium. Analysis of barley flour showed that it contained 5.7 ppm of Cr, compared with 0.21 ppm in wholemeal wheat flour, 0.11 ppm in maize starch and 0.04 ppm in success. The latter hypothesis was tested.

Adult male rats were fed on the diets containing barley or sucrose. A third group was fed on the sucrose diet, but received Cr potassium sulphate in their drinking water at a concentration calculated to provide the same quantity of absorbed Cr as from the barley diet. The results are shown in the Table.

	Absorbed Cr calculated) (µg/d)	Plasma g (mm		Water (ml	
	(calculated) (µg/d)	Mean	SE	Mean	SE
Barley	32-4	31.3	1.4	180	4.9
Sucrose	0.68	45.7*	1.6	306*	17.0
Sucrose + Cr	33.1	34-4	5.0	179	28.0

*Value differs from that for group fed on barley: *P*<0.001. *Non-fasting.

The therapeutic value of Iraqi barley may thus be accounted for largely by its high Cr content.

Effect of short-term starvation on the release of glutamine by human muscle. By S. WOOD¹, R. LEENEN², E. PULLICINO¹ and M. ELIA¹, ¹Dunn Clinical Nutrition Centre, Cambridge CB2 1QL, ²Department of Human Nutrition, Agricultural University of Wageningen, The Netherlands

Human skeletal muscle releases more nitrogen in the form of glutamine than any other amino acid (Elia *et al.* 1985, 1988). It also maintains a higher intracellular concentration of glutamine than any other amino acid. After 12–14 h of fasting (overnight fast), free glutamine concentration is about 20–25 mmol/l intracellular water, but during a further 48–60 h of fasting the concentration is reduced to about half of its original value (Magnusson *et al.* 1987). In a young adult male this loss corresponds to as much as 6 g glutamine N (equivalent to 25% of the total N excreted in the urine between 12 and 66 h of fasting). The purpose of the present study was to assess whether the depletion in the intramuscular glutamine pool is associated with increased release of glutamine into the circulation. Therefore, we measured the exchange of glutamine across forearm muscle in seven young, lean adult males on three occasions: after 12–14 h of fasting (overnight fast), after 36–40 h fasting, and after 60–64 h fasting. Glutamine and glutamate were measured enzymically in arterialized and deep venous blood from the forearm. Blood flow was measured by strain gauge plethysmography.

Despite a twofold increase in glutamine release the concentration of glutamine in arterialized blood declined (Table). These changes were associated with the expected hyperketonaemia and mild metabolic acidosis of starvation, and the decrease in the circulating glucose and insulin concentrations. There was no significant change in the circulating concentration or uptake of glutamate by the forearm.

The circulating arterialized blood concentration (µmol/l) and release (µmol/min per l muscle) of glutamine by human forearm muscle

Period of starvation (h)	12-14	36-40	60-64
Circulating concentration	613	552	510†††
Release	1.09	2.28**	2.30*

Values significantly different from the initial measurements: *P < 0.02; **P < 0.01; $\dagger \dagger \dagger P < 0.002$.

The results suggest that short-term starvation, which is associated with depletion of the intramuscular glutamine pool, results in a large and significant increase in release of glutamine from muscle. This increased release of glutamine is not reflected by the blood glutamine concentration, which decreased during the period of study.

Elia, M., Folmer, P., Schlatmann, A., Goren, A. & Austin, S. (1988). *Metabolism* (In the Press). Elia, M., Neale, G. & Livesey, G. (1985). *Clinical Science* 69, 123–133. Magnusson, K., Alvestrand, A., Ekman, L. & Wahren, Y. (1987). *Clinical Nutrition* 6, Suppl., 62. Long-term dietary intervention in multiple sclerosis patients: blood cell correlates. By R. JONES and E. GROVES, Oncology Research Unit, Radiotherapy Centre, Bristol Royal Infirmary, Bristol BS2 8ED and G. E. FITZGERALD, ARMS Research Unit, Central Middlesex Hospital, London NW10

The significance of the low levels of linoleic acid (LA) in the blood of multiple sclerosis (MS) patients (Thompson, 1966) is not understood, but this finding has promoted the suggestion that dietary supplementation with essential fatty acids (EFA) may influence the course of the disease (see Dworkin *et al.* 1984). The present study involves the implementation of a whole dietary approach rather than supplementation to provide increased EFA intake of both n-3 and n-6 fatty acids with full nutritional support and reduced saturated fat. The diet and the scoring system used to assess nutrient intake is described by Fitzgerald *et al.* (1987).

Throughout the 5-year study period, blood samples were obtained (initially from forty-eight patients) at 6-monthly, and latterly yearly intervals, corresponding with dietary assessments from which a diet score was derived. Folate, vitamin B_{12} and haemoglobin levels were measured in erythrocytes, together with electrophoretic mobility (EPM), which represents cell-surface charge, using a modification described by Zukoski *et al.* (1979) of the Field test (Field *et al.* 1977). An abnormal EPM has been found to be correlated with low levels of LA in the choline phosphoglyceride fraction of the erythrocyte membrane in MS patients (Harbige *et al.* 1986).

Dietary intake of LA and erythrocyte EPM values in MS patients completing a 5-year study

		Initial		Intermediate value (3 years)			Final value (5 years)		
	Mean	SE	n	Mean	SE	n	Mean	SE	n
EPM (% change) LA intake (g/d)	37 14·6	9·4 5·2	13 13	52 23·1	8·3 15·2	14 14	39 16·3	6∙6 6∙6	14 14

The present study shows that increased dietary intake of LA results in a normalization of the initially abnormal EPM. Correlation was strongest with LA intake, and less strong with changes in the polyunsaturated:saturated fatty acid ratio. In longer-term samples these gains were not maintained, and both erythrocyte EPM values and LA intake values fell. Thus it may be implied that erythrocyte EPM is a useful means of monitoring changes induced by increased EFA intake and the long-term study indicates that patients may find it difficult to maintain their diet.

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Zukoski, C. F., Tamblyn, C. H., Swank, R. L. & Seaman, G. V. F. (1979). In Cell Electrophoresis: Clinical Application and Methodology [A. W. Preece & D. Sobolovic, editors]. Amsterdam: Elsevier.

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Do higher vitamin A requirements in men explain the difference between the sexes in plasma provitamin A carotenoids and retinol? By D. I. THURNHAM, MRC Dunn Nutrition Laboratories, Milton Road, Cambridge CB4 1XJ and P. S. FLORA, Wolfson Research Laboratories, Queen Elizabeth Hospital, Birmingham

In a recent survey (Thurnham *et al.* 1987, 1988) of 2000 adults in Great Britain between October 1986 and September 1987, we measured the plasma concentrations of three provitamin A carotenoids: β -carotene (BC), α -carotene (AC) and β -cryptoxanthin (BCP), and two non-provitamin A carotenoids: lycopene (LYC) and lutein (LUT; March to September only). There were higher concentrations of BC, AC and BCP in women than in men throughout the year whereas LYC and LUT were not significantly different except for LYC between April and June (men > women, P < 0.001).

Carotenoid		Men				Statistical significance [†]	
(µmol/l plasma)	Median	Min	Max	Median	Min	Max	P<
β-Carotene	0.240	0.01	6.52	0.320	0.019	2.93 0	0.001
α-Carotene	0.061	0.001	0.706	0.072	0.006	1.463	0.001
β-Cryptoxanthin	0.130	0.001	1.237	0.160	0.001	1.938	0.001
Lycopene	0.250	0.007	1.219	0.250	0.010	1.320	NS
Lutein	0.292	0.027	1.105	0.286	0-057	1.494	NS
Total carotenoids	1.920	0.195	11-184	2.006	0.169	11.667	0.01
β-Carotene (%)	12.44	1.02	5 8·30	16.05	2.81	46.38	0.001
Retinol: Mean	2.205			1.895			0.001
SD	0.573			0-529			

Min, minimum; Max, maximum; NS, not significant. *944 Men and 938 women except for LUT (466 and 467 respectively). †Mann-Whitney test except for retinol (*t* test).

Carotenoids in both sexes tended to be lowest between January and March (except BCP which was highest) and highest in the last 6 months of the year. BC increased (men r 0.091, P < 0.01; women r 0.177, P < 0.001) and LYC fell with age (men r -0.256, P < 0.001; women r -0.237, P < 0.001). The difference in BC between the sexes increased with age. We suggest that the similarity between the sexes for plasma LYC and LUT indicates that the intake of total dietary carotenoids by men and women is not different, which is borne out by dietary evidence (Bull, 1985; Nelson, 1986). The lower concentration of plasma provitamin A carotenoids in men may indicate a higher utilization of dietary BC, AC and BCP for retinol synthesis in the gut and therefore less provitamin A carotenoid is able to enter the blood stream.

DIT is supported by the Department of Health and Social Security, UK.

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Thurnham, D. I., Smith, E. & Flora, P. S. (1987). 8th International Symposium on Carotenoids, Boston, Abstract 48.

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Placebo effects explained by a sex difference in vitamin A requirements and changes in plasma retinol in adults. By D. I. THURNHAM, MRC Dunn Nutrition Laboratories, Cambridge CB4 1XJ, N. MUNOZ, International Agency for Research on Cancer, Lyon, France and K. M. HAMBIDGE, Department of Pediatrics, University of Colorado, USA

In September 1983, 610 age- and sex-matched Chinese villagers were given a supplement of retinol (A, 15 mg), riboflavin (B₂ 200 mg) and zinc (Zn 50 mg) or a placebo of mannitol (M, 536 mg) once a week for 13.5 months. Measurements of plasma A, Zn and tocopherol (E) at the start and end of the study (November 1984) showed an increase of approximately 20–30% (Table) in the placebo group independent of sex, but β -carotene (BC) increased only in the women. B₂ status was measured using erythrocyte glutathione reductase (*EC* 1.6.4.2; EGRAC).

Differences (µmolll) in plasma values between September 1983 and November 1984

Group		Supple	ement			Plac	Statistical significance †		
		Mean	SD	%*	'n	Mean	SD	%*	P<
Retinol	288	0·527ª	0.437	47.7	291	0-249ª	0.358	26.6	0.001
Zinc	242	1-86ª	3.15	17.6	256	1.88ª	3-08	18·3	NS
B ₂ (EGRAC)	283	-0·408ª	0.303	-22.9	289	-0.048	0.336	-1.4	0.001
Tocopherol	288	3.49ª	4.04	27-2	291	3.50ª	3.92	27.1	NS
β-Carotene (♀)	144	0.098ª	0.183	45.8	152	0.064ª	0.203	34 ·1	NS
β-Carotene (d)	144	0.034	0.160	29.5	139	0.023	0.111	21 ·1	NS

NS, not significant. *Increase expressed as % of 1983 value. †*t* test between treatments. Significant difference between two surveys (paired *t* test): **P*<0.001.

B₂ status did not appreciably increase in the placebo group, suggesting that distribution of treatments went as planned. Quality control procedures suggested no change in method bias for A, E or BC assays between 1983 and 1984, and the different changes in BC between the sexes indicated that a method bias is unlikely. An increase in BC availability could explain the increase in plasma A in the placebo group. The seasonal increase in vegetables or the antioxidant effect of M, or both, in the placebo group and a sparing action of the supplementary retinol in the supplement group may have increased BC availability. Higher requirements for vitamin A in men than in women (Thurnham & Flora, 1988) may have led to the utilization of all the increases in E and Zn are linked to A, for differences in plasma E and Zn between the two surveys were only significant in that group whose plasma A increased (mean and sE of difference: E 4.09 (3.88) μ mol/l, *n* 484; Zn 2.12 (3.04) μ mol/l, *n* 416; both *P*<0.001, paired *t* test) and did not change in the group whose A remained the same or decreased (E 0.43 (2.88) μ mol/l, *n* 95; Zn 0.53 (3.17) μ mol/l, *n* 80; not significant) (Thurnham *et al.* 1988).

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Thurnham, D. I. & Flora, P. S. (1988). Proceedings of the Nutrition Society 47, 181A. Thurnham, D. I., Munoz, N. & Lu, J. B. (1988). European Journal of Human Nutrition (In the Press). Effects of desferrioxamine on the influence of vitamin E deficiency on rodent malaria. By A. M. OMWEGA, P. C. BATES and D. J. MILLWARD, Nutrition Research Unit, London School of Hygiene and Tropical Medicine, 4 St Pancras Way, London NW1 2PE

Vitamin-E-deficiency (-E) markedly reduces parasitaemia in the malaria-infected rat, but the severe anaemia, growth inhibition and mortality is not improved compared with heavily parasitized vitamin-E-supplemented (+E) infected rats (Omwega *et al.* 1988). This suggests that in the -E rats there is increased susceptibility to oxidant stress with erythrocyte lysis at a lower level of parasitaemia. Desferrioxamine (DFO) removes free Fe³⁺ and reduces catalytic production of free radicals. It has been used for the treatment of a number of oxidant damage-related conditions and is proposed as potential therapy against the malarial pathology due to increased free Fe³⁺ originating from erythrocyte lysis (Clark, 1984). In this study, we have investigated how DFO treatment affects the response of the vitamin-E-deficient rat infected with *Plasmodium berghei*.

Male Sprague-Dawley rats were fed on a diet containing 200 g protein/kg, with or without vitamin E (+/-E) (Omwega *et al.* 1988). After 2 weeks, a group from each dietary treatment (+/-E) was parasitized, half of which was given a daily subcutaneous injection of DFO (80 mg/kg body-weight, +/-E:P:DFO) or saline (+/-E:P) for 14 d. Uninfected animals were similarly treated, but only results for the parasitized groups are presented here (see Table).

Effect of desferrioxamine on the influence of vitamin-E-deficiency on the Plasmodium berghei-infected rat

							P	rotein s	ynthesis*	
	Body-w	rt (g)	Haemogle	obin (g/l)	Parasita	emia	Muso	cle	Live	:г
Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
+E:P	120.7	8	28.2	5.0	63-1	7	3.2	0.8	51.4	10
+E:P:DFO	139-1	12	29.9	5.5	67.3	11	2.2	0.8	72·2	14
-E:P:DFO	141-4	17	23.2	4-0	58.3	13	2.4	1.4	61.0	12
-E:P	151.0	24	31.1	7-4	27.3	5	3.4	1.5	50-9	10

*Per cent synthesized/d.

DFO increased the parasitaemia enabling the -E:P:DFO rats to accommodate as many parasites as both the +E:P:DFO and +E:P rats. However, DFO did not improve the severe anaemia, the loss of body-weight or the reduced rate of muscle and liver protein synthesis. We would assume that in the absence of free Fe³⁺, premature lysis of the -E erythrocytes does not occur, allowing increased parasitaemia. This suggests that erythrocyte lysis in the -E rat is caused by oxidative haemolysis from external, Fe³⁺-catalysed free radical production, rather than any reduced ability to cope with internal stress associated with parasite development. This points to the importance of the catalytic action of free iron (Fe³⁺) as a mediator of oxidative damage.

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Validation of a questionnaire as a method of estimating calcium intake. By J. M. LOUGHRIDGE, T. E. FOX and R. SHEPHERD, ARFC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA

In order to assess the possible requirement for dietary supplementation in females susceptible to osteoporosis, a quick method of screening is required to initially determine calcium status.

A questionnaire was designed and tested against 3-d weighed dietary records to assess Ca intake in fifty-eight subjects (twenty-nine males and twenty-nine females, mean ages 33 and 30 years respectively). The questionnaire consisted of four major food categories (dairy products, cereal products, vegetables and fish), which were divided into a total of eighteen questions in the form of boxes which asked information on frequency and quantity of foods consumed. Pre-determined estimates of portion sizes were used in the questionnaire (except where foods were consumed in the standard weight 'as purchased'); this method of estimation has been shown to give a better correlation than a quantitative method such as household measures (Cummings *et al.* 1987). Foods included were based on their frequency of consumption, and hence their contribution to total Ca intake, rather than their individual Ca content.

The responses were translated into computable data by allocating standard portion sizes for each food item and calculating the corresponding Ca content; data from other studies were used in this process, as well as actually weighing out appropriate portion sizes. Ca values were calculated using standard food tables (Paul & Southgate, 1978); the daily mean Ca intake for all subjects from the questionnaire was 1052 mg compared with 1128 mg estimated from the weighed records ($t 2 \cdot 2$, P < 0.05), supporting the findings of Stockley & Broadhurst (1987). These two values were found to correlate at r 0.63 (P < 0.001). The questionnaire gave a higher estimate of mean daily dairy Ca intake than the weighed records (770 mg against 575 mg; r 0.48) and lower mean daily non-dairy Ca intake (282 mg against 553 mg; r 0.46). To account for Ca intake from water, the non-dairy Ca intake from the questionnaire was re-calculated using water intake calculated from the weighed records (6% of total Ca intake), which gave a better correlation (r 0.51).

Subjects were ranked in ascending order of Ca intake and divided into thirds. Twenty-six subjects occurred in the same third, thirty subjects were classified into adjacent thirds and two were classified in opposite thirds.

On the basis of the results, questionnaires can provide reasonably accurate estimates of Ca intake for use in screening and epidemiological surveys.

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Paul, A. A. & Southgate, D. A. T. (1978). McCance and Widdowson's The Composition of Foods, 4th ed. London: H. M. Stationery Office.

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Certain types of dietary fibre are effective in reducing blood cholesterol levels and improving glucose tolerance. We have investigated the effect of sugar-beet fibre (SBF) on glucose tolerance, insulin, gastric inhibitory polypeptide (GIP) secretion and circulating lipids, in comparison with guar gum and wheat bran.

Six healthy volunteers consumed on three separate occasions after an overnight fast a 100 g carbohydrate meal, or the same meal supplemented with 10 g guar gum or SBF. Following both fibre-supplemented meals there were significant reductions in postprandial peak arterialized blood glucose concentrations (P < 0.01) compared with the control meal. The area under the plasma insulin curve (AUC) was significantly reduced by guar gum supplementation (P < 0.01, Student's paired t test) but not by SBF (AUC 0–180 min: control meal 8093 (se 1095) μ U/l.min, guar gum 3105 (se 388) μ U/l.min, SBF 7634 (se 1424) μ U/l.min). Plasma GIP levels were significantly reduced by guar gum (P < 0.05) and increased by SBF (P < 0.025) at 40 min when insulin and glucose levels were maximal (GIP = 1042 (se 148) pg/ml for control meal v. 747 (se 69) and 1432 (se 218) pg/ml for guar gum and SBF respectively).

SBF, guar gum and bran were each incorporated into bread such that half a loaf provided 1.29 MJ (303 kcal), 10 g protein, 67 g carbohydrate and 20 g fibre. Seven healthy volunteers consumed half a loaf of each bread as part of their habitual diet for 14 d. Food diaries (3 d) were completed whilst subjects were consuming each bread and dietary advice given to minimize any changes in total energy and fat intakes. Fasting blood samples were taken at weekly intervals for 14 d before, during and for 14 d after each fibre supplementation period. Plasma total cholesterol levels were reduced by 11.7 (sE 1.8)% (P<0.001) during guar gum supplementation and by 4.6 (sE 1.9)% (P<0.025) during SBF supplementation. Total cholesterol levels were unchanged during bran supplementation. Circulating high-density lipoprotein cholesterol, triglyceride and glucose and insulin levels were also unchanged by the fibre supplementation.

SBF has a spectrum of activity similar to that of guar gum, although its effects are less pronounced. The effect of SBF on glucose tolerance and circulating cholesterol may be due to its soluble fibre content of approximately 25% pectin. The lack of effect of SBF on post-prandial insulin secretion in spite of an improvement in glucose tolerance could be ascribed to increased secretion of the insulin-stimulating gastrointestinal hormone GIP after the SBF supplemented meal.

Influence of non-digestible polysaccharides on small-bowel cytokinetics and circulating enteroglucagon levels in the rat. By J. M. GEE and I. T. JOHNSON, Department of Nutrition and Food Quality, AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA

The polysaccharides which are classified as dietary fibre share the common characteristic of resistance to hydrolysis by endogenous digestive enzymes, but they differ strikingly in their physical properties and biological effects. In previous studies we have shown that soluble components of dietary fibre alter the gross morphology of the rat gut, and increase the rate of mucosal cell proliferation in the small intestine (Johnson & Gee, 1986). The present study was undertaken to determine whether these changes were related to increased circulating levels of the gastrointestinal peptide enteroglucagon (EG), which is a putative mucosal growth hormone (Riecken & Gregor, 1985).

Groups of ten male Wistar rats (approximately 140 g) were housed singly in wire-bottomed cages, and allowed either fibre-free or non-absorbable polysaccharide supplemented diets *ad lib*. for 14 d, during which time both food intake and faecal production were monitored. The treatment diets contained insoluble cellulose, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose (HPMC), guar gum, pectin or gum arabic (100 g/kg). On the first morning after the feeding period, the rats were killed sequentially, following an injection of vincristine sulphate (1 mg/kg body-weight). A sample of blood was collected from the vena cava for the determination of EG by radioimmunoassay. The pH of the caecal contents was also measured by direct insertion of a pH electrode. The small intestine was removed and measured, and tissue was collected from the distal ileum for the measurement of crypt cell production rate (CCPR) by the metaphase arrest technique.

Dietary supplementation with insoluble cellulose had no significant effect on circulating levels of EG. All the groups given polysaccharide gums had higher plasma EG levels than rats fed on insoluble cellulose. However, only those fed on the most viscous materials showed evidence of increased crypt cell production in the distal ileum. Stimulation of CCPR was not related to the fermentability of the polysaccharides, as judged by acidification of the caecal contents and faecal excretion.

	Plasma EG (ng/ml)		CCI (mitoses/cr		Caecal pH		
	Mean	SE	Mean	SE	Mean	SE	
Group†							
Gum arabic	1.63*	0.14	22.6NS	3.8	5.64*	0.15	
Control	0.27	0.04	20.8	1.8	6.82	0.05	
HPMC	0.56*	0.08	26.7*	2.2	7.2*	0.07	
Control	0.19	0.06	17.6	3.0	6.8	0.04	

Plasma EG, ileal CCPR and caecal pH in rats given gum arabic or HPMC

NS, not significant.

*Significantly different from their controls (P < 0.05).

[†]Control group given insoluble cellulose.

The failure of low-viscosity polysaccharides such as gum arabic to stimulate CCPR, despite increased EG levels, suggests that although the peptide may play a role in this example of diet-induced mucosal hyperplasia, the presence of additional stimuli, such as a high luminal viscosity and perhaps delayed nutrient absorption, is also necessary.

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