Proceedings of the Nutrition Society

Abstracts of Original Communications

A Scientific Meeting was held at the University of East Anglia, Norwich, 28 June–1 July 2005, when the following papers were presented.

All abstracts are prepared as camera-ready material.

The Editors of the Proceedings of the Nutrition Society *accept no responsibility for the abstracts of papers read at the Society's meetings for original communications.*

https://doi.org/10.1017/S002966510500073X Published online by Cambridge University Press

A randomised trial investigating the effects of a low-carbohydrate weight-loss regimen on cardiovascular risk factors in men. By R. BOLEY, K. HART and H. TRUBY, *Nutrition, Dietetics and Food Division, University of Surrey, Guildford, Surrey, UK, GU2 7XH*

Traditional dietary approaches are failing to impact on the obesity epidemic, therefore novel weight-loss regimens warrant further investigation.

Low-carbohydrate diets provide one dietary strategy for weight loss, yet whilst their efficacy for weight reduction has been demonstrated, further information is needed. A systemic review (Bravata *et al.* 2003) recently evaluated the efficacy and safety of low-carbohydrate diets, concluding there was insufficient evidence to make recommendations for or against their wider use.

The present study aims to comprehensively investigate the metabolic, lipaemic, haemostatic and vascular effects of a low-carbohydrate diet on cardiovascular (CV) risk in obese men. Furthermore, the additive effect of exercise will be investigated, an area highlighted by Bravata *et al.* (2003) for which evidence is currently lacking.

Sixty healthy, overweight (BMI>27 and <40 kg/m^2) men, aged 21–65 years, are being recruited to follow a low-carbohydrate diet for 6 months. Measures taken throughout the study period include anthropometrics, blood pressure, body composition, fasting insulin and glucose, lipid profile, C-reactive protein, fibrinogen, renal profile, dietary intake and physical activity and fitness. Participants are randomised to one of two intervention groups; group one will be asked to maintain current activity levels and group two will be encouraged to increase activity levels, aiming for a minimum of 30 minutes of moderate intensity exercise 3 times per week.

To date 47 subjects have commenced the study, therefore recruitment, data collection and analysis are on-going. Currently 32 subjects have had 2 month measurements taken and 22 subjects have completed the 6-month intervention, with preliminary data presented here. Paired t tests were applied to assess changes over time. Data from those subjects who had withdrawn from the study was excluded. The mean absolute weight loss achieved in the sample as a whole (group 1 and 2 combined) over 2 months was −7.9 (SD 5.15) kg (P<0.001 compared to baseline); mean waist circumference decreased significantly (P<0.001) from 110 (SD 8.8) cm at baseline to 101 (SD 8.8) cm at 6 months, and blood pressure fell from 134/85 to 123/79 mm/Hg. This fall in systolic blood pressure was significant (P=0.01). Fasting triacylglycerol (P=0.01) and insulin levels (P=0.027) were significantly reduced at 2 months compared with baseline (n 14). No significant change in overall estimated 10 year CVD risk (NCEP, 2002) was observed in subjects following a low carbohydrate diet. Fitness increased significantly in group 2 between baseline and 6 months (P=0.016) and decreased (non significantly) in group 1 over the same time period. No significant difference in the change in CVD risk was seen between subjects following a low carbohydrate diet with or without the additional exercise programme.

Results as this early stage show positive changes in several individual CVD risk factors when a low carbohydrate diet is followed with no additional benefit of an exercise intervention and no overall change in mean risk of CVD assessed as described in NCEP, 2002. However as not all data is available the study is currently underpowered for this analysis. Data collection is continuing and further information regarding the additional impact of exercise, and the changes that occur in the individual components of CV risk will be available as the study progresses.

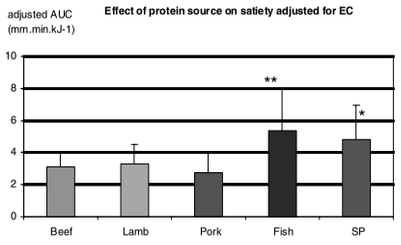
Bravata DM, Sanders L, Huang J, Krumholz HM, Olkin I & Gardner CD (2003) **289**, 1837–1850.
National Cholesterol Education Programme (NCEP) (2002) Expert Panel. detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). Final Report.

Influence of different high-protein meals on subsequent food intake. By F.A PUGLIA and C.J.K. HENRY, *Nutrition and Food Science Group, School of Biological and Molecular Sciences, Oxford Brookes University, Gipsy Lane Campus, Headington, Oxford, UK, OX3 0BP*

Obesity is a major health concern for developed and developing countries (Zimmermann-Belsing & Feldt-Rasmussen, 2004). With the obesity epidemic, energy balance has become the focal point of much research. Energy balance is the difference between energy intake (EI) and expenditure. Many factors influence EI: psychological and social pressure, energy density, and food composition. It is acknowledged that proteins are more satiating than carbohydrates and fats (Stubbs, 1995). However, there is little data on the role of protein quality on satiety and the results are inconsistent (Uhe *et al.* 1992; Lang *et al.* 1998).

The present study investigated the role of protein quality on satiety and short-term food intake. Twenty-eight healthy volunteers (aged 30.0 (SD 8.4) years; BMI 23.8 (SD 3.3) kg/m^2) were recruited to test, on separate occasions, five lunches prepared with different protein sources (beef, lamb, pork, fish and soya protein). These lunches were only matched for the amount of protein (about 75 g). Subjects recorded their satiety feelings using visual analogue scales (VAS) and their subsequent food intake in a food diary. Areas under the curve were calculated for the VAS data and dietary records were analysed using Windiet ® (Aberdeen, UK).

Analysis of the VAS showed that the five lunches had the same effect on satiety (P>0.05) and that *a priori* protein quality had little or no influence on satiety. However, once adjusted for energy content (EC), significant differences could be observed (Fig. 1). The examination of subsequent food intake revealed some interesting observations. Preliminary results showed a very strong correlation between EI at lunch and subsequent EI (Fig. 2); consumption of low-EC lunches led to the consumption of less energy at subsequent meals. These results suggest that compensatory food intake may be influenced by the energy density and the protein quality. Further investigations are needed to explore the associative or independent effects of energy density and protein quality on food intake.



*Significantly different from sources other than fish;
**significantly different only from pork.

Fig. 1. Effect of protein source on satiety.

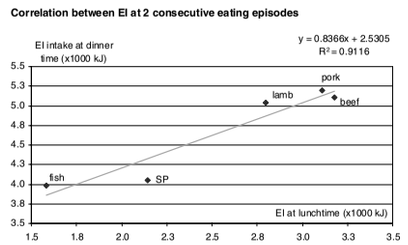


Fig. 2. Correlation between two consecutive meals.

The project is sponsored by the Meat and Livestock Commission, Milton Keynes, UK.

Lang V, Bellisle F, Oppert JM, Craplet C, Bornet FRJ, Slama G & Guy-Grand B (1998) *American Journal of Clinical Nutrition* **67**, 1197–1204.
Stubbs RJ (1995) *International Journal of Obesity* **19**, S11–S19.
Uhe AM, Collier GR & O'Dea K (1992) *Journal of Nutrition* **122**, 467–472.
Zimmermann-Belsing T & Feldt-Rasmussen U (2004) *Endocrinology* **145**, 1501–1502.

The use of ready-to-eat breakfast cereals as a meal substitute for weight loss – the impact of variety. By H.J. LIGHTOWLER[1], C.J.K. HENRY[1], J.M. WARREN[1] and D.P. RICHARDSON[2], [1]*Nutrition and Food Science Group, School of Biological and Molecular Sciences, Oxford Brookes University, Gipsy Lane Campus, Headington, Oxford, UK, OX3 0BP and* [2]*DPR Nutrition Ltd, 34 Grimwade Avenue, Croydon, Surrey, UK, CR0 5DG*

Over 90% of the British population has tried dieting at some time or other and at any one time a third of the adult population is attempting to lose weight. A recent review of the efficacy of partial meal replacement concluded that this type of intervention can safely and effectively produce significant weight loss and improve weight-related risk factors of disease (Heymsfield *et al.* 2003). Currently there is interest in the potential use of ready-to-eat breakfast cereal in weight loss and long-term weight control (Kirk *et al.* 2000). The aim of the present study was to assess the effectiveness of providing a mixed variety of ready-to-eat breakfast cereals as a meal substitute in achieving weight loss.

Fifty-four overweight subjects (BMI between 25 and 35 kg/m^2) were recruited. Forty-one subjects (75%) completed the present study; sixteen males and twenty-five females, mean age 47 (SD 12) years. Subjects were assigned to either a single cereal (Fitnesse) or variety-cereal (Fitnesse, Shredded Wheat Bitesize and Triple Berry Shredded Wheat) arm of the study. All subjects consumed their allocated ready-to-eat breakfast cereal(s) for breakfast and lunch for 2 weeks followed by *ad libitum* intake for the rest of the day. For weeks 3–6, subjects continued with their allocated breakfast. Anthropometric measurements (height, weight, waist and hip circumferences) were taken at baseline, at week 2 and at week 6. Ethical approval for the present study was obtained from the university research ethics committee at Oxford Brookes University.

The Table shows the mean change in anthropometric measurements from baseline at week 2 and week 6 for the single cereal and variety-cereal groups.

	Single cereal group				Variety-cereal group			
	Week 2		Week 6		Week 2		Week 6	
	Change	*P*	Change	*P*	Change	*P*	Change	*P*
Weight (kg)	−0.7	0.008	−0.6	0.127	−1.5	<0.001	−2.0	0.001
BMI (kg/m^2)	−0.2	0.016	−0.2	0.138	−0.5	<0.001	−0.7	0.001
WC (cm)	−0.9	0.169	−2.3	0.005	−2.0	<0.001	−3.5	<0.001
HC (cm)	−0.1	0.846	−0.6	0.243	−1.1	0.004	−1.7	0.001

WC, waist circumference; HC, hip circumference.

The results show that ready-to-eat breakfast cereals used as a meal substitute are an effective method of achieving weight loss. The mean weight loss at week 2 of the study was −1.1 (range −3.7 to +1.5) kg and the mean weight loss at week 6 was −1.4 (range −6.9 to +2.5) kg. At the end of week 2, 85% (*n* 35) of subjects had lost weight and at the end of week 6, 73% (*n* 30) of subjects had lost weight. Significantly greater weight loss was achieved in those subjects randomly assigned to the variety-cereal arm of the study than the single cereal.

In conclusion, ready-to-eat breakfast cereals used as a meal substitute resulted in significant weight loss in a 2-week study and this was sustained at 6 weeks. The provision of a variety of cereals for subjects to choose from enhanced weight loss.

This work was funded by Cereal Partners UK Ltd.

Heymsfield SB, van Mierlo CAJ, van der Knaap HCM, Heo M & Frier HI (2003) *International Journal of Obesity* **27**, 537–549.
Kirk T, Crombie N & Cursiter M (2000) *Journal of Human Nutrition and Dietetics* **13**, 277–285.

Effect of basic and advanced formulation carbohydrate–electrolyte drinks on physical and mental performance during high-intensity intermittent exercise. By G. BEGUM[1], A. CUNLIFFE[1], M. LEVERITT[1], A. ESGATE[2], M. FRANKLIN[3] and C. CHOWDREY[1], [1]*Department of Human and Health Sciences, University of Westminster, 115 New Cavendish Street, London, UK, W1W 6UW,* [2]*Department of Psychology, University of Kingston, Penrhyn Road, Kingston-upon-Thames, UK, KT1 2EE and* [3]*Research School, Biology and Molecular Sciences, Oxford Brookes University, Gipsy Lane, Oxford, UK, OX3 7JX*

There is a substantial body of evidence to support that ingestion of carbohydrate–electrolyte (CHO–elec) drinks during prolonged continuous exercise can improve physical performance. Such feedings could also improve central nervous system function via the provision of glucose or via the attenuation of rising serotonin (5-HT) levels in the brain (Blomstrand, 2001). Few studies, however, have compared the efficacy of drinks from a range of formulations on both physical and mental performance during prolonged, high-intensity intermittent activity (characteristic of sports such as football).

The present study assessed the effects of a basic drink (water, glucose and electrolytes) and a commercially formulated drink on physical and mental performance before (Pre), at half-time and after (Post) a 90 min intermittent treadmill protocol. The following measures were carried out:

(a) Objective measures: substrate availability (blood glucose), metabolite accumulation (blood lactate), biochemical correlate of central serotonin (plasma ratio of tryptophan to large neutral amino acids; Trp:LNAA), hydration status (change in body weight and plasma volume), physical performance (run to fatigue) and mental performance (short-term memory (STM), simple reaction time (SRT), choice reaction time (CRT), pattern recognition (PR), motor control, critical flicker fusion threshold (CFF));
(b) Subjective measures: perceived exertion (Borg scale) and mood state (Profile of Mood States (POMS) Questionnaire).

Twelve healthy male subjects, mean age 27 (SEM 2.1) years, were randomly allocated to four trials (no drink (ND), placebo (PLA), basic CHO–elec drink (BASIC) and a commercial (advanced) sports drink (ADV)) with a minimum of a 1-week wash-out period.

	Trp:LNAA*				SRT (ms)				CRT (ms)				CFF (%Δ from baseline)†			
Time…	Pre		Post		Pre		Post		Pre		Post		Half-time		Post	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
ND	0.005	0.001	0.012	0.002	242	12.8	232	12.1	469	21.1	445	22.0	−0.26	0.8	−1.09	1.5
PLA	0.005	0.001	0.010	0.002	233	9.6	227	9.6	468	18.1	459	20.0	−0.91	1.3	0.38	1.9
BASIC	0.004	0.001	0.008	0.001	233	12.3	222	9.1	451	19.1	460	24.2	2.74	1.0	4.32	1.0
ADV	0.005	0.001	0.007	0.001	229	8.5	215	7.4	473	16.3	460	19.3	2.93	0.5	4.81	0.7

* Significant effect of drink condition ($P<0.01$) and time ($P<0.01$).
† Baseline values (ND = 32 Hz, PLA = 32 Hz, BASIC = 33 Hz, ADV = 33 Hz). Significant effect of drink condition ($P<0.01$).

There appears to be a degree of dichotomy in the results, in which mental performance tests (CRT, STM, PR and SRT) were only marginally improved with increasing complexity of drink formulation whereas a clearer effect was observed on run to fatigue times (ND = 2.24 min, PLA = 2.69 min, BASIC = 3.13 min, ADV = 3.91 min $P<0.01$). These data suggest that CHO–elec drinks clearly improve physical performance during intermittent exercise; however, further research is required to fully elucidate the benefits of these drinks on mental performance.

Blomstrand E (2001) *Amino Acids* **20**, 25–34.

Increased wholegrain food consumption and coronary heart disease risk factors: the CHEW-IT study. By A.R. JONES[1], S. KUZNESOF[1], D.P. RICHARDSON and C.J. SEAL[1], [1]*Human Nutrition Research Centre, School of Agriculture, Food and Rural Development, University of Newcastle, Newcastle upon Tyne, UK, NE1 7RU and* [2]*DPR Nutrition Limited, 34 Grimwade Avenue, Croydon, Surrey, UK, CR0 5DG*

A growing body of evidence indicates that a diet rich in wholegrain foods (WGF) may reduce the risk of several chronic diseases including heart disease, certain cancers and type 2 diabetes (Smith *et al*. 2003). As a result of the complex nutritional characteristics of whole grains, the mechanisms by which they may exert their protective effects are diverse (Slavin, 2003). With specific reference to increased consumption of WGF and reduced risk of CHD, the possible beneficial effects include a reduction in blood pressure (BP) and the favourable modification of lipid profiles, antioxidant status and the inflammatory state. Increased whole-grain intake may also be involved in weight management thus decreasing CHD risk (Anderson, 2003). The present study examines the effects of increased WGF consumption on a number of CHD risk factors.

Subjects habitually consuming less than three servings of WGF/d were recruited into a 16-week intervention study during which they were asked to consume three servings of WGF/d for the first 8 weeks and six servings of WGF/d for the final 8 weeks. Prescribed quantities of WGF were provided to aid compliance. Food intake was assessed at baseline, 8 weeks and 16 weeks using a 4 d diary. Daily whole-grain intake was calculated using ingredient data for foods containing >10% whole grain, expressed on a DM basis. At 4-weekly intervals body weight, BMI, percentage body fat (by bioelectrical impedance) and BP were measured. Blood samples were obtained at each timepoint and plasma lipid profiles were examined using standard enzymic procedures on a clinical analyser.

Results are reported here for twenty-one subjects (fourteen females, seven males; mean age 34.8 years) who completed all elements of the study at each timepoint of the intervention. There was a significant increase in total whole-grain consumption during the study (see Table). The plasma concentrations of total cholesterol and LDL-cholesterol fell by approximately 6 and 10%, respectively, at 8 weeks. However, this reduction was not maintained when WGF consumption was increased further at 16 weeks and was not statistically significant. There were no changes observed for other measures, although there appeared to be a slight reduction in systolic BP with increased WGF consumption.

	Baseline (*n* 21)		8 weeks (*n* 21)		16 weeks (*n* 21)		
	Mean	SEM	Mean	SEM	Mean	SEM	*P**
Total whole-grain intake (g/d)	23.4	4.10	73.2	5.59	110.4	6.11	<0.001
Total cholesterol (mmol/l)	5.0	0.19	4.7	0.19	4.9	0.21	NS
Triacylglycerols (mmol/l)	1.0	0.07	1.1	0.07	0.98	0.06	NS
HDL-cholesterol (mmol/l)	1.5	0.06	1.5	0.06	1.6	0.05	NS
LDL-cholesterol (mmol/l)	3.0	0.18	2.7	0.18	2.9	0.21	NS
Body weight (kg)	68.5	2.60	68.2	2.58	68.4	2.47	NS
BMI (kg/m^2)	23.9	0.72	23.9	0.73	23.9	0.71	NS
% Body fat	25.4	1.99	26.4	1.94	25.3	2.00	NS
Systolic BP (mmHg) (*n* 20)	120.0	3.56	114.0	2.95	111.9	2.81	NS
Diastolic BP (mmHg) (*n* 20)	77.9	2.67	73.6	1.86	75.3	1.73	NS

* *P* values determined using one-way ANOVA.

The results of the present study show that US recommendations for the consumption of three servings of WGF/d can be readily achieved. Data from focus groups and the study questionnaire will explore those factors influencing whole-grain intake and the key methods by which the recommendation can be achieved. The present study, with small subject numbers, found that increased consumption of WGF had no effect on BP, indicators of obesity or lipid profile. The use of a controlled intervention of this type in a larger population is planned in order to achieve a greater understanding of the mechanisms by which WGF affect disease risk.

A.R.J. is in receipt of a BBSRC CASE Studentship with Cereal Partners, UK.

Anderson JW (2003) *Proceedings of the Nutrition Society* **62**, 135–142.
Slavin J (2003) *Proceedings of the Nutrition Society* **62**, 129–134.
Smith AT, Kuznesof S, Richardson DP & Seal CJ (2003) *Proceedings of the Nutrition Society* **62**, 455–467.

The relationship between overweight and lifestyle factors in children from the National Diet and Nutrition Survey: young people aged 4–18 years. By L.C.A. CRAIG[1,2], J. LOVE[1], B. RATCLIFFE[1] and G. MCNEILL, [1]*The Robert Gordon University, Aberdeen, UK, AB25 1HG and* [2]*University of Aberdeen, Aberdeen, UK, AB25 2ZD*

Genetic factors cannot explain the rapidly increasing prevalence of obesity being seen worldwide; therefore environmental factors need to be considered. Energy imbalance, excessive intake or inadequate expenditure, promotes weight gain and leads to the development of obesity (World Health Organization, 1998). The aim of the present study was to investigate the relationship between overweight and lifestyle factors in children from the National Diet and Nutrition Survey: young people aged 4–18 years, a nationally representative survey of the UK population carried out in 1997 (Gregory *et al*. 2000). Height and weight were measured in 1942 children. Records of weighed dietary intakes were obtained for 1701 children, and 1424 children aged 7–18 years kept a diary of their physical activities. Data on time spent in inactive pursuits were collected for 1784 children. The data were divided into overweight and non-overweight according to BMI using the international cut-offs (Cole *et al.* 2000). Logistic regression analysis was applied to compare overweight and non-overweight groups for dietary and physical activity habits adjusting for demographic confounders. The dietary (except reported dieting to lose weight) and physical activity data were split into tertiles and the Table shows the odds ratio (OR) and 95% CI for highest *v.* lowest tertiles. Analysis was carried out first for all children adjusting for age and sex then by sex and age group (4–10 years and 11–18 years). The diet score was calculated from the absolute amounts of 'healthy' or 'unhealthy' (high-fat or -sugar) foods eaten; the higher the score, the 'healthier' the diet.

			4–10-year-olds				11–18-year-olds			
	All children		Boys		Girls		Boys		Girls	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Dietary intake										
Average daily energy intake	1.6†	1.2, 2.7	2.2*	1.1, 4.5	3.3‡	1.7, 6.5	1.1	0.6, 1.9	1.1	0.6, 1.9
% Energy from fat	1.1	0.8, 1.4	1.0	0.5, 1.8	0.9	0.5, 1.5	1.2	0.7, 2.1	1.3	0.7, 2.2
% Energy from sugars	0.6‡	0.5, 0.8	0.7	0.4, 1.3	1.0	0.5, 1.7	0.6	0.4, 1.1	0.4†	0.2, 0.6
% Energy from NMES	0.6†	0.4, 0.8	0.7	0.4, 1.4	0.7	0.4, 1.2	0.7	0.4, 1.2	0.4†	0.2, 0.7
Diet score	1.5†	1.2, 2.0	1.5	0.8, 2.9	1.5	0.9, 2.5	1.4	0.8, 2.4	1.8*	1.0, 3.1
Dieting to lose weight §	4.4‡	2.8, 6.8	10.9†	1.9, 62.6	4.4	0.9, 21.0	12.6‡	4.4, 36.0	3.3‡	1.8, 6.1
Physical activity										
Calculated activity score¶	0.8	0.6, 1.0	1.1	0.5, 2.6	0.6	0.3, 1.3	0.8	0.4, 1.4	0.7	0.4, 1.2
Time spent in exercise¶	0.6†	0.5, 0.9	1.1	0.5, 2.5	0.5	0.3, 1.1	0.6	0.3, 1.1	0.6	0.4, 1.1
Time spent in inactive pursuits	1.7‡	1.3, 2.3	1.7	0.9, 3.2	1.9*	1.1, 3.5	1.6	0.9, 2.8	1.7*	1.0, 2.9

* *P* for trend <0.05; † *P* for trend <0.01; ‡ *P* for trend <0.001.
§ Yes *v.* no.
¶ Children aged 7–18 years.

Overweight was inversely associated with percentage energy from total sugars and non-milk extrinsic sugars (NMES) and positively associated with average daily total energy intake and diet score ('healthier' diet) and reported dieting to lose weight. Time spent in inactive pursuits was positively associated with overweight and time spent in exercise inversely associated with overweight. Time spent in inactive pursuits was more consistently found to be associated with overweight than time spent in physical activity, with these relationships being strongest in girls. The results suggest that energy intake and physical inactivity are important factors to target in the prevention of childhood obesity.

Cole TJ, Bellizzi MC, Flegal KM & Dietz WH (2000) *BMJ* **320**, 1240–1243.
Gregory J, Lowe S, Bates CJ, Prentice A, Jackson LV, Smithers G, Wenlock R & Farron M (2000) *National Diet and Nutrition Survey: young people aged 4–18 years. Volume 1: Report of the Diet and Nutrition Survey*. London: The Stationery Office.
World Health Organization (1998) *Obesity: Preventing and Managing the Global Epidemic. Report of a WHO Consultation on Obesity*. Geneva: WHO.

Does genetic variation modify associations between meat intake and colorectal cancer: results from a population-based, case–control study in North-East Scotland. By L.F. MASSON[1], L. SHARP[2], J. LITTLE[3], N.T. BROCKTON[1], S.C. COTTON[1], N.E. HAITES[1] and J. CASSIDY[1], [1]*School of Medicine, University of Aberdeen, Polwarth Building, Foresterhill, Aberdeen, UK, AB25 2ZD,* [2]*National Cancer Registry Ireland, Elm Court, Boreenmanna Road, Cork, Republic of Ireland and* [3]*Department of Epidemiology & Community Medicine, University of Ottawa, 451 Smyth Road, Ottawa, Canada*

Colorectal cancer incidence in Scotland is in the upper third of rates observed worldwide. Some studies suggest that intake of meat, particularly meat exposed to pyrolysis temperatures, is associated with increased colorectal cancer risk. However, the evidence is inconsistent. There is genetic variation in metabolism of the polycyclic aromatic hydrocarbons (PAH) present in meats exposed to pyrolysis temperatures, and this may account for some of the inconsistencies between studies. Cytochrome P4501A1 (CYP1A1) and glutathione S-transferases (GST) play key roles in phase I and phase II metabolism of the PAH, and the genes encoding the enzymes are polymorphic. We undertook a population-based, case–control study in Grampian to investigate the m1, m2 and m4 polymorphisms in *CYP1A1*, the *GSTM1* and *GSTT1* deletions, meat intake and colorectal cancer risk.

Eligible cases were Grampian residents diagnosed with histologically confirmed primary colorectal cancer during September 1998–February 2000. Controls were selected from the Community Health Index. Subjects completed a lifestyle questionnaire and a food-frequency questionnaire (Masson *et al.* 2003) and provided a mouthwash sample from which DNA was extracted. *CYP1A1* m1, m2 and m4 polymorphisms were detected by PCR and digestion with *Msp*I, *Bsr*D1 and *Bsa*I respectively. *GSTM1* and *GSTT1* deletions were detected directly by PCR. Reported meat intakes were adjusted for energy, and adjusted odds ratios (OR) were calculated by logistic regression in Stata.

Two hundred and sixty-four cases (62% of those eligible) and 408 controls (61%) participated. Individuals carrying the *CYP1A1* m4 variant were at significantly reduced risk of colorectal cancer (adjusted OR heterozygotes *v.* homozygous wild-types (wt)=0.31; 95% CI 0.13, 0.70). The *CYP1A1* m1, *CYP1A1* m2, *GSTM1* and *GSTT1* genotypes were not significantly associated with disease risk overall, and there was no strong association between meat intake and colorectal cancer overall. However, when subjects were categorised as low or high (below or above the median) meat consumers, there were significant interactions with the *CYP1A1* m1 ($P=0.049$), m2 ($P=0.032$) and m4 ($P=0.023$) polymorphisms, such that carriers of the m1 or m2 variants with a high meat intake had raised risk compared with those homozygous for the common alleles whose meat intake was low, and the reduced risk associated with the m4 variant was attenuated in those with high meat intake (see Table). There was no evidence of interaction between meat intake and either the *GSTM1* or *GSTT1* polymorphisms.

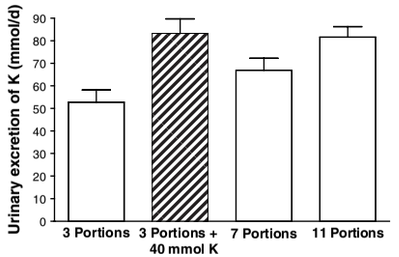
	CYP1A1 m1			*CYP1A1* m2			*CYP1A1* m4		
Meat intake	Genotype	OR	95% CI	Genotype	OR	95% CI	Genotype	OR	95% CI
Low	wt/wt	1.00	Reference	wt/wt	1.00	Reference	wt/wt	1.00	Reference
High	wt/wt	0.79	0.51, 1.24	wt/wt	0.88	0.59, 1.32	wt/wt	0.93	0.61, 1.40
Low	m1 carrier	0.61	0.29, 1.31	wt/m^2	0.44	0.11, 1.77	wt/m4	0.06	0.01, 0.51
High	m1 carrier	1.39	0.67, 2.87	wt/m^2	2.38	0.85, 6.69	wt/m4	0.57	0.22, 1.51
P (interaction)		0.049			0.032			0.023	

Our results suggest that individuals carrying the *CYP1A1* m4 variant have a reduced colorectal cancer risk, and that carriers of the *CYP1A1* m1 or m2 variants with a high meat intake have increased risk. Our results need confirmation, preferably in larger studies that have adequate power to detect gene–diet interactions.

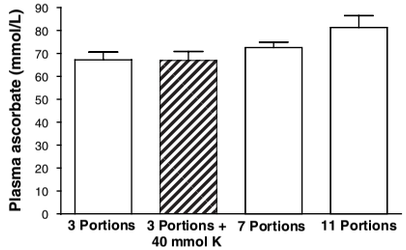
Masson LF, McNeill G, Tomany JO, Simpson JA, Peace HS, Wei L, Grubb DA & Bolton-Smith C (2003) *Public Health Nutrition* **6**, 313–321.

Urinary potassium excretion and plasma ascorbic acid concentration as biomarkers of increased intake of fruit and vegetable intake. By J.L. NICHOLAS, S.E.E. BERRY, U. MULLA, F.J. KELLY and T.A.B. SANDERS, *Nutritional Sciences Research Division, King's College London, 150 Stamford Street, London, UK, SE1 9NN*

Fruit and vegetable consumption is associated with a lower risk of CVD and cancer. Reliable biomarkers of fruit and vegetable intake are needed to evaluate the effectiveness of dietary advice. The present pilot study investigated the validity of urinary K excretion and plasma ascorbate concentrations as biomarkers of increased fruit and vegetable intake. A randomised cross-over design was used. Following a 1-week run-in on a baseline diet (K intake typical of the UK population; approximately 80 mmol/d for men and 60 mmol/d for women) providing three portions of fruit and vegetables per d, subjects were randomised to one of four orthogonal treatment sequences. The increased intakes provided seven or eleven portions of fruit and vegetables/d *v.* a reference intake of three portions/d (excluding potatoes). It was estimated that the highest intake would provide an additional K intake of 40 mmol/l. In order to ascertain whether this intake would be achieved in subjects, we examined the effect of a slow-release potassium citrate supplement (40 mmol/d) against a matching placebo and the reference diet. Subjects made a 24 h urine collection at the end of each dietary period and provided a fasting blood sample. A total of twelve subjects (three male, nine female) completed the study. The results are shown in the Figures below.



Urinary K excretion following different levels of K intake (mean with SEM).



Plasma ascorbate concentrations following different levels of K intake (mean with SEM).

K excretion increased with increasing intake of fruit and vegetables ($P=0.0006$ for linear trend) and the excretion on the intake providing an additional 40 mmol K/d was similar to that on the diet providing eleven portions/d. Plasma ascorbate concentration likewise increased with increasing fruit and vegetable intake ($P=0.0014$ for linear trend) but was unaffected by the K supplement. Urinary K excretion appeared to be a more sensitive indicator of fruit and vegetable consumption. The urinary K–creatinine excretion (data not shown) showed a similar dose–response relationship to the 24 h urinary K excretion.

The results of the present pilot study show that K excretion can be used as a reliable biomarker of fruit and vegetable intake. The K excretion on the highest intake of fruit and vegetables which was estimated to supply 40 mmol K/l was similar to that resulting from the consumption of 40 mmol/l potassium citrate.

The present study (N02030) was funded by the Food Standards Agency.

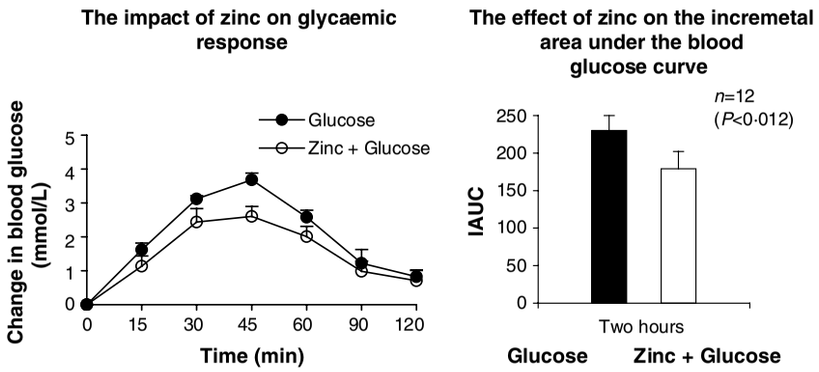
The role of zinc on glycaemic response. By T.A. SEYOUM, C.J.K. HENRY and H.J. LIGHTOWLER, *Nutrition and Food Science Group, School of Biological and Molecular Sciences, Oxford Brookes University, Gipsy Lane Campus, Headington, Oxford, UK, OX3 OBP*

Food-related, non-communicable diseases (NCD) such as obesity, heart disease and type 2 diabetes are major international public health threats. Despite global efforts to reduce the incidence of NCD, adequate treatment and interventions remain limited.

Low-glycaemic index (GI) foods have been shown to be beneficial in reducing the risk of NCD. The GI is defined as the incremental area under the blood glucose response curve (IAUC) of a 50 g carbohydrate portion of a test food expressed as a percentage of the response to the same amount of carbohydrate from a reference food taken by the same subject. Macronutrients, notably protein and fat, have been shown to influence GI. Zinc is implicated with the biosynthesis, storage and secretion of insulin. Therefore, the objective of the present study was to investigate the potential impact zinc may have on the blood glucose response curve.

The impact of 15 mg zinc gluconate (Holland & Barrett) on glycaemic response was investigated in twelve subjects – six males and six females aged between 20 and 45 years. The method used to measure glycaemic response was in line with the standard Food and Agriculture Organization/World Health Organization (1998) procedures: the test food (glucose with added Zn) was compared with a reference food (glucose) tested in equivalent amounts (50 g) of available carbohydrate with 200 ml water (Malvern still water). Blood glucose measurements were taken at fasting (0 min) and at 15, 30, 45, 60, 90 and 120 min postprandially.

The addition of 15 mg Zn to a glucose solution significantly decreased the blood glucose area under the curve for the first 45 min and 60 min by 30% ($P=0.03$) and 23% ($P=0.006$) respectively. Moreover, Zn-fortified glucose resulted in a significant 22% ($P=0.012$) decline in IAUC over the 2 h period.



The present study is the first undertaken to examine the effect of micronutrients on glycaemic response. The present results suggest that the addition of certain micronutrients may reduce the glycaemic response and thereby the GI of foods. Further research is required to understand the potential role micronutrients may play in glycaemic response and GI.

Food and Agriculture Organization/World Health Organization (1998) *Carbohydrate in Human Nutrition*. Rome: Food and Agriculture Organization.

The influence of ethnicity on the glycaemic index of foods. By A. ALDHAHERI, C.J.K HENRY and H.J. LIGHTOWLER, *Nutrition and Food Science Group, School of Biological and Molecular Sciences, Oxford Brookes University, Gipsy Lane Campus, Headington, Oxford, UK, OX3 0BP*

The glycaemic index (GI) of foods has potential implications for the prevention and treatment of major chronic diseases, including diabetes, CHD and obesity (Salmeron *et al.* 1997; Ludwig, 2000). The GI concept was developed to predict postprandial increases in blood glucose concentration after the consumption of food. The recent UN FAO/WHO consultation on carbohydrates authorised the use of the GI method for classifying carbohydrate-rich foods and recommended that the GI values of foods be used to guide food choices. These recommendations are designed for the general population without taking account of possible metabolic differences between ethnic groups. One of the questions frequently raised in relation to the GI is whether results obtained using Caucasian subjects can be applied to other ethnic groups.

Eighteen healthy adult volunteers (nine Caucasian (mean age 32 (SD 11) years; 5 female, 4 male) and nine Asian (mean age 30 (SD 3) years; 2 female, 7 male)) were recruited to investigate the ethnic differences in GI values for four foods. The method used to determine the GI values was in line with procedures recommended by the Food and Agriculture Organization/World Health Organization (1998). For each subject, height, weight, waist circumference and body composition were measured.

	Asian		Caucasian		
Test foods	Mean	SEM	Mean	SEM	*P* value
Wholemeal pitta bread	50	8	50	7	0.956
Fruit and cinnamon bread	74	10	73	12	0.956
Sultana bran cereal	65	6	62	6	0.724
Wholewheat muesli cereal	53	11	53	9	0.984

The results of the present study show that the GI values determined in the two ethnic groups were not significantly different in either a statistical or biological sense and this is consistent with a previous study conducted by Chan *et al.* (2001) in a Caucasian and Vietnamese population. The importance of the present study lies in the fact that most GI values reported in the literature come from Caucasian subjects and it is assumed to be applicable to all other ethnic group. The results confirm the validity of using GI values obtained in Caucasian to Asian subjects.

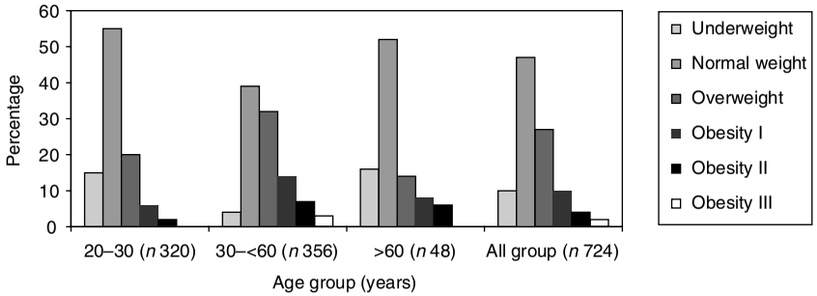
Chan HMS, Brand-Miller JC, Holt SHA, Wilson D, Rozman M & Petocz P (2001) *European Journal of Clinical Nutrition* **55**, 1076–1083.
Food and Agriculture Organization/World Health Organization (1998) *Carbohydrates in Human Nutrition*. Rome: FAO.
Ludwig DS (2000) *Journal of Nutrition* **130**, 280S–283S.
Salmeron J, Manson JE, Stampfer MJ, Colditz GA, Wing AL & Willett WC (1997) *Journal of the American Medical Association* **277**, 472–477.

The prevalence of obesity among females living in the United Arab Emirates. By L.I. SHEIKH-ISMAIL[1], C.J.K. HENRY[1], H.J. LIGHTOWLER[1] and A.O. MUSAIGER[2], [1]*Nutrition and Food Science Group, School of Biological and Molecular Sciences, Oxford Brookes University, Gipsy Lane Campus, Headington, Oxford, UK, OX3 0BP and* [2]*Directorate of Nutritional Studies, Bahrain Centre for Studies & Research, Manama, Bahrain*

The prevalence of overweight and obesity is increasing rapidly in both developed and developing countries and is emerging as a major risk factor for several chronic diseases of public health significance.

In the Middle East large shifts have occurred in dietary and physical activity patterns. These changes are reflected in nutritional and health outcomes. Rising obesity rates and high levels of chronic and degenerative diseases are observed (Galal, 2003). The United Arab Emirates (UAE) have undergone rapid development over the last 30 years following the oil boom. The effect of this on health and lifestyle of the population of the UAE is not known. In particular, the prevalence of obesity among women is not established. Therefore, the aim of the present study was to investigate the prevalence of obesity among women in the UAE.

Females aged between 20 and 90 years (*n* 724) were recruited to the present study from the seven Emirates in the UAE. Subjects were students from UAE University and members of their families. Body weight and height were measured and BMI was calculated. The cut-off points for overweight and obesity were 25 to <30 kg/m^2 and >30 kg/m^2 respectively.



The prevalence of overweight and obesity were 27 and 16% respectively. The age group between 30 to <60 years had the highest prevalence of overweight (32%) and obesity (24%). These results are in agreement with those in the same region (Gulf Countries). In Bahrain, the prevalence of overweight was 31% among females aged 30–79 years (Musaiger & Al-Mannal, 2001). In Saudi Arabia, the prevalence of overweight in women aged 30–70 years was 32%; for obesity the prevalence was 49% (Alsaif *et al.* 2002).

The results of the present survey revealed that there is a high prevalence of overweight and obesity in the UAE. Many factors such as the lack of physical activity, and rapid social and dietary changes may have contributed to this high prevalence. Further measurements, such as waist and hip circumferences, need to be carried out to ascertain the characteristics of obesity in this community.

Alsaif MA, Hakim IA, Harris RB, Alduwaihy M, Al-Rubeaan K, Al-Nuaim AR & Al-Attas OS (2002) *Nutrition Research* **22**, 1243–1252.
Galal O (2003) *Asia Pacific Journal of Clinical Nutrition* **12**, 337–343.
Musaiger AO & Al-Mannal MA (2001) *Annals of Human Biology* **28**, 346–350.

A comparison of three methods of defining obesity in children using BMI cut-offs. By L.M. BODDY[1], A.F. HACKETT[1], G. STRATTON[1], S.R. TAYLOR[2] and E. LAMB[3], [1]*Liverpool John Moores University, Faculty of Education, Community and Leisure, Barkhill Road, Liverpool, UK, L17 6BD,* [2]*Plas Coch Campus, North East Wales Institute of Higher Education, Wrexham, UK, LL11 2AW and* [3]*Leisure Services Directorate, Liverpool City Council, Millennium House, Victoria Street, Liverpool, UK, L1 6JH*

Paediatric obesity has been described as one of the most pressing public heath concerns in contemporary society (Fulton *et al.* 2001). There are conflicting reports regarding the prevalence of obesity, especially in children. These variations may be due, in part, to the cut-off points used to define BMI status. Three major sources of reference for BMI cut-off points are used, almost interchangeably, to classify childhood BMI, although there is little general agreement about which of these three methods is the most appropriate.

The three reference cut-off values (or curves) are subtly different. Such differences may, in a large population, result in a number of individuals being misclassified depending on the BMI cut-off point used. The Liverpool *Sports*Linx project annually collects data regarding the BMI from approximately 95% of the 9–10-year-old children across Liverpool. Using the data collected in 2003–2004 the prevalence of overweight and obesity was compared using the three main BMI cut-off methods. The Table displays the total number and percentage of subjects classified as either overweight or obese for both sexes in 2003–2004.

	Proportion overweight (2003–2004)				Proportion obese (2003–2004)			
	Boys		Girls		Boys		Girls	
BMI cut-off points used	Total	%	Total	%	Total	%	Total	%
UK 1990* (91st and 98th percentile)	246	12.48	280	14.06	331	16.79	287	14.41
Cole *et al.* (2000) (85th and 95th percentile)	350	17.76	465	23.34	172	8.73	210	10.54
Chinn & Rona (2002) (85th and 95th percentile)	450	22.83	474	23.8	239	12.13	206	10.34

* Child Growth Foundation (1996).

Results show marked variations in the number of children classified as obese or overweight using the three BMI cut-off values. There was a notably increased prevalence of obesity, but lower prevalence of overweight, when using the UK 1990 cut-off points (Child Growth Foundation, 1996). Furthermore, these methods do not classify boys and girls in the same way, most notably the Cole *et al.* (2000) cut-off values display marked differences in prevalence of overweight and obesity between boys and girls. In using a large sample size this study increases the available data on defining obesity in children and suggests caution when interpreting BMI data.

Child Growth Foundation (1996) *UK 1990 Growth Charts*. London: Child Growth Foundation.
Chinn S & Rona RJ (2002) *Annals of Human Biology* **29**, 306–313.
Cole TJ, Bellizzi MC, Flegal KM & Dietz WH (2000) *BMJ* **320**, 1–6.
Cole TJ, Freeman JV & Preece MA (1995) *Archives of Disease in Childhood* **73**, 25–29.
Fulton JE, McGuire MT, Caspersen CJ & Dietz WH (2001) *Sports Medicine* **31**, 153–165.

Prevalence of overweight and obesity in Afro-Caribbean schoolchildren in West London. By D. SAMANI[1], D. HAWDON[2] and H.D. McCARTHY[1], [1]*Institute for Health Research & Policy, London Metropolitan University, Holloway Road, London, UK, N7 8DB and* [2]*Shepherds Bush Healthy Living Centre, Australia Road, London, UK, W12 7PH*

Numerous studies have demonstrated a striking increase in the prevalence of overweight and obesity in the UK childhood population based upon the BMI (Chinn & Rona, 2001; McCarthy *et al.* 2003; Jebb *et al.* 2004). However, these findings relate almost exclusively to the Caucasian population. Less is known about the situation in other ethnic groups, especially in those from Afro-Caribbean background. Furthermore, little is known about upper body fatness in children in this ethnic group. The present study examined the prevalence of overweight and obesity in a group of Afro-Caribbean schoolchildren.

A total of 282 children of Afro-Caribbean background aged between 11 and 16 years, and residing in West London, participated in the study (*n* 158 boys; *n* 124 girls). Height, weight and waist circumference (WC) were measured using standardised techniques. BMI (kg/m^2) was calculated and converted to standard deviation scores based on the 1990 UK reference data (Cole *et al.* 1995). Similarly, WC was compared against the current UK WC reference data (McCarthy *et al.* 2001). As a contemporary comparison group for BMI, 1219 children (*n* 639 boys; *n* 648 girls) from the 2003 Health Survey for England (HSE) were selected for equivalent analysis (Sproston & Primatesta, 2005), where 89% of the subjects were Caucasian. The proportion of children exceeding the 91st (overweight) and 98th (obese) centiles was calculated separately in boys and girls.

In the Afro-Caribbean cohort, 26% of boys and girls exceeded the 91st centile compared with 29% of boys and 31% of girls from the HSE. In the Afro-Caribbean cohort, 13.3% of boys and 8.9% of girls were classed as obese (BMI >98th centile), compared with 12.8% of the children from the HSE. For WC, 26% of boys and 41% of girls of Afro-Caribbean background exceeded the 91st centile, whilst the equivalent figures for the 98th centile were 12.7% and 20.3% respectively.

Using the BMI as the measure, it can be concluded that the prevalence of overweight and obesity in the Afro-Caribbean children was lower than in a contemporary Caucasian cohort. This assumes, however, that a given BMI range relates to an equivalent degree of body fatness in both ethnic groups. Nevertheless, the high numbers of children exceeding the cut-offs for WC further suggest that an excess upper body fatness is seen in Afro-Caribbean children, as previously demonstrated in UK Caucasian children (McCarthy *et al.* 2003). This may be at the expense of peripheral body fat and skeletal muscle. As BMI and WC are only proxy measures of fatness, more specific measures of total and regional fat mass and fat-free mass would confirm this suggestion.

Chinn S & Rona R (2001) *BMJ* **322**, 24–26.
Cole TJ, Freeman JV & Preece MA (1995) *Archives of Diseases in Childhood* **73**, 25–29.
Jebb SA, Rennie KL & Cole TJ (2004) *Public Health Nutrition* **7**, 461–465.
McCarthy HD, Crawley HF & Jarrett KV (2001) *European Journal of Clinical Nutrition* **55**, 902–907.
McCarthy HD, Ellis SM & Cole TJ (2003) *BMJ* **326**, 624.
Sproston K & Primatesta P (2005) *Health Survey for England 2003*. London: The Stationery Office.

Reduced energy intake or increased physical activity? Effective weight-loss strategies for women. By L. KIRKWOOD, E. ALDUJAILI and S. DRUMMOND, *Centre for Nutrition and Food Research, Queen Margaret University College, Clerwood Terrace, Edinburgh, UK, EH12 8TS*

Levels of overweight and obesity in women in the UK are increasing despite the growing number of weight-management programmes offered by the commercial sector. The present study aimed to compare the efficacy of dietary advice and/or physical activity advice on weight loss in overweight Scottish women over a 12-week period. Sixty-nine women aged between 30 and 50 years were randomly assigned to one of four groups: (1) control – no advice given; (2) dietary advice only; (3) activity advice only; (4) diet and activity advice. The dietary advice consisted of an individualised energy-reduced diet which was low in fat, high in carbohydrate. The activity advice consisted of the recommendation to achieve 60 min of brisk walking over the course of the day. Dietary intakes were recorded in 4 d unweighed diet diaries and energy expended during walking activities was recorded using a Caltrac™ accelerator monitor. Anthropometric measurements, dietary intake and activity levels were recorded at baseline, 6 weeks (not reported here) and 12 weeks.

		Weight (kg)		Waist circumference (cm)		Activity (MJ)		Energy intake (MJ)		% Energy from carbohydrate		% Energy from fat	
Group	*n*	Base-line	12 weeks	Base-line	12 weeks	Base-line	12 weeks	Base-line	12 weeks	Base-line	12 weeks	Base-line	12 weeks
1	18	91.2	91.6	100.4	100.8	2.6	2.4	9.6	8.0	46.3	47.4	37.9	35.3
2	16	82.3	79.2‡	96.7	91.8†	2.6	2.2	8.6	6.6‡	47.6	49.6	35.7	30.5*
3	19	85.6	83.2†	98.8	93.2‡	2.8	3.7*	8.2	7.5	47.6	43.5	34.1	38.1*
4	16	89.1	84.9‡	103.8	97.2‡	2.7	3.2	9.6	6.7‡	50.6	52.3	33.8	28.6*

Mean values were significantly different from those at baseline: * $P<0.05$; † $P<0.005$; ‡ $P<0.001$.

Between baseline and 12 weeks, weight losses were recorded in groups 2, 3 and 4, with corresponding reductions in BMI and waist circumference. The greatest mean weight loss of 4.18 kg was recorded in group 4, together with the greatest decrease in mean waist circumference of 6.25 cm. The reduction in waist circumference may be of greater importance, in terms of associated risk factors, than a reduction in BMI (Lofgren *et al.* 2004). Percentage body fat, as estimated by electrical bioimpedance, reduced in all three intervention groups at 6 weeks (not shown). However, at 12 weeks, only groups 3 ($P<0.05$) and 4 ($P<0.01$) had maintained this reduction in body fat. Dietary analysis at 12 weeks showed a reduced daily energy intake from baseline in groups 1, 2 and 4 and a decrease in energy expenditure from walking was recorded in groups 1 and 2. Groups 3 and 4 recorded an increase in energy expenditure at 6 weeks (not shown). This was significant at 12 weeks for group 3 only. It appears that increasing physical activity without dietary advice (group 3) led to food choices which were lower in total carbohydrate (43.5%) and higher in fat (38.1%) than those at baseline. A higher consumption of fat-containing foods may have been seen as a reward for the increased physical activity levels (King, 1999).

Women are generally less physically active than men of the same age (Health Education Board Scotland, 1998) and are less likely to make changes in physical activity levels in an effort to lose or maintain weight than males (Field *et al.* 2004). The present study shows that advice to increase the amount of walking led to weight loss. However, the present study also highlights the need for a combination of diet and activity advice to be given for the most effective weight loss, body composition changes and beneficial dietary changes.

Field AE, Manson JE, Taylor CB, Willett WC & Colditz GA (2004) *International Journal of Obesity* **28**, 1134–1142.
Health Education Board Scotland (1998) *Health Education Population Study 1998.* Edinburgh: HEBS.
King NA (1999) *Proceedings of the Nutrition Society* **58**, 107–113.
Lofgren I, Herron K, Zern T, West K, Patalay M, Shachter NS, Koo SI & Fernandez ML (2004) *Journal of Nutrition* **134**, 1071–1076.

The effect of weight loss and weight maintenance on quality of life. By C. WHITE[1], A. DE LOOY[2] and S. DRUMMOND[1], [1]*Centre for Nutrition and Food Research, Queen Margaret University College, Edinburgh, UK, EH12 8TS and* [2]*University of Plymouth, Drake Circus, Plymouth, UK, PL5 3UB*

Overweight and obesity are now so common among the world's population that they are beginning to replace undernutrition and infectious diseases as the most significant contributors to ill health (World Health Organization, 2003). Obesity is associated with many health problems including CHD, diabetes, kidney failure, osteoarthritis, back pain and psychological damage. Moderate weight loss can improve these health risks, leading to a better quality of life for obese individuals.

The present study aimed to compare the effect of two types of dietary advice on quality of life throughout a 3-month weight-reduction and 9-month weight-maintenance intervention.

A total of 169 overweight or obese women were randomly assigned to: (a) group 1 – received advice to reduce energy (for 3 months only), dietary fat and sucrose; (b) group 2 – received advice to reduce energy (for 3 months only) and dietary fat only; (c) group 3 – received no nutritional advice (control).

Baseline energy and nutrient intakes were assessed using an unweighed method over 7 d and compliance with the dietary advice was assessed over 4 d after 1.5, 3, 6 and 9 months. Weight, height and percentage body fat was measured at baseline and after 3, 6, 9 and 12 months. Quality of life was assessed by the Short Form-36® Health Survey Questionnaire version 2 (SF-36®v2) (Ware *et al.* 2000) which was self-administered at baseline and after 3, 9, and 12 months. SF-36®v2 is a generic measure of subjective health status which is scored in such a way that a higher score indicates better quality of life. Mean responses to physical and mental health summary measures for each group (at baseline, after 3 and 9 months) are shown in the Table.

	Baseline						3 Months						9 Months					
	Group 1		Group 2		Group 3		Group 1		Group 2		Group 3		Group 1		Group 2		Group 3	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
n	44		43		39		44		43		39		44		43		39	
Physical component	52.3	1.0	52.4	0.9	50.6	1.0	54.3†	0.7	55.0*†	0.8	51.4	1.0	55.0	0.9	56.0*†	0.9	51.7	0.9
Mental component	46.6	1.6	48.3	1.3	47.0	1.4	49.8	1.5	51.6*	1.0	47.6	1.8	47.5	1.6	49.3	1.6	46.3	2.0

* Mean values were significantly different from baseline ($P<0.05$).
† Mean values were significantly different from control ($P<0.05$).

Both groups 1 and 2 reported a significant decrease in energy ($P<0.001$) and percentage energy from fat ($P<0.001$). Group 1 reported a significant reduction in percentage energy from sucrose after 3 months ($P<0.001$), but could not maintain that reduction at 9 months despite advice to do so. These dietary changes resulted in a significant reduction in body weight ($P<0.005$), BMI ($P<0.005$) and percentage body fat ($P<0.01$) in both intervention groups after 3 months, which was maintained after 12 months. There was no change seen in the control group over the 9 months. After 3, 9 and 12 months both intervention groups tended to report an increase in the physical component of quality of life, when compared with baseline. However, this was only significantly increased in group 2. In addition, after 3 months, group 2 reported a significant increase in quality of life in terms of the mental component. There was no significant change in quality of life in the control group over the 9 months.

The two sets of dietary advice given in the present study facilitated a significant reduction in weight over 3 months which was maintained after 12 months, and seen to have a positive effect on subjects' quality of life. In addition the less restrictive advice to reduce dietary fat only had an extra beneficial physical and psychological effect. This may promote long-term compliance.

Ware JE, Kosinski M & Dewey JE (2000) *How to Score Version 2 of the SF-36® Health Survey*. Lincon, RI: Quality Metric Incorporated.
World Health Organization (2003) *Diet, Nutrition and the Prevention of Chronic Diseases. Report of a Joint WHO/FAO Consultation*. Geneva: WHO.

A comparison of the macronutrient intake of British adults in Liverpool and Cypriot adults in Paphos, Cyprus. By A. EFSTATHIOU and S.M. MAXWELL, *School of The Outdoors, Leisure and Food, Liverpool John Moores University, IM Marsh Campus, Liverpool, UK, L18 2HE*

Mediterranean countries, such as Cyprus, traditionally had a low incidence of nutritionally related diseases (Berrino & Muti, 1989). The low incidence of disease has been related to the foods consumed, the Mediterranean diet (Ferro-Luzzi & Sette, 1989). The Mediterranean diet has a high monounsaturated:saturated fat ratio, high consumption of legumes, fruit and vegetable and moderate consumption of milk, dairy products and alcohol (Trichopoulou & Lagiou, 1997). During the last decade there has been a trend in Mediterranean countries away from the traditional way of eating due to urbanisation and the adoption of a North American way of eating and lifestyle (Serra-Majem & Helsing, 1993). In the past few years the incidence of CHD has been increasing in Cyprus (Cyprus Ministry of Health, 1995). The present study aimed to compare the macronutrient intake of Cypriot adults, living in Paphos, Cyprus and British adults, living in Liverpool, UK with respect to the Mediterranean diet.

The dietary intake of females, aged 25–55 years, was assessed using 3 d dietary diaries and analysed using Microdiet™. Thirty-two Cypriots (C) (mean age 35.5 years) and forty-two British (B) (mean age 36.8 years) from 3 socio-economic groups (professional: C: 15, B: 18; skilled: C:12, B: 16; unskilled: C: 5, B: 8) were recruited.

	Mediterranean diet	Cypriot		British	
Nutrient intake (% energy)	Mean†	Mean	95% CI	Mean	95%CI
Carbohydrate	42	49.7	47.0, 52.4	45.4*	42.8, 48.0
Protein	15	23.7	22.1, 25.4	16.9*	15.7, 17.9
Fat	42	26.2	24.8, 27.7	33.4*	30.8, 36.0
Saturated fat	9	7.2	6.4, 7.9	11.2*	9.7, 12.8
Monounsaturated fat	19	10.4	9.5, 11.2	11.3	9.9, 12.7
Polyunsaturated fat	4	2.7	2.6, 3.0	6.0*	5.2, 6.8
Alcohol	2.5	0.3	−0.1, 0.8	3.8*	2.1, 5.6

* Mean values were significantly different from those of the Cypriot subjects ($P<0.05$).
† Trichopoulou *et al.* (1993).

Differences were seen in all mean macronutrient intakes between British and Cypriot subjects with the exception of intakes of monounsaturated fats as a percentage of energy. The macronutrient intake, as a percentage of energy, of the Cypriot subjects demonstrated higher carbohydrate and protein intakes; compared with the Mediterranean diet, there was a lower fat intake (50% lower in monounsaturated fats). Very little alcohol was drunk.

It appears that Cypriot female adults are moving away from the traditional Mediterranean diet with a decrease in the monounsaturated:saturated fat ratio. Alcohol, however, is still drunk in moderation. They were consuming a diet similar in composition to that recommended in the UK. This change in dietary intake may be related to the increase in the incidence of CHD in this country.

Berrino F & Muti P (1989) *European Journal of Clinical Nutrition* **43**, 49–55.
Cyprus Ministry of Health (1995) *Risk Factors of the Cypriot Population in Relation to Coronary Heart Disease*. Nicosia, Cyprus: Ministry of Health.
Ferro-Luzzi A & Sette S (1989) *European Journal of Clinical Nutrition* **43**, 13–29.
Serra-Majem L & Helsing E (1993) *European Journal of Clinical Nutrition* **47**, Suppl. 1, 1–100.
Trichopoulou A & Lagiou P (1997) *Nutrition Reviews* **55**, 383–390.

Lifecourse influences on nutrient intake in adulthood. By A.M. CRAIGIE[1,2], A.J. ADAMSON[2], A.A. LAKE[2], M. GIBBONS[2], C. WOOD[2], J.N.S. MATTHEWS[2], A.J. RUGG-GUNN[2] and J.C. MATHERS[2], [1]*University of Dundee, Centre for Public Health Nutrition Research, Ninewells Hospital and Medical School, Dundee, UK, DD1 9SY and* [2]*University of Newcastle, Human Nutrition Research Centre, Wellcome Research Laboratories, RVI, Queen Victoria Road, Newcastle upon Tyne, UK, NE1 4LP*

A poor diet is a known risk factor for CVD and is estimated to be responsible for up to one-third of all cancer deaths (Department of Health, 2000*a*,*b*). A better understanding of the determinants of adult dietary intake may provide the basis for more effective interventions. The aim of the present study was to investigate whether knowledge of early nutrient intake and/or body size, socio-demographic or lifestyle factors between early adolescence and adulthood could help predict nutrient intake in adulthood.

The study comprised 202 individuals aged 32–33 years (mean 32.5 years) who had participated in a dietary survey of South Northumberland, UK, when aged 11–12 years (mean 11.6 years) (Hackett *et al.* 1984). At each time-point, dietary data were collected using two 3 d estimated weight food diaries with follow-up interviews. BMI was calculated from weight and height (kg/m^2). Socio-demographic and lifestyle data were collected via questionnaire. Models explaining the largest proportion of the variation (R^2) in adult nutrient intake were built additively using stepwise general linear modelling until all predictors included were significant ($P<0.05$). Social class at 12 years and 33 years was based on occupation and classified using the 1970 Registrar General and 2000 NS-SEC systems, respectively, and grouped into 'low', 'medium' and 'high'. 'Unclassified' social classes were removed from the analyses, so models involving social group refer to the remaining 184 subjects. As the BMI data were non-Gaussian, $\log_e$ transformations were used for the analyses.

	% Food energy at 33 years old			Absolute intake at 33 years old			
	Fat	Carbohydrate	Starch	Energy	Ca	Fe	Vitamin C
Intake of nutrient at 12 years	---	---	↑	↑	↑	↑	↑
Being male	↑	↓	↓	↑	↑	↑	---
Change in social group between 12 and 33 years	Down ↑ Up ↓	---	---	Down ↑ Up ↓	---	---	---
Average BMI over study ($\log_e$ geometric mean)	↓	---	---	---	---	---	---
Change in $\log_e$ BMI over study	---	↓	---	---	---	---	---
Moving away from NE England	---	---	---	---	---	↑	↑
Being a smoker in adulthood	↑	↓	---	↓	↓	↓	↓
R^2 for model	0.14	0.12	0.06	0.32	0.20	0.16	0.16

↑, Positive association; ↓, negative association; ---, factors not significant in model.

The most consistent predictors of adult nutrient intake were gender, nutrient intake at 12 years and smoking status. Males consumed more energy, fat, Ca and Fe and less carbohydrate and starch than females. Adolescent nutrient intake was a predictor of energy, starch and micronutrient intake despite the presence of confounding factors. Smokers had poorer diets with higher fat and lower micronutrient and carbohydrate intakes. While there were no associations with social group at 12 or 33 years, an upward shift in social class was associated with lower energy and fat intakes. Larger increases in BMI were associated with lower adult carbohydrate intakes while higher average BMI were associated with lower fat intakes. Finally, higher intakes of Fe and vitamin C were found in those who had moved away from North East England.

The models presented here emphasise the influence that factors present in adolescence or emerging between adolescence and adulthood can have on adult nutrient intake. In particular, they lend support to nutrition interventions targeting (i) children, since nutrient intakes appear to persist to some extent into adulthood, and (ii) smokers, given their poorer intakes of most nutrients.

The project was funded by The Wellcome Trust (057995/Z/99/Z).

Hackett AF, Rugg-Gunn AJ, Appleton DR, Eastoe JE & Jenkins GN (1984) *British Journal of Nutrition* **51**, 67–75.
Department of Health (2000*a*) *National Service Framework for Coronary Heart Disease*. London: Department of Health.
Department of Health (2000*b*) *The NHS Cancer Plan: a Plan for Investment, a Plan for Reform*. London: Department of Health.

Secular trends in whole-grain intake and sources of British adults. By C.W. THANE[1], A.R. JONES[2], A.M. STEPHEN[1], C.J. SEAL[2] and S.A. JEBB[1], [1]*MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge, UK, CB1 9NL and* [2]*Human Nutrition Research Centre, School of Agriculture, Food & Rural Development, University of Newcastle, Newcastle upon Tyne, UK, NE1 7RU*

Whole-grain food consumption has been associated with a reduced risk of diet-related chronic disease (Slavin, 2004). However, absolute intake of whole grains is seldom reported. The present study is the first to quantify whole-grain intake of British adults and to assess the change over a 14-year period.

Whole-grain intake was estimated in nationally representative samples of British adults aged 16–64 years from the 1986–1987 Dietary and Nutritional Survey of British Adults (Gregory *et al.* 1990) and 19–64 years from the 2000–2001 National Diet and Nutrition Survey (Henderson *et al.* 2002). For both surveys, 7 d weighed dietary records were used to estimate whole-grain intake and the relative contributions of different food groups. Whole-grain contents were assigned to all foods consumed with at least 10% whole-grain content. For composite foods, whole-grain content was calculated using recipe information from standard references (Food Standards Agency, 2002, plus accompanying supplements) and a specialist recipe book (Hobson, 2002). Whole-grain intake and sources were examined by age, sex, occupational social class, cigarette smoking habit, region and season.

Between 1986–1987 and 2000–2001, average whole-grain intake fell significantly (see Table). At both time points, whole-grain intake was lowest among those under 35 years and increased with age among adults surveyed in 2000–2001 ($P<0.001$; trend). Whole-grain intake was also significantly lower among smokers *v.* non-smokers at both time points (each $P<0.001$) and among those with manual *v.* non-manual occupational backgrounds (each $P<0.001$). Median whole-grain intake was lower in all regions in 2000–2001 compared with 1986–1987 except for Scotland where intake was substantially higher (15 *v.* 8 g/d, *n* 121 *v.* 175), although the difference was not statistically significant ($P=0.18$). Whole-grain intake did not differ significantly by sex or season at either time point.

	1986–1987 (*n* 2086)		2000–2001 (*n* 1692)		
	Median	Interquartile range	Median	Interquartile range	*P**
Whole-grain intake (g/d)†	16	0, 45	14	0, 36	<0.001
Whole-grain intake (g/d)‡	13	0, 39	9	0, 29	<0.001
Percentage with no whole-grain intake†	25		29		<0.001
Percentage with no whole-grain intake‡	31		39		<0.001

* 1986–1987 *v.* 2000–2001; Mann–Whitney *U* test for medians, multiple logistic regression for percentages (adjusted for the various socio-demographic and lifestyle factors).
† Considering foods with ≥10% whole-grain content.
‡ Considering only foods with ≥51% whole-grain content.

In 1986–1987 the two largest contributors to whole-grain intake were bread (54%) followed by breakfast cereals (28%), while 14 years later the contribution of breakfast cereals had risen at the expense of bread (contributing 45 and 44% respectively). Between 1986–1987 and 2000–2001, the contribution to whole-grain intake from foods containing ≥51% whole-grain content fell significantly (82 to 73%; $P<0.001$), with other sources making an increasingly important contribution to the diet.

Despite growing epidemiological evidence of health benefits associated with the consumption of whole-grain foods, average whole-grain intake is low and has declined in recent years.

This work was funded by Cereal Partners UK and the Medical Research Council.

Food Standards Agency (2002) *McCance and Widdowson's The Composition of Foods, Sixth summary edition*. Cambridge: Royal Society of Chemistry.
Gregory J, Foster K, Tyler H & Wiseman M (1990) *The Dietary and Nutritional Survey of British Adults*. London: H.M. Stationery Office.
Henderson L, Gregory J & Swan G (2002) *The National Diet and Nutrition Survey: Adults Aged 19 to 64 Years. Volume 1: Types and Quantities of Foods Consumed*. London: The Stationery Office.
Hobson W (2002) *The Classic 1000 Cake & Bake Recipes*. London: Foulsham.
Slavin J (2004) *Nutrition Research Reviews* **17**, 99–110.

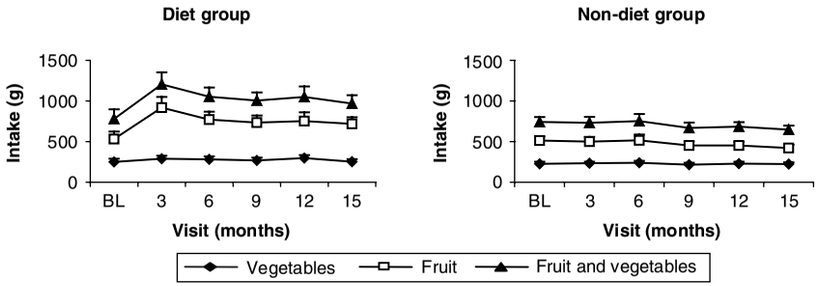
Dietary checklist detects increased fruit consumption as part of a fruit and vegetable intervention trial. By H.M. MACDONALD[1,2], A.C. HARDCASTLE[1,2], R. SANDISON[1], S.A. NEW[3] and D.M. REID[1,2], [1]*Osteoporosis Research Unit, University of Aberdeen, Woolmanhill Hospital, Aberdeen, UK, AB25 1LD,* [2]*Department of Medicine & Therapeutics, University of Aberdeen, Aberdeen, UK, AB25 2ZD and* [3]*Centre for Nutrition & Food Safety, School of Biomedical & Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH*

We previously evaluated a one-page, 3 d dietary checklist to assess fruit and vegetable intake by comparison with a 4 d food diary (Hardcastle *et al.* 2004). The aim of the present study was to assess fruit and vegetable intake using the checklist at 3-month intervals during an ongoing intervention study.

The women were recruited into the trial in October 2003 (*n* 260). Some of the study participants (*n* 65) were asked to consume a specific quantity of fruit and vegetables per d, which corresponded to the daily amount they usually consumed plus an extra three portions (diet group). The remaining women (*n* 195) were taking potassium citrate tablets or placebo as part of the double-blind intervention and were instructed not to change their usual diet (non-diet group). At 15 months, 208 women had completed checklists (diet group *n* 63; non-diet group *n* 145).

The checklist included frequency of consumption of fruits and vegetables and information on portion size that were obtained during a detailed interview with a research nurse at baseline (BL) and 3-month visits. A Photographic Atlas of Food Portion Sizes was used as a guide (Nelson *et al.* 1997). At the 6-month visit the nurse asked if portion sizes had changed. If not, portion sizes from previous visits were used. If a portion size was not given, standard portion sizes were used in the analysis.

The Figure shows daily fruit and vegetable consumption (mean values and 95% CI) for both groups at each visit. For this exercise, fruit intake included fruit juice and dried fruit, and vegetables did not include potatoes or pulses. The difference in mean daily intake of fruit and vegetables between BL and 3 months for the diet group was 390 g for fruit, and 37 g for vegetables. At 6 months the difference in fruit intake had decreased to 239 g, and from 9 months onwards the difference from BL had levelled off to 170 to 180 g. The increase in daily vegetable intake since BL remained small (+6 g to+40 g). For the non-diet group, the difference in fruit intake from BL was between −22g (at 3 months) and −75 g (at 15 months). Vegetable intake had not changed over the 15 months of the study (difference from BL, +13 g to −2 g).



The women in the diet intervention increased their fruit and vegetable intake through increased fruit consumption, which has been found in other studies (Cox *et al.* 1998). The large intake at the 3-month visit may be due to initial enthusiasm or could partly be due to a better understanding of portion size. Although the checklist may not be accurate for absolute amounts, it is a useful tool for monitoring compliance in dietary studies.

This work was funded by the Food Standards Agency (N05043). Any views expressed are the authors' own.

Cox DN, Anderson AS, Reynolds J, McKellar S, Lean ME & Mela DJ (1998) *British Journal of Nutrition* **80**, 123–131.
Hardcastle AC, Sandison R, New SA, Reid DM & Macdonald HM (2004) *Proceedings of the Nutrition Society* (In the Press).
Nelson M, Atkinson M, Meyer J. A Photographic Atlas of Food Portion Sizes. Ministry of Agriculture, Fisheries and Food. 1997.

A comparison of various existing surveys on food consumption in Scotland and their usefulness in measuring the Scottish dietary targets. By W.L. WRIEDEN[1], J. ARMSTRONG[2], K.L. BARTON[1] and H. PEACE[3], [1]*Centre for Public Health Nutrition Research, University of Dundee, Ninewells Medical School, Dundee, UK, DD1 9SY,* [2]*School of Life Sciences, Glasgow Caledonian University, UK, G4 0BA and* [3]*Food Standards Agency Scotland, 6th Floor, St Magnus House, Guild Street, Aberdeen, UK, AB11 6NQ*

Dietary targets for Scotland were published in the 1996 Scottish Diet Action Plan, Eating for Health (The Scottish Office Department of Health, 1996). These targets include a mixture of nutrient- and foods-based targets and were set for achievement in 2005. Major investments are currently being made in diet-related programmes within Scotland and, as part of this effort, it is important that progress towards meeting the targets is monitored.

The objective of the present study was to critically appraise the practical utility and validity of recent national diet, nutrition and health surveys for monitoring the Scottish Dietary Targets (SDT). The National Food Survey (NFS) data for Scotland, all years from 1994 to 2000, the National Diet and Nutrition Survey (NDNS) data for adults aged 19–64 years in 2000/2001 and the Scottish Health Survey (SHS) data 1995 and 1998 for adults aged 16–64 years were used to calculate, where possible, mean and median with interquartile range intakes for target foods and nutrients, and the proportion of subjects meeting the SDT.

		NFS 2000 (*n* 1320)	NDNS 2000 (*n* 123)		SHS 1998 (*n* 5345–7576) Frequency	
Food or nutrient	SDT	Mean	Median	Interquartile range	Median	Interquartile range
Total fat	≤35% food energy	36.3	33.0	29.7, 37.2	No nutrient data	
Saturated fat	≤11% food energy	14.8	12.6	10.6, 14.3	No nutrient data	
NNMES	No increase (% of energy)	14.0	10.7	7.18, 14.1	No nutrient data	
Breakfast cereals	34 g/d	18.4	18.9	3.0, 38.6	1.0/day	0.4, 1.0
Bread	154 g/d, mainly from wholemeal or brown	123	86.4	55.0, 123	2.5 slices/day	2.5, 4.5
Fruit and vegetables	>400 g/d	255	184	91.1, 344	2.9/day	1.8, 4.4
Oil-rich fish	88 g/week	38.9	0	0, 113	0.5/week	0.0, 1.0
Methodology		Household food consumption	7 d weighed intake		Limited food-frequency list	

NMES, non-milk extrinsic sugars.

Results from the NDNS and the SHS confirmed the majority of conclusions obtained from the NFS. None of the SDT (with exception of percentage energy from fat) had been achieved at the most recent surveys but progress was apparent in declining saturated fat intakes. Given the skewed nature of the distribution of food intakes (and some nutrients), there is a need to calculate medians. However due to the nature of the data derived from the NFS and its successor, the Expenditure and Food Survey (EFS) the household data cannot be presented in this way. The percentage of the population meeting the SDT could not be calculated from the NFS but 17% met the >400 g/d fruit and vegetables target when calculated from both the SHS 1998 and the much smaller sample of the NDNS 2000/2001. However, 15.4% of the NDNS sample and 42% of the SHS met the bread target reflecting the differences in survey methods and the fact that in most cases they were not comparable. It was concluded that none of the current surveys available provided an accurate and complete picture of the progress towards the SDT. However, the EFS should provide a more robust and longitudinal method to monitor progress together with new additional surveys where data is lacking (NMES in children and Na intake) (Food Standards Agency Scotland and Scottish Executive, 2004).

Funding provided by the Food Standards Agency Scotland is gratefully acknowledged (project no. ZS4010). Data were provided by the Department for the Environment, Food and Rural Affairs and the Food Standards Agency.

Food Standards Agency Scotland and Scottish Executive (2004) *Report of the Working Group on Monitoring Scottish Dietary Targets*. London: HM Stationery Office.
Scottish Office Department of Health (1996) *Scotland's Health a Challenge to us all. Eating for Health. A Diet Action Plan for Scotland*. London: HM Stationery Office.

An investigation into schoolchildren's knowledge and awareness of diet and dental health. By Z.A. MANAF and P.J. MOYNIHAN, *School of Dental Sciences, University of Newcastle upon Tyne, UK, NE2 4BW*

A recent Department of Health White Paper has recommended that dentists give diet and lifestyle advice as part of preventive care (Department of Health, 2004*a*). The 2003 Children's Dental Health Survey shows that there was no improvement in decay experience among 8-year-old children over the past 10 years, showing that more than half of 8-year-old children had decay into the dentine (Department of Health, 2004*b*). Diet has been identified as one of the major causes for dental caries, particularly non-milk extrinsic sugars (NMES) intake (World Health Organization, 2003). Nevertheless, more than 80% of British children aged 7 to 10 years obtain food energy from NMES at levels above recommendations (Walker *et al.* 2000), indicating a need for dietary change. However, there is a paucity of oral health education programmes aimed at altering diet to promote oral health (Kay & Locker, 1996). An important first stage in any dietary intervention is to assess current knowledge of diet and health.

With a view to designing a dietary intervention package for use in dental practice, the aim of the present research was to explore dietary knowledge in relation to dental caries among primary schoolchildren aged 8–9 years old, using a structured interview. A total of 158 subjects took part: seventy-one boys and eighty-seven girls from six state primary schools in Newcastle, from a range of socio-economic backgrounds. Children were interviewed at school by a dietitian and all of the responses were recorded on a response sheet by the investigator. The interview questions were divided into four sections to investigate their knowledge of: (1) the meaning and causes of dental decay; (2) healthy eating, in particularly foods and drinks high in NMES; (3) the recommended daily portions of fruits and vegetables; (4) food consumption habits which relate to tooth decay.

The majority of the subjects (88%) broadly understood the meaning of dental caries and 74% mentioned sugar as one of the causes of dental caries. The majority of subjects knew that bread, fruits and vegetables should be eaten more and sweetened biscuits should be eaten less, but only half of the subjects knew pasta could be eaten safely. Over 90% of the subjects knew that milk and water were safe for teeth. The majority of subjects also knew that fizzy drinks were bad for teeth. However, only 43% of subjects knew that squash drinks were bad for teeth. About half of the subjects were able to identify foods that were low or high in sugars. However, half of subjects thought that sweetened breakfast cereals were good for their teeth and a third incorrectly thought sugar-free chewing gum was bad for teeth compared with sugared chewing gum.

Only a third of subjects knew the recommendation to consume at least five portions of fruits and vegetables per d. Almost half the children did not know the meaning of 'sugar-free' on chewing gum labels. The majority of subjects incorrectly thought that it was good for teeth to eat sweets by making them last instead of eating them all at once. Approximately 70% of the subjects knew that eating sweets and drinking fizzy drinks before bed was bad for teeth but only half of these could give the correct reason why this was so.

The results of the present study show that 8–9-year-old children had a good knowledge about the meaning and causes of dental caries. Subjects also had a good knowledge of the sugar content of certain key foods but were not able to identify cordials and sweetened breakfast cereals as high in sugars and potentially harmful to teeth. Subjects had insufficient knowledge about the recommendations for fruit and vegetable consumption and of the safest pattern of consumption of sugary foods. These data will be used in future research to design a dietary intervention package for use in the dental practice.

Department of Health (2004*a*) *Public Health White Paper: Choosing Health*. London: The Stationery Office.
Department of Health (2004*b*) *Children's Dental Health in the United Kingdom 2003: Preliminary Finding*. London: Office of National Statistics.
Kay EJ & Locker D (1996) *A Systematic Review of the Effectiveness of Health Promotion Aimed at Promoting Oral Health*. London: Health Education.
Walker A, Gregory J, Bradnock G, Nunn J & White D (2000) *National Diet and Nutrition Survey: Young People aged 4 to 18 Years. Volume 2: Report of the Oral Health Survey*. London: The Stationery Office.
World Health Organization (2003) *Diet, Nutrition and the Prevention of Chronic Disease. Technical Report series* no. 916. WHO/FAO Expert Consultation. Geneva: WHO.

How do packed lunches of 7–10-year-old primary school children compare with Scottish nutritional standards for school meals? By F. ARMSTRONG and M.E. CLAPHAM, *Dietetics, Nutrition and Biological Sciences, Queen Margaret University College, Edinburgh, UK, EH12 8TS*

To improve children's diets in Scotland, the Scottish Executive has introduced nutritional standards for school meals, 'Hungry for Success' (Scottish Executive, 2003). This policy does not include those children who bring their own lunch into school. Evidence from the Scottish Executive (2001) suggest 50% of children stay for school meals. Therefore, a large proportion of children go home, eat a packed lunch or fend for themselves.

The present study assessed the nutritional intake from children's packed lunches in comparison with the Scottish nutritional standards required of school meals.

Seventeen 7–10-year-old primary school children, from Edinburgh, completed 3 d of packed lunch food diaries. The following procedure was used to ensure the children correctly recorded all food consumed. Firstly, the children were divided into groups of five. Secondly, the researcher was present at each lunchtime to enable the children to accurately record the type and quantity of food consumed. The nutrient intake of each child was then compared with the nutritional standards set by the Scottish Executive (2003).

In comparison with the standards, intakes of Na, non-milk extrinsic sugars (NMES), saturated fatty acids (SFA) and the percentage of energy from SFA and NMES, all significantly ($P \leq 0.01$) exceeded maximum standards. While intakes of Fe, folate, NSP, fruit and vegetables all significantly ($P \leq 0.001$) failed to achieve the minimum standards. However, intakes of carbohydrate, protein, vitamins A and C, Ca, and the percentage of energy from total fat and carbohydrate, met their respective standards.

From this small study, if packed lunches are to achieve recommended nutritional guidelines they need to include more NSP, folate, Fe, fruit and vegetables together with a reduction in Na, percentage energy from NMES and SFA. Thus packed lunches require nutritional improvements; however, further research is needed to establish the best ways of doing this. This will almost certainly involve schools, parents, food manufacturers and children themselves (Buttriss, 2002).

Buttriss J (2002) *Nutrition Bulletin* **27**, 275–316.
Scottish Executive (2001) School Meals in Education Authority Schools 2000–01. Edinburgh: Scottish Executive National Statistics. http://www.scotland.gov.uk/stats/bulletins/00112.pdf
Scottish Executive (2003) *Hungry For Success: A Whole School Approach to School Meals in Scotland*. Edinburgh: H.M. Stationery Office.

Assessing the impact of the National School Fruit and Vegetable Scheme on children's fruit, vegetable and nutrient intake. By J.K. RANSLEY[1], J.E. CADE[1], D.C. GREENWOOD[1], S. BLENKINSOP[2], S. SCHAGEN[2], I. SCHAGEN[2], D. TEEMAN[2], G. WHITE[2] and E. SCOTT[2], [1]*Nutritional Epidemiology Group, Centre for Epidemiology and Biostatistics, University of Leeds, 30-32 Hyde Terrace, Leeds UK, LS2 9LN and* [2]*National Foundation for Educational Research, The Mere, Upton Park, Slough, Berkshire, UK, SL1 2DQ*

Research suggests that young children do not consume enough fruit and vegetables to benefit their long-term health (Gregory *et al.* 2000). The National School Fruit and Vegetable Scheme (NSFVS) was devised as part of a wider government strategy to address children's low intakes of fruit and vegetables. The scheme provides a free piece of fruit or a vegetable to children aged 4 to 6 years each school day and aims to distribute 440 million pieces of fruit and vegetables annually to over two million children in 18 000 schools across England. Two key aims of the present research were to evaluate the impact of the NSFVS on children's: (a) consumption of fruit and vegetables; (b) intake of key nutrients.

A stratified sample of fifty-five infant and primary schools was drawn from local education authorities in the North East of England (intervention group) and matched to forty-five schools in the Yorkshire and Humberside region (comparison group). At 8 months, ninety-two schools returned data from 3404 pupils. The Child and Dietary Assessment Tool (CADET) was used to record dietary intake over 24 h and to estimate children's intake of food, energy and nutrients. The CADET was administered to pupils in Reception, Year 1 and Year 2 in three sweeps: at baseline, 3 and 8 months after the introduction of the NSFVS in the North East. Multilevel modelling (MLM) was used to assess the impact of the NSFVS while taking into account a wide range of variables simultaneously (Teeman *et al.* 2004). Estimates produced by MLM take sampling into account and allow for measurement error.

The Table shows MLM estimates of the apparent impact of the NSFVS on dietary measures.

	Reception		Year 1		Year 2	
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
Fruit (portions)	0.2	0.1, 0.4	0.3	0.1, 0.6	0	−0.2, 0.3
Vegetables (portions)	−0.2	−0.5, 0.1	−0.2	−0.5, 0.2	−0.3	−0.6, 0.1
Fruit and vegetables (portions)	0.2	0, 0.4	0.2	−0.2, 0.6	−0.2	−0.5, 0.2
Energy (MJ)	−0.03	−0.25, 0.19	0.03	−0.35, 0.4	−0.63	−1.01, 0.25
Fat (g)	−0.6	−2.2, 0.9	0.1	−2.8, 3.1	1.7	−1.3, 4.7
Salt (g)	−0.1	−0.3, 0.1	−0.2	−0.4, 0.1	−0.0	−0.3, 0.3
Carotene (% change)	14	5, 24	21	5, 40	−14	−26, 1
Vitamin C (mg)	8	3, 12	9	3, 16	−23	−32, −15
Sugars (g)	2.3	−1.9, 6.4	3.0	−3.1, 9.1	−38.2	−46.0, −30.5

At baseline pupils consumed an average of 3.4 portions of fruit, fruit juice and vegetables over a 24 h period, with girls eating on average 3.5 portions and boys 3.3 portions. The Table shows the estimated impact of the NSFVS on fruit, vegetable and nutrient intake 8 months post-intervention, by year group. The NSFVS was associated with an increase in fruit and vegetable intake across Reception and Year 1 of 0.5 and 0.7 portions at 3 months, which fell to 0.2 of a portion at 8 months. Impact on Year 2 pupils was 0.5 portions at 3 months; this fell to baseline values at 8 months. However, Year 2 pupils were no longer part of the NSFVS at this point. There was no real impact on energy or fat intake across the year groups. A negligible reduction in salt intake was observed across the groups and intake of salt and remains high following the intervention. Carotene intake has increased in Reception and Year 1 but declined in Year 2; similarly, Vitamin C intake increased in Reception and Year 1 and decreased in Year 2. There was a non-significant increase in sugar intake in Reception and Year 2. In contrast, Year 2 had a substantial decrease in sugar intake. After 8 months, the NSFVS has had a small positive overall effect on fruit and vegetable intake and little effect on children's intake of key nutrients.

The research was funded by the Big Lottery Fund.

Gregory J, Lowe S & Bates C (2001) *National Diet and Nutrition Survey: Young People Aged 4 to 18 Years. Volume 1: Report of the Diet and Nutrition Survey*. London: Office of National Statistics.
Teeman D, Blenkinsop S, Kaye J, Ransley J, Schagen I, Schagen S & White G (2004) Evaluation of the Big Lottery Fund's National School Fruit and Vegetable Scheme. First Interim Report. http://www.nof.org.uk/documents/live/5727p_nfs_first_eval.pdf

Physical activity, dietary intake and dieting behaviours of Surrey females aged 11–16 years. By S. REEVES[1], Y. JEANES[1], J. CATTERICK[2], J.A. BISHOP[2] and S.A. NEW[2], [1]*School of Human and Life Sciences, Roehampton University, London, UK, SW15 4DJ and* [2]*Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH*

It is widely known that physical inactivity is generally associated with an increased risk of being overweight or obese (Livingstone *et al.* 2003). Yet, some researchers have found that BMI is not always directly associated with habitual physical activity in school-aged children (Siegal *et al.* 2004). It is important to establish the role of physical activity and the amount of exercise required to prevent obesity and promote health in young individuals.

As part of a larger study into dietary intake and lifestyle patterns among Surrey schoolgirls, the role of physical activity was investigated. A total of 253 subjects completed the study. Each participant was asked to complete a 7 d food diary which was analysed using Diet 5 (Univation, Aberdeen) and a 7 d activity diary which was then used to calculate a Blair score (Blair *et al.* 1985). Weight and height measurements were also taken and further information on other lifestyle factors was obtained in a confidential questionnaire.

A total of 245 girls were divided into physical activity groups dependent upon their activity levels as defined by their Blair score. The main findings are outlined in the Table.

	Inactive (*n* 17)		Moderately active (*n* 84)		Active (*n* 104)		Very active (*n* 38)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Blair score	35.9	0.9	38.7	0.7	41.9	1.3	47.3†	2.3
BMI (kg/m^2)	20.6	3.1	19.5	2.9	19.2	3.1	19.3	2.6
EI:BMR	1.38	0.3	1.41	0.2	1.38	0.2	1.28	0.2
Intake (MJ/24 h)	8.0	1.8	7.9	1.2	7.7	1.4	7.2*	0.9
Fat (g)	81.7	20.7	81.4	14.5	79.6	18.9	71.8*	17.0
Protein (g)	58	15	61	10	60.9	13	53.0*	12

Mean value was significantly different from the inactive group (ANOVA): * $P<0.01$; † $P<0.001$.

The girls in the inactive group (IG) were found to consume significantly higher amounts of energy, fat and protein than those in the very active group (VAG). There were no significant differences in body weight or BMI between the groups and BMI correlated poorly with Blair score (R 0.1; $P=0.06$). However, 40% of the IG were either overweight or obese compared with 5% of the VAG. In addition, 42% of the IG reported being unhappy with their weight compared with 63% of the VAG, with 11 and 17% of the inactive and very active groups respectively currently dieting.

Further analysis of the study population is required to establish the importance of physical activity in weight control and body satisfaction.

This work was funded by a Daphne Jackson Fellowship held by J. C.

Blair S, Haskell W & Ho P (1985) *American Journal of Epidemiology* **122**, 794–814.
Livingstone M, Robson P, Wallace J & McKinley M (2003) *Proceedings of the Nutrition Society* **62**, 681–701.
Siegal S, Reyes M, Barahona E & Maline R (2004) *Medicine and Science in Sports and Exercise* **36**, S51.

Ethnic differences in fruit, vegetable and breakfast cereal consumption in girls aged 14–15 years. By A. SURUJLAL-HARRY, G.A. REES, S. BAKHSHI and A. BAKER, *London Metropolitan University, Calcutta House, Old Castle Street, London, UK, E1 7NT*

The National Diet and Nutrition Survey of young people (Gregory *et al.* 2000) reported on the eating patterns of mainly white young individuals from various socio-economic groups. There is, however, limited information on the dietary behaviour of young individuals from different ethnic minority groups in the UK. The present study focused on addressing this lack of information.

Schools in London with a high ethnic diversity of pupils were approached to take part. Out of twenty-six schools contacted, eight agreed to take part. A questionnaire on eating behaviour and psychological barriers and promoters to healthy eating was administered to girls aged 14–15 years. Areas of focus were fruit, vegetable and breakfast cereal consumption.

Six hundred and thirty-four girls completed the relevant sections of the questionnaire; 10% were mixed ethnicity, 25% were white, 31% were black and 34% were Asian. The free school meal entitlement ranged from 9 to 61% for the selected schools; the UK average is 14.8% (Department for Education and Skills, 2005).

The results for fruit consumption were: 49% of girls reported consuming two to three portions of fruit daily, 19% ate one portion per d and 32% had less than one portion per d. For vegetables, 35% reported consuming two to three portions of vegetables per d, 31% had one portion per d and 34% had less than one serving per d. The results for cereal consumption were: 19% ate no cereals, 45% ate sugar-coated cereals, 20% ate low-fibre, non-sugar coated cereals, 9% ate a mixture of low-fibre non-sugar-coated and wholegrain sugar-coated cereal and 7% ate only wholegrain cereals and oats.

Comparison of ethnic groups: ANOVA conducted on the data found that there were significant differences between the ethnic groups for fruit and vegetable consumption. *Post hoc* tests (Scheffé) revealed that white girls ate more fruit and vegetables compared with girls of black ($P=0.001$ and $P=0.006$) and mixed ethnicity ($P=0.032$ and $P=0.004$) (Table 1). For cereals, a χ^2 test revealed that there were significant differences between the groups ($P=0.001$). A large number of Asian girls ate sugary cereals (57%) (see Table 2). More white girls (14%) ate wholegrain cereals than Asians (2%).

Table 1

	White (%)		Mixed ethnicity (%)		Asian (%)		Black (%)	
Portions consumed	Fruit	Vegetables	Fruit	Vegetables	Fruit	Vegetables	Fruit	Vegetables
n	140	136	52	50	191	190	165	157
None	3	3	8	8	3	2	2	4
Less than one/week	5	1	8	6	7	7	10	13
One to two per week	4	8	10	24	7	13	15	15
One per 2 d	9	10	21	18	12	7	15	14
One per d	20	34	15	20	18	35	19	27
Two per d	27	26	19	18	32	24	21	14
Three or more per d	32	18	19	6	21	12	18	13

Table 2

	White (%)	Mixed (%)	Asian (%)	Black (%)
n	129	51	178	164
Don't eat cereals	15	23	17	24
Only sugary cereals	38	43	57	37
Sugary or low-fibre non-sugar-coated	22	20	16	23
Low-fibre non-sugar-coated and wholegrain sugar-coated cereals	11	8	8	9
Only wholegrain cereals and oats	14	6	2	7

The present study found that teenage girls of black and mixed ethnicity were less likely than white girls to eat the required amount of fruit and vegetables, and fewer Asian girls ate wholegrain and high-fibre cereals. The study provides evidence of the need for nutritional interventions to target teenage girls from ethnic minorities in the UK.

This research was commissioned by the Foods Standards Agency.

Gregory J, Lowe S, Bates CJ, Prentice A, Jackson LV, Smithers G, Wenlock R & Farron M (2000) *National Diet and Nutrition Survey: Young people aged 14–18 years. Volume 1: Report of the Diet and Nutrition Survey*. London: H.M. Stationery Office.

Department for Education and Skills (2005) Trends in Education and Skills. http://www.dfes.gov.uk

Packaged food intake by children aged 0 to 16 years (g/kg body weight). By E. FOSTER[1], J.N.S. MATTHEWS[2], J.C. MATHERS[1], P.J. MOYNIHAN[3] and A.J. ADAMSON[1], [1]*Human Nutrition Research Centre,* [2]*School of Mathematics and Statistics and* [3]*School of Dental Sciences, University of Newcastle, Newcastle upon Tyne, UK, NE1 7RU*

The term 'food-contact material' encompasses all materials which in their finished state are intended to come into contact with food. The transfer of chemicals from the food-contact material into the food is termed 'migration'. The European Framework Regulation EC no. 1935/2004 (European Parliament, 2004) states that food contact materials and articles shall not transfer their constituents to food in quantities that could endanger human health or bring about an unacceptable change in the composition of the food or deterioration of the organoleptic characteristics.

In the area of plastic materials, there is an overall migration limit of 60 mg (of substances)/kg (of foodstuff or food simulants) that applies to all substances that can migrate from the food-contact material to the foodstuff and a specific migration limit (SML) which applies to individual authorised substances and is fixed on the basis of the toxicological evaluation of the substance. The SML is generally established according to acceptable daily intake or the tolerable daily intake set by the Scientific Committee on Food. To set the limit, it is assumed that, every day throughout his or her lifetime, an individual of 60 kg eats 1 kg of food packed in plastics containing the relevant substance at the maximum permitted quantity (directive 2002/72 EC; European Union, 2002). This is equivalent to 16.7 g/kg body weight.

The current approach for the authorisation and control of substances used in food-contact materials is considered to be cautious in relation to the estimation of potential exposure of the consumer to these substances with respect to an average adult. An important issue when addressing exposure to chemical migrants in food is the food intake of children. Children are of smaller body mass than adults and may consume more packaged foods, of smaller pack size (giving a higher food-contact material : food mass ratio), and therefore they may have higher intakes per kg body weight. Although extensive data are available on the dietary intake of children, no information exists on their intake of packaged foods and no established method is available by which to measure packaged food intake.

The aim of the present study was to measure children's intakes of packaged foods per kg body weight for different types of food-contact materials. A database of packaged foods was developed to include information on food name and brand, the type of packaging (e.g. plastic, metal, glass, etc), the size of the pack, the fat content of the food as packaged and the food-contact material : food mass ratio. A 4 d estimated-weight diary was developed to collect information on food intake and food packaging. Sixty children were recruited to pilot test the diary (approximately ten in each age-group from 0 to 1, 1 to 4 (pre-school), 4 (primary) to 6, 7 to 11 (primary), 11 (secondary) to 14 and 15 to 16 years old). Fifty-nine children completed the pilot study. Results for the three youngest age groups are presented in the Table.

	Mean food intake (g/kg body weight per d)*	
Age range (years)	Total packaged foods	Food packaged in plastic
0–1	50.9	28.3
1–4	63.8	54.2
4–6	40.5	33.7

* These figures are based on data from a small number of children and should be treated with caution.

Total food intake/kg body weight was highest in those children under 1 year old, and intakes/kg body weight decreased steadily with increasing age. Intakes of total packaged foods and foods packaged in plastics/kg body weight were highest in the three youngest age groups.

These figures are based on data from a small number of children. Work is underway, with a larger number of children, to further investigate the packaged food intakes of children aged 0 to 6 years. This work will also take account of the food-contact material : food mass ratio.

This work is funded by the Food Standards Agency (A03051).

European Parliament (2004) *European Regulation (EC) no. 1935/200. Official Journal of the European Union L338/4*. Strasburg, France: European Parliament.

European Union (2002) Food Contact Materials – Emerging Issues. http://europa.eu.int/comm/food/food/chemicalsafet/foodcontact/emerging_en.htm

Dietary intake and determinants of food choice in post office shift-workers. By M. GIBBS, C. TZUNG, E. GATWOOD, J. HURLEY and L. MORGAN, *Division of Nutrition, Dietetics and Food, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey, UK, GU2 7XH*

Shiftwork is becoming an increasingly important component of working life in industrialised countries, with approximately 15% of the UK working population engaged in some form of shiftwork. One of the health impacts of shiftwork is an approximately 40% increase in CVD risk. We have proposed that this might be due in part to disordered postprandial metabolism following meals consumed at night, and have previously shown that both simulated and real shift workers (Morgan *et al*. 2003) show relatively impaired glucose and lipid tolerance following night-time meals. The magnitude of this effect will be influenced by the timing and composition of meals. The eating patterns and meal times of shift workers are disrupted by their working schedules and food choices may therefore differ from day-workers. We have previously shown that, whilst dietary macronutrient and energy intake was little affected by shift schedule in a fully-catered isolated residential offshore environment, timing of meals and consumption of beverages and caffeinated drinks was affected by shift (Gibbs *et al.* 2001; Woods *et al.* 2003). It is therefore of interest to study a catered non-isolated shift working environment, to investigate the dietary intake and determinants of food choice in night- and shift-workers and to identify whether shift or determinants of food choice impact on dietary intake.

We have recorded the determinants of food choice in forty-eight Post Office shift workers (44 male and 4 female) on three shifts (Early (E) 06:00–14:00; Late (L) 14:00–22:00; Night (N), 22:00–06:00) by questionnaire, and have investigated the dietary intake of a subset (total *n* 23: E, *n* 8, mean age 48 (range 40–61) years, BMI 26.6 (range 21.3–29.8) kg/m^2; L, *n* 8, mean age 48 (range 33–60) years, BMI 25.6 (range 23.5–30.8) kg/m^2; N, *n* 7, mean age 43 (range 25–59) years, BMI 29 (range 22.9–41.8) kg/m^2) to identify differences in daily intake by 4 d dietary recall diary. There were no significant demographic differences between the three subject groups.

Shift was found to impact significantly on determinants of food choice as workers on the L-shift preferred to bring foods from home, consuming less foods from the canteen ($P=<0.01$), citing 'convenience' and 'preferred food choice' as their reasons. All shifts, regardless of the source of food, cited 'convenience' as their main reason. However those on the E and N-shifts who consumed more foods from the workplace canteen than L shift workers, cited different secondary reasons for this: those on the E shift cited 'needing a hot meal' as a factor, while those on the N-shift cited 'no time to prepare food'.

N-shift workers were more likely to 'eat in order to maintain alertness' than those on the L-shift ($P=0.02$) and although there was no difference in consumption of coffee, those on the E-shift consumed more decaffeinated coffee than those on the L-shift or N-shift. This is in line with the increased intake of coffee-derived polyphenolic compounds on night shift reported by Woods *et al*. (2003).

There were no significant differences in overall macronutrient or energy intakes between the three shifts, however all shifts consumed greater sugar and Na than recommended intakes and less than '5-a-day' of fruit and vegetables (one-sample *t* tests, E, L, and N respectively: $P=0.02$, $P=0.002$, $P=0.001$.) Furthermore the N-shift-workers considered 'healthy eating' to be a factor in their home food choice, but not in their night-shift food choice ($P=0.01$). Profiles of the timing of nutrient intake show that the N-shift intakes a significantly higher percentage of total energy and fat during the period 21.00–07.00 hours than the E or L-shifts.

We conclude that while a catered environment is a factor in the food intake of shift workers, shift, meal timing and home environment also impact on the intake and nutritional health of shift-workers. In the present study N-shift workers consumed nearly 50% of their intake between 21.00 hours and 07.00 hours, thus the qualitative inadequacies of the diet in conjunction with the nocturnal consumption are suggested as one factor in the aetiology of CVD risk in shift-workers.

Gibbs, M, Hampton SM, Bennett G, Paul N & Morgan, LM (2002) *Proceedings of the Nutrition Society* **60**, 228A.
Morgan LM, Hampton SM, Gibbs M & Arendt J (2003) *Chronobiology International* **20**, 795–808.
Woods E, Clifford MN, Gibbs M, Hampton SM, Arendt J & Morgan LM (2003) *Proceedings of the Nutrition Society* **62**, 60A.

Salicylic acid: the spice of life: a pilot study. By G. BAXTER[1], J. PATERSON[1], A. WILES[1], A. GRAHAM[2] and R. SHIRASTAVA[3], [1]*Dumfries and Galloway Royal Infirmary, Dumfries, UK, DG1 4AP,* [2]*University of Strathclyde, Glasgow, UK, G4 0NR and* [3]*Ninewells Hospital, Dundee, UK, DD1 9SY*

Salicylic acid, a phenolic acid, is the major metabolite of aspirin, and is believed to be responsible for the anti-inflammatory action of this commonly prescribed medicine. There is a large body of evidence indicating that aspirin has a chemoprotective effect against colorectal cancer even when taken at low doses (81 mg daily) (Baron *et al.* 2003). Salicylic acid acts as a defence hormone in plants (Shirashu *et al.* 1997) and therefore it is not surprising that substantial amounts are found in foodstuffs derived from plant-based materials such as herbs, spices, fruits and vegetables (Swain *et al.* 1985). Blacklock *et al.* (2001) observed that concentrations of salicylic acid in blood from a group of vegetarians were greater than those of individuals eating an unrestricted diet (neither group had been taking aspirin). Even higher concentrations were found in the blood of a group of rural, non-aspirin-taking Indians. The incidence of colorectal cancer is very low in India and the lowest incidence rates are found in rural parts (World Health Organization, 1997). Consequently the aim of the present study was to assess whether traditional Indian dishes are rich in potentially chemoprotective salicylic acid and whether ingestion results in increased salicylic acid in blood and urine.

Various spices and Indian cooked dishes were examined. Samples of blood and urine were collected from an individual before and at intervals consuming a vindaloo curry. HPLC was used to determine the amount of salicylates in foodstuffs and in blood (Blacklock *et al.* 2001) and of salicylic acid and salicyluric acid (the major metabolite of salicylic acid) in urine (Baxter *et al.* 2002). The methods involved the extraction of the acidic and hydrophobic compounds with ethyl acetate, their separation with gradient elution and electrochemical quantification. GC-MS was used to confirm the identity of salicylic acid in spices and of salicylic acid and salicyluric acid in urine.

Substantial amounts of salicylates were measured in cumin (1629 mg/100 g), paprika (104 mg/100 g) and turmeric (350 mg/100 g) and a portion of vindaloo curry (545 g) contained 94 mg which is similar to that of a low dose of aspirin. When blood from an individual who had been fasting for 10 h before consuming a vindaloo curry were examined, the concentration of salicylic acid in serum increased by a factor of two approximately 2 h after eating the food. When urine from the same individual was examined, it was found that the salicyluric acid:creatinine ratio had increased by a factor of five approximately 4 h after taking the food. This work shows that some spices used in Indian cookery contain appreciable amounts of salicylates, and that the Indian population might be exposed on a daily basis to similar amounts of salicylic acid to that derived from a low dose of aspirin. Approximately 3% of the salicylates in a typical Indian cooked dish were found to be bioavailable. It therefore is tempting to speculate that the low incidence of colorectal cancer within the Indian population may be due to their high exposure to dietary salicylates throughout their lifetime, however, it should be noted that this study involved only one individual.

Baron JA, Cole BF, Sandler RF, *et al.* (2003) *New England Journal of Medicine* **348**, 891–899.
Baxter GJ, Lawrence JR, Graham AB & Paterson JR (2002) *Annals of Clinical Biochemistry* **39**, 50–55.
Blacklock CJ, Lawrence JR, Wiles D, Malcolm EA, Gibson IH, Kelly CJ & Paterson JR (2001) *Journal of Clinical Pathology* **54**, 553–555.
Shirashu K, Nakajaima H, Krishnamachari R & Dixon RA (1997) *Plant Cell* **9**, 261–267.
Swain R, Dutton SP & Truswell AS (1985) *Journal of the American Diabetic Association* **85**, 950–960.
World Health Organization (1997) *The World Health Report; Health Education: a Programme Review*. Geneva: WHO.

Prospective phyto-oestrogen exposure as assessed using four methods and their relationship to prostate cancer risk in the EPIC-Norfolk cohort. By Y.-L. LOW[1], J. TAYLOR[1], P. GRACE[1], A. MULLIGAN[2] and S. BINGHAM[1,2], [1]*MRC-Dunn Human Nutrition Unit, Cambridge, UK, CB2 2XY and* [2]*EPIC-Norfolk, Strangeways Research Laboratory, Cambridge, UK, CB1 8RN*

Epidemiological data on phyto-oestrogens and prostate cancer have been limited and inconsistent. This is partly due to difficulties in quantifying phyto-oestrogen exposure accurately. Phyto-oestrogen exposure is often assessed either from dietary intake collected using food-frequency questionnaires (FFQ) or food diaries, serum phyto-oestrogen levels or urinary phyto-oestrogen excretion. Little is known about the agreement between the different methods in quantifying phyto-oestrogen exposure and whether the choice of method will affect risk estimates.

The present study has two aims: (1) to study the relationship between four methods of assessing phyto-oestrogen exposure (FFQ, food diaries, urine and serum); (2) to relate these phyto-oestrogen exposures to the risk of developing prostate cancer in a nested case–control study in the Norfolk arm of the Europe Prospective Investigation into Cancer and Nutrition (EPIC) study.

Levels of seven phyto-oestrogens (daidzein, genistein, glycitein, *O*-desmethylangolensin, equol, enterodiol, and enterolactone) were measured in spot urine and serum samples from 267 men. Eighty-nine of these men later developed prostate cancer. Results were compared with those from 178 healthy men matched by age and date of recruitment. Dietary data were collected using FFQ and 7 d food diaries and isoflavone intakes were computed using an in-house database.

Urinary phyto-oestrogen concentrations (adjusted for creatinine) correlated strongly with those in serum (r 0.63 to 0.88; $P<0.001$). Urinary and serum levels correlated significantly with isoflavone intake assessed from food diaries (r 0.15 to 0.20; $P<0.05$), but not with intakes from FFQ. There was good agreement between urinary and serum daidzein and genistein levels in classifying individuals into tertiles of exposure (kappa >0.60), with more than 75% of subjects being classified in the same tertile. This was in contrast to the poor agreement between either dietary methods and urinary and serum values (kappa<0.11), with at least 60% of subjects being classified in different tertiles. Phyto-oestrogen exposure, as assessed using the four methods, was not significantly associated with prostate cancer risk.

We conclude that the choice of method to quantify phyto-oestrogen exposure can result in considerable differential classification of individuals. This may have implications on the risk estimates obtained and could potentially explain some of the inconsistent results from epidemiological studies. In addition, the present results provide no evidence that phyto-oestrogens lower prostate cancer risk. In Western populations with low habitual soya consumption, health effects of phyto-oestrogens, if any, are likely to be weak and would need larger studies to detect.

This work was funded by the UK Medical Research Council, the UK Food Standards Agency, Cancer Research UK, World Cancer Research Fund and the Agency for Science, Technology and Research, Singapore.

Diet–gene interactions: broccoli consumption and cancer prevention. By A.V. GASPER[1], A. AL JANOBI[2], J.A. SMITH[2], J. BACON[1], M.A. TAYLOR[2], C.J. HAWKEY[2], D.A. BARRETT[2], P. FORTUN[2], C. ATHERTON[2] and R.F. MITHEN[1], [1]*Institute of Food Research, Norwich Research Park, Colney, Norwich, UK, NR4 7UA and* [2]*University of Nottingham, University Park, Nottingham, UK, NG7 2RD*

Broccoli consumption is associated with reduction in risk of prostate, colorectal and lung cancer, particularly amongst individuals who have a functional allele at the GSTM1 locus (about 50% of the population) (Lin *et al.* 1998; Spitz *et al.* 2000; Joseph *et al.* 2004). The epidemiological evidence is supported by studies with animal and cell models (Gamet-Payrastre *et al.* 2000; Anonymous, 2004). The main mechanism proposed for the protective effect of crucifers is the activity of isothiocyanates (ITC) derived from the metabolism of glucosinolates that accumulate within these vegetables. Sulforaphane (SF), the major ITC derived from broccoli, is thought to be the main agent conferring protection.

The aim of the present study was to compare SF metabolism in GSTM1-null and GSTM1-positive individuals, and investigate whether a 'designer' broccoli ('super broccoli'), bred to deliver high levels of SF, can ameliorate the effect of genotype. Ethical approval for the study was obtained from the University of Nottingham Medical School Ethics Committee (ref E/10/2003). Sixteen volunteers were recruited into a randomised, cross-over dietary trial of standard broccoli, 'super broccoli' and a control of water. Blood samples were collected in lithium heparin at 0, 20, 40, 60, 100, 120, 150, 180, 210, 240, 300, 360, 420 and 480 mins after consumption of the test meal. Plasma was immediately prepared by centrifugation at 2000 g for 10 mins at 4°C and subsequently stored at −40°C until analysis. Urine was collected between 0–2, 2–4, 4–6 and 6–24 hours post consumption. We used a novel LC-MS/MS method to quantify SF and its thiol conjugates in plasma and urine.

Consumption of super broccoli led to a threefold greater exposure to SF and its metabolites compared with standard broccoli. While there were no differences in the plasma kinetics of SF between GSTM1 genotypes, positive individuals excreted less SF in urine compared with nulls, after consuming either standard ($P=0.008$) or super broccoli ($P=0.000$). GSTM1 nulls excreted 100% of ingested SF after consuming standard broccoli but only 68% after consuming super broccoli.

Following broccoli consumption, a proportion of SF is rapidly excreted via the mercapturic acid pathway, while some SF is either retained in tissues and/or excreted by another metabolic route. This hypothesis is supported by a study in rats using radiolabelled ITCs (Conaway *et al.* 2000). The amount retained depends upon GSTM1 genotype and the amount of SF consumed. Retention of SF by GSTM1-positive individuals explains the epidemiological data, in which GSTM1 positives gain greater protection than GSTM1 nulls. GSTM1-null individuals could compensate for their genotype by consuming high-glucosinolate broccoli or larger amounts of broccoli per serving.

Anonymous (2004) *IARC Handbooks of Cancer Prevention: Cruciferous Vegetables, Isothiocyanates and Indoles*. Lyon, France: International Agency for Research on Cancer.
Conaway CC, Getahun SM, Liebes LL, Pusateri DJ, Topham DK, Botero-Omary M & Chung FL (2000) *Nutrition and Cancer* **38**, 168–178.
Gamet-Payrastre L, Li P, Lumeau S, Cassar G, Dupont MA, Chevolleau S, Gasc N, Tulliez J & Terce F (2000) *Cancer Research* **60**, 1426–1433.
Joseph MA, Moysich KB, Freudenheim JL, Shields PG, Bowman ED, Zhang Y, Marshall JR & Ambrosone CB (2004) *Nutrition and Cancer* **50**, 206–213.
Lin HJ, Probst-Hensch NM, Louie AD, Kau IH, Witte JS, Ingles SA, Frankl HD, Lee ER & Haile RW (1998) *Cancer Epidemiology, Biomarkers and Prevention* **7**, 647–652.
Spitz MR, Duphorne CM, Detry MA, Pillow PC, Amos CI, Lei L, de Andrade M, Gu X, Hong WK & Wu X (2000) *Cancer Epidemiology, Biomarkers and Prevention* **9**, 1017–1020.

Broccoli consumption has no effect on xenobiotic metabolising enzymes. By A. LYNN[1], Z. FULLER[2], K. HILLMAN[3] and B. RATCLIFFE[1], [1]*The Robert Gordon University, St Andrew Street, Aberdeen, UK, AB25 1HG,* [2]*The Macaulay Institute, Craigiebuckler, Aberdeen, UK, AB15 8QH and* [3]*Scottish Agricultural College, Craibstone, Aberdeen, UK, AB21 9YA*

Brassica vegetable consumption is associated with a decreased risk of cancer (Verhoeven *et al.* 1996). Mechanisms for this are not fully understood but modulation of xenobiotic-metabolising enzymes may be important. High intakes of broccoli induce these enzymes in the liver and colon of rodents (Aspry & Bjeldanes, 1983; Vang *et al.* 2001; Keck *et al.* 2003). Many vegetables are sold frozen, but little is known of the effect of such processing on the ability of broccoli to induce enzymes. A 12 d dietary trial was conducted to compare the effect of raw and blanched frozen broccoli (*var.* Marathon) on the activity of xenobiotic-metabolising enzymes in pigs. Pigs were used because of appropriate similarities to man. Fifteen Landrace × Large White males were divided into five age- (79 (SD 3) d) and weight- (34.7 (SD 3.9) kg) matched cohorts consisting of siblings to minimise genetic variation. Pigs in each cohort were randomly assigned to one of three treatments. Each group received a standard, high-quality, cereal-based diet (control). This diet was supplemented with whole raw or blanched frozen broccoli (600 g/d). Microsomal ethoxyresorufin *O*-deethylation (EROD) and methoxyresorufin *O*-demethylation (MROD) were assayed by fluorimetric measurement of the appearance of resorufin. Cytosolic glutathione-S-transferase (GST) and quinone reductase (QR) were assayed by spectrophotometric measurement of the formation of 1-chloro-2,4-dinitrobenzene and the reduction of 2,6-dichloroindophenol respectively. Consumption of raw or blanched frozen broccoli had no significant effect on any of the enzymes (see Table), so no conclusions can be made about the effect of the freezing process on the ability of broccoli to induce enzymes.

	Hepatic GST* (nmol/min per mg protein)		Colonic GST* (nmol/min per mg protein)		Hepatic QR* (nmol/min per mg protein)		Colonic QR* (nmol/min per mg protein)		Hepatic EROD* (pmol/min per mg protein)		Hepatic MROD* (pmol/min per mg protein)	
Diet†	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Raw broccoli	2109	549.2	120	15.7	160	89.2	134	25.9	106	31.9	38	9.7
Frozen broccoli	2178	546.9	138	23.2	171	56.5	131	27.1	112	33.8	39	9.2
Control	2213	254.7	135	24.5	149	94.3	107	30.8	103	20.8	37	5.0

* All enzyme activities were determined in triplicate. Two-way ANOVA was used to assess statistical significance of differences.
† Five pigs in each diet condition.

The failure of broccoli to alter the activity of the enzymes may be explained by the amount fed or its content of glucosinolates. An intake of 600 g whole broccoli/d approximates to 5% of the dry weight of the diet, whereas studies with rodents reported enzyme induction at 10–25% (w/w) broccoli diets (Aspry & Bjeldanes, 1983; Vang *et al.* 2001; Keck *et al.* 2003). The present study used Spanish broccoli supplied by a local supermarket. Transport and distribution may result in substantial pre-retail losses of glucosinolates and may have contributed to the observed lack of effect (Vallejo *et al.* 2003). In the present study, raw and blanched frozen broccoli fed at 600 g/d, consistent with human recommendations of five portions of fruit and vegetables per d (400–600 g), did not induce xenobiotic-metabolising enzymes.

This work was supported by a grant from the World Cancer Research Fund.

Aspry KE & Bjeldanes LF (1983) *Food and Chemical Toxicology* **21**, 133–142.
Keck AS, Qiao Q & Jeffery E (2003) *Journal of Agricultural and Food Chemistry* **51**, 3320–3327.
Vallejo F, Tomas-Barberan F & Garcia-Viguera C (2003) *Journal of Agricultural and Food Chemistry* **51**, 3029–3034.
Vang O, Frandsen H, Hansen KT, Sorensen JN, Sorensen H & Andersen O (2001) *Metabolism* **50**, 1123–1129.
Verhoeven DTH, Goldbohm RA, van Poppel G & van den Brandt PA (1996) *Cancer Epidemiology, Biomarkers and Prevention* **5**, 733–748.

Possible protective effect of dietary flavonoids in colorectal cancer: a case–control study. By J.A.M. KYLE[1], L. SHARP[2], G.G. DUTHIE[1] and G. McNEILL[3], [1]*Rowett Research Institute, Aberdeen, UK, AB21 9SB,* [2]*Epidemiology Group University of Aberdeen, Aberdeen, UK, AB25 2ZD and* [3]*Department of Environmental and Occupational Medicine, University of Aberdeen, Aberdeen, UK, AB25 2ZD*

Flavonoids are phenolic compounds widely distributed in plant foods, and cellular and animal-based models have identified potential anti-carcinogenic effects such as modulation of antioxidant activity and cytokine production which could be associated with a reduced risk of cancer. The present study was carried out to explore the possibility of an association between dietary flavonoids and colorectal cancer using a case–control study design. Cases were diagnosed with colorectal cancer in 1998–2000 and were identified from histological records in the Central Pathology Department of Aberdeen Royal Infirmary. Controls were randomly selected from the Grampian Community Health Index, a list of all patients registered with a general practitioner and were frequency-matched to the cases on age and sex. Of the cases, 57% were male (51% of controls), with the mean age being 69.8 (SE 0.7) years for cases and 63.0 (SE 0.6) years for controls. Dietary flavonoid intake was assessed with a 150-item semi-quantitative food-frequency questionnaire (FFQ; Scottish Collaborative Group version 6.31) with values for five categories of flavonoids based on a recently compiled flavonoid composition database (Kyle & Duthie, 2005). A previous validation study in eighty-one women aged 19–50 years found Spearman correlation coefficients of 0.60–0.83 (all $P<0.001$) for estimates of flavonol, procyanidin and catechin intake derived from the FFQ v. 4 d weighed record, though the values for flavanones and flavones ranged from −0.16 (NS) to 0.40 ($P<0.05$) (Kyle *et al.* 2002).

A total of 261 cases (186 colon cancer and seventy-five rectal cancer) and 404 controls completed the FFQ, representing 61% of eligible subjects. The intake data were divided into quartiles and the association between intake and colorectal cancer assessed by logistic regression with adjustment for confounding variables. The analysis for flavonols, catechins and procyanidins was repeated excluding intake from black tea, which is the major dietary source of these groups in the UK. The Table shows the adjusted odds ratios (OR) and 95% CI for colorectal cancer in the four intake groups. Risk decreased significantly with increasing intake of flavonols from sources other than black tea (P for trend 0.03). There was also a borderline significant trend of increasing risk with increasing flavanone intake.

	Odds of colorectal cancer by intake quartile*							
	1 (lowest)	2		3		4 (highest)		Test for linear trend (P)
	OR	OR	95% CI	OR	95% CI	OR	95% CI	
Flavonols								
All	1.0	1.0	0.6, 1.7	1.3	0.8, 2.1	0.8	0.5, 1.3	0.37
Excluding black tea	1.0	0.8	0.5, 1.3	0.7	0.4, 1.1	0.6	0.4, 1.0	0.03
Procyanidins								
All	1.0	0.9	0.6, 1.5	1.2	0.7, 1.9	0.7	0.4, 1.2	0.19
Excluding black tea	1.0	1.7	1.1, 2.8	1.0	0.6, 1.7	1.2	0.7, 2.1	0.17
Catechins								
All	1.0	0.7	0.4, 1.1	1.3	0.8, 2.2	0.6	0.4, 1.2	0.17
Excluding black tea	1.0	1.1	0.7, 1.7	1.2	0.8, 2.0	1.0	0.6, 1.8	0.25
Flavanones	1.0	1.5	0.9, 2.5	1.4	0.9, 2.4	1.6	1.0. 2.6	0.07

* Adjusted for total energy intake, folate intake, vitamin C intake, age, sex, family history of colorectal cancer, age at diagnosis, use of aspirin or non-steroidal anti-inflammatory medication, smoking and post-school education.

Analysis of the data for individual flavonols showed an inverse association between colorectal cancer risk and intake of quercetin, which is found in high concentrations in onions (P for trend 0.01), but not kaempferol or myricetin. Larger studies in populations with differing dietary habits will be required to test these associations further.

Kyle JAM & Duthie GG (2005) Flavonoids in foods. In *Flavonoids*, 3rd ed. [JB Harborne, editor]. London: Chapman Hall. (In the Press).
Kyle JAM, Masson LF, Grubb DA, Duthie GG & McNeill G (2002) *Proceedings of the Nutrition Society* **61**, 69A.

Effect of dietary antioxidants on glutamate cysteine ligase modulator sub-unit gene expression in human THP-1 monocytic cells. By R.M. OGBORNE, S.A. RUSHWORTH, G.D. HARVEY, J.C. SCHIPPERS and M.A. O'CONNELL, *MRC Human Nutrition Research, Fulbourn Road, Cambridge, UK, CB1 9NL*

Glutathione (GSH) is the most abundant thiol antioxidant present in human cells. The rate-limiting step in the *de novo* synthesis of GSH is regulated by the heterodimeric enzyme glutamate cysteine ligase (GCL) that comprises two sub-units, GCL catalytic (GCLC) sub-unit and GCL modulator sub-unit (GCLM). Whilst GCLC expression alone is required for constitutive GSH expression, the association of GCLC with GCLM is required for higher levels of GSH synthesis in response to stress (Dickinson *et al.* 2004). Single nucleotide polymorphisms in the human GCLM promoter, resulting in decreased GSH biosynthesis, predispose to myocardial infarction (Nakamura *et al.* 2002) and result in impaired vasomotor function (Nakamura *et al.* 2003). Elevation of GCLM gene expression may therefore be protective against CVD.

Monocytes are central to the development of CVD as their migration into the artery wall is the initial step in lesion formation. Dietary antioxidants are thought to be beneficial during this initial stage. We have previously reported that the dietary antioxidant curcumin induces GCLM expression in human monocytes and THP-1 cells (Rushworth *et al.* 2004*a*,*b*). It is therefore possible that other antioxidants that are naturally present in the human diet may also induce GCLM expression in monocytic cells.

Human THP-1 monocytic cells were untreated or treated with 25–100 μM-α-tocopherol (AT), γ-tocopherol (GT), ascorbic acid (AA), dehydroascorbic acid (DHA) or resveratrol (Res) for 4, 8 or 24 h and RNA extracted. GCLM mRNA expression was measured by real-time PCR and normalised against 18s ribosomal sub-unit mRNA using the comparative cycle threshold method. The potentially cytotoxic effect of the antioxidants used was investigated by MTT assay. Cellular incorporation of AT and GT was determined by HPLC and incorporation of AA determined by fluorescence. Statistical analysis was performed using Student's *t* test.

The present study screened a range of antioxidants for their ability to induce GCLM mRNA expression in monocytic cells. The data in the Table demonstrate a trend towards antioxidant-induced GCLM mRNA expression. At the doses employed the antioxidants were not cytotoxic to THP-1 cells. To ensure that antioxidants were incorporated into the cell, uptake assays were performed. When treated with 100 μM-AT or -GT for 24 h, THP-1 cells incorporated 2.10 (SD 0.52) μM and 6.18 (SD 2.31) μM AT or GT respectively. When incubated with 100 μM-AA for 24 h, THP-1 cells incorporated 3.6 mM AA. Following treatment with 100 μM-DHA, intracellular incorporation peaked at 4 h at 2.42 (SD 0.85) mM. These data indicate that the antioxidants were successfully incorporated into THP-1 cells.

The Table shows the differential induction of GCLM mRNA expression by antioxidants in THP-1 monocytic cells (three independent experiments).

	Fold induction of GCLM mRNA above control					
Time (h)...	4		8		24	
Antioxidant	Mean	SD	Mean	SD	Mean	SD
AT (100 μM)	1.82	0.29	0.91	0.36	0.58	0.09
AA (100 μM)	1.69	1.23	1.24	0.92	1.85	2.07
DHA (100 μM)	2.00*	0.27	2.28	0.72	1.09	0.26
GT (100 μM)	2.08	0.50	1.42	0.26	0.56	0.09
Res (100 μM)	1.82	0.86	1.06	0.18	1.01	0.34

* $P<0.05$.

The level of antioxidant-induced GCLM mRNA expression detected in the present study is less than that previously described for curcumin in primary monocytes (25-fold, 15 μM; Rushworth *et al.* 2004). Further studies are required to confirm these preliminary data in primary monocytes and to fully understand the physiological consequences of antioxidant-induced GCLM expression in these cells.

Dickinson DA, Levonen AL, Moellering DR, Arnold EK, Zhang H, Darley-Usmar VM & Forman HJ (2004) *Free Radical Biology and Medicine* **37**, 1152–1159.
Nakamura S, Kugiyama K, Sugiyama S, Miyamoto S, Koide S, Fukushima H, Honda O & Yoshimura M (2002) *Circulation* **105**, 2968–2973.
Nakamura S, Sugiyama S, Fujioka K, Ogawa H & Kugiyama K (2003) *Circulation* **108**, 1425–1427.
Rushworth SA, Charalambos CA, Ogborne RM & O'Connell MA (2004a) *Proceedings of the Nutrition Society* **63**, 93A.
Rushworth SA, Charalambos CA, Ogborne RM & O'Connell MA (2004b) *Biochemical Society Transactions* Published online: http://www.biochemsoctrans.org/bst/bs2004/bs2004E104.pdf

The effect of processing of cabbage on myrosinase activity, glucosinolate concentrations and production of breakdown products. By V. RUNGAPAMESTRY[1], A.J. DUNCAN[2], Z. FULLER[2] and B. RATCLIFFE[1], [1]*The Robert Gordon University, St Andrew Street, Aberdeen, UK, AB25 1HG and* [2]*The Macaulay Institute, Craigiebuckler, Aberdeen, UK, AB15 8QH*

The cancer-protective effects of brassica vegetables may be related to their glucosinolate content (Verhoeven *et al.* 1997). Glucosinolates such as sinigrin are hydrolysed by myrosinase to yield allyl isothiocyanates, allyl cyanide and, in the presence of an epithiospecifier protein (ESP), 1-cyano-2,3-epithiopropane. Isothiocyanates are one of the main metabolites that may be implicated in chemoprotection. Earlier work indicated that losses of glucosinolates during cooking were variable and depended on cooking methods (Dekker *et al.* 2000; Verkerk, 2002). The effect of processing treatment and time on glucosinolate hydrolysis was investigated in cabbage. Cabbage (*n* 6; *var.* Marathon) was steamed or microwaved for six time intervals over 7 min. Residual glucosinolate concentrations, myrosinase activity, and concentrations of metabolites of sinigrin, formed by hydrolysis following cooking, were measured by HPLC, spectrophotometry and GC respectively. Statistical significance was determined by two-way ANOVA. There was no significant change in glucosinolate concentrations during cooking. Myrosinase activity was reduced by cooking time ($P<0.001$), cooking treatment ($P<0.001$) and an interaction between these variables ($P<0.001$). Microwaving produced an abrupt reduction of 96% in myrosinase activity after 2 min due to fast heat conduction. Conversely, the slower penetration of thermal energy to the core of cabbage during steaming produced a gradual and steady decrease in myrosinase activity over cooking time, with a 90% reduction in activity after 7 min (see Fig. 1). A significant effect of cooking treatment ($P<0.001$), cooking time ($P<0.001$) and an interaction between both factors ($P<0.001$) was found on the formation of derivatives of sinigrin after cooking. A decrease in 1-cyano-2,3-epithiopropane, with a proportionate increase in allyl isothiocyanate concentration, was observed over time in both cooking methods. This may be due to the higher heat lability of ESP compared with myrosinase. It may be that as cooking progresses and ESP is destroyed, more allyl isothiocyanate is formed than 1-cyano-2,3-epithiopropane. Allyl cyanide concentrations were reduced over cooking time. Fig. 2 illustrates the formation of derivatives of sinigrin from steamed cabbage.

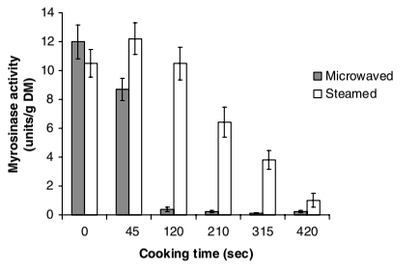


Fig. 1. Myrosinase activity in cooked cabbage.

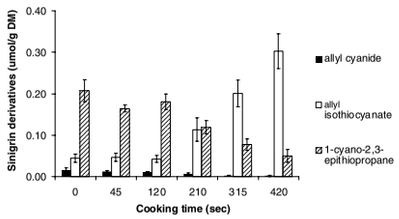


Fig. 2. Formation of sinigrin derivatives from steamed cabbage.

In the present study, the highest yield of isothiocyanates was obtained after microwaving cabbage for 2 min or steaming for 7 min. This suggests that lightly cooked cabbage may optimise the production of isothiocyanates for protection against cancer. However, further work is required to assess the uptake of these isothiocyanates *in vivo*.

This work was supported by the Food Standards Agency (project code T01027).

Dekker M, Verkerk R & Jongen WMF (2000) *Trends in Food Science and Technology* **11**, 174–181.
Verhoeven DTH, Verhagen H, Goldbohm RA, van den Brandt PA & van Poppel G (1997) *Chemico-Biological Interactions* **103**, 79–129.
Verkerk R (2002) PhD thesis, Wageningen University, Netherlands.

Folate supplementation decreases lymphocyte uracil misincorporation into DNA in healthy volunteers. By G.P. BASTEN[1], S.J. DUTHIE[2], L. PIRIE[2], N. VAUGHAN[2], M.H. HILL[1] and H.J. POWERS[1], [1]*Human Nutrition Unit, Division of Clinical Sciences (North), University of Sheffield, Northern General Hospital, Sheffield, UK, S5 7AU and* [2]*Rowett Research Institute, Division of Cellular Integrity, Aberdeen, UK, AB21 9SB*

Previous studies in cultured cells and animal models have shown that folate deficiency can alter DNA stability, DNA methylation and DNA repair capacity, which may ultimately cause chromosomal damage and malignant transformation (Blount & Ames, 1994). Some measurements of DNA instability are reported to be increased in cells from patients with cancer, and on this basis such measurements are considered as biomarkers of cancer risk. However, for such measurements to be truly informative about cancer risk they must be demonstrated to be folate-responsive in a healthy population.

Accordingly, a randomised, placebo-controlled, double-blind intervention trial of 1.2 mg folic acid for 12 weeks was carried out in sixty-five healthy volunteers. The effect of folate supplementation on potential biomarkers of folate function (plasma 5-methyltetrahydrofolate, homocysteine, methionine and whole-blood folate) and DNA stability was evaluated. Misincorporated uracil, DNA methylation, DNA repair capacity and DNA strand breakage were measured in isolated lymphocytes using modified single-cell gel electrophoresis methods (Duthie & Hawdon, 1998). Polymorphisms in the C677T methyltetrahydrofolate reductase (MTHFR) gene, important in folate metabolism, were determined by PCR. Erythrocyte folate (RCF) was determined by Abbott IMX folate ion capture assay.

Supplementation elicited a significant ($P<0.01$) improvement in folate status, measured as intracellular lymphocyte total folate, erythrocyte total folate and plasma 5-methyltetrahydrofolate, and in biomarkers of folate function (whole-blood S-adenosylmethionine (SAM), plasma total homocysteine). This was accompanied by a significant reduction in uracil misincorporation ($P<0.05$). Conversely, DNA strand breakage and global DNA methylation were not found to be sensitive to increased folic acid intake. Increasing folate status modified DNA repair capacity only in those volunteers with the lowest status at the outset. There was no effect of polymorphisms in the MTHFR gene on any of the measures. The Table shows the effect of folate supplementation on uracil misincorporation.

		Pre-intervention		Post-intervention		
Variable	Group	Median	IQR	Median	IQR	% Change
Uracil misincorporation (arbitrary units)	Folate	51	41, 60	40*	30, 46	−25†
	Placebo	47	40, 58	42.5	34, 57	−15

IQR, interquartile range.
* Significantly different from placebo ($P<0.05$).
† Significantly different from placebo ($P<0.01$).

In the present study uracil misincorporation was more sensitive to changes in folate status than other measures of DNA stability and can be considered a valid, specific and functional marker of folate status that may be relevant to cancer risk in healthy individuals.

This work was funded by the World Cancer Research Fund (www.wcrf.org.uk).

Blount BC & Ames BN (1994) *Annals of Biochemistry* **219**, 195–200.
Duthie SJ & Hawdon A (1998) *FASEB Journal* **12**, 1491–1497.

Inter-individual variation in nucleotide excision repair capacity: potential scope for dietary modulation of DNA repair. By J. TYSON[1], A. SPIERS[1], F. CAPLE[2], J.E. HESKETH[1] and J.C. MATHERS[1], [1]*Human Nutrition Research Centre, School of Clinical Medical Sciences, University of Newcastle upon Tyne, UK, NE1 7RU and* [2]*Biological and Food Sciences, Northumbria University, Newcastle upon Tyne, UK, NE1 8ST*

The integrity of the genome is under constant threat from DNA damage arising from endogenous and exogenous sources. Nucleotide excision repair (NER) is responsible for the repair of bulky DNA lesions caused by UV light, heterocyclic amines, polyaromatic hydrocarbons and many other genotoxins. Sufferers of the genetic syndrome xeroderma pigmentosa are deficient in NER and have up to a 1000-fold increased risk of developing skin cancer. More modest decreases in NER capacity have been associated with an increased risk of cancer at several sites (Lockett *et al.* 2005). Limited evidence to date suggests that dietary factors can affect NER and also other DNA repair mechanisms. Se treatment of cell lines has been seen to increase NER capacity (Seo *et al.* 2002) and, in a human study, supplementation with Kiwi fruit increased base excision repair capacity (Collins *et al.* 2003). However, little is known about the scale of inter-individual variation in NER capacity in normal healthy populations, and even less is known about the extent to which dietary factors can influence NER capacity.

To investigate inter-individual variation in NER and the possibility of modulating DNA repair through diet, forty-eight (*n* 30 female; *n* 18 male) young healthy adults (18–25 years; mean age 20 years) were recruited and given a multivitamin supplement (containing Se and vitamins A, C and E) for 6 weeks. Baseline (pre-supplementation) NER capacity was measured in lymphocytes isolated from recruits using the host cell reactivation assay which is specific for NER. Briefly, lymphocytes were isolated from whole blood and transfected with either a reporter plasmid damaged with UV light (400 J/m^2; 253 nm) or an equivalent undamaged plasmid. Transfected cells were cultured for 48 h to allow repair of the damaged plasmid before assay for reporter gene expression. NER capacity was measured as the ratio (%) of reporter gene expression in cells transfected with damaged plasmids to that of cells with the undamaged plasmids (Athas *et al.* 1991). These baseline data were used to estimate inter-individual variation and the effects of sex and age on NER capacity.

Baseline data from these forty-eight individuals show that NER capacity varied 10-fold within this young healthy population, ranging from 2.6 to 25.1% with a mean of 10.1%. There was no significant difference between sexes in NER capacity (using one-way ANOVA) nor was age associated with NER (using bivariate correlation). In case–control studies as little as a 12% decrease (patients v. controls) has been reported to be associated with an increased risk of lung cancer (Lockett *et al.* 2005). Therefore, it is possible that the inter-individual variation seen in this healthy population will be of biological significance. Both inter-individual differences in genetic make-up and variable environmental, such as diet, factors could account for this inter-individual variation, but the extent to which they contribute to NER capacity is unknown. Further investigations are currently underway to assess the effects of antioxidant supplementation, genotypes of key repair genes and dietary and lifestyle factors on NER capacity in this population.

The present study was funded by BBSRC grant 13/D15721. J. T. is funded by a BBSRC studentship. We thank J. Matta (Puerto Rico) for helpful advice on the NER assay.

Athas WF, Hedayati MA, Matanoski GM, Farmer ER & Grossman L (1991) *Cancer Research* **51**, 5786–5793.
Collins AR, Harrington V, Drew J & Melvin R (2003) *Carcinogenesis* **23**, 511–515.
Lockett KL, Snowhite IV & Hu JJ (2005) *Cancer Letters* **220**, 125–135.
Seo YR, Sweeney C & Smith SL (2002) *Oncogene* **23**, 3663–3669.

The effects of dietary folate on methylation of the *ESR1* CpG-island in a rat model of colorectal neoplasia. By L.E. BUCKLEY, N.J. BELSHAW and I.T. JOHNSON, *Institute of Food Research, Norwich Research Park, Colney Lane, Norwich, UK, NR4 7UA*

Colorectal cancer (CRC) is the second biggest cause of death from cancer in the UK. Intake of folate is strongly associated with a reduced incidence of this disease in man. Studies with animal models in which folate supplementation is coupled with carcinogen challenge go some way to support this, but have given conflicting results. A few studies suggest that folate supplementation may even promote carcinogenesis in certain circumstances. The present study aimed to further investigate the effect of folate status on the development of chemically induced aberrant crypt foci (ACF), and the methylation status of the oestrogen receptor (*ESR1*) gene in rat colonocytes. Aberrant promoter-region CpG island (CGI) methylation leads to gene-silencing (Laird, 2003), and is increasingly regarded as a contributing factor in the development of CRC, due to the silencing effect it has on tumour-suppressor genes (Belshaw *et al.* 2004). Dietary methyl donors are perceived to play an important role in the level of gene methylation, although how great an influence this is on neoplastic disease is unclear.

Weanling rats were placed on folate-free or folate-supplemented (folic acid; 8 mg/kg diet) semi-synthetic diets for periods of up to 12 months. Folate status was measured periodically by microbiological assay of plasma samples from the tail vein. CGI methylation was assessed by bisulfite modification of genomic DNA, followed by quantitative methylation-specific PCR. In some experiments folate-depleted animals were treated with 1,2 dimethylhydrazine (DMH), and sub-groups were returned to folate-supplemented diets. Animals were killed after 6 weeks and numbers of ACF were assessed.

As in man, *ESR1* CGI methylation increased significantly in colonic mucosa in an age-dependent manner when measured at 3, 6 and 12 months ($P=0.02$). Treatment with DMH appeared to increase the subsequent methylation status of ESR1 in previously folate-depleted rats. A greater number of colonic ACF were observed in rats supplemented with folic acid ($P=0.01$) than in folate-depleted rats.

The present results add to the evidence suggesting that high levels of folate supplementation may enhance the induction of colorectal neoplasia in certain circumstances. Furthermore, dietary folate status did not significantly influence the methylation of the CGI associated with the *ESR1* gene promoter, but it is not yet clear folate status affects the methylation of other genes to thereby exert a direct effect on the carcinogenic process. In view of the widespread use of folate supplements, and the introduction of folate fortification of foods in some countries, further studies on the role of folate in the regulation of DNA methylation are warranted.

Belshaw NJ, Elliot GO, Williams EA, Bradburn DM, Mills SJ, Mathers JC & Johnson IT (2004) *Cancer Epidemiology, Biomarkers and Prevention* **13**, 1495–1501.
Laird PW (2003) *Nature Reviews Cancer* **3**, 253–266.

Effects of glucosinolate breakdown products on the cell-cycle in HT29 colorectal cancer cells. By T.K. SMITH and I.T. JOHNSON, *IFRN, Norwich Research Park, Norwich, UK, NR4 7UA*

Epidemiological studies have shown that Brassica vegetables afford protection against colorectal cancer (van Poppel *et al.* 1999). Glucosinolate breakdown products are liberated from the tissues of these vegetables, during processing or consumption, by the enzymatic actions of plant myrosinase, or by the colonic microflora. One major group of glucosinolate breakdown products, isothiocyanates (ITC), have been shown to block mitosis *in vitro* (Smith *et al.* 2004) and induce apoptosis *in vivo* (Srivastava *et al.* 2003). Previously we have showed that allyl-isothiocyanate (AITC), a major breakdown product of the glucosinolate sinigrin caused loss of cell adhesion and induced a mitotic block in HT29 colorectal cancer cells, 24 h after treatment (Smith *et al.* 2004). Immunofluorescent staining of α – tubulin revealed disruption of the mitotic spindle in blocked, AITC-treated cells.

In the present study we compared the effects on the cell cycle of four ITCs (12 μM): AITC, benzyl-isothiocyanate (BITC), phenethyl-isothiocyanate (PEITC) and sulforaphane (SFN), with juice extracted from raw (SPR) and cooked (SPC) Brussels sprouts (20 μl/ml), and the antimitotic drug paclitaxel (TAX; 8 μM). We also investigated the effects of these agents on the polymerisation of isolated tubulin.

Figure 1.

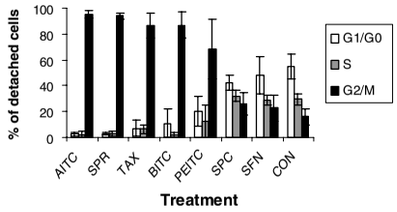
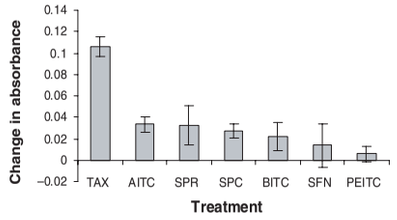


Figure 2.



All test compounds, with the exception of SFN, modified the cell cycle when compared to untreated control (CON) cells (Fig. 1). Treated cells were blocked, to varying degrees, in the G2/M phase of their cycle in the order AITC=SPR>BITC>TAX>PEITC>SPC. Sulforaphane had no effect on the cell cycle. Addition of the test compounds to isolated tubulin resulted in increased polymerisation, 60 min after addition (Fig. 2). Polymerisation was greatest after the addition of TAX, followed by AITC, SPR, SPC, BITC, SFN and PEITC. These results are consistent with the hypothesis that increased tubulin polymerisation plays an important role in the antimitotic effects of glucosinolate breakdown products. This raises the possibility that Brassica vegetable constituents might exert anticarcinogenic effects similar to those of drugs such as Taxol.

This work was supported by the Core Strategic Grant of the BBSRC.

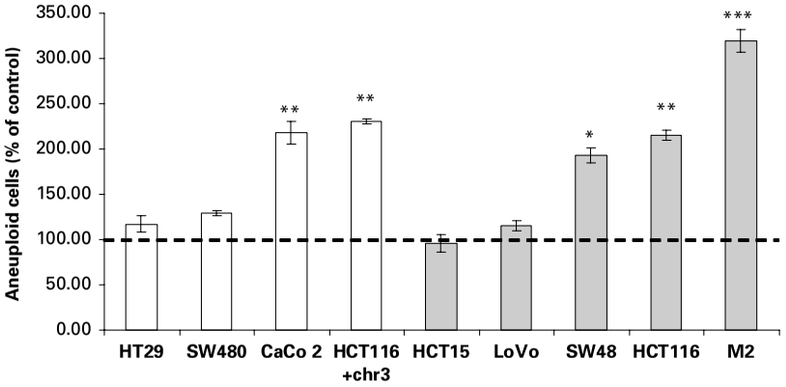
Smith TK, Lund EK, Parker ML, Clarke RG & Johnson IT (2004) *Carcinogenesis* **25**, 1409–1415.
Srivastava SK, Xaio D, Lew KL, Hershberger P, Kokkinakis DM, Johnson CS, Trump DL & Singh SV (2003) *Carcinogenesis* **24**, 1665–1670.
van Poppel G, Verhoeven DT, Verhagen H & Goldbohm RA (1999) *Advances in Experimental Medicine and Biology* **472**, 159–168.

Effect of butyrate on induction of aneuploidy in colorectal carcinoma cell lines. By J.M. COXHEAD[1], W. BAL[1], B.K. SHENTON[2], E.A. WILLIAMS[3] and J.C. MATHERS[1], [1]*Human Nutrition Research Centre, School of Clinical Medical Sciences, University of Newcastle, UK, NE1 7RU,* [2]*School of Surgical and Reproductive Sciences, University of Newcastle, UK, NE2 4HH and* [3]*Human Nutrition Unit, Clinical Sciences (North), University of Sheffield, UK, S5 7AU*

Butyrate is a SCFA endproduct of bacterial fermentation of carbohydrates in the colon and is an important energy substrate for normal colonocytes. Butyrate is also a potent anti-neoplastic agent associated with suppression of proliferation, induction of differentiation and increased apoptosis. One mechanism which may explain such anti-neoplastic effects is butyrate's ability to inhibit histone deacetylation, alter chromatin structure and hence influence gene expression (Williams *et al.* 2003).

It has been suggested that butyrate's effect on chromatin acetylation may contribute to aneuploidy (Gomez-Vargas & Vig, 2002). The present study investigated the effect of butyrate on a panel of nine colorectal carcinoma (CRC) cell lines that are either microsatellite stable (MSS; more likely to show aneuploidy due to chromosomal instability) or have microsatellite instability (MSI; more likely to show a near-diploid DNA content).

Four MSS CRC cell lines (HT29, SW480, CaCo 2, and HCT116+chr3) and five MSI CRC cell lines (HCT15, LoVo, SW48, HCT116, and M2) were cultured in triplicate for 72 h in control medium (as recommended by the European Collection of Cell Cultures) or in medium containing 1 mM-butyrate. Cells were stained with propidium iodide and subjected to fluorescence activated cell sorting to determine DNA content. Single cells were considered to show aneuploidy if their DNA content was greater than respective cells in the G2/M phase of the cell cycle. Data in the figure show aneuploidy in butyrate-treated cells as a percentage of the control (no butyrate) cells.



– – –, 100 %.

* Mean values significantly different from control: * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Some MSS (HT29 and SW480) and MSI (HCT15 and LoVo) cell lines showed no change in aneuploidy following butyrate treatment. However, the MSS cell lines CaCo 2 and HCT116+chr3 and the MSI cell lines SW48, HCT116 and M2 all showed a significant increase in aneuploidy status following 1 mM-butyrate exposure for 72 h. Investigations are currently underway to determine the underlying mechanisms responsible for aneuploidy in both MSS and MSI CRC cell lines following butyrate exposure.

Supported by BBSRC grant (D20173).

Gomez-Vargas P & Vig BK (2002) *Cancer Genetics and Cytogenetics* **138**, 56–63.
Williams EA, Coxhead JM & Mathers JC (2003) *Proceedings of the Nutrition Society* **62**, 107–115.

Response of human colonic microbiota and butyrate formation in continuous culture to a one-unit pH shift. By A.W. WALKER, S.H. DUNCAN, E.C. McWILLIAM LEITCH and H.J. FLINT, *Microbial Ecology Group, Rowett Research Institute, Greenburn Road, Aberdeen, UK, AB21 9SB*

The human gut microbiota is a complex collection of species whose activities can have profound effects upon gut health. A variety of SCFA are produced by bacterial fermentation and provide energy sources for the colonic epithelium. Butyrate in particular influences cell growth and proliferation, and plays an important role in gut health. Bacterial growth and metabolism can be expected to vary depending on the host's diet and on the location within the colon. One significant factor may be the pH of the gut lumen. This is reported to be slightly acidic at the proximal colon with a gradual increase towards pH 6.5 at the distal colon (Nugent *et al.* 2001). This may reflect more rapid carbohydrate fermentation in the proximal colon, and a lower fermentable carbohydrate:protein ratio in the distal colon (MacFarlane & Gibson, 1997).

The present study used anaerobic continuous-flow fermenters with a mixed polysaccharide substrate to investigate the effects of a one-unit pH shift from 5.5 to 6.5 upon human faecal microbial communities from two donors. Experiments were repeated at two peptide inputs (0.6 and 0.1%). Fluorescent *in situ* hybridisation (FISH), employing probes targeted at 16S ribosomal RNA, was used to monitor the microbial community structure (Walker *et al.* 2005).

Final mean butyrate concentrations at pH 5.5 (24.9 mM, high peptide; 13.4 mM, low peptide) were significantly higher than those detected at pH 6.5 (5.6 mM, high peptide; 9 mM low peptide.) At pH 5.5 and high peptide, high butyrate formation corresponded with declining acetate concentrations (Duncan *et al.* 2004). Conversely, high propionate levels (20–23 mM) were detected at pH 6.5 and high peptide conditions. FISH analysis showed that butyrate-producing *Roseburia* species (Duncan *et al.* 2002*a*) reached their highest levels at pH 5.5 (11 to 19% of total bacterial population) but were greatly diminished at pH 6.5. Another butyrate-producing species, *Faecalibacterium prausnitzii* (Duncan *et al.* 2002*b*), was also most abundant (up to 9%) at pH 5.5. The populations of these two groups correlated well with butyrate production. Conversely, *Bacteroides* species increased from approximately 20% to 80% of the total population as a result of the shift from pH 5.5 to pH 6.5 at 0.6% peptide. This correlated with the elevated propionate production.

Selected bacterial groups (% total eubacteria)	Donor 1					Donor 2				
		High peptide		Low peptide			High peptide		Low peptide	
	Start	pH 5.5	pH 6.5	pH 5.5	pH 6.5	Start	pH 5.5	pH 6.5	pH 5.5	pH 6.5
Bacteroides and *Prevotella*	4.2	32.0	83.7	13.1	33.9	12.9	10.4	81.0	14.8	4.3
Clostridial cluster XIVa	24.1	47.3	5.4	36.0	30.6	41.8	34.2	11.1	40.3	86.0
Roseburia genus	5.0	18.8	0	17.2	0	14.4	14.0	0	11.8	0
Faecalibacterium prausnitzii	4.5	7.8	1.3	3.6	2.0	11.7	3.4	1.8	9.2	0.2
Bifidobacteria	0	0	0	10.0	9.0	3.9	0	0	14.0	0.2

Dietary prebiotics such as fructo-oligosaccharides and inulin as well as resistant starch are often reported to have a butyrogenic effect. The results of the present study suggest that this might be explained by a lowering of the lumenal pH as a result of rapid bacterial fermentation of these substrates. A slight reduction in pH apparently favours the growth and metabolism of butyrate-producing bacteria such as *Roseburia* while, at the same time, limiting growth of competing *Bacteroides* species.

Duncan SH, Hold GL, Barcenilla A, Stewart CS & Flint HJ (2002*a*) *International Journal of Systematic and Evolutionary Microbiology* **52**, 1–6.
Duncan SH, Hold GL, Harmsen HJM, Stewart CS & Flint HJ (2002*b*) *International Journal of Systematic and Evolutionary Microbiology* **52**, 2141–2146.
Duncan SH, Holtrop G, Lobley GE, Calder G, Stewart CS & Flint HJ (2004) *British Journal of Nutrition* **91**, 915–923.
MacFarlane GT & Gibson GR (1997) *Gastrointestinal Microbiology*, vol. 1, pp. 269–318. London: B.A. Chapman and Hall.
Nugent SG, Kumar D, Rampton DS & Evans DF (2001) *Gut* **48**, 571–577.
Walker AW, Duncan SH, McWilliam Leitch EC, Child MW & Flint HJ (2005) *Applied and Environmental Microbiology* (In the Press).

The combined effect of flavonoids and epirubicin on *in vitro* proliferation of bladder cancer cells. By S. ELGASS, A. COOPER and M. CHOPRA, *School of Pharmacy and Biomedical Science, University of Portsmouth, Portsmouth, UK, PO1 2DT*

Epirubicin (EPI) is a drug used for the treatment of breast, nasopharyngeal and bladder cancer. The bladder is the most common site of neoplasm in the urinary system. Bladder cancer usually requires year-long treatment, commonly by way of direct instillation of the anthracycline EPI into the bladder. Chemotherapy is known to increase oxidative stress (Doroshow, 1983; Conklin, 2000). Consequently, it is suggested that antioxidant intervention alongside chemotherapy may improve the outcome of the therapy (Lamson & Brignall, 1999; Prasad *et al*. 1999; Conklin, 2000). As flavonoids have antioxidant activity, they might be of value in improving the effects of chemotherapeutic agents such as EPI.

The present study was undertaken to obtain information on the suitability of flavonoids as anti-proliferative agents, either alone or in combination with the anti-tumour drug EPI. Three flavonoid preparations, i.e. quercetin, catechin and resveratrol, were used for the experiments and their effect on the bladder cancer cell line MGH-U1 was studied at varying doses of flavonoids (0.2–40 μmol/l) and drug (0.4–180 μmol/l). Experiments involved doubling dilutions of either EPI or flavonoids alone or in combination with a constant concentration of the other compound. Cytotoxicity of the test compounds was examined by quantifying cell proliferation using MTT reagent ((3,4,5-dimethylthiazol-2-yl)-2–5-diphenyltetrazolium bromide).

At low concentrations (0.2–2 μmol/l), flavonoids on their own reduced cell proliferation (Fig. 1). However, the effect was statistically significant for catechin and resveratrol only ($P<0.05$; Mann–Whitney test). At these concentrations, none of the flavonoids showed any effect on the anti-proliferative activity of EPI (11.5 μmol/l). However, at concentrations >10 μmol/l, all flavonoids either alone or in combination with the drug significantly increased MGH-U1 cell growth. The proliferative effect was highest for catechin and lowest for resveratrol (Fig. 1).

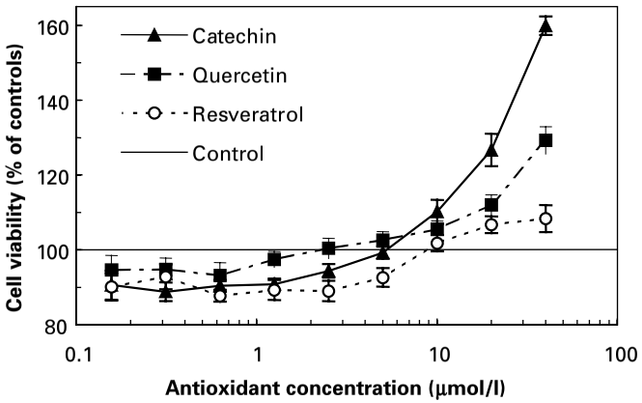


Fig. 1. The effect of the antioxidants catechin (▲), quercetin (■) and resveratrol (○) on MGH-U1 cell viability against a control of untreated cells (—). For clear presentation of data points, log-transformation of the *x* axis was performed. Anti-oxidants were used at concentrations of 0.16, 0.31, 0.63, 1.25, 2.5, 5.0, 10.0, 20.0, and 40.0 μmol/l. Values are means, with vertical bars representing standard deviations.

In conclusion, the present study found that low concentrations of flavonoids inhibit cell growth of MGH-U1 cancer cells. However, at concentrations >10 μmol/l, the effect of antioxidants (catechin, resveratrol, quercetin) with or without the anti-tumour drug EPI was enhancing rather than inhibitory on bladder cancer cell proliferation *in vitro*. This raises concerns in their use with anti-cancer drugs such as EPI.

Conklin KA (2000) *Nutrition and Cancer* **37**, 1–18.
Doroshow JH (1983) *Cancer Research* **43**, 460–472.
Lamson DW & Brignall MS (1999) *Alternative Medicine Review* **44**, 304–329.
Prasad KN, Kumar A, Kochupillai V & Cole WC (1999) *Journal of the American College of Nutrition* **18**, 13–25.

Using Raman microspectroscopy to detect lycopene uptake into prostate cancer cells *in vitro*. By A. GOYAL[1], C. HART-PRIETO[2], N. STONE[2], M. CHOPRA[1] and A. COOPER[1], [1]*School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, UK, PO1 2DT and* [2]*Biophotonics Research Group, Gloucestershire Royal Hospital, Gloucester, UK, GL1 3NN*

There is an ever-increasing focus on chemopreventative measures to curb the rising incidence of prostate cancer. The natural dietary product lycopene derived predominantly from tomatoes has been shown to accumulate in the prostate gland (Clinton *et al.* 1996). It has also been implicated in reducing the risk of developing prostate cancer in individuals consuming a diet rich in tomato products (Giovannucci *et al.* 1995). However, its exact mechanism of action in inhibiting prostatic carcinogenesis remains speculative.

Studying the mode of action, accumulation, distribution and handling of intracellular lycopene remains elusive to conventional methods of cellular imaging. Traditionally, analysis of lycopene in cells and tissues has been by means of HPLC, limited to studies on tissue extracts. Raman microspectroscopy is a novel imaging modality that overcomes this. Acquisition of Raman signals generated by the inelastic scattering of incident monochromatic light due to the vibrational distortion of molecular bonds confers a unique signature to the compound of interest, thereby allowing its detection and potential image generation.

Monolayers of malignant prostatic epithelial cells (PC3) were grown on calcium fluoride slides in Dulbecco's Modified Eagle's cell culture medium. Once the cells had reached 80% confluence they were exposed to 4.65 μmol lycopene/litre. Following an incubation period of 18 h they were imaged using Raman microspectroscopy (Renishaw Ramanoscope; Gloucester, UK) to see whether lycopene was detectable within the cells. Raman spectra were obtained from intracellular regions following excitation with 830 nm wavelength incident laser light.

Signal peaks corresponding to vibrational energy of molecular bonds within lycopene were detectable from within the cells, after background subtraction.

In conclusion, it is therefore possible to detect intracellular lycopene in malignant prostatic cells. Raman microspectroscopy offers a novel way of studying this important chemopreventative agent in its native form. Furthermore, it allows imaging of lycopene in single live cells and in real time. Determination of the exact intracellular distribution, pharmacokinetics, full imaging and even quantification is feasible using this technique.

The present study was supported by funding from Heinz Inc.

Clinton SK, Emenhiser C, Schwartz SJ, Bostwick DG, Williams AW, Moore BJ & Erdman JW Jr (1996) *Cancer Epidemiological, Biomarkers and Prevention* **5**, 823–833.
Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA & Willett WC (1995) *Journal of the National Cancer Institute* **87**, 1767–1776.

Effect of 48 h fasting upon skeletal muscle sterol regulatory element-binding protein-1c (SREBP-1c) and carbohydrate responsive element-binding protein (ChREBP) expression in human subjects. By T. BOONSONG, K. TSINTZAS, A. BENNETT and I. MACDONALD, *Centre for Integrated Systems Biology and Medicine and School of Biomedical Sciences, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, UK, NG7 2UH*

Sterol regulatory element-binding protein-1c (SREBP-1c) and carbohydrate responsive element-binding protein (ChREBP) are important in the regulation of glucose disposal in skeletal muscle, being transcription factors that regulate the expression of genes encoding enzymes of glucose metabolism and lipogenesis. SREBP-1c regulates hexokinase II (HK II), while both SREBP-1c and ChREBP regulate L-pyruvate kinase (L-PK). Animal studies suggest that SREBP-1c and ChREBP are down regulated with fasting (Horton *et al.* 1998; He *et al.* 2004). However, the role and regulation of SREBP-1c and ChREBP in human skeletal muscle is not fully understood. The present study investigated the effect of fasting and acute re-feeding on the expression of these genes in relation to changing whole-body insulin sensitivity.

Ten healthy men (age 26 (SEM 1) years; BMI 25.5 (SEM 1.2) kg/m^2) were studied before, during, and 24 h after fasting for 48 h. Subjects were initially studied 4 h after breakfast, then fasted for 48 h (only water, electrolytes and non-sugared beverages without caffeine were allowed), and finally consumed a high-carbohydrate (CHO) diet (75% CHO, 10% fat and 15% protein) for 24 h. Before and after fasting, and after re-feeding, subjects underwent insulin tolerance tests (ITT) to quantify whole-body insulin sensitivity. Muscle biopsies (*vastus lateralis*) were obtained before fasting (4 h after standard breakfast), after 24 h and 48 h of fasting, and after re-feeding for the determination of SREBP1c, ChREBP, HK II, and L-PK mRNA levels by quantitative real-time PCR using Taqman probes and normalised to α-actin.

Whole-body insulin sensitivity (calculated from the rate constant for blood glucose disappearance during ITT) decreased by 42 (SEM 5) % during fasting ($P<0.01$). Approximately one half of this reduction in insulin sensitivity was reversed after 24 h re-feeding. Fasting decreased blood glucose ($P<0.01$) and insulin ($P<0.05$) concentrations and increased plasma NEFA levels ($P<0.01$); re-feeding completely reversed these responses. SREBP-1c mRNA levels were markedly decreased after 24 h (4.44 (SEM 0.68) arbitrary units) and 48 h (4.08 (SEM 0.81) arbitrary units) fasting and increased to the pre-fasted level upon re-feeding (12.50 (SEM 2.77) arbitrary units), whereas ChREBP mRNA levels were unchanged by fasting and re-feeding. HK II mRNA levels showed a similar pattern to SREBP-1c, falling during fasting and recovering with re-feeding, while L-PK mRNA levels were unchanged in the fasting–re-feeding period. The reduction in insulin sensitivity with fasting was accompanied by a decrease in expression of SREBP-1c and HK II. However, expression of both genes was normalised by 24 h re-feeding when insulin sensitivity had not returned to normal. The metabolic responses to fasting had no effect on expression of ChREBP or L-PK.

Fasting does not have the same effect on skeletal muscle SREBP-1c and ChREBP gene expression to that reported in animals.

The present study was approved by the University of Nottingham Medical School Ethics Committee and funded in part by the BBSRC grant 42/D/15633.

Horton JD, Bashmakov Y, Shimomura I & Shimano H (1998) *Proceedings of the National Academy of Sciences* USA **95**, 5987–5992.
He Z, Jiang T, Wang Z, Levi M & Li J (2004) *American Journal of Physiology and Endocrinology Metabolism* **287**, E424–E430.

Effects of stepwise overfeeding on abdominal fat deposition and insulin sensitivity in adult lean men. By S.A. JEBB[1], M. SIERVO[1], L.J.C. BLUCK[1], A. DIXON[2] and A.M. PRENTICE[3], [1]*MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn, Cambridge, UK, CB1 9NL,* [2]*Department of Radiology, University of Cambridge, Box 219, Addenbrooke's Hospital, Hills Road, Cambridge, UK, CB2 2QQ and* [3]*MRC International Nutrition Group, London School of Hygiene and Tropical Medicine, Keppel Street, London, UK, WC1E 7HT*

Obesity is associated with an accumulation of fat and increased risk of metabolic diseases. Increases in visceral fat are associated with an increased flux of NEFA in the portal and systemic circulation, which has been linked to the pathogenesis of insulin resistance (Garg, 2004). These effects are difficult to measure in the normal course of events due to the protracted course of weight gain.

Five lean, healthy men (age 44.2 (SD 11.6) years; baseline BMI 22.1 (SD 1.4) kg/m^2) participated in a 17-week stepwise overfeeding study. A 3-week baseline period preceded three overfeeding (OF) phases (20, 40, and 60% energy intake above baseline) separated by week-long *ad libitum* phases. Body fat was assessed with a four-compartment model (Fuller *et al.* 1992) using dual X-ray absorptiometry, ^{2}H dilution and air-displacement plethysmography. A magnetic resonance imaging scan at abdominal level was performed at baseline and at the end of 60% OF to examine fat distribution. A frequently sampled intravenous glucose tolerance test (minimal model) was used to assess insulin sensitivity (S_i), glucose effectiveness (S_g), insulin secretion (AIR) and disposition index (DI).

At the end of 60% OF, weight increased by 5.98 (SD 1.37) kg ($P<0.05$) of which 55% was fat. There was a significant increase in fat in the visceral (+33 (SD 18) %; $P<0.05$) and subcutaneous depots (+18 (SD 4) %; $P<0.05$). There were no significant changes in fasting insulin and S_i. There was a trend towards an increase in S_g, AIR and DI although in this small group the change was not significant (Table).

	Baseline		OF +20%		OF +40%		OF +60%	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Weight (kg)	68.68	9.83	69.36	9.96	71.85	9.66	74.67*	10.37
Fat mass (kg)†	14.62	7.29	14.71	7.02	15.89	6.76	17.93*	7.92
Subcutaneous fat (cm^2)‡	472	91	–	–	–	–	558*	109
Visceral fat (cm^2)‡	115	20	–	–	–	–	153*	40
Fasting insulin (pmol/l)	27.40	8.76	28.60	12.65	27.60	13.16	36.20	11.86
S_i ($\times 10^{-4}$ pmol/h per l)	5.02	1.30	5.06	2.59	5.76	3.58	5.64	2.37
S_g (per h)	0.788	0.257	1.262	0.575	1.127	0.578	1.291	0.366
First phase insulin response (AIR) (pmol)	0.84	0.52	1.32	0.57	1.13	0.72	1.44	0.78
DI ($\times 10^{-4}$)§	3.77	1.54	5.76	1.72	5.01	2.30	7.83	3.5
GEZI (per min)	0.65	0.24	1.13	0.53	0.99	0.55	1.09	0.31

GEZI, glucose effectiveness zero insulin ($S_g - (S_i \times$ fasting insulin)).
* $P<0.05$. The Wilcoxon exact test was used to test for statistical significance.
† Fat mass and fat-free mass were assessed using a four-compartment model.
‡ Regional fat was assessed using magnetic resonance imaging.
§ $S_i \times$AIR.

Overfeeding in lean healthy men resulted in a significant increase in body fat, which was proportionately greater in the visceral depots. There was a trend towards an increase in insulin-independent effectors of glucose control (S_g, GEZI) and an increase in the DI driven by a change in insulin secretion (AIR) but in this small group the changes in parameters of insulin sensitivity were not statistically significant.

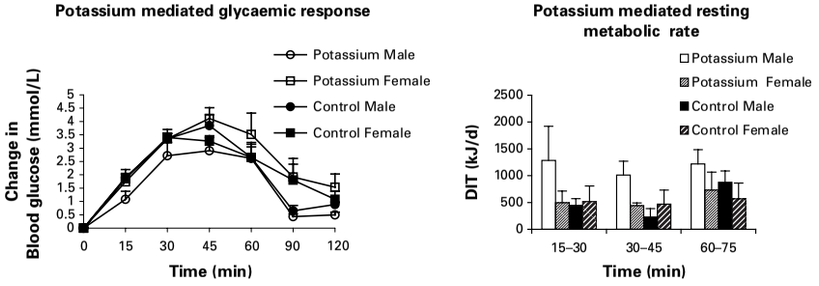
Fuller NJ, Jebb SA, Laskey MA, Coward WA & Elia M (1992) *Clinical Science* **82**, 687–693.
Garg A (2004) *Journal of Clinical Endocrinology and Metabolism* **89**, 4206–4210.

The role of potassium on blood glucose – is glycaemic response sex-specific? By T.A. SEYOUM and C.J.K. HENRY, *Nutrition and Food Science Group, School of Biological and Molecular Sciences, Oxford Brookes University, Gipsy Lane Campus, Headington, Oxford, UK, OX3 OBP*

The glycaemic index (GI) is defined as the incremental area under the blood glucose response curve (IAUC) of a 50 g carbohydrate portion of a test food expressed as a percentage of the response to the same amount of carbohydrate from a reference food taken by the same subject. Numerous factors, notably the physico-chemical nature of carbohydrate, protein and fat, have been shown to influence the digestion and absorption of glucose; thereby the blood glucose response curve and the GI. Low-GI foods have been shown to be beneficial in reducing the risk of obesity and its co-morbidities, namely type 2 diabetes and CVD. The objective of the present study was to investigate the potential impact K might have on the blood glucose response curve and to highlight the impact sex differences may have on glycaemic response.

The effect of 99 mg potassium gluconate (Holland & Barrett) on glycaemic response was investigated in twelve subjects (six male and six female aged between 20 and 45 years, with body weight (77 (±19) kg) and (58 (±8) kg respectively. All measurements were conducted according to standard Food and Agriculture Organization/World Health Organization (1998) procedures. K-fortified glucose was compared with a reference food (glucose), tested in equivalent amounts (50 g) of available carbohydrate with 200 ml water (Malvern still water). Blood glucose was measured at 0, 15, 30, 45, 60, 90 and 120 min. Concomitantly, the Deltatrac metabolic monitor (SensorMedics Corp, Yorba Linda, CA, USA) was used to measure the resting energy expenditure at 15–30, 30–45 and 60–75 min both at post-glucose and post-K-fortified-glucose absorptive stages.

The addition of 99 mg K to a glucose solution significantly decreased the blood glucose IAUC by 32% ($P=0.004$) in male subjects only. Moreover, the first 45 min post-K-fortified glucose showed a significant 26% ($P=0.049$) decline in male glycaemic response. An additional observation was a significant two-fold ($P=0.048$) increase in mean RMR as a result of the addition of K to glucose. Interestingly, in the K-treated male, the increase in resting energy expenditure coincided with the fall in IAUC.



The present study is the first undertaken to examine the effect of K on blood glucose response while considering the impact of sex differences. Further investigation is required to understand the mechanism and the potential role K may play in male and female energy and glucose metabolism during the measurement of GI.

Food and Agriculture Organization/World Health Organization (1998) *Carbohydrate in Human Nutrition*. Rome: FAO.

Postprandial metabolic responses to a low-fat low-glycaemic index diet in a simulated shiftwork environment. By N.L. HARMAN, C.W. TZUNG, S.M. HAMPTON and L.M. MORGAN, *Department of Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH*

Approximately 15% of the UK population have reported working in a shiftwork environment. Shiftwork, and in particular night-shiftwork, is associated with an approximately 40% increased risk of CVD. An important factor contributing to this increased risk could be the increased incidence of postprandial metabolic risk factors for CVD amongst shiftworkers, as a consequence of the maladaptation of endogenous circadian rhythms to abrupt changes in shift times. There is a diurnal rhythm in insulin sensitivity, with relative insulin insensitivity at night. We have previously shown that both simulated (Ribeiro *et al.* 1998) and real shiftworkers (Lund *et al.* 2001) show relatively impaired glucose and lipid tolerance if a single test meal is consumed between 00.00 and 02.00 hours (night shift), compared with 12.00–14.00 hours (day shift). A diet containing carbohydrates with a low glycaemic index is associated with increased insulin sensitivity. The aim of the present study was therefore to investigate the potential of a low-glycaemic index diet to normalise the night-time response to a meal with respect to the elevated glucose, insulin and triacylglycerol (TAG) usually observed following a meal at night.

Eight non-obese male volunteers (mean age 26 (range 24–32) years; mean BMI 21.6 (range 19.9–24.2) kg/m^2) were recruited and their metabolic and hormonal responses to test meals observed on two occasions; once during a simulated day shift (08.00–19.00 hours) and once during a simulated night shift (20.00–07.00 hours). The meal immediately preceding each study occasion was standardised. On each occasion subjects received an identical diet (two meals at 08.00 (or 20.00) hours and 13.00 (or 01.00) hours and two snacks at 10.00 (or 22.00) hours and 16.00 (or 04.00) hours) with a total energy of 7682 kJ (total energy distribution 26% fat, 17% protein, 57% carbohydrate) and an average calculated mixed GI of 38. Meal order was arranged to simulate the type of meals typically consumed at specific times of day. Venous blood was taken after an overnight fast, and for 11 h following the first meal on each occasion, for the measurement of glucose, insulin and TAG. Significant ($P<0.05$) shift×time interactions were observed for all analytes, attributable to meal order effects. Area under the curve (AUC) analysis (0–11 h) indicated the persistence of a significantly higher total AUC (TAUC) for plasma glucose at night ($P<0.01$), but no significant difference between the day and night plasma profiles for insulin and TAG, as had been observed in previous studies.

	Simulated dayshift (08.00–19.00 hours)		Simulated nightshift (20.00–07.00 hours)	
	Mean	SEM	Mean	SEM
Glucose TAUC (mmol/l×min)	3404	100	3773*	111
Insulin TAUC (pmol/l×h)	1805	218	2054	291
TAG TAUC (mmol/l×min)	799.6	71.3	756.0	69.1

* $P=0.008$.

The present study has demonstrated a partial normalisation of the previously observed raised nocturnal postprandial responses to identical meals consumed at night and during the day. Previous studies have used higher-fat, higher-energy meals. The data suggest that a small (1699 kJ) low-fat meal at night, containing carbohydrates with a lower GI, is effective in minimising inappropriate nocturnal postprandial insulin and TAG responses, but not glucose responses.

Lund J, Arendt J, Hampton SM, English J & Morgan LM (2001) *Journal of Endocrinology* **171**, 557–564.
Ribeiro D, Hampton SM, Morgan LM, Deacon S & Arendt J (1998) *Journal of Endocrinology* **158**, 305–310.

The acute effect of leptin injection on *in vivo* postprandial glycogen and lipid synthesis in rats.

By M. BASSIL, N. HWALLA and O.A. OBEID, *Department of Nutrition and Food Science, American University of Beirut, Beirut, Lebanon*

Leptin, a hormone secreted primarily by adipocytes, proportional to degree of adiposity, is known to act centrally through its receptors located mainly in the hypothalamus to regulate energy metabolism and food intake. Leptin receptors have also been isolated from peripheral tissues such as muscles, liver and adipose tissues where leptin is thought to play a role in carbohydrates and lipid metabolism independent of central involvement (Muoio & Lynis Dohm, 2002). In rats, leptin administration has been reported to enhance gluconeogenesis and inhibit glycogenolysis in liver, and enhances whole-body fatty acid oxidation and triacylglycerol hydrolysis (Muoio & Lynis Dohm, 2002). These experiments were performed following a few hours or days of leptin exposure, but the immediate effect of leptin administration on hepatic glycogen and lipid synthesis is still not clear.

The present experiment was designed to investigate the acute or immediate effect of leptin injection on hepatic glycogen and lipid synthesis *in vivo*. Twenty adult male Sprague–Dawley rats were fed *ad libitum* a semi-synthetic diet supplying 21% of energy as protein, 23% as fat (maize oil) and 56% as carbohydrate. Overnight-fasted rats were tube fed with 4 ml water containing 1.25g of the diet and immediately injected intraperitoneally (i.p.) with 4 mCi ^3H_2O. After 1 h, rats were either i.p. injected with saline (control; *n* 10) or leptin (20 µg/rat; *n* 10) and killed 1 h later. Blood, liver and epididymal fat pads (EFP) were taken for analysis as described previously (Obeid *et al.* 2000).

	Control		Leptin	
	Mean	SE	Mean	SE
Plasma glucose (mg/l)	1486	75.3	1462	33.2
Plasma triacylglycerol (mg/l)	421.0	43.5	593.0*	56.4
Hepatic fatty acid synthesis	6.63	0.54	5.88	2.11
EFP fatty acid synthesis	2.91	0.43	2.54	0.80
Glycogen content (mg/g liver)	15.57	1.08	16.73	1.79
Glycogen synthesis (mg/g liver per h)	42.88	3.35	31.46*	3.62
Glycogen via pyruvate (%)	43.00	2.54	45.17	3.81

* $P<0.05$ (*t* test).

Plasma glucose concentration was similar between the groups while that of triacylglycerol was higher in the leptin group. Both hepatic and EFP fatty acid synthesis were not affected by leptin injection. Hepatic glycogen synthesis was reduced, but content and synthesis via pyruvate (%) were not affected by leptin administration. The association between plasma triacylglycerol elevation and unaltered hepatic fatty acids synthesis indicates that such elevation may have been the result of a decrease in peripheral fatty acid uptakes.

In conclusion, leptin administration had no immediate effect on hepatic and adipose tissue fatty acid synthesis, but reduces *in vivo* postprandial hepatic glycogen synthesis and triacylglycerol uptake by peripheral tissues. The peripheral effect of leptin on glucose and fat metabolism may depend on both the type of tissues and duration of exposure.

Muoio DM & Lynis Dohm GL (2002) *Best Practice and Research. Clinical Endocrinology and Metabolism* **16**, 653–666.
Obeid OA, Powell-Tuck J & Emery PW (2000) *International Journal of Obesity* **24**, 508–513.

No acute effect of ghrelin injection on *in vivo* postprandial glycogen and lipid synthesis of rats.

By M. MRAYATI, N. HWALLA and O.A. OBEID, *Department of Nutrition and Food Science, American University of Beirut, Beirut, Lebanon*

Ghrelin, an acylated peptide originally identified in rat stomach as the endogenous ligand for the growth hormone secretagogue receptor (GHS-R), is known to act centrally through its receptors (GHS-R) located mainly in the hypothalamus and pituitary gland to regulate energy metabolism and food intake (Gnanapavan *et al.* 2002). Ghrelin receptors are also present in peripheral tissues such as liver and adipose tissues where ghrelin can act either directly on these receptors or indirectly via the hypothalamus or via pituitary hormones (Muccioli *et al.* 2002). Plasma ghrelin levels are known to be negatively correlated with BMI, body fat mass, adipocyte size, and plasma glucose levels (Muccioli *et al.* 2002). Ghrelin injection was reported to have a short-term effect on food intake, which increases only for a short period (about 2 h). However, it is not clear whether this is associated with changes in glycogen and lipid metabolism.

The present experiment was designed to investigate the acute or immediate effect of ghrelin injection on hepatic glycogen and lipid synthesis *in vivo*. Twenty adult male Sprague–Dawley rats were fed *ad libitum* a semi-synthetic diet supplying 21% of energy as protein, 23% as fat (maize oil) and 56% as carbohydrate. Overnight fasted rats were tube fed with 4 ml of water containing 1.25 g of the diet and immediately injected intraperitoneally (i.p.) with 4 mCi ^3H_2O. After 1 h, rats were either i.p. injected with saline (control; *n* 10) or ghrelin (10 µg/rat) (n 10) and killed 1 h later. Blood, liver and epididymal fat pads (EFP) were taken for analysis as described previously (Obeid *et al.* 2000).

	Control		Ghrelin	
	Mean	SD	Mean	SD
Plasma glucose (mg/l)	1375	38	1316	44
Plasma triacylglycerol (mg/l)	562	64	538	55
Hepatic fatty acid synthesis (/h)	3.04	0.22	3.36	0.11
EFP fatty acid synthesis (/h)	1.54	0.15	2.17	0.50
Glycogen content (mg/g liver)	15.18	0.92	14.33	0.87
Glycogen synthesis (/g liver per h)	38.40	3.20	37.06	2.30
Glycogen via pyruvate (%)	42.78	2.20	44.03	2.50

Plasma glucose concentration was similar between the groups. Hepatic and EFP fatty acid synthesis were not affected by ghrelin injection. Similarly, hepatic glycogen synthesis and content, as well as the glycogen via pyruvate (%), were not affected by ghrelin administration. Ghrelin administration does not seem to alter the partitioning of fuels between lipid and glycogen.

In conclusion, ghrelin does not have an immediate effect on *in vivo* postprandial hepatic glycogen and lipid synthesis. Partitioning of fuels between lipid and glycogen may not be involved in the increase in food intake following ghrelin administration.

Gnanapavan S, Kola B, Bustin SA, Morris DG, McGee P, Pairclough P, Bhattacharya S, Carpenter R, Grossman AB & Korbonits M (2002) *Journal of Clinical Endocrinology and Metabolism* **87**, 2988–2991.
Muccioli G, Tscho M, Papotti M, Deghenghi R, Heiman M & Ghigo E (2002) *European Journal of Pharmacology* **440**, 235–254.
Obeid OA, Powell-Tuck J & Emery PW (2000) *International Journal of Obesity* **24**, 508–513.

Inhibition of stearoyl CoA desaturase activity induces hypercholesterolaemia in cholesterol-fed hamsters. By H.M. SIMS[1], C. MAJOR[1], A.L. LOCK[2], D.E. BAUMAN[2] and A.M. SALTER[1], [1]*Division of Nutritional Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire, UK, LE12 5RD and* [2]*Department of Animal Science, Cornell University, Ithaca, NY 14853, USA*

Stearoyl CoA desaturase (SCD) plays an important role in regulating tissue fatty acid composition primarily by converting stearic acid to oleic acid (*cis*-9 18:1) and has been reported to be required for normal synthesis of cholesteryl ester in the liver (Miyazaki *et al.* 2000). In the present experiment we have investigated the effect of inhibiting SCD on plasma and lipoprotein cholesterol levels in hamsters fed diets with and without added dietary cholesterol.

Male Golden Syrian Hamsters (n 8 per group) were fed chow-based diets supplemented with 20% butter (CON). Further groups were fed the same diet supplemented with 0.2% cholesterol (CH), 0.5% sterulic acid (SA) or both (CH+SA). Sterculic acid, a cyclopropenoic fatty acid, is a known inhibitor of SCD activity. Diets were fed for 4 weeks after which animals were killed and blood and tissues collected. Lipids were extracted from liver and analysed for fatty acid composition by GC of fatty acid methyl esters. Total plasma cholesterol and non-esterified cholesterol were determined enzymically using commercial kits. The amount of cholesterol in the esterified form was calculated as the difference between these two values. Plasma lipoproteins were separated by preparative ultracentrifugation and cholesterol determined as above. Hepatic cholesterol and cholesteryl ester were separated by TLC before enzymic determination. Data were analysed by two-way ANOVA with significant effects of cholesterol (CH), sterculic acid (SA) or an interaction between the two (CH × SA) reported.

	CON	CH	SA	CH+SA	SED	Significant effects
Hepatic C18:0 (%)	18.8	14.1	24.3	22.0	2.2	CH*, SA***
Hepatic C18:1 (%)	26.8	34.3	20.1	25.0	2.7	CH**, SA***
Plasma cholesterol (mM)	4.71	9.87	4.04	15.07	0.44	CH×SA***
Plasma free cholesterol (mM)	1.49	2.64	1.26	6.06	0.307	CH×SA***
Plasma cholesteryl ester (% of total)	68.52	73.26	68.76	59.96	1.50	CH×SA***
LDL-cholesterol (mM)	0.24	1.63	0.20	5.59	0.34	CH×SA***
HDL-cholesterol (mM)	2.67	3.52	2.31	3.73	0.32	CH***
Hepatic free cholesterol (mg/liver)	8.3	14.2	6.3	12.8	1.1	CH***, SA*
Hepatic cholesteryl ester (mg/liver)	5.7	102.8	1.7	40.7	6.73	CH×SA***

SED, Standard error of the difference.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Changes in hepatic 18:0 and *cis*-9 18:1 were indicative of increased SCD activity with CH-feeding and inhibition by SA. Total plasma cholesterol was increased by CH with a small increase in the relative proportion of cholesteryl ester. SA had no effect of plasma cholesterol when fed alone, but caused a dramatic increase when fed together with CH. This was associated with a relative decrease in the proportion of cholesteryl ester. CH feeding increased both LDL- and HDL-cholesterol but the interactive effect of CH and SA was restricted to the LDL fraction. CH feeding increased hepatic non-esterified and esterified cholesterol with a much larger effect on the latter. SA significantly reduced the increase in hepatic cholesteryl ester associated with CH feeding.

The present studies support the finding that SCD plays an important role in regulating hepatic cholesterol esterification even when significant amounts of oleic acid are present in the diet. Low SCD activity compromises the ability to store dietary cholesterol as cholesteryl ester and, as a result, dramatically increases LDL-cholesterol.

The present study was supported in part by the UK BBSRC.

Miyazaki M, Kim Y-C, Gray-Keller MP, Attie AD & Ntambi JM (2000) *Journal of Biological Chemistry* **276**, 30032–30138.

Interactive effects of dietary cholesterol and inhibition of stearoyl CoA desaturase activity on lipogenic gene expression. By K. RYAN[1], C. MAJOR[1], A.L. LOCK[2], D.E. BAUMAN[2] and A.M. SALTER[1], [1]*Division of Nutritional Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire, UK, LE12 5RD and* [2]*Department of Animal Science, Cornell University, Ithaca, NY 14853, USA*

Stearoyl CoA desaturase (SCD) introduces a double bond between carbons 9 and 10 of fatty acids. Preferred substrates include the CoA derivatives of palmitic (16:0), stearic (18:0) and vaccenic (*trans*-11 18:1) acids. Conversion of stearic acid to oleic acid (*cis*-9 18:1), the predominant MUFA in the body, appears to be the primary function of the enzyme. It has recently been shown that inhibition of SCD activity results in reduction of body fat in mice. Dietary cholesterol is known to increase SCD gene expression, while sterculic acid, a cyclopropenoic fatty acid, is a known inhibitor of enzyme activity. In the present experiment we have investigated the effects of adding cholesterol and/or sterculic acid to the diet of hamsters, on body weight and the expression of lipogenic genes in liver.

Male Golden Syrian hamsters (*n* 8 per group) were fed chow-based diets supplemented with 20% butter (CON). Further groups were fed the same diet supplemented with 0.2% cholesterol (CH), 0.5% sterculic acid (SA) or both (CH+SA). Body mass and food intake were monitored throughout the experiment and feed efficiency was calculated as change in body weight (g)/food intake (g). Diets were fed for 4 weeks after which animals were killed and organ weights recorded. Livers were snap-frozen in liquid N_2 and stored at −80 °C until determination of mRNA concentration. The steady-state mRNA concentration for acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), lipoprotein lipase (LPL) and SCD were determined by real-time PCR. Data were analysed by two-way ANOVA with significant effects of cholesterol (CH), sterculic acid (SA) or an interaction between the two (CH×SA) reported.

	CON	CH	SA	CH+SA	SED	Significant effects
Body weight (g)	127.3	126.8	129.5	108.5	4.5	CH×SA**
Efficiency (g/g)	0.036	0.048	0.046	−0.075	0.021	CH×SA***
Adipose tissue (% body weight)	1.58	1.61	1.48	1.09	0.12	CH × SA*

SED, Standard error of the difference.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

No significant effect of CH or SA alone was seen on body weight, feed efficiency or perirenal adipose tissue weight. However, when CH and SA were fed together significant reductions were seen in all three parameters, with animals actually losing weight.

Data for hepatic mRNA concentrations (relative to β-actin mRNA concentration) are shown below. Feeding CH and SA together reduced ACC mRNA relative to the other diets. Feeding CH decreased FAS mRNA but increased LPL and SCD mRNA, compared with CON and SA. The effects of CH were further amplified when SCD was inhibited by SA.

	CON	CH	SA	CH+SA	SED	Significant Effects
ACC	2.42	2.11	3.28	0.78	0.536	CH×SA**
FAS	2.68	1.38	3.42	0.50	0.408	CH×SA**
LPL	0.66	2.08	1.05	3.13	0.391	Ch***, SA*
SCD	0.59	1.85	0.71	2.85	0.253	CH×SA*

SED, Standard error of the difference.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

We conclude that in the hamster, inhibiting SCD has little effect of body fat deposition unless cholesterol is also present in the diet. When this is the case, there appears to be a specific reduction in the expression of ACC and FAS genes in the liver. By contrast, LPL and SCD expression are increased in response to dietary cholesterol, an effect which is amplified by inhibition of SCD activity.

The present study was supported in part by the UK BBSRC.

Influence of palmitic acid-rich triacylglycerols on postprandial activation of factor VII. By S.E.E. BERRY[1], G.J. MILLER[2] and T.A.B. SANDERS[1], [1]*Nutritional Sciences Research Division, King's College London, 150 Stamford Street, London, UK, SE1 9NN and* [2]*Medical Research Council Cardiovascular Group, Wolfson Institute, St Bartholomew's and the Royal London School of Medicine and Dentistry, Charterhouse Square, London, UK, EC1M 6BQ*

It has previously been demonstrated that altering the triacylglycerol (TAG) structure of stearic acid-rich fats influences postprandial lipaemia and the activation of FVII (Sanders *et al.* 2003). This research has been extended to ascertain if manipulating the TAG structure of palmitic acid-rich fats has similar effects on postprandial FVII activated (FVIIa) concentrations.

Two studies were conducted using a similar test meal model. Healthy male subjects (age 20–50 years) were fed test meals containing 50 g test fat in a randomised cross-over design. Plasma FVIIa concentrations were measured at fasting and 3 and 6 h postprandially. The first study compared palm oil, consisting predominantly of the TAG species 1-palmityl, 2-oleyl, 3-palmityl glycerol with randomly interesterified (randomised) palm oil (n 20). The second study compared randomised palm oil with high-oleic sunflower-seed oil (n 18). Data were log-normalised before statistical analysis by repeated-measures ANOVA. Results are shown in the Figs. 1 and 2.

Fig. 1.

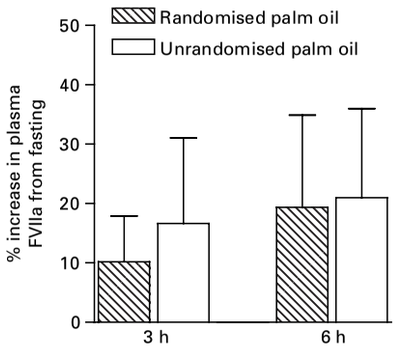
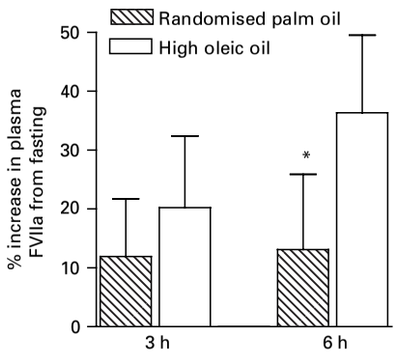


Fig. 2.



Change in FVIIa concentration following test meals (geometric mean with 95% CI).

Fig. 1. Randomised *v.* unrandomised palm oil (n 20); no significant differences between meals.

Fig. 2. Randomised palm oil *v.* high-oleic sunflower-seed oil (n 18); * $P<0.05$ compared with the corresponding high-oleic oil value (paired t test).

As expected, FVIIa concentrations increased postprandially ($P<0.01$ at 3 h and 6 h in both studies). The postprandial increase following randomised palm oil tended to be lower compared with unrandomised palm oil, but did not achieve statistical significance. However, the increase in FVIIa was lower at 6 h following randomised palm oil compared with high-oleic sunflower-seed oil (ANOVA; meal ×time interaction $P=0.043$; diet effect $P=0.02$).

These results are consistent with our previous observations (Oakley *et al.* 1998) that saturated fatty acid-rich TAG do not increase activation of FVII more than MUFA-rich TAG.

Oakley FR, Sanders TA & Miller GJ (1998) *American Journal of Clinical Nutrition* **68**, 1202–1207.
Sanders TA, Berry SE & Miller GJ (2003) *American Journal of Clinical Nutrition* **77**, 777–782.

Systematic review of published trials on the effects of *n*-3 long-chain polyunsaturated fatty acids on depressed mood. By K.M. APPLETON[1], R.C. HAYWARD[1], D. GUNNELL[2], T.J. PETERS[3], P.J. ROGERS[1], D. KESSLER[3] and A.R. NESS[4], [1]*Department of Experimental Psychology, University of Bristol, Bristol, UK, BS8 1TN,* [2]*Department of Social Medicine, University of Bristol, Bristol, UK, BS8 1TN,* [3]*Academic Unit of Primary Health Care, Department of Community Based Medicine, University of Bristol, Bristol, UK, BS8 1TN and* [4]*Unit of Paediatric and Perinatal Epidemiology, University of Bristol, Bristol, BS8 1TN*

Several lines of evidence suggest a relationship in man between dietary intake of n-3 long-chain PUFA (n3FAs) and depressed mood. The present review aimed to identify and combine all published randomised controlled trials (RCT) investigating the effects of dietary supplementation with n3FAs on depressed mood.

Published RCT were identified by searching eight databases (MEDLINE, EMBASE, PsycInfo, CINAHL, Biosis, AMED, Cochrane Controlled Trials Register, Cochrane Database of Systematic Reviews) over all years of records until October 2003. Identified articles were independently assessed for inclusion in the review by two researchers using six inclusion criteria: published in English; exposure was n3FAs or fish; outcome measures included depressed mood; study of human participants; included a comparison group; reported a trial. Data were abstracted independently from each identified trial by three researchers.

Eleven relevant RCT were identified. Trials ranged in size, participant number, participant population, n3FA intervention, and measure of depressed mood. Most of the trials were small, of short duration and used different combinations of different doses of n3FAs in varied groups of participants. Trials also ranged in assessed quality. Data from eight trials reporting mean and standard deviation data were formally combined by meta-analyses using random and fixed effects models.

The pooled standardised difference in mean outcome from a fixed effects model was 0.03 (95% CI −0.11, 0.18) standard deviations in those receiving n3FA supplementation compared with those receiving placebo. The pooled standardised difference in means using a random effects model was 0.54 (95% CI 0.08, 1.00) standard deviations. There was strong evidence of heterogeneity between studies (I^2 37%; $P<0.001$) and clear funnel plot asymmetry. The pooled difference was strongly influenced by the results of one large trial where depressed mood was not a primary outcome measure. This trial pulled the pooled standardised difference in means towards the null, but removal of this trial did not reduce heterogeneity. Heterogeneity was reduced by analysis only by studies conducted in participants with clinical depression, where n3FA provided a beneficial effect on depressed mood compared with placebo.

The pooled estimate from the fixed effects model provides no evidence of a beneficial effect of n3FAs on depressed mood, whereas the combined estimate from the random effects model does suggest a beneficial effect. The size of this latter effect, however, is small and of limited clinical significance. Moreover, evidence of heterogeneity and funnel plot asymmetry suggests that the combined estimates should be interpreted with caution. There are several causes of heterogeneity and funnel plot asymmetry, the most usual of which is publication bias. If publication bias exists, random effects models, which place greater weight on small studies, tend to produce estimates of effect size that are biased in the direction of the positive effects of the small published studies. For this reason, more credence is given here to the results of the fixed effects model.

Thus, the evidence available does not support the use of n3FAs to improve depressed mood. Marked heterogeneity and funnel plot asymmetry are consistent with publication bias in favour of smaller studies that report beneficial effects. Large well-conducted trials are required to clarify the effect of n3FAs on depressed mood.

This work was funded by the Food Standards Agency, UK Government (grant NO5038) and The University of Bristol, UK.

Meal fatty acid composition and postprandial vascular reactivity. By C.K. ARMAH, L. JAMES, I. DOMAN and A.M. MINIHANE, *Hugh Sinclair Unit of Nutrition, University of Reading, UK, RG6 6AP*

Decreased smooth-muscle tone and vascular reactivity is associated with the development and progression of atherosclerosis and hypertension and increased risk of CVD. A number of recent studies have demonstrated that increased meal fat content is associated with decreased postprandial vascular reactivity, although the impact of meal fatty acid composition remains largely unknown. Chronic fish oil feeding has been repeatedly shown to improve endothelial function and vascular tone (Khan *et al.* 2003). However, the acute impact of fish oil fatty acids on postprandial vascular reactivity is unknown and is the subject of the present investigation.

Twenty healthy males (age 18–70 years) completed an acute test meal cross-over study, where they attended the Clinical Investigation Unit on two separate occasions, consuming either a placebo or fish oil test meal in random order. The high-fat test meals, consisted of 73 g white bread, 30 g strawberry jam and an oil-containing chocolate shake made with either 40 g mixed fat (palm olein–soyabean oil; 80:20 w/w) with a fatty acid profile representative of a typical UK diet, or 31 g mixed fat and 9 g fish oil providing 5.4 g EPA plus docosahexaenoic acid (DHA). On each visit volunteers attended the unit in a fasting state, and vascular reactivity was measured at baseline and at 2 and 4 h post-breakfast. The vascular reactivity of the forearm microvasculature was determined by laser Doppler iontophoresis, where blood flow in response to transdermally administered acetylcholine (endothelial-dependent vasodilator; Ach) and sodium nitroprusside (endothelial-independent vasodilator; SNP) was assessed by laser imaging. In addition, blood samples were collected at 0 and 4 h for the determination of blood lipids, insulin and circulating measures of oxidative stress and endothelial function (data not shown).

No significance changes in postprandial vascular reactivity or impact of fat type were evident 2 h post-breakfast. However, at 4 h post-breakfast, Ach- and SNP-induced reactivity decreased by 2.7 (NS) and 8.6% (NS) following the placebo meal, and increased by a highly significant 27.1 ($P<0.05$) and 46.8% ($P<0.05$) following the fish oil-containing meal. A 0–4 h 67 and 81% increase in fasting triacylglycerol (TG) levels were evident following the fish oil and placebo test meals respectively, with the inter-group differences failing to reach significance. However, significant correlations between 4 h endothelial-dependent reactivity and fasting TG were observed ($P<0.05$).

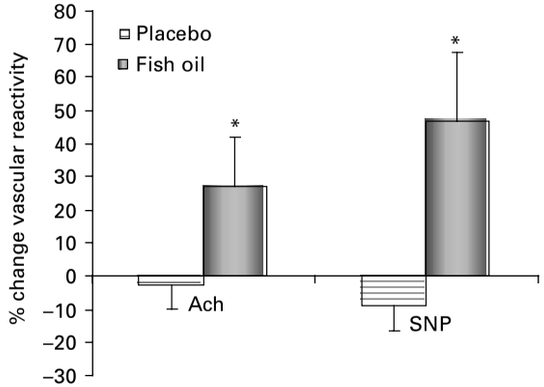


Figure: Vascular reactivity 4 h post-breakfast
* indicates significantly different from baseline

The present study provides novel findings which suggest that meal fatty acid composition is an important determinant of vascular reactivity, with fish oil fatty acids resulting in significant increases in postprandial vascular tone. The effect was greatest with respect to endothelial-independent vascular reactivity, indicating a direct effect of fish oil fatty acids on smooth-muscle action. Further studies are needed to elucidate potential mechanisms.

Khan F, Elherik K, Bolton-Smith C, Barr R, Hill A, Murrue I & Belch JJ (2003) *Cardiovascular Research* **59**, 955–962.

The bioavailability of stearic acid-rich triacylglycerol from shea butter. By L. CHEN, T.A.B. SANDERS and S.E.E. BERRY, *Nutritional Sciences Research Division, King's College London, 150 Stamford Street, London, UK, SE1 9NH*

We have previously reported that the structure of stearic acid-rich triacylglycerols from cocoa butter influences the extent of postprandial lipaemia (Sanders *et al.* 2001, 2003). Randomisation of cocoa butter was found to decrease postprandial lipaemia compared with unrandomised cocoa butter. However, the cocoa butter substitute shea butter, which consists mainly of 1-, 3- distearyl, 2- oleyl glycerol decreased postprandial lipaemia to the same extent as randomised shea butter (Berry & Sanders, 2003). These responses could be explained by differences in the bioavailability of the fats. The present report describes measurements of the digestibility of the unrandomised shea butter and randomised shea butter.

Healthy male subjects (n 16) were fed 30 g test fat per d for a 3-week period in a randomised, cross-over study. At the end of each intervention a 3 d faecal collection was made for analysis of total faecal fatty acids and fatty acid composition, by GLC using heptadecanoic acid (17:0) as an internal standard. The results are shown in the Table.

	Randomised shea butter				Unrandomised shea butter			
	g/d	SD	wt%	SD	g/d	SD	wt%	SD
Palmitic acid (16:0)	0.4	0.2	12.0	4.3	0.5	0.2	15.9	6.3
Stearic acid (18:0)	2.1	1.4	50.9	16.0	1.9	1.0	49.4	13.0
Oleic acid (18:1)	0.5	0.6	11.6	7.1	0.4	0.3	10.7	6.0
Linoleic acid (18:2)	0.3	0.3	8.8	6.5	0.2	0.2	6.6	3.3
Others	0.6	0.5	16.7	5.3	0.6	0.3	17.3	4.3
Total fat	3.9	2.6			3.5	1.7		

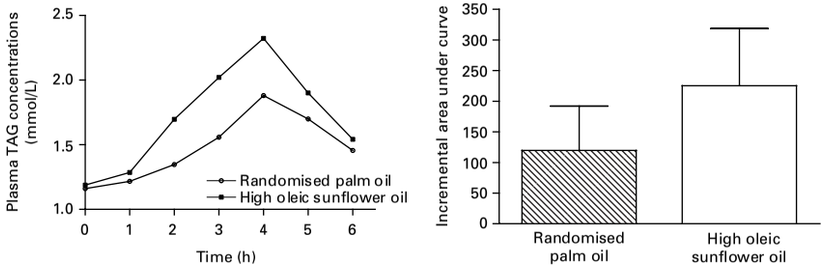
Total fat excretion was within the normal range (<5 g/d) and there were no significant differences in total fat excretion (g/d) and fatty acid composition (wt%) between test fats. However, the proportion of stearic acid (approximately 50%) was higher than other fatty acids and was presumably derived from the test fat. These results indicate that both randomised and unrandomised shea butter are relatively well digested at intakes likely to be consumed in the human diet. These findings are similar to those reported for cocoa butter (Shahkhalili *et al.* 2000) and do not support the view that the fat in chocolate has a low bioavailability.

Berry SE & Sanders TA (2003) *Proceedings of the Nutrition Society* **62**, A47.
Sanders TA, Berry SE & Miller GJ (2003) *American Journal of Clinical Nutrition* **77**, 777–782.
Sanders TA, Oakley FR, Cooper JA & Miller GJ (2001) *American Journal of Clinical Nutrition* **73**, 715–721.
Shahkhalili Y, Duruz E & Acheson K (2000) *European Journal of Clinical Nutrition* **54**, 120–125.

Acute effects of randomised palm oil *v.* high-oleic sunflower-seed oil on postprandial lipaemia. By R.J. WOODWARD, C. YEOH, T.A.B. SANDERS and S.E.E. BERRY, *Nutritional Sciences Research Division, King's College London, 150 Stamford Street, London, UK, SE1 9NH*

The positional distribution of fatty acids within dietary triacylglyerols (TAG) may influence the magnitude and duration of postprandial lipaemia. We have previously reported that the TAG structure of stearic acid-rich fats influences the level of postprandial lipaemia (Sanders *et al.* 2001, 2003). More recently we reported similar effects following palmitic acid-rich fats (Berry & Sanders, 2004), consistent with observations by Yli-Jokipii *et al.* (2001), whereby randomly interesterified palm oil reduced postprandial lipaemia compared with unrandomised palm oil. The process of randomisation is being widely used by the food industry as an alternative to the partial hydrogenation of fats, as it changes the physical properties of the fat without the generation of *trans* fatty acids.

The aim of the current study was to investigate the effects of randomised palm oil, the high-oleic sunflower-seed oil on postprandial lipaemia. A randomised cross-over design was used to compare the effects of test meals containing 50 g test fat in eighteen healthy male subjects aged 18–60 years. Plasma TAG, total fatty acid, glucose, and insulin concentrations, lipoprotein and hepatic lipase activity and chylomicron TAG composition were determined over a 6 h period. The changes in plasma TAG are shown below.



Following the randomised palm oil the postprandial increase in plasma TAG was significantly lower (47% lower incremental area under curve) compared with that following the high-oleic sunflower-seed oil (ANOVA; diet effect $P=0.031$). The fatty acid composition of the chylomicron TAG broadly reflected that of the test fat. There were no significant differences between test meals in changes in glucose, insulin, serum total and HDL-cholesterol concentrations and lipase activity.

Analysis of the physical properties of the test fats using NMR revealed that there was 15.2% solid fat at 37 °C in the randomised palm oil but at the same temperature all of the high-oleic sunflower-seed oil was liquid. These results are consistent with our findings with stearic acid-rich fats, which indicates that the solid fat content at body temperature influences the extent of postprandial lipaemia.

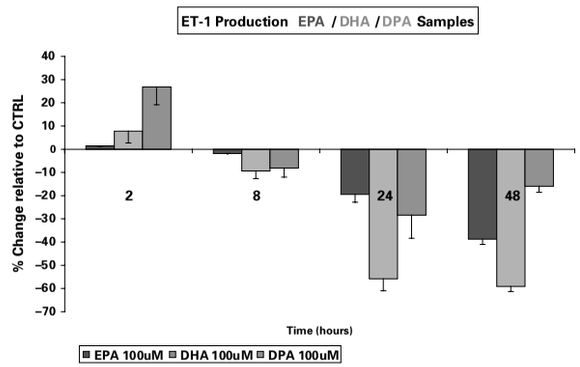
Berry SE & Sanders TA (2004) *Proceedings of the Nutrition Society* **63**, A67.
Sanders TA, Berry SE & Miller GJ (2003) *American Journal of Clinical Nutrition* **77**, 777–782.
Sanders TA, Oakley FR, Cooper JA & Miller GJ (2001) *American Journal of Clinical Nutrition* **73**, 715–721.
Yli-Jokipii K, Kallio H, Schwab U, Mykkanen H, Kurvinen J, Savolainen MJ & Tahvonen R (2001) *Journal of Lipid Research* **42**, 1618–1625.

The impact of fish oil fatty acids on endothelial function: a series of cell culture experiments. By M. VASSILIADOU, E. OLANO-MARTIN and A.M. MINIHANE, *Hugh Sinclair Unit of Nutrition, The University of Reading, Reading, UK, RG6 6AP*

The balance between locally produced vasoconstricting and vasodilating agents in the micro-environment of the arterial lining determines vascular tone. Decreased vascular reactivity is associated with the development and progression of atherosclerosis and hypertension and increased risk of CVD. Endothelial-derived factors such as NO, endothelin 1 (ET-1), prostaglandins and endothelial-derived hyperpolarising factor are important effectors of the reactivity of the underlying smooth muscle cells and therefore vascular tone. Chronic feeding studies in experimental animals and human subjects indicate that diets enriched with fish oils improve endothelial-dependent vascular tone (Khan *et al.* 2003). However, the underlying molecular mechanisms and the impact of the individual fish oil fatty acids, EPA, docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) remain uncertain. Therefore the aim of the current series of experiments was to investigate the impact of EPA, DPA and DHA on endothelial cell gene expression and ET-1 and NO secretion profiles.

EAHy926 endothelial cells were supplemented with EPA, DPA and DHA (10 μM, 25 μM and 100 μM) for 2, 8, 24 and 48 h. Following fatty acid exposure the cells were stimulated with thrombin for a further 24 h. Experiments were conducted in triplicate on two separate occasions. RNA was isolated using a mammalian RNA isolation kit (Sigma, Poole, Dorset, UK) and expression of ET-1, endothelial NO synthase (eNOS), vascular cell adhesion molecule 1, endothelin-converting enzyme and β-actin at 8 and 24 h measured by real-time PCR. Cell fatty acid composition was determined by GC of the cell lipid extract and ET-1 and NO secretion were assessed by determining the concentration in the cell-culture medium using commercially available kits.

The expected dose- and time- (0–48 h) dependent increases in cell EPA, DPA and DHA were evident following supplementation with the respective fatty acid, with maximum cellular levels evident after 24 h of fatty acid exposure. Consistent with the literature, no increase in cellular DHA was observed following EPA or DPA supplementation. All three fatty acids resulted in a dose-dependent decrease in ET-1 (vasoconstrictor) gene expression and protein levels, with the greatest reductions evident following DHA exposure (see Figure).



Although no dose effect was evident, EPA, DPA and DHA also resulted in increases in eNOS gene expression and NO (vasodilator) secretion.

The current series of studies indicates that the previously described positive effect of fish oil fatty acids on endothelial dependent vascular reactivity may be in part attributable to an effect of EPA, DPA and DHA on ET-1 and NO production, with DHA emerging as the most potent fatty acid. Further experiments are currently underway to investigate further mechanisms.

Khan F, Elherik K, Bolton-Smith C, Barr R, Hill A, Murrue I & Belch JJ (2003) *Cardiovascular Research* **59**, 955–962.

Influence of ultraviolet-B *v.* an increased dietary intake of cholecalciferol on serum 25-OH vitamin D concentrations. By T.A.B. SANDERS[1], A.J. CLODE[1], L.A. LINCOLN[1] and S. WALKER[2], [1]*Nutritional Sciences Research Division, King's College London, 150 Stamford Street, London, UK, SE1 9NN and* [2]*Department of Environmental Dermatology, St John's Institute of Dermatology, St Thomas' Hospital, Lambeth Palace Road, London, UK, SE1 7EH*

A reappraisal of vitamin D requirements is needed because recent evidence suggests that poor vitamin D nutriture increases the risk of developing cancer and CVD (Holick, 2004). Vitamin D is synthesised in the skin by the action of UV-B radiation (approximately 295–315 nm) from summer sunlight, but can also be obtained from the consumption of oily fish, eggs, liver, meat and fortified foods (for example, margarine). The UK Dietary Reference Values Panel concluded that there was no need to specify a dietary requirement for vitamin D for normal adults and that the requirement could be met by UV-B exposure during the summer months, which would result in the accumulation of sufficient stores to tide individuals through the winter months (November to March), when there is no synthesis of vitamin D. Serum 25-OH vitamin D (25-OH-D) concentrations indicate the cumulative effects of sunlight and dietary intake of vitamin D. The UK National Dietary and Nutritional Survey of British Adults 2000 (Henderson *et al*. 2004) found that about 25% of men and women had low serum 25-OH-D levels between January and March. Subjects with higher dietary intakes of vitamin D had higher serum 25-OH-D levels, which would imply that differences in dietary intake of vitamin D could explain some of the variation. We undertook a pilot study in healthy men and women (aged 18–40) to compare the effects on serum 25-OH-D of a daily supplement of 10 µg vitamin D provided as cod-liver-oil capsules (Seven Seas Ltd) with the effect of a single exposure to UV-B during the month of February in subjects who had low UV-B exposure and did not use vitamin D supplements or consume oily fish more than twice weekly.

Baseline serum 25-OH-D concentrations were determined by radio-immunoassay on two occasions 1 week apart and the subjects were then randomly allocated to either the vitamin D supplement or to a single sub-erythemal UV-B dose of 80 mJ/cm^2 equivalent to about 10–15 minutes of noonday summer sunlight in the UK. Further blood samples were obtained 1, 3 and 7 d post-exposure. The results are shown in the Table.

	Vitamin D			UV-B		
Serum 25-OH-D (ng/ml)	*n*	Mean	SD	*n*	Mean	SD
Baseline	6	18.8	4.9	10	23.6	7.7
1 d post-exposure	6	19.9*	4.9	8	25.6	9.2
3 d post-exposure	6	20.8	5.8	9	28.1†	8.0
7 d post-exposure	6	21.3*	5.3	10	28.9†	7.8
Change (day 7 – baseline)	6	2.5	1.9	10	5.3	3

* $P<0.05$.
† $P<0.01$ compared with baseline (paired *t* test).

Serum vitamin D concentrations increased by 13% and by 22% in the vitamin D-supplemented and UV-B groups respectively. The difference between treatments approached statistical significance ($P=0.059$). These results suggest that physiological doses of vitamin D taken daily do improve vitamin D status in subjects not exposed to UV-B but that UV-B exposure has a far more potent effect. However, as UV-B exposure is associated with increased risk of skin ageing and cancer, further work is required to ascertain how much UV-B exposure is required to maintain vitamin D status and how much additional dietary vitamin D is needed to prevent serum 25-OH-D falling below desirable levels.

Henderson L, Bates CJ, Prentice A, Birch M, Swan G & Farron M (2004) *The National Diet and Nutrition Survey: Adults aged 19 to 64 years. Summary Report. Volume 5*. London: The Stationery Office.
Holick MF (2004) *American Journal of Clinical Nutrition* **80**, 1678S–1688S.

Estimation of net acid excretion indirectly (NAE_{ind}) and net endogenous non-carbonic acid production (NEAP) in the Irish population: analysis of the North-South Ireland Food Consumption Survey (NSIFCS). By R.H.T. GANNON[1], E.M. HANNON[2], S.A. NEW[1] and A. FLYNN[2], [1]*Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH and* [2]*Nutrition Sciences, Department of Food Science and Technology, University College Cork, Republic of Ireland*

Acid–base homeostasis is critical to health. Negative health effects of increases in acid loading and acidosis has been shown, such as growth retardation of babies and several nephrological diseases, including renal insufficiency (Manz, 2001). There is also evidence demonstrating that high estimates of NEAP are associated with higher bone turnover (Macdonald *et al*. 2005).

The aims of the present study were to estimate the acid–base-generating potential of the diet in a representative sample of Irish adults (*n* 1379) between the ages of 18 and 64 years. Two estimates of acid–base balance were calculated from food diaries using the formulae of Remer (net acid excretion, estimated indirectly; NAE_{ind}) and Frassetto (net endogenous non-carbonic acid production; NEAP). A further aim was to identify the main food sources contributing to both the acid and alkali load of the diet. NAE_{ind} and NEAP values were calculated using $\sum$ (protein (SO_4)+P+Endogenous organic acids) – (Mg+K+Ca) and protein:K ratio respectively from 7 d food records (Frassetto *et al.* 1998; Remer *et al.* 2003). (Endogenous organic acids = 41 × body surface area (m^2)/1.73 (m^2).)

	Quartiles of NAE_{ind} (*n* 328)								Quartiles of NEAP (*n* 345)							
	1		2		3		4		1*		2		3		4	
Mean daily intake (g)	Mn	95% CI	Mn	95% CI	Mn	95% CI	Mn	95% CI	Mn	95% CI	Mn	95% CI	Mn	95% CI	Mn	95% CI
Fruits	132	168, 209	107	118, 143	92	109, 14	68	93, 118	138	177, 211	120	137, 166	89	104, 129	64	78, 95
Vegetables and vegetable dishes	117	128, 150	102	111, 126	101	103, 12	107	107, 122	120	131, 153	114	118, 133	101	108, 121	95	96, 109
Meats and meat products	126	126, 144	147	149, 166	170	171, 19	230	228, 250	127	128, 147	164	165, 180	172	178, 197	201	203, 224
Fish and fish products	14	21, 27	17	21, 27	18	23, 29	21	25, 33	18	23, 29	17	23, 29	19	23, 3	17	22, 29

Mn, median. Krushal Wallis test was used for the analysis * *n* 344.

	All		Men (662)								Women (717)							
	18–64 years		18–64 years		18–35 years		36–50 years		51–64 years		18–64 years		18–35 years		36–50 years		51–64 years	
Age group ...	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
NEAP*	44.9	9.3	45.8	9.8	45.3	9.6	46.3	9.7	45.9	10.2	44.0	8.7	43.5	9.2	44.1	8.0	44.8	9.0
NAE_{ind}†	49.4	13.8	55.4	14.9	55.3	15.1	56.5	14.6	54.0	15.2	44.2	10.2	42.7	10.5	44.9	9.7	45.4	10.2

Mann Whitney test was used for the analysis *NEAP given in g/mEq per d. †Mean NAE_{ind} given in mEq/d.

Higher consumption of fruit, vegetables and vegetable dishes was associated with a lower acid load indicative of the base-generating potential of fruit ($P<0.001$) and vegetables ($P<0.01$). Conversely, an inverse relationship was seen with higher intakes of both meats and meat products impacting more on dietary acid load ($P<0.001$). Higher consumption of fish and fish products showed a weak association with NAE_{ind} but not NEAP. As can be seen from the data estimates, both NAE_{ind} and NEAP are relatively similar. Estimates of NAE_{ind} and NEAP remained constant across the age groups for both men and women, with women having slightly lower values for NAE_{ind} ($P<0.001$) and NEAP ($P<0.01$). Estimates of NEAP in this Irish population are somewhat lower than those found in the British elderly population (≥65 years) (Gannon *et al.* 2004). Further analyses of the NSIFCS with comparisons of the National Diet and Nutrition Survey (19–64 years) are currently underway.

R. H. T. GANNON is a recipient of a University of Surrey PhD Scholarship.

Frassetto LA, Todd KM, Morris RC Jr & Sebastian A (1998) *American Journal of Clinical Nutrition* **68**, 576–583.
Gannon RHT, Millward DJ, Macdonald HM, Frassetto LA, Remer T & New SA (2004) *Proceedings of the Nutrition Society* **63**, 7A.
Macdonald HM *et al.* (2005) *American Journal of Clinical Nutrition* (In the Press).
Manz F (2001) *European Journal of Nutrition* **40**, 189–199.
Remer T, Dimitriou T & Manz F (2003) *American Journal of Clinical Nutrition* **77**, 1255–1260.
www.iuna.net North/South Ireland Food Consumption Survey (NSIFCS).

Relationship between dietary measures of potential renal acid load and urinary pH. By A.A. WELCH[1], S.A. RUNSWICK[2], A.A. MULLIGAN[1], K.T. KHAW[1] and S.A. BINGHAM[2], [1]*Department of Public Health and Primary Care, Strangeway's Research Laboratory, University of Cambridge, Cambridge, UK, CB1 8RN and* [2]*MRC Dunn Human Nutrition Unit, Cambridge, UK, CB2 2XY*

It is thought that dietary acid–base balance is important to bone health (New, 2004). The dietary potential renal acid load (PRAL) can be calculated by taking into account the mineral and protein composition of foods, the average intestinal absorption rates of the nutrients, S metabolism and urinary excretion of organic acids (Remer & Manz, 1995). Dietary PRAL has been related to urinary pH but it was not known whether measures from casual (spot) urine samples would provide similar results to 24 h urine collections (Michaud *et al.* 2003). A negative PRAL and more alkaline urine pH should be protective for bone.

The purpose of the present study was to investigate whether PRAL was related to urinary pH in a UK population and how an estimate of PRAL from a 7 d diary compared with estimates from a food-frequency questionnaire (FFQ) and measures of pH from casual and 24 h urine collections. The participants were a sub-sample from the EPIC-Norfolk cohort study of diet and chronic diseases in men and women (*n* 25 000) aged 40–79 years in Norfolk, UK.

A casual urine sample, a 7 d diary and an FFQ were collected at a baseline health check. A sample of 363 men and women who had provided 24 h urine samples (an average of 1 year after the baseline health check) in whom 7 d diaries were fully coded were used in the present study. pH was measured in casual urine samples using AMES multiple reagent strips and in 24 h urine collections with a Jenway 3310 pH meter (Barloworld). Dietary PRAL was divided into quintiles. Means, standard deviations, correlations and regression of PRAL against urine pH were calculated. The β coefficient shown in the Table estimates the change in urine pH per quintile of PRAL intake or change in pH or dietary PRAL by method. Statistics were performed using STATA V8 (Stata Corp., release 8).

	Men (*n* 165)		Women (*n* 198)	
	Mean	SD	Mean	SD
Age (years)	56.4	9.3	54.4*	9.5
Height (cm)	174.4	6.9	162.2**	6.6
Weight (kg)	78.9	10.2	66.3**	10.0
PRAL 7 d diary (mEq/d)	0.45	10.27	–4.74**	11.20
PRAL FFQ (mEq/d)	−5.18	12.35	−7.63	15.28
Urine pH (casual)	6.0	0.8	6.1	0.8
Urine pH (24 h)	5.98	0.58	6.26**	0.64

Mean values were significantly different from those for men: * $P<0.05$, ** $P<0.001$.

	Men (*n* 165)			Women (*n* 198)		
Regression	β	SE	*P*	β	SE	*P*
PRAL 7 d diary with pH (casual)	−0.35	0.15	0.020	−0.21	0.12	0.070
PRAL FFQ with pH (casual)	−0.33	0.14	0.020	−0.06	0.13	0.649
PRAL 7 d diary with pH (24 h)	−0.66	0.19	<0.001	−0.38	0.15	0.011
PRAL FFQ with pH (24 h)	−0.53	0.18	0.004	−0.47	0.16	0.003
Urine pH (24 h) with pH (casual)	0.46	0.11	<0.001	0.17	0.09	0.065
PRAL 7 d diary with PRAL FFQ	0.40	0.08	<0.001	0.46	0.06	<0.001

There was an inverse relationship of between 0.06 and 0.66 urine pH units per quintile of dietary PRAL, which was significant except for estimates of PRAL and casual urine samples in women. The magnitude of the relationship is stronger for men than women and with the 7 d diary than the FFQ, except for comparison of the FFQ and 24 h urines in women. These data indicate that as dietary PRAL decreases, urinary pH becomes more alkaline. While the strongest associations are with 7 d diary PRAL and 24 h urine pH, even casual urine pH may be a useful indicator of dietary PRAL.

Michaud DS, Troiano RP, Subar AF, Runswick S, Bingham S, Kipnis V & Schatzkin A (2003) *Journal of the American Dietetic Association* **103**, 1001–1007.
New SA (2004) *Osteoporosis International* **15**, 679–688.
Remer T & Manz F (1995) *Journal of the American Dietetic Association* **95**, 791–797.

No evidence for a negative association between bone mineral status and indirect estimates of renal net acid excretion in adolescents. By F. GINTY, C.J. PRYNNE, G. MUNIZ-TERRERA, G.D. MISHRA, A. PRENTICE, M.A. O'CONNELL, *MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge, UK, CB1 9NL*

A high dietary acid load is caused by a greater intake of S amino acid- and P-containing foods, relative to alkali-forming foods such as fruit and vegetables. Increasing dietary acid load has been shown to increase urinary Ca losses and bone resorption, and thus may negatively affect bone health. We have recently shown that bone mineral content (BMC) at the whole body, spine and hip was positively associated with fruit and vegetable intake in 16–18-year-old boys and girls (Prynne *et al.* 2005). The aim of the present study was to determine whether the positive relationships with fruit and vegetable intake were attributable to lower dietary acid load.

BMC, bone area (BA) and bone mineral density (BMD) were measured at the whole body, hip and spine by dual energy X-ray absorptiometry in adolescent boys (*n* 111) and girls (*n* 101). Height and weight were also measured and information on health and lifestyle and physical activity was determined by questionnaires. Diet was assessed by 7 d food diaries and renal net acid excretion (RNAE) was estimated from dietary intakes using the formulae of Remer (net acid excretion (NAE_{ind}); (Remer *et al.* 2003) and Frassetto (net endogenous acid production (NEAP); Frassetto *et al.* 1998). Biochemical markers of bone resorption (C-terminal telopeptides of type I collagen; S-CTX) and formation (N-terminal propeptides of type I pro-collagen (P1NP) and total osteocalcin (total OC)), and bone metabolism regulation (osteoprotegerin; OPG) were measured in plasma.

Using multiple regression analysis, no significant relationships were found between bone mineral measurements or metabolism markers and NAE_{ind} or NEAP in boys; however, osteocalcin was significantly inversely associated with NAE_{ind} ($P=0.01$). In girls, significant positive relationships were found between BMC, before size-adjustment, at the whole body (+17.9 (SE 5.8) %; $P=0.003$), total hip (+20.2 (SE 6.3) %; $P=0.001$) and trochanter (+26.8 (8.8) %; $P=0.003$) and NAE_{in} (the percentage coefficients are interpreted as the percentage increase in BMC associated with a 100% increase in NAE_{ind}). However, after adjustment for bone and body size (i.e. size-adjusted BMC) the relationships with BMC were not significant. No significant relationships were found with bone metabolism markers.

These results do not provide evidence for a negative association between BMC (after adjustment for size) and RNAE in younger age groups. Although NAE_{ind} and NEAP are strongly correlated, they are subject to different interpretation due to the strong association between dairy product intake and NAE_{ind}, but not NEAP. Strategies for improving dietary acid–base balance may be better served by promoting increased consumption of fruit and vegetable intake, rather than decreasing potentially acid foods, of which dairy products may be a significant contributor.

Supported by the Food Standards Agency (project award N05044).

Frassetto LA, Todd KM, Morris RC Jr & Sebastian A (1998) *American Journal of Clinical Nutrition* **68**, 576–583.
Prynne CJ, Mishra GD, O'Connell MA, Muniz G, Prentice A & Ginty F (2005) *Proceedings of the Nutrition Society* **64**, 92A.
Remer T, Dimitriou T & Manz F (2003) *American Journal of Clinical Nutrition* **77**, 1255–1260.

Fetal programming of adult disease may be induced by oxidative damage and alterations in antioxidant activity. By C. LILLEY and S.C. LANGLEY-EVANS, *School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough, UK, LE12 5RD*

A well-established animal model exists to investigate the effects of supplying a low-protein diet during gestation on adult disease risk in the offspring (Langley-Evans *et al.* 1994). However, it has been suggested that the content of other nutrients in the diet may be responsible for the programming effects rather than the protein balance itself (Langley-Evans, 2000). One concern that has been raised is that the methionine content of the protein-deficient diet (9% protein) is excessive, and this may exert programming effects via an imbalance in components of the activated methyl cycle (Petrie *et al.* 2002).

The present study aimed to assess whether the low-protein diet causes raised homocysteine (hcy) levels in the maternal and fetal circulations of rats due to excess methionine in the diet, and whether production of reactive oxygen species by hcy programmes disease by causing cell injury during fetal development. Four different gestational time points were investigated (days 4, 10, 18 and 20) to reflect different stages of pregnancy development. Pregnant Wistar rats were fed either a control diet (180 g casein/kg diet; *n* 8 per time point), or a low-protein (LP) diet (90 g casein/kg diet; *n* 8 per time point) throughout the entire pregnancy duration. Liver and plasma were used for analysis from all dams, but only from fetuses at day 18 and day 20. Liver protein and protein carbonyl content, antioxidant activity, DNA:protein ratio and plasma hcy levels were determined in all samples.

	Day 18				Day 20			
	Control†		LP†		Control†		LP†	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Protein (mg/g liver)	89.95*	3.93	82.59*	5.98	139.3*	9.41	206.6*	22.87
Protein carbonyls (nm/mg protein)	0.0172	0.0016	0.0195	0.0015	0.0191	0.0018	0.0206	0.0042
GP (units/mg protein per g liver)	5.34	0.35	5.61	0.42	4.23	0.90	2.31	0.19
SOD (units/mg protein per g liver)	0.803	0.068	0.747	0.060	0.438	0.044	0.328	0.043
Catalase (units/mg protein per g liver)	0.416*	0.064	0.381*	0.028	1.107*	0.098	1.589*	0.212

* $P<0.05$ for gestational age × maternal diet (ANOVA).
† *n* 10 to 14 per group.

Studies of fetal livers taken at days 18 and 20 of gestation suggest that protein content rises dramatically in the LP group compared with controls between these two time points ($P=0.01$). This was not paralleled by an increase in the level of protein carbonyls, which provide an indication of oxidative damage. By day 20, there was less glutathione peroxidase (GP) and superoxide dismutase (SOD) activity in the LP livers, although this was not significant. There was significantly greater catalase activity in these livers ($P=0.017$). The data suggest that between days 18 and 20 of gestation the fetus increases its protein stores in the liver, and the LP fetus salvages more than controls. Despite 67.5% more protein being stored in LP livers compared with controls, there was not an associated increase in oxidative damage, and this could be due to the up regulation in catalase activity in these animals. These results do not support the hypothesis that adult disease is programmed by oxidative damage during fetal development.

Langley-Evans SC (2000) *International Journal of Food Science Nutrition* **51**, 11–17.
Langley-Evans SC, Phillips GJ & Jackson AA (1994) *Clinical* Nutrition **13**, 319–324.
Petrie L, Duthie SJ, Rees WD & McConnell JML (2002) *British* Journal *of Nutrition* **88**, 471–477.

Exposure to a maternal low-protein diet in pregnancy programmes altered expression of sterol regulatory element-binding protein-1c and carbohydrate responsive element-binding protein in the offspring. By A.M. ERHUMA[1], D. SCULLEY[2], R. PLANT[2], A.M. SALTER[2], S.C. LANGLEY-EVANS[2] and A.J. BENNETT[1], [1]*School of Biomedical Sciences, Queen's Medical Centre and* [2]*Centre for Reproduction and Early Life, University of Nottingham, Nottingham, UK, NG7 2UH*

Nutrient restriction in pregnancy has been shown to programme diseases in adult life, including the metabolic syndrome (obesity, diabetes and hypertension) (Gluckman & Hanson, 2004). The mechanistic basis of this programming has not yet been established (Vickers *et al.* 2000). Sterol regulatory element-binding protein-1c (SREBP-1c) and carbohydrate responsive element-binding protein (ChREBP) belong to a basic helix-loop-helix leucine zipper family of transcription factors that play a crucial role in controlling fatty acid and glucose metabolism (Kosaku *et al.* 2002). It is not known how maternal protein restriction impinges on SREBP-1c and ChREBP expression. The aim of the present study was to investigate whether maternal protein restriction is associated with changes in gene expression involved in lipid and carbohydrate homeostasis in the offspring.

Pregnant Wistar rats were randomly allocated to five treatment groups. A control group was fed a 180 g casein/kg diet and four other experimental groups were fed a 90 g casein/kg, low-protein diet (LP) during the first (LP early), second (LP mid) and third week (LP late) or throughout pregnancy (LP all). On delivery of litters all dams were transferred to standard laboratory chow and the same diet was used to wean the offspring at 4 weeks of age. The offspring were killed at the age of 9 months. Plasma glucose, triacylglycerol and cholesterol concentrations were analysed and SREBP-1c and ChREBP mRNA expression in liver, soleus muscle and perirenal fat were measured using real-time RT-PCR.

There were no differences in plasma glucose, triacylglycerol and cholesterol levels between the experimental and control groups. On the other hand, expression of SREBP-1c and ChREBP in liver tissue were significantly suppressed by 85 and 70% respectively in all experimental groups relative to control animals ($P<0.0001$). In adipose tissue, in particular in the LP early group, SREBP-1c was increased ($P<0.0001$). There were no changes in gene expression in muscle tissue. SREBP-1c expression was correlated with liver size; a larger liver weight per body weight predicting greater expression (r 0.305; $P=0.005$). The Table shows mRNA expression normalised for 18S RNA expression in liver, muscle and adipose tissue. Data are for *n* 7–8 animals per group.

Tissue . . .	Liver				Adipose tissue				Muscle			
Maternal group	SREBP-1c		CHREBP		SREBP-1c		CHREBP		SREBP-1c		CHREBP	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	1.29	0.08	1.85	0.12	0.61	0.18	0.59	0.22	0.22	0.04	0.31	0.04
LP early	0.17*	0.08	0.47*	0.12	1.58*	0.19	0.88	0.23	0.17	0.04	0.28	0.04
LP mid	0.19*	0.08	0.54*	0.12	0.44	0.17	0.71	0.21	0.16	0.04	0.22	0.04
LP late	0.33*	0.08	0.72*	0.12	0.74	0.17	0.84	0.21	0.23	0.04	0.27	0.04
LP all	0.16*	0.08	0.45*	0.12	0.49	0.17	0.60	0.21	0.17	0.04	0.33	0.04

* Significantly different to control group ($P<0.05$).

We conclude that maternal protein restriction causes suppression of SREBP-1c and ChREBP expression in liver and increased expression in adipose tissue with no changes in muscle. Despite the suppression of SREBP-1c and ChREBP expression in liver, the animals were normoglycaemic and had normal lipid profiles. Further work is needed to investigate the changes of downstream targets of SREBP-1c and ChREBP, such as mRNA levels of fatty acid synthase, acetyl Co-A carboxylase and L-type pyruvate kinase respectively. These studies may provide important indicators of how exposure to prenatal undernutrition may have long-term effects on energy balance and the metabolism of lipids and glucose.

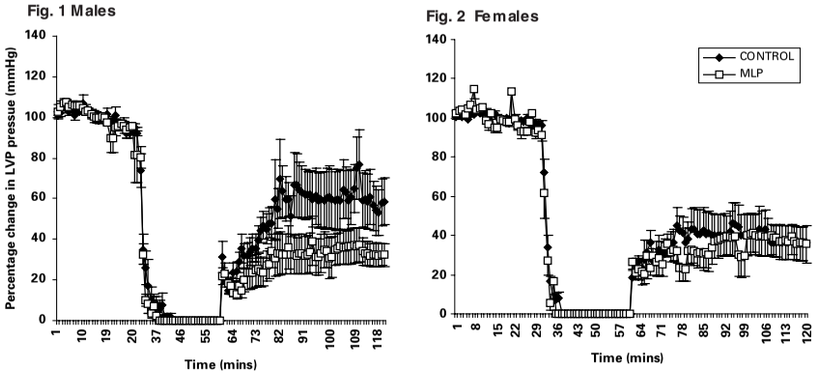
A. M. E. is in receipt of a Libyan Government postgraduate studentship. The present study was supported by a grant from the British Heart Foundation.

Gluckman PD & Hanson MA (2004) *Trends in Endocrinology Metabolism* **5**, 183–187.
Kosaku Y, Hiromi Y& Takumi K (2002) *Biochemical Pharmacology* **63**, 2075–2080.
Vickers MH, Breier BH, Cutfield WS, Hofman PL & Gluckman PD (2000) *American Journal of Physiology* **279**, E3–E7.

Prenatally programmed hypertension and its effects on the left ventricular pressure (LVP) function of the rat heart following ischaemia reperfusion. By M.J. ELMES[1], D.S. GARDNER[2] and S.C. LANGLEY-EVANS[1], *Centre for Reproduction and Early Life,* [1]*Division of Nutritional Sciences and* [2]*School of Human Development, University of Nottingham, Loughborough, Leicestershire, UK, LE12 5RD*

Hypertension is a major risk factor for IHD. An age-specific increase of 20 mmHg (systolic) or 10 mmHg (diastolic) blood pressure above average doubles the risk of IHD and represents a common cause of death among Western populations over the age of 50 years. Fetal undernutrition is a well-established risk factor for hypertension; rats subject to protein restriction *in utero* have elevated systolic blood pressure in adult life (Langley-Evans *et al.* 1996). At present it is not known whether these hypertensive animals are more susceptible to ischaemia–reperfusion injury.

Pregnant Wistar rats were fed either a control (CON) 180 g casein/kg or low-protein (LP) 90 g casein/kg diet throughout pregnancy with water available *ad libitum*. At birth litters were weaned onto standard laboratory chow (200 g protein/kg). At 4 weeks of age systolic blood pressure was determined by tail-cuff plethsmography. At 6 months of age rats were anaesthetised by 3% isofluorane in 2 litres O_2/min and killed by cervical dislocation. Hearts were rapidly excised, cannulated via the aorta to Langendorff perfusion apparatus, and perfused at a constant pressure (Palmer *et al.* 2004) with oxygenated Krebs–Henseleit buffer in a coronary retrograde fashion at 37 °C. A latex balloon was inserted into the left ventricle (LV) and adjusted to an end diastolic pressure of 5–8 mmHg. LV and perfusion pressure were constantly measured and recorded. All hearts were subjected to 30 min baseline recording before 30 min ischaemia and 60 min reperfusion. Baseline LVP data shows a sex and dietary treatment interaction but no significance between CON and LP exposed male and female rats. Ischaemia-reperfusion decreased LVP function in the hearts of both sexes (Figures 1 and 2). The effect was similar between female CON (n 7) and LP (n 6) groups but in males LVP function was decreased in the LP (n 7) group when compared to controls (n 7), ($P=0.057$).



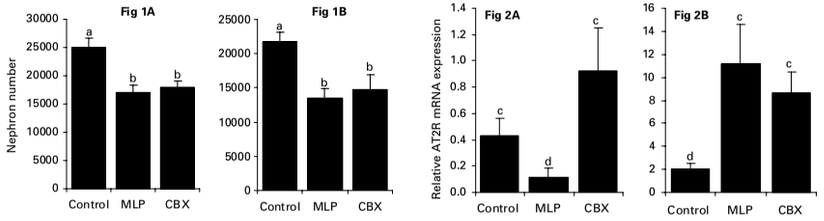
In conclusion, exposure to a restricted protein intake during fetal life has a sex specific effect on the LVP function of the adult rat heart. The LVP function post ischaemia was significantly higher in CON-fed males than those on a LP diet, but this dietary effect was absent in female rats. It is evident that the recovery in LVP function following reperfusion-ischaemia in male offspring is impaired by maternal protein restriction during pregnancy. As a result male hearts may be predisposed to cardiac dysfunction after such insults.

Langley-Evans S, Phillips GJ, Beneddiktsson R, Gardner DS, Edwards CR, Jackson AA & Seckl JR (1996) *Placenta* **17**, 169–172.
Palmer ES, Hadziahmetovic M, Veci T & Angelos MG (2004) *Resuscitation* **62**, 97–106.

Sex-specific up regulation of angiotensin II receptor (AT_2R) mRNA expression in hypertensive rats exposed to a low-protein diet *in utero*. By S. McMULLEN and S.C. LANGLEY-EVANS, *Division of Nutritional Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, UK, LE12 5RD*

The feeding of a low-protein (LP) diet during rat pregnancy consistently programmes high blood pressure in the offspring. This effect is replicated by exposing the developing fetus to excess glucocorticoids (GC), by administration of dexamethasone or carbenoxolone (CBX) during pregnancy. Over-exposure to maternal GC is therefore thought to play a central role in the nutritional programming of hypertension. However, our previous work has indicated that, whilst the reduction in nephron number is GC-dependent, the modulation of angiotensin receptor (ATR) expression is GC-independent (McMullen & Langley-Evans, 2005). The present study examined the differential effects of prenatal LP and CBX treatment on nephron number and renal ATR mRNA expression in the offspring.

Pregnant Wistar rats were allocated to one of three treatment groups: control (180 g casein/kg, saline; n 6), LP (90 g casein/kg, saline; n 6) or CBX (180 g casein/kg, CBX; n 6). Subcutaneous saline or CBX (12.5 mg/kg per d) injections were administered on days 14–21 of pregnancy. CBX inhibits 11β-hydroxysteroid dehydrogenase, increasing the passage of GC across the placenta. At birth, litters were transferred to standard laboratory chow and culled to a maximum of eight pups. Kidneys were collected from 4- and 20-week-old offspring. Nephron number was counted using a maceration method. Renal ATR mRNA expression was assessed by real-time RT-PCR and normalised to β-actin.



Data are means with their standard errors.
Fig. 1. Nephron number in offspring at 4 (A) and 20 (B) weeks; a>b, $P<0.001$.
Fig. 2. AT_2R mRNA expression in female offspring at 4 (A) and 20 (B) weeks; c>d, $P<0.01$.

The reduction of nephron number consistently shown in LP offspring was replicated in the CBX-exposed offspring at 4 (see Fig. 1A) and 20 weeks (see Fig. 1B). In this respect, the nutritional and steroid-based models appear similar, supporting the notion that the nutritional programming of nephron number at least is mediated by an increased exposure to maternal GC. However, the down regulation of AT_2R expression in 4-week-old LP female offspring (see Fig. 2A; $P<0.005$) was not observed in CBX offspring. In contrast, CBX programmed an increase in AT_2R expression at the same time-point. At 20 weeks of age, AT_2R expression was up regulated in both the LP and CBX female offspring (see Fig. 2B). AT_2R expression is known to be up regulated in adult life in response to renal injury, promoting remodelling via apoptotic and proliferative pathways (Zhang *et al.* 2004). The up regulation of AT_2R expression in the LP females is secondary to the onset of hypertension and may constitute a compensatory response to protect the kidney from the progressive cycle of hypertension and renal injury. The absence of such a response in the male LP offspring may explain their increased susceptibility to and faster progression towards renal disease and hypertension.

S. McM. is supported by the British Heart Foundation.

McMullen S & Langley-Evans SC (2005) *American Journal of Physiology* **288**, R85–R90.
Zhang SL, Guo J, Moini B & Ingelfinger J (2004) *Kidney International* **66**, 2818–2892.

Age-related loss of renal function is driven by programmed oxidative processes in the rat. By D.V. SCULLEY and S.C. LANGLEY-EVANS, *School of Biosciences, University of Nottingham, Sutton Bonington, Leicestershire, UK, LE12 5RD*

The quantity of nephrons in the kidney is vital to its effective function. A reduction in nephron number has been implicated as a cause of hypertension as the kidney struggles to perform its filtration rate (Mackenzie & Brenner, 1995). Previous research has indicated that maternal diet may influence the number of nephrons present in offspring (Langley-Evans, 2001). Offspring from mothers who were fed a low-protein (MLP) diet during pregnancy displayed a reduction in nephron numbers and an increase in hypertension in maturity. In the present study, we investigated the effects of a MLP diet (90 g protein/kg) on nephron number and glutathione redox status. A low-protein diet was administered to maternal rats either throughout pregnancy (3 weeks gestation) or during the first (LP early), second (LP mid) or third (LP late) week only. At 4 weeks of age, nephron number in control rats (maternal 180 g protein/kg diet) was significantly higher compared with those exposed to a MLP diet ($P<0.01$). However, the nephron profile at 9 months did not show a similar pattern. Nephron number in the control group had reduced significantly from 17 978 to 13 269 ($P=0.005$). Significant reductions were also observed in the LP early ($P=0.029$), LP mid ($P<0.001$) and LP late ($P=0.02$). These reductions, whilst significant, were not as severe as those observed in the control group. No significant reduction was apparent in the LP all group.

GSH and GSSG concentrations were determined to evaluate the potential role of reactive oxygen species in the degradation of nephrons. No significant differences were found between any of the control or MLP groups. However, GSH concentrations were lower in all groups, but significantly reduced at 9 months compared with 4 weeks only in the control group ($P=0.002$) and LP early ($P=0.002$). Similarly, no differences were observed in GSSG concentration between the maternal diet groups. GSSG concentrations were significantly lower at 9 months compared with 4 weeks in the control ($P<0.001$), LP all ($P=0.002$), LP early ($P=0.002$), and LP mid ($P=0.014$).

	Nephron number (nephrons/kidney)				Glutathione (μmol/g tissue)				GSSG (μmol/g tissue)			
	4 weeks		9 months		4 weeks		9 months		4 weeks		9 months	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	17978	1060	13269	1141	2.338	0.16	1.6346	0.14	0.8850	0.09	0.4033	0.06
LP all	14486	1030	12978	877	2.502	0.20	2.0762	0.16	0.9428	0.09	0.5507	0.07
LP early	15745	1515	10872	1459	3.049*	0.21	2.0625	0.16	0.9161	0.06	0.6128	0.05
LP mid	18537	1581	8355*	822	2.323	0.17	2.2232*	0.12	0.9471	0.13	0.5483	0.06
LP late	15918	1502	11010	889	2.489	0.15	2.2768*	0.10	0.7218	0.05	0.6590*	0.66

n 14–20.
*Significantly different from control ($P<0.05$).

In agreement with previous studies, we found that nephron number was reduced in offspring exposed to a MLP diet with the exception of the LP mid group and the data are therefore consistent with the hypothesis that prenatal undernutrition impairs renal development. At 9 months, despite the rapid reduction in number, the nephrons of the control group remained higher than the LP groups. The reduction in kidney GSH at 9 months is indicative of a decline in antioxidant activity leading to increased free radical-mediated oxidative damage. These oxidative processes could cause the depletion of nephrons, a situation that would be exacerbated by the reduction in nephron numbers at birth, leading to a greater risk of hypertension in maturity. Oxidative processes are clearly important in ageing of the kidney and may be influenced by maternal nutrition in pregnancy.

The present study was supported by a grant from the British Heart Foundation.

Langley-Evans SC (2001) *Proceedings of the Nutrition Society* **60**, 505–513.
Mackenzie HS & Brenner BM (1995) *American Journal of Kidney Diseases* **26**, 91–98.

Changes in iron status throughout pregnancy in an ethnically diverse population. By L. BROUGH and G. REES, *Institute of Brain Chemistry and Human Nutrition, London Metropolitan University, 166–220 Holloway Road, London, UK, N7 8DB*

Hackney in the East End of London has an ethnically diverse population with high levels of social and economic deprivation. Previous research here has shown many women to have low intakes of micronutrients, including Fe, which have been linked to adverse pregnancy outcomes (Doyle & Rees, 2001). Studies have shown low Fe status in the first trimester (Brooke, 2001) and postpartum (Doyle *et al.* 2001), but no data exist concerning Fe status throughout pregnancy in this population.

In a study concerning nutrition throughout pregnancy, 402 women were recruited from the Homerton Hospital, Hackney, UK. The participants were ethnically diverse comprising: Caucasian (38.6%); Asian (10.4%); African (27.6%); West Indian (16.4%); others (7.0%). Women were recruited at their first (booking) antenatal appointment between 5 and 17 weeks gestation.

Venous blood samples were taken with participants' consent at booking, 26 and 34 weeks gestation and analysed at the hospital. Hb was measured on samples of EDTA-stabilised whole blood using a Coulter STKS analyser. Serum ferritin was measured using a DPC Immulite 2000 analyser, a fully automated immunoassay analyser. Both analysers routinely undergo quality checks.

The WHO defines anaemia for pregnant women as Hb <110 g/l; at booking, 26 and 34 weeks the number of anaemic women were 12.6, 52.3 and 45.0%, respectively. ANOVA showed significant differences between ethnicities at booking ($P<0.001$) and 26 weeks ($P=0.052$) only. *Post hoc* analysis (Tukey–HSD) showed the Africans had significantly lower mean Hb than the Caucasians at booking ($P<0.001$) and 26 weeks ($P=0.046$) and the Asians at booking ($P=0.001$).

Serum ferritin <15 μg/l indicates low Fe stores. At booking, 11.1% of women had low Fe stores; at 26 and 34 weeks this had increased to 49.1 and 55.7%, respectively. Fisher's exact test showed ethnic disparities between those replete or deficient at 26 weeks ($P=0.041$) and 34 weeks ($P=0.005$). ANOVA showed significant ethnic disparities in serum ferritin at 26 weeks ($P=0.005$) and 34 weeks ($P<0.001$). Tukey–HSD showed at 26 weeks West Indians had higher serum ferritin levels (calculated from $\log_{10}$-transformed data) than Caucasians ($P=0.013$) and Asians ($P=0.030$); at 34 weeks Caucasians had lower serum ferritin levels than West Indians ($P=0.001$) and Africans ($P=0.004$).

	Booking (5–17 weeks)			26 weeks			34 weeks		
Hb (g/l) at ...	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Caucasian	153	122*†	9.1	121	110‡	9.4	131	112	11.7
Asian	42	124†	9.7	34	109	10.5	34	111	11.2
African	106	117*	10.1	80	107‡	9.1	96	110	10.4
West Indian	63	119	10.1	55	108	10.4	58	109	10.6
Other	26	121	11.3	21	111	8.8	23	111	11.8
Total	390	120	10.0	311	109	9.7	342	111	11.1

	Booking (5–17 weeks)			26 weeks			34 weeks		
Serum ferritin (μg/l) at ...	*n*	%<15 μg/l	Mean	*n*	%<15 μg/l	Mean	*n*	%<15 μg/l	Mean
Caucasian	137	10.9	38.7	40	60.0	11.8§	40	70.0	10.0¶**
Asian	41	9.8	42.2	12	66.7	10.5‖	9	77.8	10.7
African	91	13.2	48.0	32	37.5	18.5	28	39.3	17.3**
West Indian	55	10.0	43.2	14	21.4	26.2§‖	13	23.1	21.9¶
Other	26	11.5	39.8	8	62.5	12.7	7	71.4	11.0
Total	350	11.1	42.2	106	49.1	14.9	97	55.7	13.2

Significantly different: * $P<0.001$; † $P=0.001$; ‡ $P=0.046$; § $P=0.013$; ‖ $P=0.030$; ¶ $P=0.001$; ** $P=0.004$.

Low Fe status is a problem for this population that increases as pregnancy progresses. Clear ethnic differences exist for Hb and serum ferritin. The present study corroborates previous work showing Africans have lower Hb but higher serum ferritin than Caucasians (Perry *et al.* 1992). Further research is needed to better understand ethnic disparities and also interventions are required to prevent Fe deficiency in pregnancy.

The authors gratefully acknowledge financial support from the Mother and Child Foundation.

Brooke ZM (2001) Nutrition and Pregnancy. PhD Thesis, University of North London.
Doyle W & Rees G (2001) *Nutrition and Health* **15**, 213–218.
Doyle W, Srivastava A, Crawford MA, Bhatti R, Brooke Z & Costeloe KL (2001) *British Journal of Nutrition* **86**, 81–87.
Perry GS, Byers T, Yip R & Margen S (1992) *Journal of Nutrition* **122**, 1417–1424.

Hepcidin pro-hormone ELISA kit: a reproducibility study. By H.J. MOHAMED[1], S.F.A. BRUGGRABER[2], C.A. GEISSLER[1] and J.J. POWELL[2], [1]*Department of Nutrition and Dietetics, King's College, London, UK and* [2]*MRC Human Nutrition Research, Cambridge, UK*

Hepcidin pro-hormone, or pro-hepcidin, is an eighty-four-amino acid precursor for the putative hepcidin signal peptide (Ganz, 2003). It is proposed that pro-hepcidin, like hepcidin, is involved in the regulation of Fe metabolism (Kulaksiz *et al.* 2004). A competitive-based ELISA has been developed and commercialised (DRG Diagnostics, Marburg, Germany) to measure pro-hepcidin in human blood samples. However, the reproducibility and reliability of this assay was questioned by researchers investigating Fe metabolism (Kemna *et al*. 2005). Hence, the aim of the present study was to assess the reproducibility of this pro-hepcidin assay using human serum samples from iron supplementation study. Blood samples were obtained from studies investigating the tolerance to $FeSO_4$ supplementation in Fe-deficient and anaemic subjects. A total of forty-one serum samples were measured in duplicate on two hepcidin pro-hormone ELISA plates simultaneously (batch number 12K084). Intra-assay variation was high on both plates, A and B. Correlation of the absorbance of the intra-assay sample duplicates gave r values of 0.74 and 0.70, respectively, for plates A and B ($P<0.0001$). Inter-assay comparison (i.e. plate A *v*. plate B) of the pro-hepcidin concentrations derived from the mean absorbance of the intra-assay sample duplicates gave the correlation r 0.8 ($P<0.0001$). A Bland & Altman plot showed that the inter-assay variation depended on concentration; the variation increased at higher pro-hepcidin concentrations (see Fig. 1). In addition, paired t tests showed the inter-assay results were significantly different at all concentrations ($P<0.01$) and even at concentrations below 250 ng/ml ($P<0.01$). We conclude that the pro-hepcidin ELISA kit shows poor reproducibility at both low and high concentration. These results have been discussed with the manufacturer.

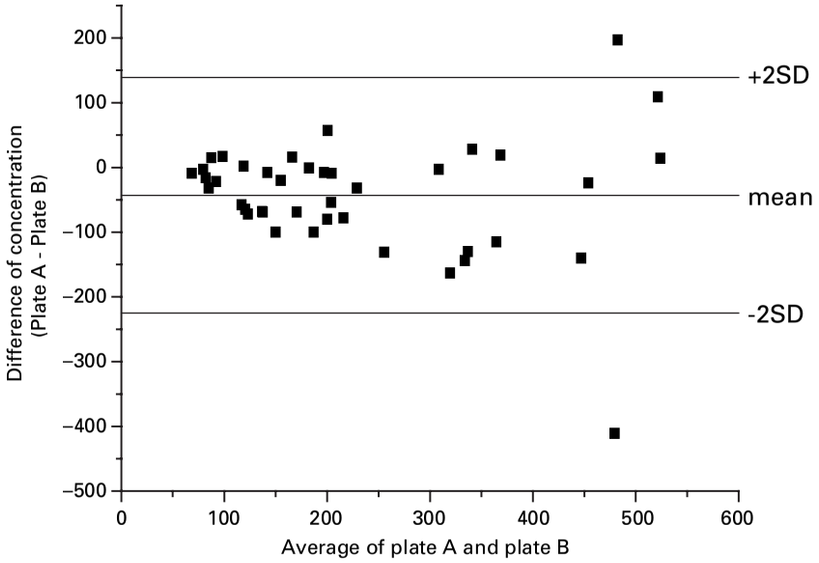


Fig. 1. Bland & Altman plot on inter-assay analysis.

We gratefully acknowledge IDS Limited (Boldon, UK) for providing the ELISA kit. We would like to thank Dr Lomer and Ms Hutchinson, the leading researchers in the studies from which these samples were collected. H. J. M. is funded by the Public Service Department of Malaysia.

Ganz T (2003) *Blood* **102**, 783–788.
Kemna E *et al.* (2005) Blood – pre-published online May 10, 2005.
Kulaksiz H, Gehrke SG, Janetzko A, Rost D, Bruckner T, Kallinowski B & Stremmel W (2004) *Gut* **53**, 735–743.

The efficacy of low-dose folic acid supplementation on endothelial function using pulse wave analysis. By P. CUMMINS[1], R. ARMSTRONG[2], D. NEWBY[3], R. WILSON[3] and I. DAVIDSON[1], [1]*Queen Margaret University College, Clerwood Terrace, Corstorphine, Edinburgh, EH12 8TS,* [2]*Chief Scientist Office, St Andrews House, Regent Road, Edinburgh, EH1 3DG,* [3]*Centre for Cardiovascular Sciences, Royal Infirmary of Edinburgh, 51 Little France Crescent, Edinburgh, EH16 4SB*

The measurement of endothelial function has been widely used in folic acid intervention studies as a prognostic marker of cardiovascular outcome (Verhaar *et al*. 1999; Woo *et al.* 2002). Such studies have demonstrated that high doses of folic acid can improve endothelial function. Few studies have investigated the potential of low-dose folic acid supplementation and it remains unclear whether folic acid mediates this endothelial effect by reducing homocysteine levels or by another cardio-protective property, independent of homocysteine lowering (Moat *et al.* 2004). The present study investigated the efficacy of low-dose folic acid supplementation on endothelial function in both healthy and dyslipidaemic men.

A double-blind parallel controlled trial was employed where both control (n 8 placebo; n 8 folic acid) and dyslipidaemic (n 7 placebo; n 7 folic acid) subjects were randomly allocated to either low-dose folic acid (0.4 mg) or placebo for 6 weeks. Endothelial function was measured using a non-invasive function test called pulse wave analysis. This function test measured indices of arterial stiffness including pulse wave velocity (PWV) and augmentation index (AIx) and AIx corrected to a heart rate of 75 beats per min (AIx@75), in response to an endothelial-dependent (salbutamol) and endothelial-independent (GTN) agent. Serum folate, total homocysteine and a lipid profile were also measured before and after treatment. A Mann–Whitney U test was carried out to measure the differences between study variables for placebo and folic acid groups before and after supplementation and all values are represented as median 25th percentile, 75th percentile. $P<0.05$ was considered significant.

	Control Placebo		Control Folic acid		Dyslipidaemic Placebo		Dyslipidaemic Folic acid	
	Week 0	Week 6	Week 0	Week 6	Week 0	Week 6	Week 0	Week 6
Homocysteine	9.3	8.6	10.7	9.2	10.0	10.4	11.1	9.1
(μmol/L)	8.1, 9.7	7.8, 10.0	10.4, 13.0	8.1, 9.8	6.6, 14.3	7.4, 13.0	8.5, 11.4	7.7, 9.5
Folate (μg/L)	5.8	5.4	6.0	13.8	6.5	5.9	7.4	12.9
	4.2, 7.8	4.0, 7.2	4.9, 7.8	12.3, 20.0	4.7, 6.6	5.3, 7.3	4.6, 7.7	8.6, 15.9
AIx@75 - GTN	−13.0	−18.0	−14.5	−15.0	−13.5	−14.0	−16.0	−19.0
response (%)	−18.3, −11.3	−21.5, −10.8	−19.0, −10.3	−19.3, −12.3	−16.0, −9.5	−18.0, −8.0	−21.0, −10.0	−24.0, −15.0
AIx@75 -	−7.5	−7.0	−1.0	−4.5	−11.5	−6.0	−5.0	−6.0
Salbutamol response (%)	−12.5, −2.8	−15.0,−4.3	−5.3, 1.8	−11.3, −3.0	−13.8, −0.25	−8.0, −3.0	−9.0, −2.0	−13.0, −5.0

Supplementation with folic acid significantly increased serum folate ($P=0.001$) and decreased homocysteine levels ($P=0.027$) amongst control subjects. Despite this, no significant improvement in PWV or the endothelial-dependent and -independent responses for AIx and AIx@75 amongst control subjects was evident. There was no reduction in homocysteine in the dyslipidaemic group ($P=0.084$), despite a significant increase in serum folate levels ($P=0.002$). This group also exhibited no significant change in PWV or AIx in response to the endothelial-dependent and independent agents; however, there was a notable trend towards a reduction in AIx@75 ($P=0.07$) following the administration of the endothelial-dependent agent salbutamol. As expected, folic acid exhibited no treatment effect on lipid levels for both control and dyslipidaemic groups.

Despite a significant reduction in homocysteine levels and increase in serum folate, the lack of response to improve PWV or the endothelial-dependent and independent responses for AIx or AIx@75 may highlight the lack of endothelial dysfunction amongst healthy individuals. The dyslipidaemic group exhibited a trend towards an improvement in the salbutamol response for AIx@75 ($P=0.07$), despite a non-significant treatment effect on homocysteine levels. These findings suggest that low-dose folic acid does exhibit a beneficial effect on the endothelium, independent of homocysteine lowering. Further studies are needed to establish the optimal dose of folic acid needed to ameliorate endothelial dysfunction.

Moat, S.J., Lang, D., McDowell, I.F., Clarke, Z.L., Madhavan, A.K., Lewis, M.J., & Goodfellow, J (2004) *The Journal of nutritional biochemistry* **15**, 64–79.
Verhaar MC, Wever RM, Kastelein JJ, van Loon D, Milstien S, Koomans HA & Rabelink TJ (1999) *Circulation* **100**, 335–338.
Woo KS, Chook P, Chan LL, Cheung AS, Fung WH, Qiao M, Lolin YI, Thomas GN, Sanderson JE, Metreweli C & Celermajer DS (2002) *American Journal of Medicine* **112**, 535–539.

Dynamics of flavonoid consumption in the Australian population. By S.M. SOMERSET and L. JOHANNOT, *School of Public Health, and Heart Foundation Research Centre, Griffith University, Meadowbrook, Australia*

Flavonoids are a group of about 5000 polyphenolic compounds found ubiquitously in plant foods. Six major flavonoid classes are recognised: anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols and isoflavones (Hu & Willett, 2002; Ross & Kasum, 2002). *In vitro* and *in vivo* studies indicate that dietary flavonoids affect the risk of diseases such as cancer and CHD in human subjects. To date, few population-based flavonoid consumption studies have been published. Estimates of flavonoid intake have been published for the USA (Sampson *et al.* 2002), Denmark (Justesen *et al.* 1997), Holland (Hertog *et al.* 1993) and Finland (Kumpulainen, 2001), France (Commenges *et al.* 2000), Greece (Lagiou *et al.* 2004), Japan (Arai *et al.* 2000) and Spain (Garcia-Closas *et al.* 1999), although dietary intake methodologies vary substantially. The present study provides an Australian perspective to international comparisons, especially in relation to variations in sources and intakes of flavonoids according to age.

Flavonoid consumption was estimated by combining 24 h recall data from the National Nutrition Survey 1995 (Australian Bureau of Statistics, 2001) in a representative sample (n 13 858) of the Australian population aged 2 years and over with USDA flavonoid composition data. Mean adult total flavonoid intake (>18 years) was 454 mg/d (93% being flavan-3-ols). Tea was the major dietary flavonoid source in Australia. Apple was the highest quercetin source until age 16–18 years, after which onions became a more prominent source. Hesperetin consumption varied according to orange intake. Apple, apricot and grapes were the major sources of epicatechin and catechin for children, but wine became a more prominent source in adulthood. The main source of malvidin was wine. Grapefruit was the major source of naringenin in older age groups, whereas orange was the major source in individuals up to 24 years of age. Apigenin intake was comparatively higher in Australia (compared with USA and Denmark), possibly due to leaf and stalk vegetables, and parsley consumption. Consistency in dietary methodologies, survey tools validated specifically for flavonoid intakes, and enhanced local food composition data for flavonoid content would facilitate international comparisons of flavonoid intake.

Australian Bureau of Statistics (2001) *National Nutrition Survey 1995 (NNS95)*. Canberra: Australian Bureau of Statistics.
Arai Y, Watanabe S, Kimira M, Shimoi K, Mochizuki R & Kinae N (2000) *Journal of Nutrition* **130**, 2243–2250.
Commenges D, Scotet V, Renaud S, Jacqmin-Gadda H, Barberger-Gateau P & Dartigues JF (2000) *European Journal of Epidemiology* **16**, 357–363.
Garcia-Closas R, Gonzalez CA, Agudo A & Riboli E (1999) *Cancer Causes and Control* **10**, 71–75.
Hertog MGL, Hollman PC, Katan MB & Kromhout D (1993) *Nutrition and Cancer* **20**, 21–29.
Hu FB & Willett WC (2002) *JAMA* **288**, 2569–2578.
Justesen U, Knuthsen P & Leth T (1997) *Cancer Letters* **114**, 165–167.
Kumpulainen JT (2001) Intake of flavonoids, phenolic acids and lignans in various populations. In *Third International Conference on Natural Antioxidants and Anticarcinogenic Food, Health And Disease (NADH)* [S Voutilainen and JT Salonen, editors]. Helsinki, Finland: Kuopion Yliopisto.
Lagiou P, Samoli E, Lagiou A, Peterson J, Tzonou A, Dwyer J & Trichopoulos D (2004) *Cancer Causes and Control* **15**, 67–72.
Ross JA & Kasum CM (2002) *Annual Review of Nutrition* **22**, 19–34.
Sampson L, Rimm E, Hollman PC, de Vries JH & Katan MB (2002) *Journal of the American Dietetic Association* **102**, 1414–1420.

Dietary flavonoid intakes in early postmenopausal Scottish women. By A.C. HARDCASTLE[1,2], J.A.M. KYLE[3], G. DUTHIE[3], G. McNEILL[2], D.M. REID[1,2] and H.M. MACDONALD[1,2], [1]*Osteoporosis Research Unit, University of Aberdeen, Woolmanhill Hospital, Aberdeen, UK, AB25 1LD,* [2]*College of Medicine, University of Aberdeen, Aberdeen, UK, AB25 2ZD and* [3]*Rowett Research Institute, Aberdeen, UK, AB21 9SB*

Flavonoids are potentially bioactive polyphenols that are ubiquitously found in plants and are therefore an integral part of the human diet. Main subclasses of flavonoids include flavonols (quercetin, kaempferol and myricetin), flavones, flavanones (hesperidin and naringin), procyanidins and catechins. Studies in Holland and Scotland have shown that the catechinsaccount for most of the flavonoid intake with onions, apples and black tea as major sources (Kyle *et al.* 2002). Using food-frequency questionnaires (FFQ) from over 10 000 individuals from Finland, flavonoid intake was estimated as 24.2±26.7 mg/d (4.0 mg flavonols, 20.1 mg flavanones and <0.1 mg flavones) (Knekt *et al.* 2002). The aim of the present study was to estimate flavonoid intake in a population of Scottish early postmenopausal women.

The subjects were 3236 women (mean age 54.7±2.2 years) that had been recruited in 1990–3 for the Aberdeen Prospective Osteoporosis Screening Study, the majority of whom returned in 1997–2000 for a second visit and completed a validated FFQ (Bodner *et al.* 1998). The diets were analysed for flavonoid intake using a database developed at the Rowett Research Institute (Kyle *et al.* 2002). The flavonoid contents of items that were in our FFQ but not in the Rowett database (for example, swede) were obtained from the US Department of Agriculture database. We made the assumption that the flavonoid concentration in satsumas, tangerines and clementines was similar to that of oranges. Although this FFQ has not been specifically validated for flavonoids, a similar FFQ has been validated (Kyle *et al.* 2002) and our own validation work is in progress using 4 d food diaries.

Preliminary data for the dietary intakes of flavonoids in our population show that the flavanones accounted for 39% of the total mean intake of 200 mg/d (see Table). Some classes of flavonoids were totally missing from the diet of a small number of women (flavonols n 4; flavanones n 9).

	Mean intake (mg/d)	SD	Median	Minimum (mg/d)	Maximum (mg/d)
Flavonols	22.8	11.7	21.6	0.5	92.2
Procyanidins	23.9	13.7	22.3	<0.1	104.3
Catechins	74.7	53.0	69.9	0.3	319.6
Flavanones	78.6	60.4	60.8	0	651.6
Total flavonoids	200.0	110.0	185.2	4.3	905.1

The daily flavanone intake in our population, although higher than that found in other studies, is equivalent to that obtained from one and a half oranges. Dutch subjects were consuming <0.5 oranges/d (Knekt *et al*. 2002). We previously reported a link with fruit intake and bone mineral density in a subgroup of this population (Macdonald *et al*. 2001), which may partly be attributable to acid-balancing properties. However, it is possible that the beneficial effect of fruit and vegetables may be due to other components such as vitamins and flavonoids. Hesperidin has been shown to protect against bone loss in rats (Horcajada & Coxam, 2004). The absorption and subsequent distribution, metabolism and excretion of flavonoids in man is not well understood and further work is required to determine whether these compounds play a definitive role in human health.

This work was funded by a Food Standards Agency postgraduate scholarship. Any views expressed are the authors' own.

Bodner CH, Soutar A, New SA, Scaife AR, Byres M & Henderson GD (1998) *Journal of Human Nutrition and Dietetics* **11**, 373–380.
Horcajada M-N & Coxam V (2004) *Nutritional Aspects of Osteoporosis*, pp. 109–126 [P Burckhardt, B Dawson-Hughes and R Heaney, editors]. Amsterdam: Elsevier.
Knekt P, Kumpulainen J, Jarvinen R, Rissanen H, Heliovaara M, Reunanen A, *et al.* (2002) *American Journal of Clinical Nutrition* **76**, 560–568.
Kyle JAM, Masson LF, Grubb DA, Duthie GG & McNeill G (2002) *Proceedings of the Nutrition Society* **61**, 69A.
Macdonald HM, Downie FH, New SA, Moore F, Grubb DA & Reid DM (2001) *Proceedings of the Nutrition Society* **60**, 202A.

Use of stable isotopically labelled compounds to investigate the digestive fate of glucosinolates in brassica vegetables. By Z. FULLER[1], N.P. BOTTING[2], J.J. MORRISON[2], C. BOTTING[2] and A.J. DUNCAN[1], [1]*The Macaulay Institute, Craigiebuckler, Aberdeen, UK, AB15 8QH and* [2]*University of St Andrews, St Andrews, UK, KY16 9AJ*

Consumption of brassica vegetables has been linked to a reduced incidence of colon cancer (Verhoeven *et al.* 1996), although not all studies demonstrate a reduction in risk (for example, Michels *et al.* 2000). Brassica vegetables contain glucosinolates (inert thioglucosides) which are hydrolysed by plant myrosinase following tissue damage (for example, cutting or chewing). A variety of breakdown products are produced depending on the hydrolysis conditions. Isothiocyanates predominate under pH-neutral conditions, nitriles under acidic conditions and cyanoepithioalkanes are formed in the presence of epithiospecifier protein. Isothiocyanates are thought to be responsible for the cancer-protective action of brassica vegetables but nitrile compounds are more associated with harmful effects (Ahmed & Farooqui, 1982). It is possible that the varying proportions of these products arising following brassica consumption may be related to the inconsistency in reports from epidemiological studies. Enhancing isothiocyanate and reducing nitrile formation may maximize the health benefits of consuming brassica vegetables.

Ingested isothiocyanates form conjugates with glutathione and are excreted in urine as mercapturic acids which are used as biomarkers of isothiocyanate uptake. Urinary biomarkers for nitrile uptake following brassica ingestion have not previously been reported. We have synthesised stable isotopically labelled (incorporating five ^{2}H atoms) phenethyl glucosinolate (PEG; found in watercress) and its breakdown products, phenethyl isothiocyanate (PEITC) and phenethyl nitrile (PEN) and used them to identify urinary biomarkers of PEITC and PEN metabolism.

Male, Rowett Hooded Lister rats (*n* 3 per compound) were maintained in metabolism cages and offered glucosinolate-free feed. Rats received PEG, PEG+myrosinase in feed (PEG+M), PEITC or PEN (150 μmol/kg LW) by tube feeding on two separate occasions separated by 7 d. Each animal separately received labelled and unlabelled versions of one compound. Urine was collected on dry ice for 24 h following tube feeding. Urinary metabolites of the compounds were identified by MS.

Preliminary data demonstrated that this is a suitable method for identifying mercapturic acid production following PEG, PEG+M and PEITC administration. Unlabelled phenethyl mercapturic acid (*m/z* 327, 349 and 365) and labelled phenethyl mercapturic acid (*m/z* 332, 354 and 370) were identified in urine after treatment with unlabelled and labelled compounds respectively and can be identified by the increase in mass (+5 mass units) following administration of stable isotopically labelled compounds.

Administration of unlabelled PEN resulted in excretion of an unidentified urinary metabolite (*m/z* 384 and 400). Use of stable isotopically labelled PEN permitted identification of this product as a metabolite of PEN, since a related compound (*m/z* 389 and 405) was found after treatment with labelled PEN.

Work is ongoing to separate and identify the PEN metabolite. Development of a quantitative analytical method will permit evaluation of the digestive fate of the major metabolites of glucosinolates consumed by humans in brassica meals. This information would enable formulation of appropriate dietary advice for humans to maximise isothiocyanate uptake and the resultant cancer-protective benefits.

The authors gratefully acknowledge the financial support of project code T01027 by the Food Standards Agency.

Ahmed EA & Farooqui MYH (1982) *Toxicology Letters* **12**, 157–163.
Michels KB, Giovannucci E, Joshipura KJ, Rosner BA, Stampfer MJ, Fuchs CS, Colditz GA, Speizer FE & Willett WC (2000) *Journal of the National Cancer Institute* **92**, 1740–1752.
Verhoeven DTH, Goldbohm RA, van Poppel G, Verhagen H & van den Brandt PA (1996) *Cancer Epidemiology, Biomarkers and Prevention* **5**, 733–748.

The bioavailability of riboflavin from foods. By N.R. BULLOCK[1], J.R. DAINTY[2], D. HART[2], P. FINGLAS[2] and H.J. POWERS[1], [1]*Human Nutrition Unit, Division of Clinical Sciences (North), University of Sheffield, UK, S5 7AU and* [2]*Nutrition Division, IFR, Norwich, UK, NR4 7UA*

Riboflavin (vitamin B_2) is essential for several key redox reactions in the body, mediated through its biologically active forms –FAD and FMN. It is found predominantly in meat and dairy products, green leafy vegetables and yeast extract. Limited data are available on the absorption of dietary riboflavin by human subjects at relevant physiological doses. The aim of the present study was to examine the bioavailability and pharmacokinetics of riboflavin from two sources – milk and spinach – using a dual stable-isotope technique. Twenty healthy female subjects (18–65 years of age) were recruited. Each was randomised to receive either ^{13}C-labelled riboflavin in milk or endogenously labelled ^{15}N-riboflavin in spinach (in the form of spinach soup). Following a 4-week washout period, the alternative meal was given. In addition to the oral dose, each volunteer was administered a simultaneous intravenous (IV) infusion of ^{13}C-labelled riboflavin. Blood samples were taken at regular intervals, up to a maximum of 24 h. Plasma flavins were measured by HPLC (riboflavin, FAD and FMN). The erythrocyte glutathione reductase activation coefficient (EGRAC) assay was performed on washed erythrocytes to determine riboflavin status. Plasma flavins were separated via MS to analyse the relative contributions from the oral and IV doses. Total urine output was recorded in the three 24 h periods covering the study and analysed for excreted flavins. EGRAC values were significantly lower on the first visit when compared with the second, independent of randomisation group. Peak response in plasma riboflavin was clearly discernable between 30 and 40 min after ingestion of the test dose. There was no observed difference in the time taken to reach peak riboflavin level, or the height of the peak, between the milk and spinach doses. The data suggest the appearance of a secondary plasma FMN peak that may correspond to reconversion of the IV dose following initial uptake from the systemic circulation.

Mechanisms involved in benzoic acid absorption by human intestinal epithelial cells. By E.L. HAUGHTON[1], M.N. CLIFFORD[1] and P.A. SHARP[1,2], [1]*School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH and* [2]*Division of Nutritional Sciences, King's College London, UK, SE1 9NH*

Epidemiological evidence suggests that the consumption of diets rich in fruit and vegetables are associated with a decreased risk of developing chronic diseases. Much work in this field has focused on the protective effects of dietary polyphenols, which are readily utilised by the colonic microbiota producing a substantial range of metabolites including a number of aromatic and phenolic acids (for a review, see Clifford, 2004). Our current research has focused on benzoic acid and its mono-hydroxy derivatives which are both present in the diet and produced through the action of the gut flora (Clifford *et al.* 2000). Previous work has shown that salicylic acid (2-OH benzoate) is transported in a pH-dependent manner and is inhibited by benzoic acid, suggesting that a common transport mechanism may account for the uptake of this family of compounds (Takanaga *et al.* 1994). It has been suggested that the monocarboxylate transporter MCT1, which is expressed in intestinal tissue (Tamai *et al.* 1995; Ritzhaupt *et al.* 1998) and has transport affinity for benzoic acid (Tamai *et al.* 1995), is a likely candidate for the uptake of these compounds from the gut lumen.

Studies employed human intestinal Caco-2 cells grown on glass coverslips. For measurement of proton-dependent (hydroxy)benzoate uptake, cells were loaded with the pH-sensitive dye BCECF (5 μM, provided as the membrane permeant acetoxymethyl ester). Uptake was assessed, using a Varian Eclipse Spectrofluorimeter, by measuring fluorescence emission at 530 nm following dual excitation at 450 and 495 nm. Baseline fluorescence was measured in either HEPES-buffered saline (pH 7.5) or MES-buffered saline (pH 6.5) for 1 min. Changes in fluorescence following addition of benzoic acid or its mono-hydroxy derivatives (1 mM) to the cuvette were monitored over the next 5 min. Data (means with their standard errors) are presented as changes in fluorescence (ΔF) in arbitrary units (a.u.). Statistical analysis employed either Student's unpaired *t* test or one-way ANOVA where appropriate.

Preliminary studies revealed that the uptake of benzoic acid (1 mM) was strongly pH dependent (ΔF at pH 7.5, 0.04 (SE 0.02) a.u.; ΔF at pH 6.5, 0.43 (SE 0.05) a.u.; $P<0.005$). Benzoic acid, 2-OH benzoate, 3-OH benzoate and 4-OH benzoate all evoked changes in intracellular acidification that were of a similar magnitude and not significantly different from each other ($P>0.05$; one-way ANOVA), suggesting that all compounds might be substrates for the same transporter. MCT1 expression in undifferentiated Caco-2 cells (7 d) was significantly lower than in fully differentiated cells (21 d). Consistent with these observations, benzoic acid-induced changes in BCECF fluorescence were significantly higher in fully differentiated cells (ΔF 7 d, 0.21 (SE 0.10) a.u.; ΔF 21 d, 0.59 (SE 0.07) a.u.; $P<0.01$). Taken together these data strongly suggest that benzoic acid and its monohydroxy-derivatives are taken up by intestinal epithelial cells via MCT1. Given that salicylates are members of this family of compounds and are known to regulate cell signalling pathways it is possible that the generation of (hydroxy)benzoates by the gut microflora may be important for normal gut health.

Clifford MN (2004) *Planta Medica* **70**, 1103–1114.
Clifford MN, Copeland EL, Bloxsidge JP & Mitchell LA (2000) *Xenobiotica* **30**, 317–326.
Takanaga H, Tamai I & Tsuji A (1994) *Journal of Pharmacy and Pharmacology* **46**, 567–570.
Tamai I, Takanaga H, Maeda H, Sai Y, Ogihara T, Higashida H & Tsuji A (1995) *Biochemical and Biophysical Research Communications* **214**, 482–489.
Ritzhaupt A, Wood IS, Ellis A, Hosie KB & Shirazi-Beechey SP (1998) *Journal of Physiology* **513**, 719–732.

Comparison of methods to determine salicylic acid content of fruit juices. By A. WOOD[1], G. BAXTER[2], J. PATERSON[2], F. THIES[3] and G. DUTHIE[1], [1]*Rowett Research Institute, Aberdeen, UK, AB10 6UX,* [2]*Dumfries and Galloway Royal Infirmary, Dumfries, UK, DG1 4AP and* [3]*College of Medicine and Life Sciences, University of Aberdeen, Aberdeen, UK, AB25 2ZD*

In vitro studies suggest that salicylic acid moderates key events implicated in early disease development including oxidation, inflammatory pathways, immune responses and cell-cycle progression. Salicylic acid is present in plants where it functions as a hormonal mediator of the systemic acquired resistance response. It is therefore likely to be present in a wide range of plant-based products of dietary relevance. This has led to the suggestion that the recognised effects of consuming fruit and vegetables on lowering disease risk may be due in part to salicylates in plant-based foods (Paterson *et al.* 2001). However, estimated daily intakes of natural salicylates range widely from 0 to 200 mg/d, confounding assessment of whether there are sufficient in fruit and vegetables for therapeutic significance. Disparity in reported dietary values may reflect, in part, differences in sensitivities between the various methodologies used for salicylate determination. To investigate this possibility, the present study has compared two methods for the determination of salicylic acid in commercially available fruit juices.

A commonly used UV spectrophotometric method (Nordic Committee on Food Analysis, 1999) involved addition of NaCl and CaO to 100 ml samples of fruit juice, followed by acidification, addition of ferric chloride solution and determination at 480 nm. A validated electrochemical detection procedure (Baxter *et al.* 2001) required acidification of only 0.5 ml samples of fruit juice, elution with a ternary gradient programme and detection at an oxidation potential of +1.1 V, peak identities being confirmed by GC-MS. Each sample was analysed in triplicate.

Fruit juice	UV spectrophotometric (mg/100 ml)	Electrochemical (mg/100 ml)
Grove Fresh organic apple	5.76	0.109
Tropicana orange	2.57	0.064
Ocean Spray cranberry	4.51	0.099
ASDA cranberry	3.98	0.079
ASDA 100% apple	4.30	0.094
Aspirin 75 mg (per tablet)	49.04	49.44

Salicylic acid was present in all of the fruit juices but values were markedly greater by UV spectrophotometric determination ($P<0.0004$). The reason for the disparity between methods is unclear. However, the UV spectrophotometric method relies on ferric ions binding with phenol and carboxyl groups of salicylic acid and therefore reaction with structurally related compounds may artefactually increase absorbance leading to overestimation of dietary intakes of salicylic acid. Similar values for salicylate content of aspirin by both methods also suggests the presence of potentially confounding factors in juices. Consequently, the wide range of salicylate values recorded for similar plant-based food items may reflect the methodology used as well as other factors such as varietal differences, growing conditions and storage.

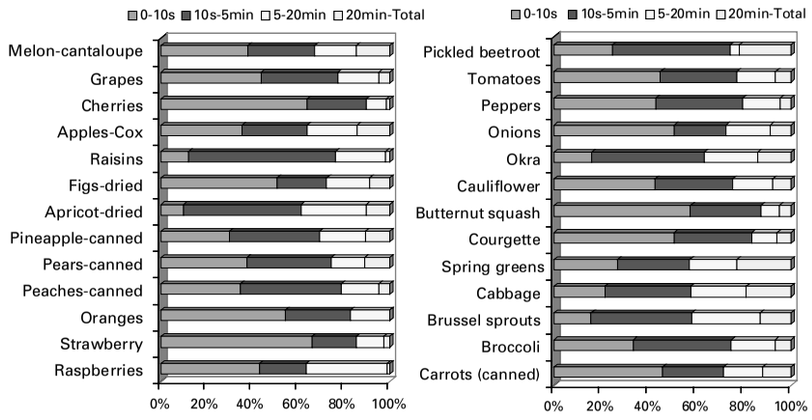
Funded by the Food Standards Agency Postgraduate Scholarship, NHS Dumfries and Galloway and the Scottish Executive Environment and Rural Affairs Department.

Baxter GJ, Graham AB, Lawrence JR, Wiles D & Paterson JR (2001) *European Journal of Nutrition* **40**, 289–292.
Nordic Committee on Food Analysis (1999) *Salicylic Acid in Foods. Qualitative and Quantitative Analysis*, no. 3, 3rd ed. Oslo: Nordic Committee on Food Analysis (NMKL).
Paterson JR & Lawrence JR (2001) *Quarterly Journal of Medicine* **94**, 445–448.

A method for characterising carbohydrate bioavailability of fruit and vegetables. By K.N. ENGLYST, M.E. QUIGLEY and H.N. ENGLYST, *Englyst Carbohydrates, 2 Venture Road, Chilworth Science Park, Southampton, UK, SO16 7NP*

The concept of bioavailability is useful for evaluating and describing the role of carbohydrates in nutrition, with its implications for obesity-related diseases. An important aspect of carbohydrate bioavailability is the rate and extent that starch and sugars are released and absorbed from the food matrix. In conjunction with human physiology studies, we have previously developed *in vitro* methodology for characterising starch digestibility (Englyst *et al.* 1999). Many fruit and vegetables do not have a starch component, and for these products carbohydrate release is linked more with the disruption of the encapsulation effect of the food matrix. The aim of the present study was to investigate the properties of fruit and vegetables that determine the rate at which sugars are released from the food matrix and to characterise these with a modified working protocol of our existing *in vitro* procedure.

The principle features of the modified procedure are: (1) a sample preparation that closely resembles the food matrix disruption that occurs during buccal mastication (chewing); (2) a greater emphasis on the initial phase of carbohydrate release, as it was recognised that there were implications for both exposure of sugars to dental enamel in the mouth, as well as the rate that sugars become available for absorption in the small intestine; (3) a timed incubation with an HCl solution (pH 1.5), reflecting the fact that the food matrix disruption of these products is a gastric, rather than intestinal, event. The figure shows the release characteristics for a few example products.



The present study supports the role of the food matrix in moderating the release of intrinsic sugars. The rate at which carbohydrates were released differed between products, and is an important determinant of their glycaemic index values. The assay, which includes values for individual sugars, provides an important tool for mechanistic studies elucidating the physiological properties of dietary carbohydrates and their impact on obesity and related diseases.

The present study was funded by the FSA: project no. NO 5052.

Englyst KN, Englyst HN, Hudson GJ, Cole TJ & Cummings JH (1999) *American Journal of Clinical Nutrition* **69**, 448–454 (1999).

Effect of dietary fat and fibre on the bioavailability of lycopene from tomato soup. By M. CHOPRA[1], K. COOPER[2], F. COSSOR[1], N. KAYANI[1], and D.I. THURNHAM[2], [1]*School of Pharmacy and Biomedical Science, University of Portsmouth, Portsmouth, Hampshire, UK, PO1 2DT and* [2]*Northern Ireland Centre for Diet and Health, University of Ulster, Coleraine, UK*

The effect of fat on absorption of carotenoids is now well documented (Jayaragan *et al.* 1980; Sies & Stahl, 1998). However, there is limited information on whether a fibre-rich diet can also affect the absorption of carotenoids from the diet. The present study was undertaken to investigate whether the fibre-rich diet can affect bioavailability and plasma levels of lycopene after consumption of tomato soup with either a fat- or fibre-rich meal.

In preliminary experiments, changes in plasma levels of lycopene after consumption of 300 ml/d of tomato soup for 1 week were determined in different age groups (18–30 years, 31–50 years and 51–70 years) and these were not significantly different.

Subsequently, ten healthy subjects (*n* 5 male, *n* 5 female; age range 35–65 years) were supplemented with Heinz cream of tomato soup (300 ml) plus olive oil (20 ml) and white bread for 1 week. After a 2-week washout period, subjects (only nine subjects returned) were supplemented with soup (300 ml) with two to three slices of wholemeal bread (providing 6–9 g fibre/d). For both studies meals were served to subjects (except for weekends) and every effort was made to ensure that subjects consumed the same number of bread slices for both studies. A significant increase in plasma lycopene was observed in both supplementation weeks, i.e. after supplementation of soup with white or wholemeal bread (see Table). There was no significant change in plasma lipid levels during the study period.

The Table shows changes in plasma lycopene, antioxidant activity and lipid levels in nine subjects following consumption of tomato soup, a part of a fat- or fibre-rich meal.

	Plasma FRAP (antioxidant activity) (μmol/l)		Total cholesterol (mmol/l)		Triacylglycerols (mmol/l)		Lycopene (μmol/l)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	1081[a]	191	4.81	0.68	1.15	0.53	0.86[a,c]	0.36
Soup+olive oil	1223[b,c,*]	185	4.67	0.74	1.54	0.68	1.11[b,c,†]	0.28
Baseline 2	1197[c]	163	5.07	0.97	1.17	0.45	1.00[a,c]	0.49
Soup+fibre	1164[a,b,c]	179	4.77	0.73	1.25	0.48	1.28[b,†]	0.41

[a,b,c] Mean values within a column with unlike superscript letters are significantly different: * $P<0.05$; † $P<0.01$ (Wilcoxon paired rank test).

In conclusion, the consumption of tomato soup with white or wholemeal bread does not affect the changes in plasma lycopene levels but supplementing soup with additional olive oil may affect plasma antioxidant activity measured with the FRAP assay.

We would like to thank our volunteers for their participation. The present study was funded by Heinz UK. F. C. was supported by The Wellcome Trust.

Sies H & Stahl W (1998) *Proceedings of the Society for Experimental Biology and Medicine* **218**, 121–124.
Jayaragan P, Reddy V & Mohanram M (1980) *Indian Journal of Medical Research* **71**, 53–56.

Association between body weight and calcium intake in Surrey females aged 11–16 years. By Y.M. JEANES[1], S. REEVES[1], J. CATTERICK[2], J.A. BISHOP[2] and S.A. NEW[2], [1]*School of Human and Life Sciences, Roehampton University, London, UK, SW15 4DJ and* [2]*Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH*

Observational studies have shown an inverse association between dietary Ca intake and body weight in adults and children (Davies *et al.* 2000; Skinner *et al.* 2003). However, to date there are a limited number of studies that have investigated the association between Ca or dairy product intake and body weight in adolescents, with some reporting a negative correlation (Novotny *et al.* 2004) and others reporting no association (Cvijetic *et al.* 2003; Phillips *et al.* 2003).

As part of a larger study into dietary intake and lifestyle patterns among schoolgirls in Surrey, UK, aged 11–16 years, we aimed to examine the association between body weight and Ca intake. Surrey schoolchildren (n 250) completed a 7 d food diary. Weight and height measurements were taken and BMI and BMI z-score were calculated. Mean daily intakes of food and nutrients were assessed using Diet 5 (Univation, Aberdeen, UK).

A positive correlation between Ca intake and body weight (r 0.16; $P<0.012$) and energy intake (r 0.61; $P<0.01$) were found. However, there were no significant correlations between Ca intake or the Ca : protein ratio and BMI. There was no difference in body weight or BMI of subjects when divided into quartiles of Ca intake as indicated in the Table. Additionally there was no association between anthropometric measurements and energy-adjusted calcium intake. The majority of subjects (77 %) were not meeting the reference nutrient intake for Ca (800 mg/d).

	1st (n 63)		2nd (n 62)		3rd (n 62)		4th (n 63)	
Ca quartile ...	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Weight (kg)	48.1	9.2	49.2	9.7	51.1	9.5	51.5	9.5
BMI (kg/m^2)	19.1	2.6	19.3	2.8	19.5	4.0	19.7	2.7
BMI z-score	0.0	0.8	0.7	0.9	0.1	0.9	0.7	0.8
Energy intake (MJ/d)*	6.8	0.9	7.6	1.2	7.8	1.2	8.9	1.4
Ca intake (mg/d)*	455.5	61.7	605.9	42.1	718.7	30.9	967.1	136.4
Protein intake (g/d)*	50.8	9.4	61.0	1.3	60.6	11.5	69.7	11.4
Ca : protein (mg/g)*	9.2	1.8	10.2	1.9	12.3	2.6	14.2	2.3

*Significant difference between quartiles ($P<0.05$).

The present results support the findings of Phillips *et al.* (2003), who conducted a longitudinal study and reported no association between consumption of dairy products and BMI in girls aged 8–12 years followed for 4 years. Similarly, Cvijetic *et al.* (2003) found no correlation between body weight and Ca intake in postpubertal adolescents. In contrast, however, Skinner *et al.* (2003) reported a negative correlation between Ca intake and body weight in pre-adolescent girls. Adolescents are understudied in this potentially important area of weight control, they are a challenging population to study as it is a period of rapid growth and puberty which have an obvious effect on body composition. Intervention studies are required to clarify fully the relationship between Ca intake and dairy product consumption and body-weight regulation.

The present study was funded by a Daphne Jackson Fellowship held by J. C.

Cvijetic S, Baric IC, Bolanca S, Juresa V & Ozegovic DD (2003) *Journal of Clinical Epidemiology* **56**, 591–597.
Davies KM, Heaney RP, Recker RR, Lappe JM, Barger-Lux MJ, Rafferty K & Hinders S (2000) *Journal of Clinical Endocrinology and Metabolism* **85**, 4635–4638.
Novotny R, Daida YG, Acharya S, Grove JS & Vogt TM (2004) *Journal of Nutrition* **134**, 1905–1909.
Phillips SM, Bandini LG, Cyr H, Colclough-Douglas S, Naumova E & Must A (2003) *International Journal of Obesity Related Disorders* **27**, 1106–1113.
Skinner JD, Bounds W, Carruth BR & Ziegler P (2003) *Journal of the American Dietetic Association* **103**, 1626–1631.

The efficacy of low-dose folic acid supplementation on the percentage of circulating platelets *ex vivo* in healthy and dyslipidaemic men. By P. CUMMINS[1], R. ARMSTRONG[2], D. NEWBY[3], R. WILSON[3] and I. DAVIDSON[1], [1]*Queen Margaret University College, Clerwood Terrace, Corstorphine, Edinburgh, EH12 8TS,* [2]*Chief Scientist Office, St Andrews House, Regent Road, Edinburgh, EH1 3DG and* [3]*Centre for Cardiovascular Sciences, Royal Infirmary of Edinburgh, 51 Little France Crescent, Edinburgh, EH16 4SB*

There has been a lot of interest in the homocysteine-lowering effect of folic acid and its potential role in the treatment of CVD. It has been previously suggested that a folate-deficient diet may stimulate a prothrombotic response, due to the concomitant rise in homocysteine levels (Durand *et al.* 1996, 1997). This platelet-related risk has never been demonstrated in human subjects and it remains to be investigated whether the homocysteine-lowering effect of folic acid may also decrease this thrombotic tendency. The present study measured the potential anti-platelet effect of low-dose folic acid supplementation by measuring sensitive markers of platelet activation, namely platelet p-selectin expression and platelet monocyte binding (PMB) in both healthy and dyslipidaemic men.

A double-blind parallel controlled trial was employed where both control (n 8 placebo, n 8 folic acid) and dyslipidaemic (n 7 placebo, n 7 folic acid) subjects were randomly allocated to either low-dose folic acid (0.4 mg) or placebo for 6 weeks. Platelet p-selectin expression and PMB were measured using two-colour flow cytometry in whole blood. Serum folate, total homocysteine and a lipid profile were also analysed before and after treatment. All values are represented as median 25th percentile, 75th percentile. $P<0.05$ was considered significant.

This folic acid control group experienced a significant reduction in homocysteine levels ($P=0.027$) and increase in serum folate ($P=0.001$) amongst control subjects. Folic acid supplementation also exhibited a significant reduction in PMB ($P=0.025$), but had no treatment effect on platelet p-selectin expression.

	Control Placebo		Control Folic acid		Dyslipidaemic Placebo		Dyslipidaemic Folic acid	
	Week 0	Week 6	Week 0	Week 6	Week 0	Week 6	Week 0	Week 6
P-selectin	8.3	9.2	7.2	4.6	9.7	6.8	8.7	9.0
	6.4, 10.2	7.1, 12.7	6.2,9.7	3.8, 8.6	5.7, 10.6	5.1, 10.4	6.4, 10.6	5.5, 10.0
PMB	21.7	16.3	16.8	13.0	18.7	23.0	20.0	18.6
	13.6, 23.0	11.0, 22.1	13.0, 22.5	11.8, 16.5	11.1, 23.1	18.8, 24.0	15.1, 23.1	13.0, 23.6

In the dyslipidaemic group there was no significant reduction in homocysteine levels ($P=0.084$), despite a significant increase in serum folate levels ($P=0.002$). There was no difference in either p-selectin expression or PMB following folic acid supplementation. As expected, folic acid exhibited no treatment effect on lipid levels for both control and dyslipidaemic groups.

The findings from the present study show that the homocysteine-lowering effect of folic acid may also be associated with a reduction in PMB. This was not apparent for p-selectin expression; although p-selectin may not be an ideal marker for the detection of circulating activated platelets (Michelson *et al.* 1996). Further studies are needed to establish the association between folic acid supplementation and platelet activity.

Durand P, Lussier-Cacan S & Blache D (1997) *Federation of American Societies for Experimental Biology* **11**, 1157–1168.
Durand P, Prost M & Blache D (1996) *Atherosclerosis* **121**, 231–243.
Michelson AD, Barnard MR, Hechtman HB, MacGregor H, Connolly RJ, Loscalzo J & Valeri CR (1996) *Proceedings of the National Academy of Sciences* **93**, 11877–11882.

Colon mucosal folate is responsive to folic acid supplements and correlates with plasma folate. By M.H. HILL[1], J.C. MATHERS[2], E.A. WILLIAMS[1], A. SPIERS[2], Y. DUCKWORTH[2], M. WELFARE[2] and H.J. POWERS[1], [1]*Human Nutrition Unit, University of Sheffield, The Northern General Hospital, Sheffield, UK, S6 7AU and* [2]*Human Nutrition Research Centre, University of Newcastle, Newcastle upon Tyne, UK, NE1 7RU*

Data from epidemiological studies suggest that folate status is a determinant of risk for colorectal cancer (CRC). Of interest therefore is the relevance of measurements of plasma or erythrocyte folate, being conventional markers of folate status, to folate concentration in the target tissue. It is also important to know the extent to which folate concentration in colon mucosa is responsive to changes in folate intake. A randomised placebo-controlled trial was carried out in healthy patients and those with adenomatous polyps, to examine the effects of folate and riboflavin supplementation on conventional markers of folate and riboflavin status as well as colon mucosal folate. Effects of the C677T polymorphism in methylene tetrahydrofolate reductase (MTHFR) were also examined. Blood and biopsy samples were collected at baseline and subjects were randomised to receive either placebo, 400 µg folic acid, 1.2 mg folic acid or 400 µg folic acid plus 5 mg riboflavin, for an average of 50 d.

Ninety-five healthy controls (38 males) and 102 polyp patients (60 males), age range 40–87 y, completed the intervention. Supplementation with folic acid elicited a significant dose-dependent increase in plasma folate (measured as 5-methyltetrahydrofolate; 5MTHF), and a reduction in plasma homocysteine (tHcy). Riboflavin status (erythrocyte glutathione reductase activation coefficient; EGRAC), showed the anticipated improvement in response to riboflavin supplementation, but no significant interaction with folate intake in determining folate status. Colon mucosal folate (measured as 5MTHF) was strongly correlated with plasma folate at baseline ($P<0.001$) and inversely correlated with plasma tHcy ($P=0.009$). Colon mucosal folate showed a significant increase in response to 400 µg folic acid relative to the placebo ($P<0.05$) but no further increase at the higher folic acid dose. The magnitude of the response in colon mucosal folate was strongly correlated with the increase in plasma folate and the decrease in plasma tHcy. An interaction between genotype and histology occurred in determining plasma folate response to supplements. The table show post-intervention biochemistry according to treatment group.

	Placebo		Low folate		High folate		Low folate plus riboflavin	
Variable	Median	Range	Median	Range	Median	Range	Median	Range
Plasma 5MTHF (nM)	38.9[a]	0–122	79.7[b]	11–146	123.3[c]	13–1237	83.7[b,c]	31–131
Mucosal 5MTHF (nmol/g)	0.70[a]	0–2.3	1.33[b]	0–2.7	1.38[b]	0–3.9	1.35[b]	0–6.8
PlasmatHcy (µM)	12.7[a]	7–42	11.1[b]	5–16	10.5[b]	7–29	10.8[b]	6–25
EGRAC	1.37[a]	1.16–1.95	1.34[a]	1.05–1.92	1.37[a]	1.0–2.04	1.17[b]	1.06–1.70

[a,b,c] Median values within a row with unlike superscript letters are significantly different, having been corrected for age, sex, smoking, histology and baseline biochemistry (ANOVA (log-transformed data), Tukey's test).

These results support the use of plasma folate as a surrogate for colon mucosal folate concentration but also suggest that the relationship may be weaker at folate intakes not achievable by diet alone.

The present study was supported by the Food Standards Agency (N12004/6).

Iron intakes of rural Gambian women and children. By C.J. PRYNNE, A.A. PAUL, R.K. KEY, L.M.A. JARJOU and G.R. GOLDBERG, *MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge, UK, CB1 9NL and MRC Keneba, West Kiang, The Gambia*

Anaemia is a widespread problem in Africa that is associated with high levels of morbidity and mortality especially in young children and pregnant women. There are several causes, mostly co-existing, which include disease, particularly malaria, intestinal parasite infestation and diet. Fe-deficiency anaemia is commonly attributed to a diet based mainly on plant foods as predominates in most of Africa. However, there is very little information on dietary intakes of Fe probably, due to the paucity of data on the Fe content of African foods.

In common with many other African countries the Gambian diet is based on a staple cereal, usually rice, accompanied by sauces based on leaves with or without groundnuts. Meat is rarely eaten but fish, fresh or dried, often is a component of the sauces. Over many years we have assembled an extensive database of compositions of Gambian foods based on analyses of energy, protein, Ca, Zn and carotene and also computations from recipes gathered in The Gambia. This database includes a very wide range of cooked, mixed dishes (Prynne *et al.* 2002). In parallel with database development a very large number of dietary records have been collected. To date it has not been possible to assess Fe intakes because this nutrient was not included in the database. Using appropriate published sources of information on the Fe content of most of the ingredients of Gambian foods, together with the recipes previously assembled, Fe values have now been assigned to most of the commonly eaten dishes. Using this new information, 2 d dietary records collected on three separate occasions from eighty-five women of reproductive age (1995–1999) and from 160 children aged 8–15 years surveyed annually over a 3-year period (1995–1997) (Prynne *et al.* 2002) have been coded and analysed for total Fe intake and principal sources of Fe.

	Children (*n* 160)		Women (*n* 85)	
Fe intake (mg/d)	Dry season	Wet season	Dry season	Wet season
Mean	33.8	25.3	36.6	27.9
SD	23.2	16.1	22.6	21.8
Median	23.6	20.5	22.6	20.7
Range	6.7–170	1.0–153	1.0–251	1.4–225
Food and Agriculture Organization recommendation	18–65		30–59	

Mean Fe intakes in both the women and the children were high but within the range of requirements for Fe of low bioavailability recommended by the Food and Agriculture Organization (www.fao.org). Similar intakes have been reported for women in Nigeria (Mbofung & Atinmo, 1985). Fe intake was also very skewed as the greatest contribution to total Fe came from millet, which, unlike rice, was not universally eaten. Bulrush millet has a high Fe content (Nordeide, 1995) but the availability is low due to the high level of phytate. The other principal source is boiled rice but in this case the Fe content derives from the local salt. Leaves have a high Fe content but the quantity eaten is small in comparison with cereal consumption. These diet records date back to the 1990s. More recent observation has reported that less millet is being eaten in The Gambia now. However, it could still be used as a rich source of Fe if it is subjected to fermentation which, by destroying the phytate, renders the Fe more available (Makokha *et al.* 2002).

The high Fe intakes do not accord with the low Fe status reported from The Gambia (Bah *et al.* 2001) which is probably only partly due to the low availability of Fe from predominantly plant sources. In addition there are several other factors which may affect Fe status, such as underlying disease, particularly malaria, and genotype.

Bah A, Semega-Janneh I, Prentice A & Bates CJ (2001) *Nationwide Survey on the Prevalence of Vitamin A and Iron Deficiency in Women and Children in the Gambia*. The Gambia: National Nutrition Agency and MRC.
Makokha AO, Oniang'o RK, Njoroge SM & Kamar OK (2002) *Food and Nutrition Bulletin* **23**, 241–245.
Mbofung CM & Atinmo T (1985) *British Journal of Nutrition* **53**, 427–439.
Nordeide M (1995) *The Composition of Malian Foods*. Programme de Reserche SSE Bamako & University of Oslo.
Prynne CJ, Paul AA, Dibba B & Jarjou LMA (2002) *Journal of Food Composition and Analysis* **15**, 349–357.

The effects of pro-inflammatory cytokines on iron absorption by human intestinal epithelial cells. By D.M. JOHNSON[1] and P.A. SHARP[2], [1]*School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH and* [2]*Division of Nutritional Sciences, King's College London, UK, SE1 9NH*

The anaemia of chronic disease (ACD) is the most common form of anaemia in hospitalised patients and is characterised by hypoferraemia and elevated body Fe stores. The progression of ACD is mediated by the release of a cocktail of pro-inflammatory cytokines (for example, IL-1, IL-6 and TNFα) that leads to tissue Fe sequestration (for a review, see Weiss, 2002). Our recent work and that of others suggest that intestinal Fe absorption may be influenced by cytokine action (Han *et al.* 1997; Johnson *et al.* 2004). The purpose of the present study was to compare the actions of individual pro-inflammatory cytokines on intestinal Fe absorption in order to determine whether they operated through a common regulatory pathway.

Studies were performed on the human intestinal Caco-2 cells grown for 21 d on semi-permeable membranes in bicameral chambers (Transwell; Corning Life Sciences, Hertfordshire, UK) to allow them to fully differentiate. Before experimentation, cells were placed in serum-free medium in the presence or absence of TNFα or IL-6 (10 ng/ml), and added to the basolateral chamber of the Transwell plate for the final 24–72 h of the culture period. Fe transport across cell monolayers was determined using a previously published protocol (Tandy *et al.* 2000). Separate plates of cells were used for Western blotting to determine the effects of pro-inflammatory cytokines on Fe transporter protein expression. Data are means and SE. Statistical analysis was performed using Student's unpaired *t* test.

Fe uptake across the apical membrane of the Caco-2 cell monolayer was significantly decreased following exposure to TNFα (control, 1.00 (SE 0.04) nmol/cm^2 per h;+TNFα, 0.66 (SE 0.03) nmol/cm^2 per h, n 6; $P<0.001$). In contrast there was no effect of IL-6 on Fe uptake (control, 1.12 (SE 0.07) nmol/cm^2 per h;+IL-6, 1.02 (SE 0.04) nmol/cm^2 per h, n 4; $P<0.21$). Western blotting for DMT1, the intestinal apical membrane Fe transporter protein, revealed a significant decrease in TNFα-treated cells (control, 90.1 (SE 5.2) arbitrary units (a.u.);+TNFα, 57.1 (SE 4.4) a.u., n 4; $P<0.01$) but expression was unchanged in cells stimulated with IL-6 (control, 84.5 (SE 4.6) a.u.;+IL-6, 79.7 (SE 6.8) a.u., n 6; $P<0.57$). Expression of the basolateral efflux transporter IREG1 was not significantly altered by either cytokine compared with the untreated control groups. In TNFα-treated cells, ferritin protein levels were elevated (64 (SE 10) %; $P<0.02$) compared with untreated cells, and this was associated with a decrease in the rate of Fe release into the basolateral medium. In contrast, no significant changes in cell ferritin or Fe efflux were observed in IL-6-treated cells.

Taken together these data suggest that the intestinal response to the cocktail of cytokines released as a consequence of inflammation or infection is not uniform. TNFα acts directly on the epithelial cells to regulate the flow of Fe across the intestinal epithelium, whereas IL-6 appears to have no direct regulatory activity on Fe uptake. However, IL-6 may influence intestinal Fe metabolism indirectly through its extra-intestinal actions. This possibility is currently under investigation.

Han O, Failla ML & Smith JC (1997) *Journal of Nutritional Biochemistry* **8**, 585–591.
Johnson D, Bayele H, Johnston K, Tennant J, Srai SK & Sharp P (2004) *FEBS Letters* **573**, 195–201.
Tandy S, Williams M, Leggett A, Lopez-Jimenez M, Dedes M, Ramesh B, Srai SK & Sharp P (2000) *Journal of Biological Chemistry* **275**, 1023–1029.
Weiss G (2002) *European Journal of Clinical Investigation* **32**, Suppl. 1, 70–78.

Selenium-enriched yeast as a nutritional supplement: bioavailability, toxicology, efficacy and new identification of an anti-cancer component. By M.P. RAYMAN[1] and H. GOENAGA INFANTE[2], [1]*School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH and* [2]*LGC Limited, Queens Road, Teddington, Middlesex, UK, TW11 OLY*

Se-enriched yeast (Se-yeast) is the product of the aerobic fermentation of *Saccharomyces cerevisiae* in a Se-enriched medium. It is the commonest form of Se used to supplement dietary intake of this important trace mineral. Though reputable manufacturers apply a considerable number of quality-control measures in its production, concerns have been expressed that Se-yeast supplements are poorly characterised and could potentially cause the build up of Se in tissues to toxic levels (Rayman, 2004). However, of about twelve supplementation studies, none has shown evidence of toxicity even up to an intake level of 800 μg Se/d over a period of years.

The bioavailability of Se-yeast has been found to be higher than that of inorganic Se sources in all but one study; it has been shown to resemble that of wheat Se rather than inorganic Se (selenate) in its effect on plasma Se. Absorption and retention of manufactured Se-yeast were measured in male subjects fed ^{77}Se-labelled SelenoPrecise™ yeast, as 90 and 75% respectively though laboratory-prepared Se-yeast gave a lower absorption and retention, 53.5 and 59.3% respectively. The reasons for the different results between the two studies may relate to the strain of the yeast and, more importantly, to the difference between the processes used to prepare the Se-yeast.

Se-yeast is capable of increasing the activity of the selenoenzymes. Intervention studies have shown the benefit of this form in cancer prevention, immune response and HIV infection. It is the only form of Se to date to have shown efficacy in human anticancer intervention studies. Although the major component of Se-yeast has been identified as selenomethionine (SeMet), accounting for 54–74% of total Se in commercial Se-yeast, it is possible that this supplement may contain other Se species with more potent cancer-preventive activity. Examples of such species are seleno-methyl-seleno-cysteine (SeMC) or its storage form, γ-glutamyl-SeMC, both of which are thought to be metabolised to methyl selenol, a potent anti-carcinogenic metabolite of Se found in *Allium* and *Brassica* vegetables.

Despite a large number of studies, information on the identity of the molecules incorporating or binding Se in yeast is still scarce, mostly because of the complexity of the matrix and the low concentration of the Se compounds (Uden *et al.* 2004). However, advances in speciation methodology in recent years have allowed us now to identify and quantify of a number of Se species in Se-yeast samples from different sources. We have used complementary HPLC methods combined with on-line ICP-MS and electrospray MS/MS for Se speciation analysis of Se-yeast. Extraction of the Se compounds from the complex matrix following enzymic digestion with proteolytic enzymes allowed full identification and quantification of SeMC as a minor species (Goenaga Infante *et al.* 2004). Accelerated solvent extraction with water only, allowed γ-glutamyl-SeMC in the Se-yeast aqueous extract to be identified, on the basis of retention time, molecular mass determination and product-ion pattern. Though the amounts of SeMC and γ-glutamyl-SeMC were low, representing 5 and 7% of the extracted Se respectively, these are significant findings that may be relevant to the effectiveness of Se-yeast in cancer prevention.

Goenaga Infante H, O'Connor G, Rayman M, *et al.* (2004) *Journal of Analytical Atomic Spectrometry* **19**, 1529–1538.
Rayman MP (2004) *British Journal of Nutrition* **92**, 557–573.
Uden PC, Boakye HT, Kahakachchi C, *et al.* (2004) *Journal of Analytical Atomic Spectrometry* **19**, 65–73.

Dietary selenium intake and plasma glutathione peroxidase status in healthy British adults. By E. PATERSON, J. CARSON-LONG, M. GORDON and J. LOVEGROVE, *School of Food Biosciences, The University of Reading, Whiteknights, PO Box 226, Reading, Berkshire, UK, RG6 6AP*

The aim of the present research was to establish dietary intake of Se and plasma glutathione peroxidase (GPx) status in healthy British adults participating in a randomized, controlled, cross-over dietary intervention investigating the effect of carotenoid consumption on disease biomarkers. Thirty-six volunteers with low fruit and vegetable intake aged between 20 and 67 years completed the study. Three-day diet records, completed by participants at the beginning of each leg of a 23-week cross-over intervention study, were analysed for mean daily macro- and micronutrient intake using nutrition analysis software (Diet Cruncher, Dunedin, NZ) with McCance and Widdowson food composition database (FSA, London, UK). Plasma aliquots from subject blood samples collected at the beginning of each leg of the study were analysed for the selenoenzyme GPx using an EIA kit (Oxis Research, Portland, OR, USA). The results showed that mean daily energy intake was 9097 (SD 1264) and 7812 (SD 1197) kJ for males (*n* 12) and females (*n* 24) respectively. Mean daily Se intake for males and females was 43 (SD 4) and 46 (SD 3) μg/d. The 25th percentile for Se intake was 29 and 36 μg/d for males and females respectively. Caucasian's (*n* 29) tended to have lower dietary Se intakes than non-Caucasian's (*n* 7) with values of 42 (SD 5) and 54 (SD 6) μg/d respectively (P=0.060). Plasma GPx levels were 5.4 (SD 0.5) μg/ml for males (*n* 12) and 5.1 (SD 0.4) μg/ml for females (*n* 24). Mean plasma GPx values were not significantly different between males and females.

Dietary Se intake in the UK has been falling over the last 25 years (Jackson *et al.* 2004) and Se intakes could be as low as 30–40 μg/d (Rayman, 1997). Our data indicate low dietary Se intakes, similar to those reported for current British adults. However, plasma GPx levels in our subjects were normal and were similar to those reported elsewhere for males and females (Rush & Sandiford, 2003). The low Se intakes found in our subject's supports evidence that British Se intakes remain low: the functional consequences of which have been reported by other researchers (Jackson *et al.* 2004). Rayman (1997) advocated for government intervention, but this was declined because of insufficient evidence of adverse health consequences (Department of Health, 1998). We observed normal plasma GPx status in our subjects; however, we suggest that continued monitoring of dietary Se status using several biomarkers combined with a multicentred approach is warranted.

We gratefully acknowledge Unilever Bestfoods for funding for this research.

Jackson MJ, Dillon SA, Broome CS, McArdle A, Hart CA & McArdle F (2004) *Proc Nutr Soc* **63**, 513–517.
Rayman M (1997) *BMJ* **314**, 387–388.
Rush JW & Sandiford SD (2003) *Clin Biochem* **36**, 345–351.
Department of Health (1998) Food Safety Information Bulletin no. 93.London: The Stationary Office.

Fruit and vegetable consumption and bone mineral status in adolescents. By C.J. PRYNNE, G.D. MISHRA, M.A. O'CONNELL, G. MUNIZ, A. PRENTICE and F. GINTY, *MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge, UK, CB1 9NL*

Osteoporosis is a significant public health problem resulting in fractures and increased morbidity and mortality in older men and women. Maximising peak bone mass in young adult life may reduce the risk of developing the disease. There is increasing evidence for positive effects of fruit and vegetable intake on bone health in older age groups. The aim of the present study was to investigate whether a similar positive relationship was evident in 16–18-year-old boys and girls.

Bone mineral measurements were made of the whole body, hip and spine by dual energy X-ray absorptiometry in male (*n* 111) and female (*n* 101) students from Cambridge, UK. Information on health and lifestyle and physical activity was obtained by questionnaire. Each completed a 7 d food diary, recording their food and drink consumption in household measures. The diaries were coded and analysed using in-house programs to calculate the intake of fruit, including fruit juices, and vegetables, excluding potatoes. Multiple regression analysis was used to examine the relationship between bone mineral content (BMC), with and without adjustment for bone and body size (i.e. size-adjusted BMC (SA-BMC), bone mineral density (BMD) and fruit and vegetable intake.

	Boys (*n* 111)						Girls (*n* 101)					
	Fruit			Fruit and vegetables			Fruit			Fruit and vegetables		
	r	SD	*P*	*r*	SD	*P*	*r*	SD	*P*	*r*	SD	*P*
Whole body												
BMC	+3.0	0.9	0.002	+9.2	2.8	<0.001	+2.2	1.2	NS	+5.2	2.2	0.02
BMD	+1.5	0.5	0.003	+4.3	1.2	<0.001	+1.4	0.6	0.03	+2.9	1.1	0.01
SA-BMC	+0.5	0.4	NS	+0.4	0.4	NS	+0.9	0.5	NS	+1.9	0.9	0.05
Spine												
BMC	+3.2	1.1	0.005	+7.8	2.5	0.002	+3.2	1.4	0.03	+8.9	2.6	0.001
BMD	+1.9	0.7	0.006	+4.3	1.5	0.005	+2.3	0.9	0.01	+5.8	1.7	0.001
SA-BMC	+1.3	0.6	0.04	+2.6	1.4	0.07	+1.7	0.8	0.04	+4.9	1.6	0.003
Femoral neck												
BMC	+3.3	1.0	0.001	+10.3	2.3	<0.001	NS			NS		
BMD	+3.1	0.8	0.001	+7.6	1.7	<0.001	NS			NS		
SA-BMC	+2.5	0.7	0.001	+5.9	1.7	0.001	NS			NS		

BMC and BMD were adjusted for supplement use, smoking, physical activity, age, Ca and energy intake, and age at menarche (girls only). SA-BMC is BMC adjusted for height, weight, bone area and the aforementioned potential confounding variables. Results shown are from parsimonious regression models. Analyses were carried out on naturally logged data. Results show the regression coefficient (and SD) × 100, which equates to the percentage increase for a 100% increase in fruit and vegetable intake.

The present study is the only known study of bone mineral status where fruit and vegetable intake has been assessed from a 7 d food diary. Mean intakes of total fruit and vegetables (excluding potatoes) were 309 and 296 g/d for boys and girls respectively and 26% of the boys and 21% of the girls consumed more than 400 g/d. A significant positive relationship was found between spine SA-BMC and fruit intake in both boys and girls, and with femoral neck SA-BMC in boys only. Although no significant relationships were found with vegetable intake alone, the magnitude of the effect increased when fruit and vegetables were combined.

These results are in partial agreement with a recent study where heel BMD was positively associated with fruit intake in 12–15-year-old adolescent girls, but not boys (McGartland *et al.* 2004). Similarly to the spine and femoral neck, the heel contains a high proportion of trabecular bone, which has a high turnover rate and is subject to more degradation over the lifecycle. Our findings suggest that higher fruit and vegetable intake may positively affect bone mineral accrual at potentially vulnerable skeletal sites in adolescent boys and girls. Based on parallel investigations, the mechanism does not appear to be related to greater dietary alkalinity (Ginty *et al.* 2005). Higher intakes of fruit and vegetables may also be indicators of other diet and lifestyle characteristics beneficial to bone health.

Supported by the Food Standards Agency (project award N05044).

Ginty F, Prynne CJ, Muniz-Terrara, Mishra GD, Prentice A & O'Connell MA (2005) **64**, 80A.
McGartland CP, Robson PJ, Murray LJ, *et al.* (2004) *American Journal of Clinical Nutrition* **80**, 1019–1023.

The effect of frequent feeding on bone turnover in human subjects. By A.L. BONNETT[1], K.L. PROCTER[1], F. GOSSIEL[1], C.A. HARRISON[2], M.E. BARKER[1] and A. BLUMSOHN[1], [1]*Human Nutrition Unit of Clinical Sciences (North), University of Sheffield, Sheffield, UK, S5 7AU and* [2]*Skin Research Group, Division of Clinical Sciences, University of Sheffield, Sheffield, UK*

Bone turnover shows a marked circadian rhythm (amplitude approximately 100%) with peak in bone resorption in the early morning and trough in the late afternoon (Blumsohn *et al.* 1994). This circadian rhythm may be largely driven by nutrient intake, with bone resorption decreasing rapidly after intake of food or oral glucose (Bjarnason *et al.* 2002), and being attenuated by fasting (Schlemmer & Hassager, 1999; Bjarnason *et al.* 2002). In animal studies, feeding a meal in four portions instead of one blunts bone resorption and causes an increase in bone mass (Muhlbauer & Fleisch, 1995; Muhlbauer & Li, 1997).

On this basis, frequent feeding has been investigated for the first time in a pilot study on eight healthy adults (4 male, 4 female) between the ages of 22 and 41 years. Women were all premenopausal, not pregnant and had regular menstrual cycles. Participants were excluded for bone disease (or recent fracture), malignant disease, nutrient absorption problems, or consumption of drugs known to affect bone turnover. Participants were also excluded if their daily regime did not fit into the normal environmental light-dark cycle. This was a two-way crossover study. Volunteers were studied on two occasions in a randomised order. On one occasion (frequent feed), they were fed eight, identical small meals every 2 h from 09.00 to 23.00, and on the other occasion, three meals (control; 10.00, 15.00 and 18.00). The total food consumption of each participant was controlled during each circadian study, being of equivalent energy (12.28 MJ/d ±2% males, 9.33 MJ/d ±2% females) and macronutrient composition (15% protein ±2, 55% carbohydrate ±3 and 30% fat ±3). The aims of the imposed regimes were to identify whether a frequent feeding pattern could influence the circadian rhythm of bone turnover, and, in addition, reduce daily bone resorption in healthy human subjects.

Significant circadian rhythms were observed in two biochemical markers of bone resorption: urinary NTX (amplitude 90% control, 86% frequent) and serum β-CTX (amplitude 120% control, 103% frequent), but not in parathyroid hormone (PTH) secretion. Whilst there was a blunting of the nocturnal rise in bone resorption, no significant effect of frequent feeding has been seen on the pattern of circadian variation observed in u-NTX and s-β-CTX. There was also no significant difference between the circadian rhythms observed in PTH secretion.

Frequent feeding did, however, have a significant but very small effect in reducing daily bone resorption as indicated by u-NTX ($P=0.023$; paired t test) and s-β-CTX ($P=0.049$; paired t test). There was also a significant reduction ($P=0.019$) in PTH levels when comparing the control to frequent feeding pattern.

After confirmation of these findings in a larger study, a frequent feeding regimen may therefore be able to help in attainment of peak bone mass in adolescents, and reduce age-associated bone loss in the elderly.

Bjarnason NH, Henriksen EE, Alexandersen P, Christgau S, Henriksen DB & Christiansen C (2002) *Bone* **30**, 307–313.
Blumsohn A, Herrington K, Hannon RA, *et al.* (1994) *Journal of Clinical and Endocrinological Metabolism* **79**, 730–735.
Mühlbauer RC & Fleisch H (1995) *Journal of Clinical Investigation* **95**, 1933–1940.
Mühlbauer RC & Li F (1997) *Biomedicine and Pharmacotherapy* **51**, 360–363.
Schlemmer A & Hassager C (1999) *European Journal of Endocrinology* **140**, 332–337.

Sources of dietary alkali in the UK diet: potential renal acid load (PRAL) of commonly consumed hot and cold beverages. By J. CATTERICK[1], R.H.T. GANNON[1], D. LOVELL[2], H.M. MACDONALD[3], D.J. MILLWARD[1] and S.A. NEW[1], [1]*Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences,* [2]*Postgraduate Medical School, University of Surrey, Guildford, UK, GU2 7XH and* [3]*Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen, UK, AB25 2ZD*

There is a growing interest in the importance of acid–base homeostasis to overall health, with a particular focus on the positive role of the dietary alkali supply to skeletal integrity (Arnett, 2003; Lin *et al.* 2003). Dietary acidity is particularly influenced by the level of potential renal acid load (PRAL), which is a predictor of the dietary component of net acid excretion (Remer *et al.* 2003). Identification of foods and beverages which provide a supply of dietary alkali remains a priority.

The aim of the present study was to determine the level of PRAL in commonly consumed hot and cold beverages in the UK. Using the McCance and Widdowson's food composition tables sixth edition (Food Standards Agency, 2002), PRAL was calculated using the appropriate formulae:

$$PRAL_{FULL} = \Sigma((\text{protein } (SO_4) + P + Cl) - (Na + Mg + K + Ca))$$

$$PRAL_{SHORTENED} = \Sigma((\text{protein } (SO_4) + P) - (Mg + K + Ca))(\text{Remer \& Maz, 1995}).$$

Beverages (100 g)	Energy (kJ)	Protein (g/100g)	Element content (mg/100 g)						PRAL (mEq/100 g)	
			Na	K	Ca	Mg	P	Cl	$PRAL_{FULL}$	$PRAL_{SHORTENED}$
Semi-skimmed milk	46	3.5	43	156	120	11	94	87	0.7	0.2
Tea with milk	5	0.4	4	40	12	3	11	9.6	−0.3	−0.4
White coffee (instant)	5	0.5	5	50	13	4	12	9	−0.6	−0.6
Low-fat Horlicks	50	1.9	110	120	90	7	0	0	−7.4	−2.9
Original Horlicks(with skimmed milk)	82	4.2	93	215	178	14	117	77	−2.4	−0.7
Fruit squash diluted	19	0.02	8	5.4	1.2	0.2	0.4	0.8	−0.4	−0.1
Pure orange juice	36	0.5	10	150	10	8	13	9	−2.9	−2.7
Pure apple juice	38	0.1	2	110	7	5	6	3	−2.2	−2.2

As shown in the Table above, fruit juice and hot beverages containing potassium bicarbonate had a substantive negative PRAL and hence were suppliers of dietary alkali. $PRAL_{full}$ is a more accurate indicator of alkaline load when sodium chloride is not the only source of sodium, whereas $PRAL_{shortened}$ is a useful indicator of alkaline load when sodium chloride is the major source of sodium, or when chloride is not given in the composition of food tables. Further work is required to determine the impact of alkali-forming food products on estimates of net endogenous acid production in habitual diets.

This study was funded by GlaxoSmithKline. RHTG is recipient of a University of Surrey PhD scholarship.

Arnett TR (2003) *Proceedings of the Nutrition Society* **62**, 511–520.
Food Standards Agency (2002) *McCance and Widdowson's The Composition of Food, Sixth summary edition*. Cambridge: Royal Society of Chemistry.
Lin PH, Ginty F, Appel LJ, Aickin M, Bohannon A, Garnero P, Barclay D & Svetkey LP (2003) *Journal of Nutrition* **133**, 3130–3136.
Remer T & Manz F (1995) *Journal of American Dietetic Association* **95**, 791–797.
Remer T, Dimitriou T & Manz F (2003) *American Journal of Clinical Nutrition* **77**, 1255–1260.

Do prostasomes play a role in lycopene delivery? By A. GOYAL, M. CHOPRA, G. DELVES and A. COOPER, *Department of Biomedical Sciences, St Michael's Building, University of Portsmouth, UK, PO1 2DT*

Prostasomes are cellular exosomes liberated from the prostatic epithelium into semen. They are multilamellar lipid vesicles, whose membranes have at least 130 proteins embedded in them (Utleg *et al.* 2003). Their composition, as lipid or protein, is distinct from the plasma membranes of the cells from which they derive. They are known, in global terms, to have antioxidant activity (Saez *et al.* 1998), although there is uncertainty about how this is mediated. Analogous exosomes are liberated *in vitro* by prostatic epithelial cells in culture (Nilsson *et al.* 1999), and also into the local tissue environment by prostate cancer metastases (Sahlen *et al.* 2004). In disease, antioxidants can counter the carcinogenic or inflammatory activities of free radicals. Antioxidant chemoprevention can be mediated by dietary supplementation. Giovannucci *et al.* (1995) demonstrated an inverse relationship between the risk of prostate cancer and the consumption of a tomato-enriched diet. The protective effect is thought to be mediated by the powerful antioxidant lycopene. Antioxidant activity in semen is physiologically important. Its absence is a major cause of idiopathic male infertility. Lycopene is present in seminal plasma, however its levels are significantly decreased in infertile men (Palan & Naz, 1996). Furthermore, oral lycopene supplementation has been shown to improve various sperm parameters in idiopathic male infertility (Gupta & Kumar, 2002).

It is reasonable to hypothesise that a highly lipophilic antioxidant such as lycopene, which is known to accumulate in the prostate gland, (Clinton *et al.* 1996) might easily be carried by prostasomes. The present study applies a reductionist approach to the possibility of prostasomal antioxidant activity being mediated through lycopene, by assessing the intrinsic ability of these particles to absorb and hold lycopene, and to interact with components of the male genital tract.

Prostatic epithelial cells accumulate lycopene *in vitro* (Hwang & Bowen, 2004) but its export has not been demonstrated. Prostasomes are known to fuse with spermatozoa (Arienti *et al.* 1997) however any interactions with differentiated, somatic, anchorage-dependent cells have not been addressed. In this study human virally immortalised prostatic epithelial (PNT2) and human umbilical vein endothelial (HUVEC) cells were cultured as monolayers. Exosomes were harvested from conditioned medium by ultracentrifugation. Seminal prostasomes were acquired by ultracentrifugation and gel chromatography (Ronquist & Brody, 1985). The fluorescent aliphatic lipid-like marker PKH26 was used to trace prostasomal membrane traffic. Lycopene was delivered solubilised and analysed by HPLC.

In this study, *in vitro* prostasome analogues shed from lycopene-loaded cells contained lycopene. Seminal prostasomes accumulated lycopene from an *in vitro* milieu. Calculating vesicle-bound concentrations is difficult, but approximately 20–80 ng lycopene/mg exosomal protein has been determined. Prostasomes adhere to and donate lipid to PNT2 and HUVECs. We have demonstrated this by the transfer of PKH26, from prostasomes to cells by fluorescence microscopy.

Summarising, prostasomes and analogous vesicles take up lycopene and donate lipid to epithelial and endothelial cells. This forms the conceptual basis for a functional antioxidant delivery system, potentially capable of impinging not only on fertility but also prostatic disease. Prostasomes shed from micrometastases potentially affect cell kinetics and the endothelial activation required for angiogenesis and progression to frank tumour.

This study was supported by funding from Heinz Inc.

Arienti G, Carlini E & Palmerini CA (1997) *The Journal of Membrane Biology* **155**, 89–94.
Clinton SK, Emenhiser C, Schwartz SJ, Bostwick DG, Williams AW, Moore BJ & Erdman JW Jr (1996) *Cancer Epidemiology, Biomarkers and Prevention* **5**, 823–833.
Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA & Willett WC (1995) *Journal of National Cancer Institute* **87**, 1767–1776.
Gupta NP & Kumar R (2002) *International Urology and Nephrology* **34**, 369–372.
Hwang ES & Bowen PE (2004) *Journal of Medicinal Food* **7**, 284–289.
Nilsson BO, Lennartsson L, Carlsson L, Nilsson S & Ronquist G (1999) *Upsala Journal of Medical Sciences* **104**, 199–206.
Palan P & Naz R (1996) *Archives of Andrology* **36**, 139–143.
Ronquist G & Brody I (1985) *Biochimica et Biophysica Acta* **822**, 203–218.
Saez F, Motta C, Boucher D & Grizard G (1998) *Molecular Human Reproduction* **4**, 667–672.
Sahlen G, Ahlander A, Frost A, Ronquist G, Norlen BJ & Nilsson BO (2004) *Prostate* **61**, 291–297.
Utleg AG, Yi EC, Xie T, Shannon P, White JT, Goodlett DR, Hood L & Lin B (2003) *Prostate* **56**, 150–161.

The protective effect of the isoflavone phyto-oestrogen daidzein against formation of oxysterols in rat liver and brain after acute alcohol dosage. By J. ADACHI[1], M. SATO[1], N. YOSHIOKA[1], Y. UENO[1], C.I. LIN[2], M.C.Y. WONG[2], V.R. PREEDY[2] and H. WISEMAN[2], [1]*Department of Legal Medicine, Kobe University Graduate School of Medicine, Kusunoki-cho 7 Chuo-ku Kobe 6500017, Japan and* [2]*Nutritional Sciences Research Division King's College London, Franklin-Wilkins Building, 150 Stamford Street, London, UK, SE1 9NH*

Ethanol (alcohol) has been shown to have deleterious effects in various organs, including the brain and liver. Part of this damage may be due to oxidative stress and related perturbations in biomembranes. For example, Adachi *et al.* (2000) has shown that ethanol increases cholesterol hydroperoxides and oxysterols in rat skeletal muscle, possibly reflecting sarcolemmal damage. We hypothesised that acute ethanol consumption would also increase oxysterols in rat brain and liver. Furthermore, we also hypothesised that the perturbations can be protected against by pre-treatment with the isoflavone phyto-oestrogen, daidzein.

To test this, male Wistar rats (body weight approximately 0.1 kg) were divided into four groups (n 6), which were: saline (control; group A); daidzein (group B); ethanol (group C); daidzein+ethanol (group D). Each group was given pre-treatment with either carrier (20% fat emulsion) or daidzein (100 mg/kg body weight, intraperitoneally) for 2 d. Saline (0.15 mol NaCl/l (1 ml/0.1 kg body weight), intraperitoneally) or ethanol (75 mmol/kg body weight, intraperitoneally) was administered 1 h after the second pre-treatment injection. Rats were killed 24 h after ethanol or saline dosage and brain and liver samples were dissected out for subsequent analysis. 7α- and 7β-Hydroxycholesterol (7α-OH and 7β-OH), and 7-ketocholesterol (7-keto) were analysed by HPLC with UV detection.

The result of the present study showed that in brain there was no significant effect of either ethanol or daidzein on oxysterols. In the liver, acute ethanol dosage had no significant effect on either 7α-OH or 7β-OH ($P>0.05$; NS). However, the mean concentration of 7-keto increased significantly from 1129 nmol/g (group A) to 1462 nmol/g (group C; group A *v.* group C, $P=0.004$). There was no effect of daidzein *per se*, but it significantly ameliorated the ethanol-induced increase in 7-keto, i.e. the concentration in group D was 1180 nmol/g ($P=0.012$, compared with group C).

In conclusion, the present study showed that oxysterol 7-keto concentration increased in rat liver indicative of oxidative stress. Daidzein showed a protective effect against ethanol-induced perturbations in the liver.

We wish to thank Hangzhou FST, Republic of China, for their generous gift of phyto-oestrogen.

Adachi J, Asano M, Ueno Y, Niemelä O, Ohlendieck K, Peters TJ & Preedy VR (2003) *Journal of Nutritional Biochemistry* **14**, 616–625.

The effect of acute alcohol dosage on the perturbation of antioxidant capacity in the rat liver.

By M.C.Y. WONG[1], R. HUNTER[1], R. SRIRAJASKANTHAN[1], K. TRICK[2], M.R. LABBE[2], V.R. PREEDY[1] and H. WISEMAN[1], [1]*Nutritional Sciences Research Division, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London, UK, SE1 9NH and* [2]*Bureau of Nutritional Sciences, Health Canada, Ottawa, Canada K1A 0L2.*

Alcohol administration has previously been shown to induce oxidative stress in liver as reflected by increased hydroxycholesterol level (Ariyoshi *et al.* 2002). We hypothesised that 24 h acute ethanol dosage would be a potential model to induce oxidative stress in the liver as reflected by measures of total antioxidant capacity. To test this, we dosed rats acutely with alcohol (75 mmol ethanol/kg body weight) and compared the effects with D-galactosamine (GalN) at 1 g/kg body weight, which has previously been shown to induce oxidative stress in liver (Sun *et al.* 2003). Male Wistar rats (0.1 kg body weight) were assigned to four groups in two separate experiments. In study 1, the groups were: saline (0.15 mol NaCl/l; control; group A) and ethanol (group B). In study 2, the groups were saline (control; group C) and GalN (group D). Animals were injected intraperitoneally (single dose) in the morning, food was withdrawn and the rats were killed 24 h later. Blood and liver samples were collected for subsequent analysis for total antioxidant capacity by the ferric reducing antioxidant power (FRAP) assay and total superoxide dismutase (SOD) activity.

	Control		Treated (ethanol)			Control		Treated (GalN)		
	Mean	SEM	Mean	SEM		Mean	SEM	Mean	SEM	
FRAP value of plasma (μM/μl)	59	3	55	2	NS	60	3	75	6	*
FRAP value of liver (μM/mg tissue homogenate)	1229	116	1220	167	NS	1047	115	1213	115	NS
Total SOD activity of liver homogenate (U/mg protein)	41	4	41	6	NS	37	1	46	3	†

Data are means with their SEM (n 6). Differences between means were analysed by Student's t test within the same experimental group.
NS, $P>0.05$.
* Difference $P<0.05$ when compared with corresponding control rats.
† Difference $P<0.01$ when compared with corresponding control rats.

The results showed that 24 h after ethanol injection, neither plasma nor liver homogenate showed a significant alteration in antioxidant capacity in terms of both FRAP value and SOD activity. However, the same exposure time of GalN treatment increased both FRAP values of plasma and liver total SOD activity significantly. In conclusion the data suggest that 24 h acute ethanol does not perturb the overall antioxidant capacity of the liver, though there may be specific effects related to selective sites within the liver or even different biochemical targets, such as lipid peroxidation.

Ariyoshi K, Adachi J, Asano M, Ueno Y, Rajendram R & Preedy VR (2002) *Free Radical Research* **36**, 661–666.
Sun F, Hamagawa E, Tsutsui C, Sakaguchi N, Kakuta Y, Tokumaru S & Kojo S (2003) *Biochemical Pharmacology* **65**, 101–107.

The application of 'Lab-on-a-Chip©' for proteomic studies in screening metabolic perturbations.

By C.I. LIN[1], M.C.Y. WONG[1], N.J. WHITEHEAD[1], V.R. PREEDY[1] and H. WISEMAN[1], [1]*Nutritional Sciences Research Division, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London, UK, SE1 9NH*

Delineating the multiple changes of protein expression in diseases is a fundamental need in the proteomic era. Capillary electrophoresis (Lab-on-a-Chip, Agilent Technologies, Inc., Palo Alto, CA, USA) enables profiling proteins to be rapidly determined and provides information on both protein mass and quantity, i.e. ten protein profiles can be obtained within 1 h by using capillary electrophoresis. Potentially, it is very useful for screening samples to determine if further, more in-depth, proteomic studies should be undertaken, i.e. by surface-enhanced laser desorption ionisation (SELDI). The objective of the present study is to investigate the applicability of capillary electrophoresis for profiling rat livers exposed to alcohol.

Male Wistar rats were divided into four groups (n 6) and were treated with either saline (0.15 mol NaCl/l, intraperitoneally) or ethanol (75 mmol/kg body weight, intraperitoneally). Rats were killed 2.5 or 24 h after injection and liver samples were dissected out for subsequent analysis. The groups were: saline (control, 2.5 h; group A); ethanol (2.5 h; group B); saline (control, 24 h; group C); ethanol (24 h; group D). Samples of liver homogenate were analysed using two different types of protein chip targeting a dynamic range of 5–50 kDa and 14–210 kDa (referred to as Chip50 and Chip200, respectively).

The results of the present study showed that twenty-two and eighteen individual protein peaks were identified using Chip50 and Chip200, respectively. Using Chip50, we showed that acute 2.5 h ethanol exposure had no significant effect of the relative abundance (percentage of the total protein pool) of any of the proteins. However, the 24 h ethanol treatment protocol was shown to decrease the relative abundance of 23.7 kDa proteins and increase the abundance of 46.5 kDa proteins. Thus, in group C, the relative abundance of 23.7 kDa proteins was 5.10% compared with 3.93% in group D (C *v.* D; $P=0.036$). Corresponding values for the 46.5 kDa proteins were 3.25% (group C) and 5.50% (group D; group C *v.* group D, $P=0.02$). With the Chip200 only the 25.8 kDa proteins were shown to alter, from 8.10% (group A) to 4.98% (group B; group A *v.* group B, $P=0.016$) after acute 2.5 h ethanol exposure.

The present study showed that the pattern of protein expression in the rat liver was altered by acute alcohol dosage. However, the relatively small number of peaks determined suggests that capillary electrophoresis technology may not be a suitable technique for elucidating changes in the individual patterns. Further work is required to characterise and identify changes in expression of affected proteins using SELDI.

α-Tocopherol does not ameliorate acute *in vivo* formation of malondialdehyde–protein adducts in liver and brain after D-galactosamine administration. By M.C.Y. WONG[1], O. NIEMELA[2], S. PARKKILA[2], R. RAJENDRAM[1], V.R. PREEDY[1] and H. WISEMAN[1], [1]*Nutritional Sciences Research Division, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London, UK, SE1 9NN and* [2]*EP Central Hospital Laboratory, Seinajoki, Finland*

Formation of protein adducts, such as the malondialdehyde–protein adduct has previously been shown to be a biomarker of oxidative stress induced by ethanol administration (Niemela *et al.* 1994). However, there are few studies that have attempted to use nutritional components to ameliorate protein adduct formation. We hypothesised that D-galactosamine (GalN) administration would induce oxidative stress and thus malondialdehyde–protein adducts while α-tocopherol (ATC), a well-known antioxidant, would ameliorate this.

To test this, male Wistar rats (0.1 kg body weight) were ranked and assigned to four groups (n 6 rats per group) as follows: carrier+saline (group A); carrier+GalN (group B); ATC+saline (group C); ATC+GalN (group D). The protocol involved a pre-treatment with either carrier or ATC followed 1 h later by treatment with either GalN or saline. Rats were then killed after 23 h. The carrier was Intralipid (20% fat emulsion). ATC was dissolved in the carrier and injected at a dose of 30 mg/kg body weight. GalN was dissolved in saline (0.15 mol NaCl/l) and injected at a dose of 1 g/kg body weight. All rats were injected intraperitoneally. Rats were killed 24 h after the first injections. Livers and brains were dissected for subsequent analysis of MDA adduct by immunohistochemistry.

Tissues sections were stained for malondialdehyde–protein adduct using the biotin–streptavidin complex method. The intensity of the staining was scored on a scale of 0 (no reaction) to 5 (strong reaction). The scores were ranked and analysed using 2×2-way ANOVA, followed by a *post hoc* LSD (Least Significant Difference) test, where $P<0.05$ was considered to be significant (see Table).

		Mean MDA adduct score	
Group	Treatment	Liver	Brain
A	Carrier+saline	0.58	1.50
B	Carrier+GalN	2.17*	1.83
C	ATC+saline	0.83	1.50
D	ATC+GalN	1.58*	1.50

ANOVA showed a significant effect ($P<0.001$) due to GalN for liver but not the brain (n 6).
* Mean values were significantly different when compared with group A ($P<0.001$; LSD analysis).

The result of the analysis showed that GalN administration induced marked increases of malondialdehyde–protein adducts formation in liver but not brain, suggesting that the latter tissue is more protective against GalN-induced oxidative stress. Moreover, the degree of malondialdehyde–protein adduction in liver was not significantly ameliorated by ATC pre-treatment.

Niemela O, Parkkila S, Yla-Herttuala S, Halsted C, Witztum JL, Lanca A & Israel Y (1994) *Laboratory Investigation* **70**, 537–546.

The mRNA levels of brain antioxidant enzymes are not altered by galactosamine-induced metabolic and oxidative stress. By M.C.Y. WONG[1], T. NAKAHARA[2], K. HASHIMOTO[2], M. HIRANO[2], R. DUFFY[1], R. RADENDRAM[1], V.R. PREEDY[1] and H. WISEMAN[1], [1]*Department of Nutrition and Dietetics, Health and Life Sciences, King's College London, Franklin-Wilkins Buildings, 150 Stamford Street, London, SE1 9NH and* [2]*Hizen National Mental Hospital, Kanzaki, Saga 842–0104, Japan*

Various studies have shown that there are significant changes in brain tissue as a consequence of metabolic and oxidative stress. In some instances this may arise via organ damage (i.e. hepatic changes) or generation of free radicals *per se*. For example, the activities of brain superoxide dismutase (SOD) and catalase decease 24 h after dosage with carbon tetrachloride with concomitant increases in glutathione peroxidase (GPx) activities (Szymonik-Lesiuk *et al.* 2003). We hypothesised that (i) there may also be concomitant perturbations in the levels of mRNA levels encoding antioxidant enzymes in metabolic and oxidative stress induced by D-galactosamine (GALN) and (ii) such changes may also be ameliorated with α-tocopherol, a well-characterised antioxidant.

To test this hypothesis, male Wistar rats (0.1 kg body weight) were ranked and assigned to four groups (n 6 rats per group) as follows: carrier+saline (group A); carrier+GALN (group B); α-tocopherol (ATC)+saline (group C); ATC+GALN (group D). The protocol involved a pre-treatment for 1 h followed by treatment for 23 h. The carrier was Intralipid (20% fat emulsion). ATC was dissolved in the carrier and injected at a dose of 30 mg/kg body weight. GALN was dissolved in saline (0.15 mol NaCl/l) and injected at a dose of 1 g/kg body weight. All rats were injected intraperitoneally. Rats were killed 24 h after the first injections and brains were dissected for subsequent analysis of mRNA encoding a number of antioxidant enzymes, specifically, mitochondrial Mn-containing SOD (Mn-SOD), cytosolic Cu/Zn-containing superoxide (Cu/Zn-SOD), catalase and GPx. Levels of mRNA were assayed by semi-quantitative reverse transcription-PCR with an endogenous internal standard, GAPDH.

The Table shows brain mRNA levels in GALN-dosed rats. All data are expressed relative to GAPDH mRNA (n 6). Differences between means were analysed by two-way ANOVA followed by the Least Significant Difference (LSD) test using the pooled estimate of variance (which also included data not presented here).

	Mean mRNA level (arbitrary units)			
mRNA	Controls	GALN	ATC	ATC+GALN
Mn-SOD	0.57	0.57	0.56	0.63
Cu/Zn-SOD	1.26	1.25	1.35	1.32
Catalase	1.10	1.13	1.05	1.14
GPx	1.14	1.19	1.00	1.13

There were no significant effects of GALN or ATC ($P>0.05$ in all instances).

The results (see Table) showed that there was no significant effect of GALN on antioxidant mRNA levels in the brain or amelioration with ATC. This suggests that the brain is a well protected against metabolic and oxidative stress or that the hitherto-reported changes in enzyme activity due to carbon tetrachloride may be perturbant specific.

Szymonik-Lesiuk S, Czechowska G, Stryjecka-Zimmer M, Slomka M, Madro A, Celinski K & Wielosz M (2003) *Journal of Hepato-Biliary-Pancreatic Surgery* **10**, 309–315.

The mRNA levels of brain antioxidant enzymes in supplemental antioxidant therapy: a comparison of phyto-oestrogens and α-tocopherol. By M.C.Y. WONG[1], T. NAKAHARA[2], K. HASHIMOTO[2], M. HIRANO[2], R. HUNTER[1], R. SRIRAJASKANTHAN[1], V.R. PREEDY[1] and H. WISEMAN[1], [1]*Department of Nutrition and Dietetics, Health and Life Sciences, King's College London, Franklin-Wilkins Buildings, 150 Stamford Street, London, UK, SE1 9NH and* [2]*Hizen National Mental Hospital, Kanzaki, Saga 842–0104, Japan*

Phyto-oestrogens, such as genistein and daidzein, have been shown to protect against oxidative stress *in vitro* by increasing antioxidant enzymes such as superoxide dismutase and catalase (for example, see Rohrdanz *et al.* 2002; Choi *et al.* 2003). These changes also include increases in the mRNA levels encoding antioxidant enzymes (Rohrdanz *et al.* 2002). We hypothesised that a treatment regimen encompassing phyto-oestrogen supplementation would also increase brain antioxidant enzymes at the molecular level. To test this, we investigated the effects of supplemental daidzein and genistein on the mRNA encoding mitochondrial Mn-containing superoxide dismutase (Mn-SOD), cytosolic Cu/Zn-containing superoxide (Cu/Zn-SOD), catalase and glutathione peroxidase (GPx). For comparative purposes we also investigated the effects of α-tocopherol (ATC), a well-characterised antioxidant.

Male Wistar rats (about 0.1 kg body weight) were ranked and assigned to four groups (*n* 6 rats per group) as follows: carrier (group A); carrier+daidzein (group B); carrier+genistein (group C); carrier+ATC (group D). The carrier was Intralipid (20% fat emulsion). Daidzein, genistein and ATC were mixed with the carrier and homogenised ultrasonically before injection at a dose of 100 mg/kg body weight (phyto-oestrogens) or 30 mg/kg body weight (ATC). All rats were injected intraperitoneally for 4 d with an accumulated dose of 400 mg/kg (phyto-oestrogens) and 120 mg/kg (ATC). At the end of the study, rats were killed, and brains were dissected and frozen in liquid N_2 for subsequent measurement of mRNA. Mn-SOD, Cu/Zn-SOD, catalase and GPx mRNA were assayed by semi-quantitative reverse transcription-PCR with an endogenous internal standard, GAPDH.

The table shows brain mRNA levels in phyto-oestrogen treated rats. All data are expressed relative to GAPDH mRNA (n 6–8). The data are analysed using one-way ANOVA, followed by a *post hoc* Least Significant Difference (LSD) test, where $P<0.05$ is considered to be significant.

	Mean mRNA level (arbitrary units)			
mRNA	*Carrier*	Daidzein	*Genistein*	ATC
Mn-SOD	0.64	0.66	0.70	0.64
Cu/Zn-SOD	1.04	1.04	1.06	1.10
Catalase	1.21	1.24	1.41	1.68***
GPx	1.17	1.15	1.25	1.37*

* Difference $P<0.05$ when compared with the control group (Carrier).
*** Difference $P\leq0.001$ when compared with the control group (Carrier).

Neither daidzein nor genistein affected Mn-SOD, Cu/Zn-SOD, catalase or GPx mRNA levels ($P>0.05$ in all instances). Here we show for the first time that ATC supplementation increases the antioxidant potential of the brain as reflected by elevated catalase and GPx mRNA levels. However, the failure to see similar increases with phyto-oestrogens is unexpected, possibly reflecting the fact that the present studies were carried out *in vivo* whereas other reports showing beneficial effects of daidzein and genistein employed isolated cell systems.

We wish to thank Hangzhou FST, Republic of China, for their generous gift of phyto-oestrogen.

Choi C, Cho H, Park J, Cho, C & Song Y (2003) *Bioscience, Biotechnology and Biochemistry* **67**, 1916–1922.
Rohrdanz E, Ohler S, Tran-Thi QH & Kahl R (2002) *Journal of Nutrition* **132**, 370–375.

A qualitative study on the main determinants of job satisfaction for postgraduate nutritionists. By J. EARLAND, *School of Health and Social Sciences, Coventry University, Priory Street, Coventry, UK, CV1 5FB*

The present study is centred on postgraduate nutrition education within the UK. Qualitative data were collected using in-depth interviews with seventeen graduates of two Masters courses in human nutrition, between 1 and 3 years after completing their studies. The graduates were selected using a purposeful sampling technique called maximum variation sampling (Patton, 2002), which was designed to ensure that a range of occupations was included in the final sample. Only one participant was male which reflects the fact that the majority of students on most nutrition courses in the UK are female. A range of issues was explored in this research, including job satisfaction. Specifically, interviewees were asked what they particularly liked and disliked about the job they were doing and their future career plans.

The majority of participants were employed within the field of nutrition or in research. The types of occupations or organisations represented included industry (*n* 2), academia (*n* 4), local and national government (*n* 3), international non-government organisations (*n* 2) and UK-based charities (*n* 3). Although the roles and responsibilities of those interviewed were very varied, it was possible to identify a number of common factors associated with job satisfaction. The main ones were: using nutritional knowledge or other aspects of their Masters education; being busy; having plenty of opportunities for continuing professional development and colleagues being friendly and supportive. Based on these criteria, eight of the participants were satisfied with the work they were doing. Conversely those who were dissatisfied were either not using their nutritional knowledge or found the work difficult, had little support in the workplace and felt that there were few opportunities for career progression. Five interviewees fell into this category, with the remaining four falling between the two ends of the spectrum. Consequently a number of interviewees were looking for a more suitable position. An interesting finding was that these graduates were more likely to get to the interview stage than when applying for their first job, even if they were not in a nutrition position.

It is evident that both academic and personal factors were associated with the degree of job satisfaction amongst these postgraduates. Similar factors have been identified in other studies. For example, in a study of dietitians in the USA, lack of promotion opportunities was associated with job dissatisfaction (Pless *et al.* 1998). The role of staff development in increasing job satisfaction and retaining staff has also been recognised (Lee & Bruvold, 2003). Opportunities for the postgraduates in the present study being able to use their nutrition knowledge could be equated with the level of professional practice achieved in the ward for nurses (Adams & Bond, 2000), which was found to be positively correlated with job satisfaction.

The findings from the present study can be used by nutritionists when seeking suitable employment and by employers to enhance job satisfaction and retention rates amongst their staff.

Adams A & Bond S (2000) *Journal of Advanced Nursing* **32**, 536–543.
Lee CH & Bruvold NT (2003) *International Journal of Human Resource Management* **14**, 981–1000.
Patton MQ (2002) *Qualitative Research and Evaluation Methods*, 3rd ed. London: Sage Publications.
Pless AM, Wolman PG, Stallings SF & Goodner CH (1998) *Journal of the American Dietetic Association* **98**, Suppl. 1, A26.

Can peer education empower individuals from low-income backgrounds with diabetes? By L.P. McCAUGHEY, L. FREAR, C. WHITHAM, J. THOMAS and J.E. CADE, *Nutritional Epidemiology Group, Centre for Epidemiology and Biostatistics, The University of Leeds, 30–32 Hyde Terrace, Leeds, UK*

The rate of diabetes is increasing worldwide. In the UK there are approximately 1.8 million individuals living with diabetes, about three in every 100 individuals; a further 1 million are expected to be diagnosed by 2010 (Sicree *et al.* 2003). It has been recognised that adoption of self-management skills by an individual with diabetes is necessary to enable them to manage their diabetes. A diabetic patient on average spends 3 h annually with a health professional, so it is important for individuals with diabetes to understand and take responsibility for their condition.

The Expert Patients Programme (EPP) is a new approach developed by the National Health Service (NHS) to help individuals living with long-term chronic diseases to become better educated in 'self-managing' their long-term health problems.

We have developed a peer-led diabetes-specific module within the EPP. This involves seven weekly group sessions led by a layperson with diabetes who has been trained to deliver the EPP. The standard NHS EPP 6-week sessions precede the final diabetes-specific session. The present study was designed to assess whether the peer-led diabetes-specific EPP has an effect on the patients' self-management skills. In a randomised controlled trial, 319 individuals from a low-income area in East Lancashire were randomly assigned to an intervention group (n 112; sixty males and fifty-two females) and a control group (n 127; sixty-nine males and fifty-eight females) with a mean age of 65.8 years. Data were collected at baseline and 6 months, on lifestyle and empowerment outcomes by questionnaire, and a 3 d food diary and clinical measurements were also taken. Empowerment scores were calculated (see Table) from using questions from a previously validated questionnaire (Anderson *et al.* 2000). Scores for each subscale range from a minimum of one to a maximum of five, five showing the greatest empowerment. Differences between the intervention and control group were determined by an independent t test. The audit of diabetes dependent quality of life (ADDQOL) questionnaire (Bradley *et al.* 1999) was used to measure individuals' perceptions of the impact of diabetes on their quality of life. The combined score for impact on life ranged from −9 (maximum negative impact of diabetes) to +9 (maximum positive impact on diabetes).

	EPP		Control		Unadjusted			Adjusted for possible baseline imbalance		
Empowerment scores at 6 months	Mean	*n*	Mean	*n*	Effect size	95% CI	*P*	Effect size	95% CI	*P*
Psychosocial	3.6	51	3.5	95	0.06	−0.18, 0.30	0.61	0.01	−0.24, 0.27	0.91
Readiness to change	3.6	52	3.5	94	0.08	−0.15, 0.32	0.51	0.01	−0.22, 0.26	0.88
Setting and achieving goals	4.0	55	4.2	97	−0.18	−0.41, 0.06	0.13	−0.3	−0.07, −0.52	0.01
ADDQOL	−1.5	54	−1.3	98	−0.21	−0.84, 0.41	0.50	−0.01	0.59, 0.39	0.69

Differences between EPP and control groups at 6 months were small and generally not statistically different. Protein intake was lower in the EPP group and a greater goal-setting ability was perceived by the control group. In the short term it appears that the diabetes-specific EPP does not show any major short-term benefits in terms of dietary change for individuals with diabetes. Further follow-up at 12 months is being carried out.

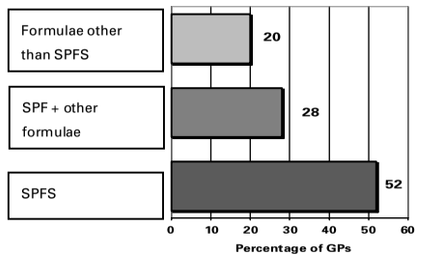
Funded by the Food Standards Agency.

Anderson RM, Funnell MM, Fitzgerald JT & Marrero DG (2000) *Diabetes Care* **23**, 739–743.
Bradley C, Todd C, Gorton T, Symonds E, Martin A & Plowright R (1999) *Quality of Life Research* **8**, 79–91.
Sicree R, Shaw J & Zimmet P (2003) *Diabetes Atlas*, vol. 2, pp. 17–71 [Gan, editor]. Brussels: International Diabetics Federation.

General practitioners' prescribing practices for children with cows' milk allergy or intolerance. By H. DUNLOP and S. LENNIE, *The Robert Gordon University, St Andrews Street, Aberdeen, UK, AB25 1HG*

Isoflavone phyto-oestrogens, present in soya, are structurally similar to mammalian oestrogen and can bind to its receptors. The potency of these phyto-oestrogens in man is unknown; however, infants may be more susceptible to changes in sex hormones that may occur compared with adults. Abnormalities in sexual development have been observed in animals fed phyto-oestrogen-rich diets (Bennetts *et al.* 1946; Setchell *et al.* 1987; Sharpe *et al.* 2002), and this has raised concern regarding soya consumption in human infants. In response to this, the British Dietetic Association Paediatric Group (2004) issued a position statement discouraging the use of soya protein formulae (SPF) in infants with atopy or cows' milk protein (CMP) allergy, particularly in the first 6 months of life.

General practitioners (GPs) are recognised as the 'gatekeepers' to health care and, as such, must be aware of current healthcare standards, guidelines and recommendations. The potential risks associated with phyto-oestrogens in SPF were explained in the *BMJ* (Essex, 1996), and the Chief Medical Officer sent an urgent message to all GPs in 1996 advising them on SPF. The present study investigated the awareness shown by Aberdeen GPs about these potential risks, and questioned them about which infant formulae they prescribe for children with CMP allergy or intolerance. A postal questionnaire was administered to all GPs within the Aberdeen area (thirty-three practices) requesting information concerning the number of cases of CMP allergy and intolerance treated in the previous year, their prescribing practices for this patient group, and factors influencing their choice of formula. GPs were also asked if they were aware of the current debate regarding SPF, the main issues of the debate, and whether these deterred them from prescribing SPF. The response rate was 34.5% (n 59). Only 36% of respondents had treated a child with CMP allergy or intolerance in the previous year. Most GPs (80%) reported they would include SPF in their formula prescription for these conditions. The prescription practices of GPs can be seen in Figure 1 below.



No significant difference was observed in prescribing practices for whey hydrolysed, casein hydrolysed or SPF between GPs who had treated a child in the last year and those who had not. However, for elemental formula, 50% of GPs who had treated a child in the previous year prescribed this formula whereas only 19% of those who had not treated such a child would prescribe this type of formula (P=0.027). The advice of health visitors (28%) and paediatricians (28%) were most commonly cited as factors influencing prescription of infant formula by GPs. Relatively few GPs (21%) were aware of the debate regarding SPF, and only one identified the potential risk of sexual development problems. The majority of GPs (71%) reported updating their knowledge of nutritional issues less than once per year. It was concluded that GPs in Aberdeen are unaware of the recent concerns regarding SPF usage in human infants. Further research should establish the prescription practices and recommendations of other health professionals involved in treating these patients.

Bennetts HW, Underwood EJ & Shier FL (1946) *Australian Veterinary Journal* **22**, 2–12.
British Dietetic Association Paediatric Group (2004) *Paediatric Group Position Statement on the use of Soya Protein for Infants.* http://members.bda.uk.com/Downloads/paedstatementsoyaprotein.pdf
Essex C (1996) *BMJ* **313**, 507–508.
Setchell KDR, Zimmer-Nechamias L, Cai J & Heubi JE (1987) *Lancet* **350**, 23–27.
Sharpe RM, Martin B, Morris K, *et al.* (2002) *Human Reproduction* **17**, 1692–1703.

Diet and asthma and atopy risk in Northern Irish primary school children. By P.M.L. SKIDMORE[1], J.W.G. YARNELL[2], M.R. STEVENSON[2], J. MACMAHON[2], M. SHIELDS[2] and A. EVANS[2], [1]*School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, UK, NR4 7JT and* [2]*School of Clinical Medicine, Queen's University Belfast, Belfast, UK, BT12 6BT*

Asthma and atopy are becoming increasingly common in the UK and this increase in prevalence has coincided with changing dietary patterns, leading to decreased intakes of fruit and vegetables and increased fat intake. Diet is now considered to be one of the environmental factors potentially implicated in the aetiology of asthma. Previous research has focused mainly on the effects of dietary components such as fatty acids and antioxidants, or particular foods, such as fruit and vegetables, (Kalantar-Zadeh *et al.* 2004; McKeever & Britton, 2004) and not on the diet as a whole.

We surveyed a sample of boys and girls aged 5 to 8 years (n 2373), representative of the general population of Northern Ireland, to investigate asthma and allergy. A food-frequency questionnaire using questions based on previously validated studies was also completed and a dietary index (DI) was calculated from this and converted to Z scores, in which a positive score reflects a healthier diet. Principal Components Analysis (PCA) with varimax rotation was performed with 22 of the standardised foods. No account was taken of confounding factors. Of the children, 24.5% had been diagnosed with or treated for asthma, with 8.3% of all subjects suffering more than four asthma attacks in the past 12 months, 6.2% experiencing sleep disturbance more than one night per week and 5.0% experiencing moderate disruption of daily activities. Of the children, 27.6% had suffered from hay fever or eczema. A higher percentage of boys were diagnosed with or treated for asthma (6.2%) than girls (22.4%; $P=0.02$) and a higher percentage of boys experienced more asthma-related symptoms than girls. There was a statistically significant difference in the mean DI score for boys (–0.07) and girls (0.08) (95% CI −0.27, −0.03; Student's t test).

The relationship between frequency of food consumption and asthma and atopy was investigated using the χ^2 test. Those children who drank pure fruit juice had a lower prevalence of asthma diagnosis or treatment (22.7%) than those who did not (27.6%; $P=0.004$), and were less likely to have four or more asthma attacks in the past 12 months (7.8% compared with 9.9%; $P=0.05$), or to have at least one night of sleep disturbance per week (4.9% compared with 8.7%; $P<0.001$), or to limit their daily activities (4.7% compared with 6.5%; $P=0.05$) but the opposite relationship was seen for fresh fruit consumption with a higher percentage of children who ate two or more portions of fruit per d having at least four or more asthma attacks in the past 12 months (9.7% compared with 7.5%; $P=0.04$), and at least one night of sleep disturbance per week (7.7% compared with 5.4%; $P=0.02$). They also had a higher prevalence of eczema (25.9% compared with 22.5%; $P=0.04$). Higher consumption of green vegetables, salad, beans and peas was also associated with sleep disturbance due to asthma. Those children who ate cakes and biscuits every day had a higher prevalence of asthma diagnosis or treatment (26.4% compared with those who did not, 23.1%; $P=0.05$). They also had a higher prevalence of eczema (25.9%) compared with those who did not (22.5%; $P=0.01$). Of the children who did not drink milk, 42.1% had been treated for or diagnosed with asthma compared with 24.5% who drank milk ($P=0.09$). Those children who consumed non-processed meat up to 5 d a week had a lower prevalence of asthma treatment or diagnosis (23.1%) than those who consumed it 6 or 7 d/week (26.9%; $P=0.02$). There were no significant differences in mean DI score and any measure of asthma or atopy. These results are preliminary and we are also in the process of investigating if overall diet types produced by principal components analysis are related to asthma and atopy symptoms.

With PCA three dietary components emerged – a healthy diet, an intermediate diet and an unhealthy diet. Those children who had at least one night of sleep disturbance per week had a higher score on the intermediate diet pattern (0.24) than those who did not (–0.02, $P=0.004$). Those children who had eczema had a lower score for the intermediate diet pattern (–0.08) than those who did not (0.03, $P=0.039$). Those children who had been diagnosed with asthma had a higher score for the unhealthy diet pattern (0.07), than those who had not (0.02, $P=0.048$).

Preliminary results of the present study indicate that while individual foods in the diet are associated with asthma and atopy prevalence and severity, with pure fruit juice consumption in particular associated with lower levels of asthma and asthma events. Analysis using assessment of food choice by PCA has indicated that unhealthy dietary practices may only have limited association with asthma and atopy.

McKeever TM & Britton J (2004) *American Journal of Respiratory and Critical Care Medicine* **170**, 725–729.
Kalantar-Zadeh K, Lee GH & Block G (2004) *Medical Hypotheses* **62**, 280–290.

Dietary characteristics of gastroenterology patients. By S. JARVIS[1], A. SPIERS[2], Y. DUCKWORTH[2], M. WELFARE[2], H.J. POWERS[1], J.C. MATHERS[2] and E.A. WILLIAMS[1], [1]*Human Nutrition Unit, University of Sheffield, Northern General Hospital, Sheffield, UK, S5 7AU and* [2]*Human Nutrition Research Centre, School of Clinical Medical Sciences, University of Newcastle, Newcastle upon Tyne, UK, NE1 7RU*

Colorectal cancer (CRC) is the fourth most common cancer in the world, and diet has been strongly implicated in the aetiology of the disease. There is convincing evidence that diets high in vegetables protect against CRC, whilst high alcohol and red meat consumption are thought to increase the risk of CRC. Adenomatous polyps are often used as surrogate biomarkers of CRC risk. An analysis of the dietary intakes of gastroenterology patients with different bowel pathologies may therefore reveal dietary factors associated with disease progression.

Volunteers over the age of 40 years attending out-patient endoscopy clinics at North Tyneside General Hospital for the investigation of gastrointestinal symptoms were provided with a food-frequency questionnaire (FFQ) to assess their habitual diet. The FFQ was a modified version of the previously validated EPIC FFQ (Bingham *et al.* 1994). Minor modifications to the original questionnaire were made to reflect the diet of the local population. Cross-check questions were included on fruit and vegetable and alcohol consumption. Two-hundred and thirteen volunteers completed the FFQ. Nutrient analysis was performed using an in-house SPSS database developed at the University of Newcastle. Volunteers were categorised into normal, polyps and CRC according to the diagnosis following endoscopy procedures and biopsy histopathology. Patients with diverticulitus and inflammatory bowel disease were excluded from the analysis. Data were analysed by one-way ANOVA.

As previously reported the FFQ was found to overestimate fruit and vegetable intake. The mean number of portions of fruit and vegetables reported to be consumed per week when analysed by the FFQ was 47 (SD 24.8) compared with 13 (SD 8.3) portions reported by the cross-check question. However, regression analysis revealed a strong positive correlation between the two reporting methods ($r=0.53$, $P<0.001$). Despite apparent overestimation of certain nutrients, ANOVA showed no significant differences between the patient groups in the intake of total energy, protein, fat or NSP, nor was there any significant difference in BMI. The number of portions of fruit and vegetables reported to be consumed was lowest in the cancer group, although differences between the groups just failed to reach significance ($P=0.055$). The number of units of alcohol consumed per week was significantly different between the groups ($P=0.046$). However, there were no significant differences between any of the dietary variables when age and sex were included as co-variates in the analysis.

	Normal (n 85)		Polyps (n 92)		Cancer (n 36)	
Variable	Mean	SD	Mean	SD	Mean	SD
Energy (MJ/d)	11.8	3.81	12.4	3.69	13.5	5.23
Protein (g/d)	115	37.9	117	39.9	118	39.1
Carbohydrate (g/d)	375	138.8	387	130.6	406	178.4
NSP (g/d)	26	10.9	26	11.5	24	9.5
Total fat (g/d)	92	35.0	97	34.9	109	54.2
Units of alcohol/week	9	9.7	12	14.9	15	16.0
% Energy derived from alcohol	4.3	5.87	5.4	7.54	6.1	8.25
Total number of portions of fruit and vegetables consumed/week (reported by FFQ)	51	25.6	47	25.4	39	19.1
Total number of portions of fruit and vegetables consumed/week (reported by cross-check question)	15	10.0	12	7.3	11	5.8

Values were not significantly different when corrected for age and sex (ANOVA).

There was no evidence from the present study that the habitual diet of CRC patients was significantly different from that of other patient groups attending gastroenterology clinics for the investigation of abnormal bowel symptoms.

This work was funded by the Food Standards Agency (N12004/6).

Bingham SA, Gill C, Welch A, Day K, Cassidy A, Khaw KT, Sneyd MJ, Key TJA, Roe L & Day NE (1994) *British Journal of Nutrition* **72**, 619–643.

Water intake and risk of breast cancer: a case–control study. By M.E. BARKER, S.A. HUSSAIN, J.M. RUSSELL and J.D. STOOKEY, *Human Nutrition Unit, University of Sheffield, Herries Road, Sheffield, UK, S5 7AU*

Several studies have shown positive associations between diuretic beverage consumption and breast cancer risk (van der Brandt, 1995). Water consumption has been linked to reduced breast cancer risk in a pilot case–control study (Stookey *et al.* 1997). The study showed that plain water consumption (versus not drinking plain water) was associated with a 4.7 times reduction in risk of breast cancer. The main aim of the present study is to extend the work of the previous study and further investigate the hypothesis that water intake is inversely related to breast cancer risk.

The present study had a hospital-based case–control design. Subjects were recruited consecutively between April and August 2000 from two large hospital trusts in Sheffield, UK. Cases (*n* 47) were women with newly diagnosed, histological confirmed breast cancer. Controls (*n* 96) were subjects with non-neoplastic conditions and no history of malignancy, drawn from the same hospitals covering five surgical categories. The mean age of cases and controls were 56.5 and 49.6 years, respectively. A validated semi-quantitative food-frequency questionnaire was completed by the individual to assess their usual dietary intake of the previous year (Spence *et al*. 2002). Particular emphasis was placed on beverage consumption. Exposure to established risk factors was ascertained by interview. Anthropometric measurements (height and weight) were recorded. One researcher carried out the study procedure. Multiple logistic regression was used to ascertain the association between water consumption and risk of breast cancer controlling for major risk factors for breast cancer. The water intake variable was categorised into two groups: never, occasional or regular drinkers; heavy drinkers (more than four glasses of plain water per d).

In logistic regression analyses, water drinking appeared strongly, inversely and significantly associated with breast cancer risk. Adjusted for age, alcohol and tea consumption, height, family history, hormone replacement therapy, contraceptive pill use, menstrual status, and weight, the effect of heavy water drinking on breast cancer risk was 0.25 (95% CI 0.07, 0.93; $P<0.05$). The association of water intake and breast cancer risk appears to be modified by menopausal status. A greater protective effect was observed among postmenopausal women (odds ratio 0.20 (95% CI 0.04, 0.97); $P<0.05$). The OR for premenopausal women was 0.34 (95% CI 0.02, 6.08; $P<0.05$).

Heavy water drinking was associated with lower odds of breast cancer in the present study. A four-fold reduction in breast cancer risk was observed among all women who consumed on average four glasses of plain water per d. These results parallel those described by Stookey *et al.* (1997). These results also concur with the theory that dehydration is an aetiological factor in the greater incidence of breast cancer amongst airline attendants (Barker & Stookey, 1998). Water intake as a risk factor for breast cancer merits further study.

Barker ME & Stookey JD (1998) *Lancet* **352**, 1388–1390.
Spence M, Cade JE, Burley VJ & Greenwood DC (2002) *Proc Nut Soc* **61**, 117A.
Stookey JD, Belderson PE, Russell JM & Barker ME (1997) *Cancer Epidemiology, Biomarker and Prevention* **6**, 657.
van der Brandt PA (1995) *American Journal of Epidemiology* **141**, 907–915.

Validity of nutritional assessment methods in long-term haemodialysis patients. By S. SMITH[1], D.A.S. JENKINS[2] and H.I.M. DAVIDSON[1], [1]*Dietetics, Nutrition and Biological Health Sciences, Queen Margaret University College, Corstorphine Campus, Edinburgh, UK, EH12 8TS and* [2]*Fife NHS Trust Renal Unit, Whitefield Road, Dunfermline, UK, KY12 0SU*

Long-term haemodialysis patients face significant risks of CVD and/or protein–energy malnutrition (Stenvinkel *et al.* 1999), which in turn may influence morbidity (in particular quality of life and functional ability) and mortality in this group (Laws *et al.* 2000). The quantification of body composition *per se* in relation to such risk is vital in order to ascertain the subsequent effects of possible intervention studies. However, the most appropriate clinical measurements likely to achieve this, in addition to the timing of such measurements relative to the dialysis procedure, remain equivocal.

The present study aimed to examine a variety of anthropometric measurements along with dual-frequency whole-body bioelectrical impedance analysis (BIA), which can be used to estimate lean tissue mass (LTM) and fat mass (FM) in clinical practice and compared these measures with those obtained from dual-energy X-ray absorptiometry (DXA). It also examined the effects of timing on measurements obtained both immediately post-dialysis and during the interdialytic period. The results reported are part of a larger intervention trial.

Anthropometric and BIA measurements were taken post-dialysis and the following interdialytic (non-dialysis) day at the same time as patients attended for a whole-body DXA scan (height, weight, BMI, mid-arm circumference (MAC), triceps skinfold (TSF), arm muscle circumference (AMC), arm muscle area (AMA), calf circumference (CC), calf skinfold (CSF), calf muscle circumference (CMC), handgrip dynanometry (HGD)). All anthropometric measurements were taken by a trained anthropometrist (S. S.) following standard methods. DXA scans were conducted using a GE Lunar Prodigy scanner in the Radiology Department to quantify lean tissue mass (DLTM) and FM (DFM) and BIA measurements were conducted using the Body Stat dual frequency (5 kHz, 200 kHz) analyser following standard protocols.

Nineteen (nine male, ten female) long-term stable haemodialysis patients (>6 months) consented to participate in this part of the present study. The mean age of the group was 54.1 (SD 10.9) years with a BMI of 25.5 (SD 4.75) kg/m^2. Simple regression analysis was performed using DXA as the dependent variable on all measures and is summarised in the table.

	Post-dialysis		Interdialytic	
	R^2	P	R^2	P
DLTM: AMC	0.279	<0.05	0.020	<0.05
DLTM: AMA	0.212	<0.05	0.194	NS
DLTM: CC	0.507	<0.01	0.507	<0.01
DLTM: CMC	0.699	<0.01	0.694	<0.01
DLTM: HGD	0.394	<0.01	0.481	<0.01
DLTM: BIALTM	0.945	<0.01	0.922	<0.01
DFM: BIAFM	0.953	<0.01	0.866	<0.01
DFM: MAC	0.845	<0.01	0.836	<0.01
DFM: TSF	0.403	<0.01	0.597	<0.01
DFM: CSF	0.277	<0.05	0.253	<0.05
DFM: BMI	0.845	<0.01	0.832	<0.01

These results suggest that there are differences in the ability of nutritional assessment methods to predict LTM and FM and may be in relation to the timing of the dialysis procedure. Therefore measurements and timing should be standardised. Overall BIA measures post-dialysis appeared to be the most valid predictors of LTM and FM.

Laws RA, Tapsell LC & Kelly J (2000) *Journal of Renal Nutrition* **10**, 139–147.
Stenvinkel P, Heimburger O, Paultre F, Diczfalusy U, Wang T, Begluand L & Jogestrand T (1999) *Kidney International* **55**, 1899–1911.

Validation of the caffeine assessment tool. By K. CURRAN, S.M. BOYLAN, J.E. CADE and S.F.L. KIRK, *Nutritional Epidemiology Group, Centre for Epidemiology and Biostatistics, The University of Leeds, 30–32 Hyde Terrace, Leeds, UK, LS2 9LN*

Exposure to caffeine during pregnancy has been associated with an increased risk of spontaneous abortion and low birth weight (Wisborg *et al.* 2003). The Leeds CARE study is exploring the possible link between maternal caffeine intake and pregnancy outcome. Caffeine is assessed using the caffeine assessment tool (CAT), a detailed questionnaire assessing habitual caffeine intake during pregnancy, involving recall of consumption of specific food and drinks. The CAT being evaluated was obtained at approximately 28 weeks gestation and recalled caffeine consumption during weeks 13–20 and 21–28 of pregnancy. To examine whether the CAT accurately reflects actual intake, twenty-nine subjects with mean age 29.7 years participated. Seventeen women were contacted by telephone at approximately 18 weeks gestation and asked about their intake of tea, coffee, cola and energy drinks in the last 24 h (current report). This information was compared with a 24 h diet recall completed at approximately 17 weeks gestation and the CAT relating to weeks 13 to 20 of gestation. These 17 subjects along with a further 12 were also telephoned at approximately 26 weeks gestation and asked the same questions regarding their current caffeine intake. This information was compared with intakes recalled in the CAT for weeks 21 to 28 of pregnancy and a further diet recall completed at 28 weeks gestation.

	Mugs or cups			
	Mean	SD	Correlation with current report estimate	Significant *P* value
Coffee				
Current report	1.65	2.5	–	
CAT (13–20 weeks)	0.91	1.18	0.85*	0.01
Diet recall	1.00	1.56	0.49	
Tea				
Current report	1.29	1.72	–	
CAT (13–20 weeks)	2.22	2.16	0.86*	
Diet recall	1.64	1.77	0.92*	0.01
Coffee				
Current report	2.07	2.90	–	
CAT (21–28 weeks)	0.65	0.74	0.85*	
Diet recall	0.44	0.53	0.62	
Tea				
Current report	1.90	2.72	–	
CAT (21–28 weeks)	2.13	2.05	0.95*	0.01
Diet recall	1.56	1.67	0.73	

*Correlation is significant at $P<0.01$ (two-tailed).

Coffee and tea intake in the CAT (13–20 weeks) and CAT (21–28 weeks) strongly correlates with the current report taken within the same time period. Both the current report and diet recall assess intakes over a short period of time. However, it is the CAT that reflects habitual caffeine intake.

These preliminary results would suggest that the CAT is an accurate and reflective measure of caffeine intake in pregnant women. To date the CAT is the most detailed assessment of caffeine intake during pregnancy. Along with a detailed exploration in interindividual caffeine metabolism, the CAT will prove a valuable tool in the exploration of caffeine's role in pregnancy outcome.

The CARE study is funded by FSA grant T01033.

Wisborg K, Kesmodel U, Bech BH, Hedegaard M & Henriksen TB (2003) *BMJ* **326**, 420–422.

Validity and reliability of a short questionnaire for assessing impact of cooking skills interventions. By K.L. BARTON, A.S. ANDERSON, W.L. WRIEDEN and R. BAXTER, *Centre for Public Health Nutrition Research, University of Dundee, Ninewells Hospital and Medical School, Dundee, UK, DD1 9SY*

The CookWell programme (Valentine *et al.* 2002) was developed by the University of Dundee as a transferable, community-based food skills intervention aimed at promoting increased consumption of starchy foods, fish, vegetables and fruit, and decreased consumption of fat in adults living in areas of social deprivation. The impact of the programme was extensively assessed by intensive research methods but it is recognised that for wider use in the community a simple evaluation tool is desirable such as that available for use in schools (Anderson *et al.* 2002).

The present study aimed to assess the validity and reliability of a short questionnaire designed to measure the impact of cooking skills interventions on (selected) food choices, cooking confidence, and knowledge and attitudes (about food preparation, recommended fruit and vegetable consumption and food safety). The present study also assessed the feasibility of using the questionnaire in a community food skills intervention.

A working draft of the questionnaire was compiled and checked for content, clarity and layout. Content validity of the questionnaire was assessed by a panel of independent public health nutritionists (n 16) and community development workers (n 12), who scored each of the seventeen items in relation to: clarity; appropriateness; cognitive complexity; relevance. Responses were collated and the questionnaire amended as appropriate. Face validity was assessed by a group of adults, who completed the questionnaire and were interviewed with regard to question comprehension. No individual reported problems with questions or words that they did not understand. The short completion time (5–10 min) indicated that the questionnaire could be administered by a community worker if literacy problems exist.

Repeat reliability was assessed in adults attending community-based classes, who completed the questionnaire twice, 1 week apart. Correlation analysis was carried out and Cronbach's Alphas (Bland & Altman, 1997) were computed to assess repeat reliability and internal consistency. Seventy-four adults completed the questionnaire at time 1 and fifty-seven at time 2. Cronbach's Alphas for confidence and knowledge sections were 0.86 and 0.84 respectively, showing good internal consistency. Correlation between time 1 and time 2 was significant ($P<0.01$) for all questions with correlation coefficients ranging from 0.46 to 0.91.

The feasibility of the final questionnaire was assessed in a community setting to evaluate the first year of the West Lothian 3-year 'Get Cooking' project. Thirteen adults completed the questionnaire at time 1 and eleven at time 2. No assistance was requested by individuals when filling in the questionnaire, but a few questions were not fully completed. It was noted that more detailed instruction for the class tutor would improve the completion rate for the individual questions and that more guidance was required for matching subject identification numbers on the questionnaires pre- and post-intervention.

The final evaluation tool comprised nineteen questions within five topic sections: meal preparation; confidence in cooking and tasting; usual food consumption patterns; knowledge about fruits and vegetables; knowledge of good food-safety practices. This tool provides a standardised method of evaluating cooking skills interventions which could assist in the evaluation of multi-centre community-based cooking interventions.

An instruction sheet and pre- and post-intervention questionnaires are available on the Food Standard Agency website (www.food.gov.uk).

Funding from the Food Standards Agency is gratefully acknowledged.

Anderson AS, Bell A, Adamson A & Moynihan P (2002) *Public Health Nutrition* **5**, 497–503.
Bland JM & Altman DG (1997) *BMJ* **314**, 572.
Valentine K, Longbottom P, Wrieden WL, Anderson AS & Dowler E (2002) *Proceedings of the Nutrition Society* **61**, 157A.

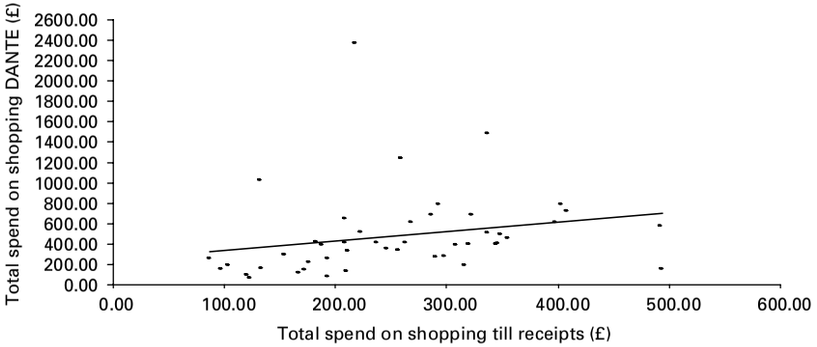
Validation of the integral costing of DANTE – Diet and Nutrition a Tool for Evaluation. By C. OYSTON, J.K. RANSLEY, J.D. THOMAS and J.E. CADE, *Nutrition Epidemiology Group, Centre for Epidemiology and Biostatistics, University of Leeds, 30–32 Hyde Terrace, Leeds, UK, LS2 9LN*

A central focus of nutrition research is the analysis of food intake. Nutrient and food intake is patterned socio-economically and economic factors determine the amount of money available to spend on food. The Expenditure and National Food Survey 2002–03 indicated that, of the total food purse, 80% of food and drink expenditure took place at large supermarket chains (Office of National Statistics, 2004).

All receipts issued at the point of purchase itemise the price of all food bought and this provides a convenient and accurate record of what is spent on food in retail outlets.

Using data from the Supermarket Nutrition Information Project (Ransley *et al.* 2003), the present study aims to validate a method of calculating the cost of food: (i) purchased by families over 28 d; (ii) eaten over 4 d.

Itemised till receipts collected for forty-four families recruited from the Tesco Clubcard database were used to calculate the exact cost of food purchased over a 28 d period. The same data were entered into DANTEan in-house ACCESS-based dietary analysis programme for the entry for food diary information, which provides a breakdown of nutrients, and costs computed using data from a variety of sources. In addition, price data from DANTE was used to calculate the cost of 4 d food diaries.



A significant positive correlation was found between DANTE-calculated totals for Tesco till receipts and actual costs from Tesco till receipt totals adjusted for inflation (r 0.394; $P<0.01$). A significant positive correlation was also found between total household shopping cost (from all shops and supermarkets) calculated in DANTE and total household shopping cost from till receipts adjusted for inflation (r 0.505; $P<0.01$). DANTE-calculated costs for 494 individual food diaries and pocket books (food eaten away from home, which was not prepared at home) indicated that a mean of £3.04 was spent on food per d. A significant positive correlation (r 0.233; $P<0.01$) was found between 1 d food diary cost and 1 d till receipt cost when divided by household number.

In conclusion, the costing element incorporated into DANTE allows the nutritional profile of diets to be estimated along with the cost.

Acknowledgements to Kevin Tarbutt for cost data collection.

Office of National Statistics (2004) *Family Spending. A report on the 2002–2003 Expenditure and Food Survey*. London: TSO.
Ransley JK, Donnelly JK, Botham H, Khara TN, Greenwood DC & Cade JE (2003) *Appetite* **41**, 141–148.

How much does what we eat cost? Validation of a new database approach to estimating food costs. By A. SMYTH, J.E. CADE and J. THOMAS, *Nutritional Epidemiology Group, Centre for Epidemiology and Biostatistics, University of Leeds, 30–32 Hyde Terrace, Leeds, UK, LS6 9LN*

Dietary choices are primarily influenced by factors such as cost, taste and convenience (French, 2003). Economic factors influence what we eat and how much money is spent on food. However, existing databases of nutrients do not include cost information to allow costs of food diaries to be estimated. We have added costs per 100 g of foods to our in-house dietary analysis program (Dietary and Nutrient Tool for Evaluation; DANTE). Costs of 4200 foods based on the McCance and Widdowson food composition tables have been added. Costs were obtained in 2004 from a number of sources including the website of UK retailer Tesco.

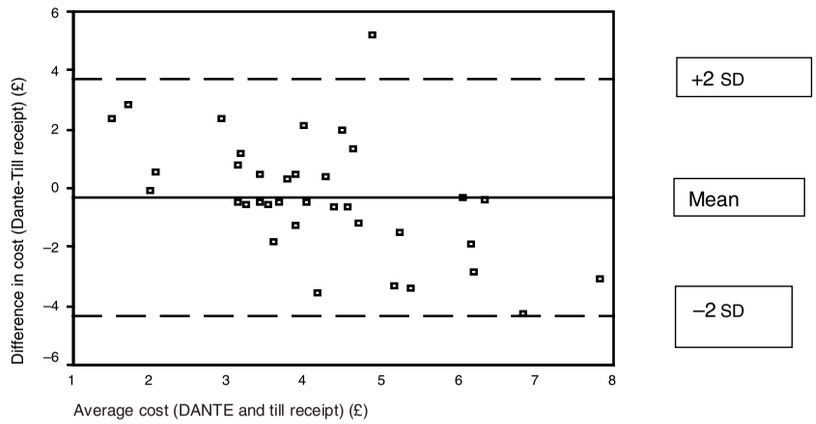


Fig. 1. Difference of estimated and actual costs against mean diary and till receipt costs – Bland-Altman analysis.

The purpose of the present study was to validate DANTE costs as a means of costing different eating habits in comparison with the actual amount of money being spent on food shopping. The sample for the present study was drawn from the UK Women's Cohort Study (UKWCS). Fifty single, middle-class women who reported shopping for themselves alone agreed to take part. The response rate was 72%, a sample size of thirty-six. The women's age range was 52–81 years. Four-day food diaries and 2 weeks of food shopping till receipts were collected. The 4 d food diaries were analysed using DANTE costs. The cost information from the diaries was compared with the till receipt using a paired t test.

The results showed that the average daily cost estimated from the food diaries, £4.05 (SD 1.22), and the actual costs from the till receipts, £4.35 (SD 2.15), were similar. When comparing the methods, using a Bland–Altman analysis (see Fig. 1) there was good agreement between the two methods, the mean difference between the methods was close to zero. These results suggest that food diary-based costs are a good method to estimate the amount of money being spent on food shopping.

The UKWCS is funded by the World Cancer Research Fund. Thanks to Rank Prize Funds for support for Kevin Tarbutt for cost database development.

French SA (2003) *Journal of Nutrition* **133**, Suppl., 841S–843S.