Proceedings of the Nutrition Society

Abstracts of Original Communications

A Scientific Meeting was held at the Aberdeen Exhibition and Conference Centre, Aberdeen, UK, 3–6 July 2006, 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.
Programming effects of folic acid supplementation in rat pregnancy on blood pressure and abdominal fat deposition. By S.C. Langley-Evans, A Hasse and S.F. Engeham, School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough, UK, LE12 5RD

It is widely accepted that nutritional insult during fetal life has the capacity to permanently programme tissue structure and function. In the rat, fetal exposure to a maternal low-protein (MLP) diet has been shown to promote redistribution of fat in the offspring at 9 and 18 months (Bellinger et al. 2006) and a sex-specific increase in blood pressure (McMullen & Langley Evans, 2005). The addition of folic acid to the mother’s diet has been suggested to modify the programming effects of MLP, specifically reversing changes in DNA methylation status (Lillycrop et al. 2005).

Twenty-four virgin female Wistar rats were mated at a weight of 180–220 g. Upon confirmation of mating the rats were fed one of four diets: control (CP; 180 g casein/kg diet with 1 mg folic acid/kg; n = 6), control with folate (CPF; 180 g casein/kg diet with 5 mg folic acid/kg; n = 6), MLP (90 g casein/kg diet with 1 mg folic acid/kg; n = 6) or low protein with folate (MLPF; 90 g casein/kg diet with 5 mg folic acid/kg; n = 6). All animals were transferred to the same standard laboratory chow diet upon delivery of pups, and the litters were culled to a maximum of eight pups, with four males and four females where possible. The pups were weaned onto standard laboratory chow at 21 d of age. At 4 weeks of age the blood pressure of all offspring was measured using a tail-cuff method. At this point, half of the animals were culled and organ weights recorded, including perirenal and gonadal fat pad weights. The remaining animals were retained for analysis at 13 weeks of age. Statistical analysis utilised a mixed models analysis to take into account that the study included related animals from the same litters. Post hoc tests were not carried out due to significant interaction between all factors.

Unexpectedly, at 4 weeks of age the systolic blood pressures were similar in males and females and across all of the maternal dietary groups. Perirenal fat deposition in the offspring, expressed as percentage body weight, was increased in offspring exposed to the maternal low-protein diet compared to the control diet. As early as 4 weeks of age (Fig. 2), the addition of folate to the maternal diet caused a decrease in deposition of perirenal fat in all female offspring. Male offspring of the 18% casein diets showed an increase in perirenal fat, and there was no change in male offspring of the 9% casein diet. There was no effect of folate supplementation in the offspring at 4 weeks, although folate showed a decrease in gonadal fat deposition in females at 13 weeks (Fig. 1).

Rice cereal was the most common first food reported to be introduced (85%). The majority (72%) would advise a particular order of introduction of foods, with cereals being the first, followed by either vegetables or fruits, while meat was typically the last to be introduced. The results, however, did not provide a convincing standard practice for the age to introduce specific complementary foods.

Research pertaining to nurses’ knowledge of infant feeding has been primarily focused in the area of breast-feeding, with little in the literature on complementary feeding. One recent study in the UK that evaluated the knowledge of complementary-feeding recommendations in a group of paediatric nurses indicated poor recognition and limited understanding of current infant-feeding recommendations (Williams & Pimminington, 2003). The importance of timely and accurate dissemination of infant-feeding recommendations to relevant healthcare professionals should be recognised, in order to provide up-to-date and consistent information to parents and caregivers.

The aims of the study were to (a) determine whether non-dietetic healthcare professionals are familiar with the current international and national infant-feeding guidelines and (b) identify the current practices relating to the introduction of complementary foods.

A total of 320 self-administered questionnaires were sent to the nursing managers of nine Singhealth Polyclinics during the first week in October 2005, who subsequently distributed to eligible staff. Completed questionnaires were returned via internal mail to the principal investigator by 1 November 2005. The response rate was 50% (n = 224); of these questionnaires, four were incomplete and were discarded. There were forty-one doctors (19%), twelve pharmacists (5%), eight pharmacy technicians (4%) and 159 nurses (72%).

There were three questions on infant-feeding recommendations. A score was given to each correct answer, and a median score was computed for each profession. Both the doctor and pharmacy groups scored lower than the nurses (P = 0.001). Female staff scored significantly higher than male staff (P = 0.002). No statistical differences were found for place of education, length of experience, marital status, whether they have children or the amount of time spent working with infants.

The Table shows the number of respondents (% ) providing correct answers to questions on infant-feeding recommendations.

<table>
<thead>
<tr>
<th>Questions</th>
<th>Doctor (n = 41)</th>
<th>Pharmacy (n = 22)</th>
<th>Nurse (n = 159)</th>
<th>Total (n = 222)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO</td>
<td>22</td>
<td>7</td>
<td>35</td>
<td>105</td>
</tr>
<tr>
<td>Singapore</td>
<td>16</td>
<td>3</td>
<td>30</td>
<td>97</td>
</tr>
<tr>
<td>Age of introduction of complementary foods</td>
<td>18</td>
<td>14</td>
<td>25</td>
<td>78</td>
</tr>
<tr>
<td>Respondents with three correct answers</td>
<td>7</td>
<td>17</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>Median score</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
<td></td>
</tr>
</tbody>
</table>

Rice cereal was the most common first food reported to be introduced (85%). The majority (72%) would advise a particular order of introduction of foods, with cereals being the first, followed by either vegetables or fruits, while meat was typically the last to be introduced. The results, however, did not provide a convincing standard practice for the age to introduce specific complementary foods.

The level of knowledge on infant nutrition was found to be inadequate. Doctors and pharmacists were less familiar with the current infant-feeding recommendations as compared with nurses. Regular updates may be necessary to improve the level of knowledge. A standard age of introduction of specific complementary foods could not be identified due to the irregularities in practice. However, it may not be clinically important in light of the recent suggestion that the order of introduction of complementary foods is not important.

Effect of consumption of five portions of fruit and vegetables as juice shots on risk factors for cardiovascular disease. By M. CLEGG and A. SHAFAT, Department of Physical Education and Sport Sciences, University of Limerick, Republic of Ireland

By T.W. GEORGE, E. PATERSON, S. WAROONPHAN, M.H. GORDON and J.A. LOVEGROVE, School of Food Biosciences, The University of Reading, Whiteknights, P.O. Box 226, Reading, Berks, UK, RG6 6AP

The current increase in obesity prevalence has been documented as a primary health concern in recent times. Results of studies examining various aspects of obesity and obesity risk factors have been inconsistent. Rapid DE may reflect a reduced satiety signal leading to increased food intake. Also, selective exposure to fruit and vegetables for high fat content may further increase DE. The aim of the present study was to investigate mouth to caecum transit time (MCTT), macronutrient diet intake and plasma carotenoids, cholesterol, ghrelin, Willebrand factors, and plasma lipids in adult men. Sixteen healthy males consented to participate in the present study, approved by the University Research Ethics Committee; eight obese (31.0 (SD 8.7) years, 113.3 (SD 14.2) kg, 179.4 (SD 4.0) cm, BMI 35.2 (SD 3.6) kg/m²) and eight lean controls, age and height matched (30 (SD 7.4) years, 77.6 (SD 6.3) kg, 77.6 (SD 4.5) cm, BMI 23.3 (SD 1.6) kg/m²). Dietary intake was recorded for 1 week before the first test session using a weighed food diary. MCTT was evaluated using the lactulose H2 breath test. Breath H2 of at least 3 parts per million for three consecutive readings (Bond et al. 1975) was examined with SPSS (version 11.0; SPSS Inc., Chicago, IL, USA) using repeated-measures ANOVA and t-tests.

The high-fat meal had shorter MCTT than the low-fat meal for both the obese group (low-fat 96.3 (SD 24.0) min, high-fat 57.5 (SD 18.1) min) and the lean group (low-fat 103.1 (SD 39.7) min, high-fat 72.5 (SD 26.8) min). The obese group consumed a higher percentage of dietary fat as part of total energy intake (obese 38.3 (SD 7.5) %; lean 28.6 (SD 5.9) %) and consequently lower dietary carbohydrate (obese 40.2 (SD 6.7) %; lean 50.7 (SD 5.9) %) in both groups. Plasma ghrelin levels differed between the baseline and the 60-minute postprandial (P = 0.005) and between the two groups (P = 0.03) for the low fat meal. No differences were found for satiety. The high-fat meal had a faster transit time possibly due to the lower weight and volume in comparison with the low-fat meal. The results indicate that MCTT is faster in obese males whereby the test procedure was replicated using the alternative test meal. Statistical significance (P < 0.0005; see Figure). The obese group had shorter MCTT (P = 0.005) and consequently lower dietary carbohydrate intake than the lean group. Further work on GE and MCTT regulation of fats in obesity is being investigated in our laboratory.

Overall, the present study provided evidence that consumption of five portions of fruit and vegetables in the form of juice shots increased dietary carotenoids, antioxidants and carotenoid absorbance capacity. Further work on dietary carotenoids and their bioavailability is required. The results indicate that MCTT is faster in obese males and whereby the test procedure was replicated using the alternative test meal. Statistical significance (P < 0.0005; see Figure). The obese group had shorter MCTT (P = 0.005) and consequently lower dietary carbohydrate intake than the lean group. Further work on GE and MCTT regulation of fats in obesity is being investigated in our laboratory.
Nutritional status and subsequent all-cause mortality in men and women over 75 years old living in the community. By X. JIA1, G. McNEILL1 and L.S. AUCOTT1, 1Department of Environmental and Occupational Medicine, University of Aberdeen, Foresterhill Road, Aberdeen, UK, AB25 2ZP and 2Department of Public Health, University of Aberdeen, Foresterhill, Aberdeen, UK, AB25 2ZD.

In the present study, we prospectively investigated the relationships between Fe, vitamin B12, folate, vitamin C and vitamin D status and subsequent all-cause mortality in 208 men and 197 women aged 75 years or over living in the community. The participants were recruited in 1999–2000 when health and lifestyle questionnaires were completed and blood samples were taken for analysis of serum ferritin, serum vitamin B12, erythrocyte folate, plasma vitamin C and serum 25-hydroxycholecalciferol (25-OHD) (McNeill et al. 2002). Mortality records were checked with the General Register Office (Edinburgh, UK) in December 2005 after a median of 69.2 months follow up. Overall, seventy-one men had died, of whom 32.8% died from IHD, 11.9% from cerebrovascular disease and 30.0% from cancer; fifty-eight women had died, of whom 25.9% died from IHD, 24% from cerebrovascular disease and 15.5% from cancer.

Individuals were divided into six specific tertiles of blood levels of each nutrient. The Cox proportional hazard model was used to estimate the hazard ratios (Parmar & Machin, 1995) (see Table). Logistic regression was used to assess the trend of association across the tertiles. There was no clear pattern of association between vitamin B12 or folate status and mortality. There was a tendency for men in the highest tertile of vitamin C and women in the highest tertile of ferritin to have the lowest risk of death compared with those in other tertiles, but the trends were not significant. Vitamin D status was inversely related with mortality in both men and women and the trends were both significant (P for trend: men 0.05; women 0.01). Participants in the lowest tertile of vitamin D status had a nearly two-fold higher risk of death compared with those in the highest tertile.

<table>
<thead>
<tr>
<th>Nutritional status Range in tertile</th>
<th>Men (n=208)</th>
<th>Women (n=197)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum vitamin B12 (pmol/l) 199–299</td>
<td>1.28 0.70, 2.32</td>
<td>1.23 0.62, 2.44</td>
</tr>
<tr>
<td>Serum vitamin B12 (pmol/l) 299–380</td>
<td>1.42 0.70, 2.58</td>
<td>0.91 0.46, 1.81</td>
</tr>
<tr>
<td>Serum vitamin B12 (pmol/l) 380–460</td>
<td>1.42 0.70, 2.58</td>
<td>0.91 0.46, 1.81</td>
</tr>
<tr>
<td>Plasma vitamin C (μmol/l) 23.9–49.1</td>
<td>1.36 0.92, 3.39</td>
<td>1.28 0.79, 2.12</td>
</tr>
<tr>
<td>Plasma vitamin C (μmol/l) 50.2–68.9</td>
<td>1.36 0.92, 3.39</td>
<td>1.28 0.79, 2.12</td>
</tr>
<tr>
<td>Plasma vitamin C (μmol/l) 69.0–87.0</td>
<td>1.36 0.92, 3.39</td>
<td>1.28 0.79, 2.12</td>
</tr>
</tbody>
</table>

- *P<0.05 (Cox proportional hazard model, two-sided). Results were adjusted for factors that significantly contributed to survival: (1) age; (2) taking five or more kinds of medicine; (3) self-perceived health status; (4) existing heart diseases and/or diabetes at baseline.

Of the men, 12.1% were vitamin C supplement users; 14.2% women were users. Vitamin D supplement use was 24.2% in men and 29.7% in women. Including use of supplement (comprising the corresponding nutrient) or frequency of being outdoors in sunny weather into hazard models used in the Table did not change the results substantially. When including ‘frequency of doing physical activity outdoors’ into hazard models used in the Table, there was still a trend that participants with higher vitamin D status had lower hazard ratios, but the trend in men was not significant any more (P for trend: men 0.16; women 0.03).

The results suggest that low vitamin D status may increase the risk of death independently. Further research is needed to assess the effect of vitamin D status on survival in old individuals.

α-Tocopherol is a fat-soluble vitamin with an essential role in the protection of cell membranes from lipid peroxidation. The richest sources of α-tocopherol are vegetable oils, while meats such as pork, beef and lamb also contribute significantly to daily intakes. The amount of α-tocopherol consumed does not always accurately reflect the amount which is available for absorption and utilisation. α-Tocopherol bioavailability has been shown to be influenced by the levels and composition of fats in the diet, fibre, alcohol and pectin (Cohn, 1997). As traditional methods for the assessment of α-tocopherol bioavailability are both time consuming and expensive, the development of a reliable in vitro method for assessing biological availability would greatly enhance our understanding of the factors influencing the absorption of this vitamin.

The aim of the present study was to determine the proportion of biologically available α-tocopherol from a range of cooked and uncooked processed meat products. Biological availability describes the proportion of a nutrient which is packaged into micelles and is thereby made available for absorption in the gastrointestinal tract. The meat products selected were (a) sausages, (b) low-fat sausages, (c) prepacked, sliced luncheon roll and (d) prepacked, sliced pork, onion and tomato roll. Following a homogenisation step, meat samples were subjected to an in vitro digestion procedure involving incubation with the enzyme pepsin for 1h at pH 2, followed by a 3.5h incubation with bile salts and pancreatin at a pH of 7.8 (Garrett et al. 2000). The micelle fraction, contained in the aqueous phase, was isolated using ultracentrifugation at 50,000 rpm for 95 min. α-Tocopherol was extracted from both the homogenised, undigested meat and the micelle fraction using hexane and samples were analysed by HPLC.

Assessment of the micellarisation of α-tocopherol from processed meat products using an in vitro model. By O. KENNY, Y.C. O’CALLAGHAN and N.M. O’BRIEN, Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland.

α-Tocopherol values in the undigested meat samples ranged from 0.18 mg/100 g in the cooked low-fat sausage to 0.37 mg/100 g in luncheon roll. The α-tocopherol content in the micelle fraction of the various meats ranged from 0.09 mg/100 g in luncheon roll to 0.12 mg/100 g in cooked sausage. The percentage micellarised ranged from 24% in luncheon roll to 57% in the cooked sausage. Cooking of the sausages appeared to enhance the micellarisation of α-tocopherol. Although pork, onion and tomato roll and luncheon roll were found to have the highest content of α-tocopherol, the biological availability of α-tocopherol in these products is lower, possibly due to the presence of some ingredient which hampers the micellisation or promotes the degradation of α-tocopherol.

The present study was funded by the Department of Agriculture and Food, Dublin, under the Food Institutional Research Measure.
The impact of increased wholegrain food consumption on daily intake of nutrients. By A.R. JONES, S. KUZNESOFT, D.P. RICHARDSON and C.J. SEAL. Human Nutrition Research Centre, School of Agriculture, University of Nottingham, University of York, UK.

Increased wholegrain food (WGF) consumption is associated with a reduced risk of several chronic diseases such as CVD, type 2 diabetes and certain cancers (Smith et al. 2003). Due to the complex nutritional profile of whole grains, the mechanisms by which they exert their protective effect are poorly understood. However, it is suggested that the range of nutrients and phytoprotective substances naturally abundant in whole grains may work in synergy to confer their health benefits (Seal, 2006). The present study examines the effect of increased WGF consumption on daily nutrient intakes.

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 and the Photographic Atlas of Food Portion Sizes to assess portion size (Nelson et al. 1997). Whole-grain intake was calculated using ingredient data for foods containing ≥10% whole grain, expressed on a DM basis. Nutrient intakes were calculated using Windiets (Univation Ltd, Aberdeen, UK) and together with whole-grain intake data were assessed using the general linear model procedure of repeated-measures analysis in SPSS (SPSS Inc., Chicago, IL, USA).

Results are shown for twenty-six subjects (ten males, sixteen females; mean age 33.0 years) who completed all elements of the study at each time point of the intervention.

<table>
<thead>
<tr>
<th>Daily intake</th>
<th>Baseline</th>
<th>8 weeks</th>
<th>16 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-grain intake (g)</td>
<td>27.46</td>
<td>79.57</td>
<td>109.52</td>
</tr>
<tr>
<td>Energy (MJ)</td>
<td>10.05</td>
<td>70.04</td>
<td>10.04</td>
</tr>
<tr>
<td>% Energy from fat</td>
<td>15.4</td>
<td>15.6</td>
<td>15.6</td>
</tr>
<tr>
<td>% Energy fromCHO</td>
<td>48.15</td>
<td>48.14</td>
<td>49.14</td>
</tr>
<tr>
<td>% Energy from alcohol</td>
<td>6.09</td>
<td>6.09</td>
<td>6.09</td>
</tr>
<tr>
<td>NSP (g)</td>
<td>16.10</td>
<td>18.08</td>
<td>20.11</td>
</tr>
<tr>
<td>Mg (mg)</td>
<td>340.20</td>
<td>371.66</td>
<td>389.18</td>
</tr>
<tr>
<td>K (mg)</td>
<td>4.03</td>
<td>5.03</td>
<td>5.02</td>
</tr>
<tr>
<td>Na (mg)</td>
<td>3281.236</td>
<td>3096.196</td>
<td>3070.171</td>
</tr>
<tr>
<td>Ca (mg)</td>
<td>973.620</td>
<td>980.517</td>
<td>883.459</td>
</tr>
<tr>
<td>Fe (mg)</td>
<td>15.10</td>
<td>15.07</td>
<td>15.07</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>2.01</td>
<td>2.01</td>
<td>2.01</td>
</tr>
<tr>
<td>Thiamin (mg)</td>
<td>2.01</td>
<td>2.01</td>
<td>2.01</td>
</tr>
<tr>
<td>Total folate (ug)</td>
<td>306</td>
<td>280</td>
<td>312</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>128</td>
<td>134</td>
<td>127</td>
</tr>
</tbody>
</table>


Whole-grain intake increased significantly during the intervention. There was a trend towards decreasing total energy intake and percentage energy from fat with increasing whole-grain intake. NSP intake increased as expected with higher whole-grain intake. Na intake tended to fall with increasing whole-grain intake. In contrast, Mg intake was significantly higher from baseline to 16 weeks.

The results of the present study show that the US recommendation to consume three ounce-equivalents of WGF per d can be readily achieved and exceeded through substitution of refined-grain foods for whole-grain alternatives. The substitution caused a modest reduction in total energy intake and fat intake, and resulted in favourable changes in nutrient composition, particularly increased NSP and Mg intake and lower Na intake. The results also suggest that increased WGF consumption does not significantly affect intake of those micronutrients readily fortified in refined products such as breakfast cereals.

Lack of effect of increased fruit and vegetable intake on plasma 8-isoprostane F₂α concentrations. By U. MULLA, S.E.E. BERRY, R. GRAY and T.A.B. SANDERS, Nutritional Sciences Research Division, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London, UK.

Increased fruit and vegetable (F&V) consumption is associated with a lower risk of CVD. It has been argued that some of the protective effect may be mediated by antioxidant material present in F&V. Reactive oxygen species (ROS) are believed to play an important part in vascular endothelial pathophysiology and hypertension. 8-Isoprostanes are a family of metabolites arising from the oxidation of arachidonic acid by ROS. 8-Isoprostane F₂α is currently regarded as the most reliable marker of in vivo ROS production and non-enzymic lipid peroxidation (Morrow, 2005). The present study reports plasma 8-isoprostane F₂α concentrations determined using a very specific assay in pre- and mild hypertensive subjects recruited into a randomised controlled trial of increased F&V consumption (ISRCTN50011192; www.controltrials.com (DRFRUTINVEG)). Subjects consumed four diets of low (three portions/d), medium (about five portions/d) or high (about nine to eleven portions/d) F&V intake for 6-week periods. On the lowest intake of F&V subjects received an additional 40 mmol K (K₂CO₃) per day.

Fasting blood samples were collected at the end of each treatment period into 4.5 ml citrate-containing vacutainers, indomethacin (0.2 mm final concentration) was added to inhibit cyclo-oxygenase and the sample was chilled to 4°C for 30 min. After centrifugation the plasma was separated and 5 ml of 50% butylated hydroxytoluene was added per ml plasma as an antioxidant. The plasma was stored at −80°C pending analysis and samples from each subject were analysed in the same batch to minimise analytical variation. Following alkaline hydrolysis of a known volume of plasma in the presence of a four times 1H-labelled internal standard, 8-isoprostane F₂α was isolated by immunoaffinity purification and then subjected to esterification with pentfluorobenzoyl bromide, followed by silylation with BSTFA. The resulting derivatives were separated and analysed on an Agilent Technologies 6890N network gas chromatograph system equipped with 7683 series autoinjector, PTV (Gerstel) inlet and capillary column. The GC–MS was operated in negative chemical ionisation mode using methane as the reagent gas with selective ion monitoring of ions 569 and 573 corresponding to the carboxylate anion (M−181). The method of internal standardisation was used for quantification. The results for twenty-six subjects are shown in the Table, adjusted for age and sex.

Lack of effect of increased fruit and vegetable intake on plasma 8-isoprostane F₂α concentrations.

<table>
<thead>
<tr>
<th>Level of F&amp;V intake</th>
<th>Mean (ng/ml)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low + placebo</td>
<td>4.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Low + 40 mmol K₂CO₃</td>
<td>4.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Medium</td>
<td>4.5</td>
<td>1.5</td>
</tr>
<tr>
<td>High</td>
<td>4.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Repeated-measures ANOVA.

There were no statistically significant differences between the treatments.

Plasma 8-isoprostane F₂α concentrations did not change with increasing intakes of F&V. These preliminary results do not support the hypothesis that high intakes of F&V reduce lipid oxidation in vivo in subjects with pre- or mild hypertension.

Effects of exercise on energy compensation in healthy sedentary volunteers. By C. MARTINS, H. TRUBY and L. MORGAN, School of Biomedical and Molecular Sciences, University of Surrey Guildford, UK, GU2 7XH

Both physical activity and food intake impact on energy balance and their relationship therefore needs to be examined. We have previously shown in a cross-sectional study that sedentary males are unable to compensate for previous energy intake (EI) compared with active males (Long et al. 2002). The present study investigates the effects of a 6-week moderate exercise programme (four times/week, 30 min/session, 65–75% maximal heart rate) on appetite regulation in a group of normal-weight sedentary volunteers. It tests the hypothesis that exercise improves appetite regulation by increasing the sensitivity of compensation for previous EI.

EI at a buffet meal (and after that until breakfast next day; cumulative EI) was measured following high-energy preloads (HEP; 2540 kJ (607 kcal)) and low-energy preloads (LEP; 1030 kJ (246 kcal)) in twenty-five healthy volunteers (eleven men, fourteen women; aged 30 (so 12) years; BMI 22.7 (so 2.3) kg/m²) at baseline and after the 6-week exercise intervention.

Buffet EI after the two preloads was not significantly different at baseline in either men or women. However, after the exercise intervention EI after the LEP was significantly higher than after the HEP in both men and women. Overall, buffet EI was significantly lower after the HEP compared with LEP at baseline and the significance of the difference increased with exercise. The Table shows EI (kJ) at a buffet lunch after HEP and LEP at baseline and after the exercise intervention.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>Men</td>
<td>Women</td>
<td>All</td>
<td>Men</td>
<td>Women</td>
<td>All</td>
<td>Men</td>
</tr>
<tr>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td>HEP</td>
<td>3075*</td>
<td>2840</td>
<td>128</td>
<td>2464</td>
<td>870</td>
<td>2745***</td>
<td>3176*</td>
<td>107</td>
</tr>
<tr>
<td>LEP</td>
<td>3615*</td>
<td>2310</td>
<td>1314</td>
<td>1312</td>
<td>2067</td>
<td>1387</td>
<td>3659***</td>
<td>117</td>
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<tr>
<td></td>
<td></td>
<td>517</td>
<td>317</td>
<td>212</td>
<td>117</td>
<td>4255*</td>
<td>115</td>
<td>3029</td>
</tr>
</tbody>
</table>

Mean values were significantly different between conditions: *P<0.05, ***P<0.001.

The exercise programme also resulted in a better longer-term energy compensation, based on cumulative EI. A significant increase in the difference in cumulative EI (kJ) after each preload was observed overall (n 25) following the exercise intervention (–13 (so 1877) v. 1218 (so 2171); P<0.05).

This approach showed significance in men (–439 (so 2456) v. 1452 (so 2732); P=0.076), but not in women (318 (so 1318) v. 1033 (so 1720); P>0.250).

These findings confirm the original results from our cross-sectional study and emphasise a role for exercise in improving the accuracy of compensation for previous EI, especially in men. Further studies are needed to clarify the mechanisms whereby exercise improves energy compensation and the reasons for the sex differences elucidated.


Visual estimation of nutritional status in older subjects by carers, using comparative silhouettes of body shapes. By C.R. HANKEY, L. WATSON and W.S. LESLIE, Human Nutrition Section, University of Glasgow Division of Developmental Medicine, Glasgow Royal Infirmary, Glasgow, UK, G31 2ER

Older individuals are at risk from undernutrition for various reasons, including loss of appetite and impaired taste perception (Committee of Medical Aspects of Food Policy, 1991; Caroline Walker Trust, 1995). To prevent or manage undernutrition it is essential that care staff are able to recognise it, as it is often undetected and untreated (Todorovic, 2001). The aim of the present study was to discover whether care staff could correctly assess BMI of residents using silhouette photographs. These have only been used in the identification of overweight and obesity.

Care-home staff (n 27) were asked to classify the BMI of residents, for whom they cared, using a set of silhouette photographs for both males and females (Han & Lean, 1998). Six body silhouettes ranged from a BMI of 18 to 29 kg/m² and 20 to 38 kg/m² for adult males and females respectively (12 in total). Data were collected for seventy-five residents (fourteen males) who were subsequently weighed, knee height measured and their BMI calculated. Mean BMI for men was 27.0 (range 17.6–32.9) kg/m² and for women was 25.1 (range 16.5–40.3) kg/m².

Using silhouettes, care staff were able to correctly identify the BMI of residents in 47% of all estimates. Of the estimates, 24% over-classified and 29% under-classified. The majority of the accurate assessments were in residents with a BMI of 20 kg/m² or less. The strength of agreement as measured by kappa for males was 0.169 (poor) and for females 0.296 (fair). Weighted kappa value were 0.464 (moderate) and 0.558 (moderate) respectively.

<table>
<thead>
<tr>
<th>Silhouette BMI (kg/m²)</th>
<th>Correct classification</th>
<th>Over-classification</th>
<th>Under-classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>18</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>7</td>
<td>17</td>
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<td></td>
<td>29</td>
<td>3</td>
<td>18</td>
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<tr>
<td>Females</td>
<td>20</td>
<td>69</td>
<td>0</td>
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<td></td>
<td>24</td>
<td>43</td>
<td>17</td>
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<td></td>
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<td>33</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>12</td>
<td>0</td>
</tr>
</tbody>
</table>

Mean values were significantly different between conditions: *P<0.05.

Silhouettes show promise as a tool to assist the identification of undernutrition in care-home residents by carers, most often untrained in nutrition. Further research is required to determine the validity of these findings and ultimately define a role for these tools in the care of the older person.

Supplement use and comparative differences in nutritional status amongst patients with multiple sclerosis. By H. BENNEWITH1, B. WELLER2, C. O’LEARY3, O’RELLY4, A. DUNCAN5, L. WOOD1, L. PAUL1 and A. PAYNE1, 1Glasgow Caledonian University, Glasgow, UK, G4 OBA, 2Institute of Neurological Sciences, Western General Hospital, Edinburgh, UK, EH3 9RT, 3Institute of Neurological Sciences, Southern General Hospital, Glasgow, UK, G51 4TF and 4Trace Element and Micronutrient Unit, Department of Clinical Biochemistry, Glasgow Royal Infirmary, Glasgow, UK, G4 OSF

Whilst there is no conclusive evidence to support the use of high-dose nutritional supplements as a therapy for multiple sclerosis (MS), speculation regarding the possible benefits of a variety of supplements may lead to patients using them as a potential therapy for MS (Schwarz & Lewelling, 2005). This study aims to assess the level and type of supplement usage in a cohort of patients with MS and compare the nutritional status of supplement using (US) and non-supplement using (NSU) patients.

Patients attending MS outpatient clinics were asked for information on current usage of nutritional and non-nutrient supplements. The number and type of supplements currently being taken were recorded. Patients were asked to refrain from taking any supplements on the day of the study. Blood nutrient levels of a range of vitamins and minerals and other nutritional indicators were measured.

Twenty-nine male and seventy-six female patients (n 105) with an age range 20–75 years participated in the study. Fifty-seven patients (54%) reported taking supplements at the time of participation, a level that may be higher than in the general population, (National Diet and Nutrition Survey, 2004). The preparations used ranged from one to thirteen per d with a mean intake of 3.26 (SD 2.806) per d. Single nutrients and fish oils were most popular, taken by thirty-eight (6%) and thirty-six (63%) of the SU group respectively. This contrasts with findings in general-population studies where fish oils and combined multivitamin and mineral supplements were found to be most popular, (National Diet and Nutrition Survey, 2004). While those in the SU group were found to have higher blood nutrient levels for all nutrients measured, two independent sample t and Mann Whitney U tests identified significantly higher levels for the nutrients shown in the Table.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>SU</th>
<th>NSU</th>
<th>SU</th>
<th>NSU</th>
<th>P value</th>
<th>Reference ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D (nmol/l)</td>
<td>70.86</td>
<td>49.70</td>
<td>5.83</td>
<td>3.32</td>
<td>0.01</td>
<td>25–170</td>
</tr>
<tr>
<td>Vitamin E (μg/ml)</td>
<td>30.50</td>
<td>26.19</td>
<td>6.45</td>
<td>5.33</td>
<td>0.00</td>
<td>15–48</td>
</tr>
<tr>
<td>Vitamin B6 (μmol/l)</td>
<td>84.93</td>
<td>45.55</td>
<td>11.97</td>
<td>7.32</td>
<td>0.01</td>
<td>20–140</td>
</tr>
<tr>
<td>Folate (ng/ml)</td>
<td>10.34</td>
<td>6.57</td>
<td>0.78</td>
<td>0.69</td>
<td>0.00</td>
<td>2.7–34.0</td>
</tr>
<tr>
<td>Erythrocyte folate (ng/ml)</td>
<td>369.5</td>
<td>267.9</td>
<td>20.34</td>
<td>14.91</td>
<td>0.00</td>
<td>160–714</td>
</tr>
</tbody>
</table>

Supplement use is common amongst patients with MS and the types of supplement chosen may differ from general population choices. This may reflect a perception of nutrition and supplement use, as a self-selected therapy to promote well-being in MS. While we were unable to measure intake of nutrients from dietary sources in this study, results suggest that high levels of supplement use may have a significant effect on the nutritional status of MS patients.

Exercise induced anorexia: a role for PYY, GLP-1 and PP? By C. MARTINS, L.M. MORGAN and M.D. ROBERTSON, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK

The present study investigated the acute effects of exercise on subjective appetite, energy intake (EI) and postprandial levels of appetite-related hormones and metabolites.

Ghrelin, peptide YY (PYY), glucagon like-peptide-1 (GLP-1), pancreatic polypeptide (PP), insulin, glucose, NEFA and triacylglycerols (TG) were measured in fasting and postprandially (over a 3 h period) in twelve healthy volunteers with normal physical activity levels (six males) (Age: 25.9 ± 4.6 years, BMI: 22.0 ± 3.2 kg/m²), using a randomised cross-over design. At 1 h after a standardised breakfast, subjects cycled for 1 h at 65% maximal heart rate or rested. Subjective appetite was assessed throughout the study using visual analogue scales and subsequent food intake at a buffet meal was measured at the end (3 h post-breakfast; 1 h post-exercise).

Exercise significantly increased mean PYY, GLP-1 and PP levels. No significant effect was observed on postprandial levels of ghrelin. During exercise hunger scores were significantly decreased; however, this effect disappeared in the post-exercise period. Exercise significantly increased absolute EI, but produced a significant decrease in relative EI after accounting for the energy expended during exercise. Hunger scores and PYY, GLP-1 and PP levels showed an inverse temporal pattern during the 1 h exercise/control intervention.

In conclusion, acute exercise, of moderate intensity, temporarily decreased hunger sensations and was able to produce a short-term negative energy balance. This impact on appetite and subsequent energy homeostasis was not explained by changes in postprandial levels of ghrelin, however, "exercise-induced anorexia" may potentially be linked to increased PYY, GLP-1 and PP levels.

Caffeine intake during pregnancy. By S.M. BOYLAN, S.F.L. KIRK, D.C. GREENWOOD and J.E. CADE, Centre for Epidemiology and Biostatistics, University of Leeds, UK, LS2 9LN

Studies on the effects of caffeine on health, particularly in relation to pregnancy outcome, have suffered from a number of methodological flaws due to inaccurate or biased assessment of caffeine intake. The aim of the present study was to assess caffeine intake prospectively throughout pregnancy using a detailed, yet practical, questionnaire which assesses caffeine intake from all sources of caffeine along with potential confounders associated with pregnancy outcome.

This questionnaire, known as the Caffeine Assessment Tool (CAT) was administered at three time points throughout pregnancy in a sample of 250 healthy pregnant women attending the Leeds General Infirmary antenatal clinic (mean gestation at recruitment was 17 weeks). An algorithm was developed so that caffeine intake (mg/d) could be calculated for each woman. Multivariate analyses were conducted to explore the relationship between caffeine intake and nausea, smoking and alcohol intake.

The women were mainly Caucasian (88%) with a mean age of 30 years. The mean caffeine intake during pregnancy was 167 mg/d, which is lower than that currently advised for pregnant women (<300 mg/d; Committee on Toxicity of Chemicals in Foods, Consumer Products and the Environment, 2001). Caffeine intake reduced markedly at the beginning of pregnancy, but increased again towards the end of pregnancy. The Figure shows mean caffeine intake (mg/d) before, during and post-pregnancy.

During the first trimester, women who were nauseous had an average caffeine intake which was two-thirds of that consumed by women who were not nauseous (weeks 1–4 and 5–12 of pregnancy; \( P = 0.02 \) and \( P = 0.04 \), respectively). Smokers were significantly associated with caffeine intakes two to three times higher than non-smokers (weeks 5–12, 13–20, 21–28 of pregnancy; \( P = 0.03 \), \( P = 0.01 \), \( P = 0.009 \), respectively). There were weak, yet significant correlations between caffeine and alcohol intakes during the first seven months of pregnancy (\( P = 0.001 \) to \( P = 0.03 \)).

The present study is the first to assess caffeine intake in such detail among pregnant women and is now being used in a larger study in Leeds and Leicester investigating the impact of caffeine on birth weight. The CAT, along with a more detailed exploration of the interindividual variations in caffeine metabolism, may provide a conclusive answer to whether there is any link between caffeine and birth weight.

The present study is funded by the Food Standards Agency (FSA) grant 70103. Thanks to the Caffeine And Reproductive Health (cARE) study team: V.A. Dolby, S.M. Chell, B.E. Longshaw, D. Camidge, K. Curran, T. Moore, J.D. Thomas and K.L.M. White, and S. Shires for the laboratory analysis.


Tracking patterns of fruit and vegetable intakes from childhood to adulthood and their relationships with social class: longitudinal tracking from the 1946 British Birth Cohort. By Y. CHEN1, A.M. STEPHEN1, A.J.E. CADE, MANDER1, C.J. PRYNNE1 and M.E.J. WADSWORTH2. 1MRC Human Nutrition Research, Elsie Waldowson Laboratory, Fulbourn Road, Cambridge, UK, CB1 9NL and 2MRC National Survey of Health and Development, Department of Epidemiology and Public Health, University College London Medical School, London, UK, WC1E 6BT

For studies relating early life exposures to later diseases, an important assumption of the stability of risk factors over time can be examined by tracking subjects’ exposure status through repeated assessments. For fruit and vegetables, tracking analysis is particularly important because there are many international programmes aiming to increase children’s fruit and vegetable intake, and the rationale for these programmes rests largely on the premise that good health habits formed during childhood will be maintained into adulthood. There are very few studies where individual fruit and vegetable intakes have been tracked (for example, Wang et al. 2002); most of these have short follow-up periods and/or inaccurate dietary information and/or limited tracking methods.

The present study tracked fruit and vegetable intakes of subjects through ages 4, 36 and 43 years in the MRC National Survey of Health and Development (1946 British Birth Cohort). Dietary intakes at age 4 years were measured by 24 h recalls and adult dietary information was collected using 5d food diaries. Fruit and vegetable intakes were divided into high and low consumption groups based on median values. Tracking patterns of men and women were studied separately using agreement analyses when tracking two time points (a value of \( >0.5 \) indicates tracking) and Generalized Estimating Equations (GEE) when tracking all the three ages (an odds ratio of \( >1 \) indicates tracking). The roles of factors such as social class, region of residence and marital status on tracking patterns were also investigated.

The Table shows that for men and women overall, fruit intakes tracked through the three ages, but vegetable consumption did not, and there was a stronger tracking for women. Region of residence was only a confounder for tracking from age 4 to 36/43; marital status did not have any impact (data not shown). However, social class was a strong predictor of tracking. The Figure shows that fruit tracking from age 4 to 36 in children of non-manual social class fathers tracked high consumption but not low consumption, indicating that low consumers at age 4 must have increased fruit intakes. Children of manual social class fathers tracked low consumption but not high consumption, indicating that low consumers at age 4 stayed low while high consumers at age 4 decreased fruit intakes relative to others by age 36. Hence non-manual social classes were choosing a healthier diet but the reverse was happening in the manual social classes. The identification of social class as a strong effect modifier suggests that fruit and vegetable promotion programmes should target lower social classes at a very early age and continue throughout life.

Effect of short-term high- and low-glycaemic-index diets on subjective measures of appetite, and plasma glucose, insulin and ghrelin concentrations in healthy male subjects. By D. GRIGOROPOULOU, A. PARKER, C.A.E. EDWARDS, D. MALKOVA and S. HIGGINS, Human Nutrition, Division of Developmental Medicine, University of Glasgow, Glasgow, UK. G3 8SJ

The obesity epidemic is a growing concern worldwide. Current dietary recommendations advise a low-fat diet for the prevention of chronic disease and obesity (Department of Health, 1991). This is controversial, as with the reduction of dietary fat there tends to be an increase in carbohydrate, principally from refined starchy foods that have a high glycaemic index (GI) and are associated with poor body-weight control (Brand-Miller et al. 2002). On the other hand, low-GI meals have been shown to prolong feelings of satiety as they are digested and absorbed more slowly, resulting in more gradual blood glucose and insulin responses (Ludwig, 2002). Recently, interest has grown in the role of ghrelin, a peptide released from the gut and thought to be related to satiety. It is not known if ghrelin is influenced by GI. Thus, the aim of the present study was to investigate the short-term effects of high- and low-GI diets on subjective feelings of appetite, as well as on plasma glucose, insulin and ghrelin concentrations in healthy male volunteers.

Thirteen healthy males (age 23.3 (SD 2.9) years; BMI 22.5 (SD 1.5) kg/m²) participated in a randomised, cross-over study, in which they followed isoenergetic high-carbohydrate (70% energy from carbohydrate), high (GI = 77) and low (GI = 35) GI diets for 3 d with a 2-week washout period. Subjects were supplied with detailed dietary advice and all food items. On the third day of each dietary intervention, subjects participated in a trial in which blood samples were taken and appetite questionnaires completed before and every 30–60 min for 3 h after breakfast and lunch. Appetite was assessed using a validated questionnaire (Flint et al. 2000). Blood glucose concentrations were determined using an enzymic colorimetric method (GOD-PAP, Sigma, UK), and plasma insulin (Ultramensitive ELISA, Mercodia, Sweden) and ghrelin (Phoenix, Belmont, CA, USA) concentrations were analysed using commercially available methods.

Flavonoids are bioactive polyphenols that are an integral part of the human diet. Little is known about their role in the body. Interest has grown in the role of ghrelin, a peptide released from the gut and thought to be related to satiety. It is not known if ghrelin is influenced by GI. Thus, the aim of the present study was to investigate whether dietary flavonoid and flavanone intake is associated with bone mineral density (BMD) and bone turnover in a large group of Scottish women.

The subjects had been recruited in 1990–3 for the Aberdeen Prospective Osteoporosis Screening Study, and the majority of them returned 6.3±0.6 years later (mean age at baseline 54.7 (SD 2.2) years). They had bone density scans of the lumbar spine (LS) and hip (FN) (Norland XR26/36 DXA) and completed a food-frequency questionnaire (FFQ) at both visits. They provided second early morning fasted urine samples for analysis of free pyridinoline (fPYD) and deoxypyridinoline (fDPD) which were measured by HPLC and are expressed as ratios relative to creatinine (Cr).

The diets were analysed for flavonoid intake using a food composition database (Kyle & Duthie, 2006) developed for a similar version of our FFQ. We have now validated our FFQ for use with flavonoids in early postmenopausal Scottish women. Participation in a trial in which blood samples were taken and appetite questionnaires completed before and every 30–60 min for 3 h after breakfast and lunch. Appetite was assessed using a validated questionnaire (Flint et al. 2000). Blood glucose concentrations were determined using an enzymic colorimetric method (GOD-PAP, Sigma, UK), and plasma insulin (Ultramensitive ELISA, Mercodia, Sweden) and ghrelin (Phoenix, Belmont, CA, USA) concentrations were analysed using commercially available methods.

As expected, the area under concentration vs. time curve (AUC) for glucose and insulin after breakfast (P = 0.002 for glucose; P = 0.003 for insulin) and lunch (P = 0.076 for glucose; P = 0.001 for insulin) were lower during the low-GI trial compared with the high-GI trial. The AUC after the second meal was significantly greater for satiety (P = 0.03) and fullness (P = 0.014) during the low-GI trial. There was no significant difference in AUC values after either meal for ghrelin between the trials. The findings of this short-term experimental study show that a low-GI diet is associated with lower blood glucose and insulin responses and is more satiating than a high-GI diet. However, in this study this effect does not appear to be mediated by ghrelin.

Dietary flavanones are associated with increased bone mineral density and reduced bone resorption in early postmenopausal Scottish women. By A.C. HARDCASTLE1,2, J.A.M. KYLE2, G. DUTHIE3, G. McNEILL7, W.D. FRASER4, D.M. REID1,2 and H.M. MACDONALD3, 4Osteoporosis Research Unit, University of Aberdeen, Woodmanhill Hospital, Aberdeen, UK, AR25 1LD; School of Medicine, University of Aberdeen, Aberdeen, UK, AR25 2ZD; Rowett Research Institute, Aberdeen, UK, AR25 9SB and 4Department of Clinical Biochemistry, Royal Liverpool University Hospital, Liverpool, UK. L6 3GA

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There is increasing evidence that fruit and vegetables are important for bone health. One hypothesis is that they provide alkaline metabolites to balance the acid-generating Western diet. Net endogenous acid production is thought to be associated with bone health in different ways and further work is required to elucidate its role.

The present study was funded by a Food Standards Agency postgraduate scholarship. Any views expressed are the authors’ own.

1Centre for Public Health Nutrition Research and 2Department of Surgery and Molecular Oncology, University of Dundee, Ninewells Hospital and Medical School, Dundee, UK, DD1 9SY

There is now convincing evidence for a decreased risk of diabetes in adults who are physically active and can maintain a normal BMI. Achievement of these goals in the general population is also likely to result in a small reduction of chronic-diet-related diseases including CVD and certain cancers. The aim of the present study was to review the design and implementation details of successful lifestyle intervention programmes (in body weight and physical activity) which have produced clinically relevant reductions in markers of type 2 diabetes. These results could then be used to inform cancer risk-reduction intervention programmes aimed at increasing activity and weight management.

Details of relevant studies were obtained from computerised searches of the bibliographic PubMed base. Keywords used in the search were ‘type 2 diabetes prevention’ combined with ‘lifestyle interventions’, ‘lifestyle change’, ‘insulin resistance’ and ‘diabetes prevention program’. Only studies with a follow-up of at least 12 months were included. Lead authors were also contacted to identify specific details on intervention design when these were not described in the literature.

Five successful prevention studies carried out in high-risk individuals were used to inform the review, namely the Diabetes Prevention Programme (Knowler et al. 2002), the Diabetes Prevention Study (Tuomilehto et al. 2001), the Da Qing IGT and Diabetes Study (Pan et al. 1997), the Xendos study (Torgersen et al. 2004) and the Oslo Diet and Exercise Study (Torgersen et al. 1997).

All studies were designed as randomised control trials and included educational, motivational and behavioural approaches. Main goals of the lifestyle interventions were to reduce initial body weight and to perform at least 150 min moderate physical activity per week.

Behavioural theories, feedback, social support, and provision of food and physical activity-related activities and aids were used in some but not all studies. All studies had one-to-one individual approaches through some also had group approaches for food-based (for example, shopping and cooking) or physical activity group sessions. Intervention visits varied from a minimum of three to a maximum of fifty personal contacts over a 1-6 year period. The interventions were delivered by a range of healthcare professionals, though it is not clear if key individuals were involved. All interventions used additional educational material to support professional consultations, though these are barely described. Evidence of the use of behaviour theory in intervention design and implementation was limited and where stated seemed to focus on improving self-efficacy.

A focus on diet, body weight and physical activity has been successful in achieving a reduction in the incidence of type 2 diabetes. The minimum intensity (dose) and duration of intervention required to achieve lifestyle goals is unclear. Reporting and describing the implementation of lifestyle interventions was insufficient for replication. There was little evidence of the importance of social-psychological theory-based, behaviourally focused approaches to inform intervention strategies.

Achieving reduction in body weight and increased activity is a major part of public health policy. Identifying the key characteristics of clinically relevant lifestyle intervention programmes offers a unique and potentially cost-effective approach to aim the design of chronic disease-reduction programmes. A minimum standard for reporting implementation procedures (educational, behavioural and motivational) would enable wider dissemination of relevant techniques and strategies.

Funding from The Scottish Cancer Foundation is gratefully acknowledged.

Glycaemic control has been shown to be improved with low-glycaemic index (GI) diets in subjects with type 2 diabetes (Wolever & Mehling, 2002) and recommendations for a reduction in carbohydrates of high GI in the diabetic diet have been made (Willett, 2002). Although GI for individual foods are known, the application of GI in the context of mixed meals and diets merits further investigation (Flint et al. 2002). In the present study, the Food Multimix (FMM) concept was employed in order to produce food recipes of high fibre, low total sugar, low fat and moderate protein content using commonly consumed and plant-based food ingredients in the UK (Tables 1 and 2). A food multimix is a blend of locally-available, affordable, culturally-acceptable and commonly-consumed foodstuffs mixed proportionately, drawing on the ‘nutrient strengths’ of each component of the mix in order to optimise the nutrient value of the end product without the need for external fortification.

FMM of predicted low GI were formulated and subjected to proximate analyses to determine total dietary fibre (AOAC official method 991.42), fat (AOAC official method 922.06), protein (modified Kjeldahl method) moisture and ash content. Total carbohydrate was obtained by derivation and energy content calculated using the Atwater factors.

Results of experimental analyses showed energy density of 16.19 (so 0.50) kJ/g; total carbohydrate content was 61.77 (so 3.49)g/100g product, of which 78.39 (so 8.39) % was complex carbohydrates and sugar content of 5.05 (so 2.95) g; fibre content was 9.44 (so 4.45) g, providing 31.47% of the recommended daily fibre intake for diabetes. Protein was 13.20 (so 0.72) g; fat content was 9.69 (so 0.83) g. The percentage contributions of carbohydrate, fat and protein to total energy were 63.83 (recommended > 55%); 22.53 (recommended <30%) and 13.64 (recommended 10-15%) respectively. The mean predicted GI calculated was 49.08 (so 0.22), thus making it a low-GI product. One of the FMM selected for tests of glycaemic response in human volunteers confirmed its low GI properties, the findings of which have been reported elsewhere (Trowse et al. 2006).

These findings suggest that it is possible to formulate low-GI foods containing high proportions of fibre and complex carbohydrates, moderate amounts of protein and with a low sugar and fat content. These FMM have the potential to aid glycaemic control in human subjects and may benefit type 2 diabetics. Further feeding trials to test their clinical efficacy among diabetics is envisaged.

Table 1. Sample food ingredients (purchased from UK supermarkets) selected for FMM formulation

<table>
<thead>
<tr>
<th>Nutrient source</th>
<th>Examples of food groups at commodities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staples selected</td>
<td>Irish potatoes, brown rice, pearled barley, wholegrain wheat (high-fibre, high-complex-carbohydrate sources)</td>
</tr>
<tr>
<td>Protein sources selected</td>
<td>Soybeans, kidney beans, chick peas, lentils dried peas (avoidance of high-fat animal protein sources)</td>
</tr>
<tr>
<td>Vitamin and mineral sources selected</td>
<td>Spinach, tomatoes, carrots, apples, dates</td>
</tr>
<tr>
<td>Natural and oil-sources selected</td>
<td>Nuts (almonds, groundnuts), seeds (sunflowers, sesame)</td>
</tr>
</tbody>
</table>

Table 2. Summary of criteria for design of FMM for management of type 2 diabetes mellitus

<table>
<thead>
<tr>
<th>FMM design criteria</th>
<th>Rationale for criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy density &lt;48 kJ/g (4 kcal/g)</td>
<td>To ensure low-energy density to aid glycaemic control and weight loss in obese subjects</td>
</tr>
<tr>
<td>55% energy from complex carbohydrate</td>
<td>To keep GI low to aid glycaemic control</td>
</tr>
<tr>
<td>&lt;25% energy from fat</td>
<td>To maintain low energy density</td>
</tr>
<tr>
<td>51% energy from fibre content 2.5 g/100 g FMM</td>
<td>To maintain low fat content, energy density and minimise total nutrient breakdown</td>
</tr>
<tr>
<td>To provide 30% recommended fibre intake/100 g and aid glycaemic control without inhibiting digestion and absorption of other key nutrients</td>
<td></td>
</tr>
<tr>
<td>Sugar content &lt;8 g/100 g FMM</td>
<td>To maintain low GI</td>
</tr>
<tr>
<td>Vitamins and minerals providing &gt;60% RNI/100 g</td>
<td>To meet basic nutrient requirements in a balanced diet and control complications</td>
</tr>
</tbody>
</table>

Age-related changes in taste and effect on food supplement palatability. By K.W.C. LAW, M.A. GOSNEY, and O.B. KENNEDY, 1Hugh Sinclair Human Nutrition Unit, School of Food Biosciences, and 2Institute of Health Sciences, University of Reading, PO Box 225, Reading, UK, RG6 6AP

Malnutrition among the elderly is reported to be as high as 60% (National Institute for Health and Clinical Excellence, 2006). Oral nutritional sip-feed supplements (ONS) are often prescribed to the malnourished elderly in order to improve their nutritional and clinical status. However, previous research has shown these ONS are poorly consumed and often wasted (Gosney, 2003), with sweetness being identified as one of the factors responsible for dislike of ONS. The present study investigated if differences in sweetness thresholds, liking of sweetness and overall liking of ONS existed between young and elderly adults. Thirty-six young adults (age 18–33 years) and forty-eight healthy elderly (free-living and independent; age 63–85 years) took part in the present study. Detection and recognition threshold levels, basic taste identification and 'just about right' (JAR) of sweetness tests were examined.

Evaluation of three ONS (chocolate, vanilla, strawberry) and sucrose solutions for sweetness intensity, hedonic sweetness, overall hedonic and rank preference tests were performed.

<table>
<thead>
<tr>
<th>Threshold and JAR</th>
<th>YM</th>
<th>YF</th>
<th>EM</th>
<th>EF</th>
<th>Overall young</th>
<th>Overall elderly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection</td>
<td>1.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Recognition</td>
<td>3.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.88&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>JAR</td>
<td>4.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.82&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

YM, young males; YF, young females; EM, elderly males; EF, elderly females.

<sup>a,b</sup>Mean values within a row with unlike superscript letters were significantly different (determined by Fisher’s least significant difference (LSD) test (<0.05)).

Significant differences were found in both detection and recognition thresholds and correctly identifying taste between young and the elderly. Dislike of ONS was found, the degree of which varied across flavours, sexes and age groups. Results of the present study indicate that ONS may need to be reformulated depending on target group (age and sex) to increase acceptance and increase consumption. This could be especially beneficial amongst malnourished elderly, so that required nutrients could be delivered to them in a more palatable form.


Food group intake in a sample of primary school children participating in the Phunky Foods programme. By P.G. LOCK and J.E. COCKROFT, 1Life and Health Sciences, University of Ulster, Coleraine Campus, Cromore Road, Co. Londonderry, UK, BT52 1SA and 2Purely Nutrition Ltd, 46 Cheltenham Mount, Harrogate, UK, HG1 1DL

The prevalence of overweight and obesity in school-age children in England and Wales has doubled over the last 10 years. The prevalence of obesity in children aged between 2 and 10 years now stands at over 15.5% (Sproston & Primatesta, 2002). In order to reverse the current negative trend in the UK it is essential that successful strategies for improving the diets of children are developed. The aim of the present study was to describe the baseline diets of a sample of primary school children participating in Phunky Foods, an in-school healthy eating and physical activity programme. The research team visited seven randomly selected primary schools in the Yorkshire region before they started to teach the Phunky Foods programme. Four key stage 1 classes and three key stage 2 classes were identified to take part in the research programme. Prior to the research team visiting the children, an inducement letter was sent out to the parents/guardians of the children informing them of the evaluation that was going to take place.

The letter contained contact details of the research team if the parents had any questions or queries. Children were asked to complete a 24 h food and physical activity diary using both words and pictures to describe all food and drink consumed over a 24 h period. The children were also asked to take a copy of the Phunky Food frequency questionnaire (PFFQ) home for parental completion. The data presented here are taken from the results of the PFFQ (n=57). Out of the 7 schools that visited, 173 pupils were seen by the research team and a total of 57 Phunky Foods Frequency questionnaires were filled out and returned (33% response rate).

Individual foods from the PFFQ were categorised into food groups based on the ‘Balance of Good Health’ and the frequency of daily eating occasions for each food group was calculated. The Table below shows mean and median daily frequencies of consumption for each of the five food groups of the ‘Balance of Good Health’.

| Number of eating occasions foods from each group were consumed on a daily basis |
|--------------------------------|----------------|------------|------------|----------------|--------|
|                                | Mean          | Median     | 25th Percentile | 75th Percentile |
| Bread, other cereals and potatoes | 3.4           | 3.5        | 2.5         | 4.4            |
| Milk and dairy                 | 2.9           | 2.7        | 1.4         | 4.5            |
| Food containing fats, and food and drinks containing sugar | 5.1           | 5.3        | 3.8         | 6.5            |
| Meat, fish and alternatives    | 2.1           | 1.6        | 1.2         | 2.7            |
| Fruit and vegetables           | 5.9           | 4.9        | 2.9         | 8.0            |

Results show that in this sample of primary school children reported average intakes of fruits and vegetables, and milk and dairy products, are meeting current recommendations of ‘5-a-day’ and ‘3-a-day’ respectively. However, consumption of bread, potatoes and cereal products is relatively low and the number of eating occasions per day for foods high in fat and sugar is startlingly high in comparison. Small dietary changes in this sample of children could very easily shift their diets in a healthier direction. Future results will inform us as to whether the Phunky Foods programme can help children to achieve this desired shift and consume diets more in line with the ‘Balance of Good Health’.

Effects of alcohol consumption on weight gain in middle-aged women. By A. McAuley, V.J. Burley, J.D. Thomas, E.F. Taylor and J.E. Cade, Centre for Epidemiology and Biostatistics, University of Leeds, UK. IS6 9LN

There have been many previous studies exploring the relationship between alcohol consumption and weight gain. Despite its high energy content of 30 kJ (7.1 kcal)/g, it is still controversial whether moderate amounts of alcohol represent a risk factor for weight gain and obesity. One such study (Suter, 2005) found that alcohol energy counts more in moderate non-daily alcohol consumers than in (daily) heavy consumers. With this in mind, we aimed to investigate the effects of alcohol consumption on weight gain, using the women in the UK Women’s Cohort Study (UKWCS).

To do this, 33,929 middle-aged women who took part in the UKWCS were used. The UKWCS is a national, 10-year investigation of diet and cancer in women initially aged 35–69 years of age. The baseline data on the cohort were obtained with a 217-item food-frequency questionnaire, with additional questions on health and lifestyle. A second contact was undertaken (phase 2) 2–5 years after baseline, with all the women being sent a 4d food diary and a further health and lifestyle questionnaire. For the present study, 13,110 diaries were used to conduct analyses on change in weight.

A cross-sectional analysis was carried out to explore the impact of alcohol intake on weight at baseline followed by linear regression modelling to assess the effect of ethanol intake on weight on weight change adjusting for age, baseline BMI, total energy intake and smoking. Ethanol consumption was divided into quartiles according to level of intake. Baseline comparisons showed non-significant differences between ethanol groups and weight gain (see Table).

<table>
<thead>
<tr>
<th>Alcohol (units)</th>
<th>Mean weight baseline (kg)</th>
<th>Mean weight phase 2 (kg)</th>
<th>Mean weight gain (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>66 (14)</td>
<td>67 (14)</td>
<td>1.4 (6.2)</td>
</tr>
<tr>
<td>&lt;2</td>
<td>66 (12)</td>
<td>66 (12)</td>
<td>1.5 (5.0)</td>
</tr>
<tr>
<td>2 to 3</td>
<td>64 (11)</td>
<td>65 (11)</td>
<td>1.4 (4.8)</td>
</tr>
<tr>
<td>&gt;3</td>
<td>65 (11)</td>
<td>67 (12)</td>
<td>1.6 (5.0)</td>
</tr>
</tbody>
</table>

On analysis of weight change from baseline to phase 2, it was found that the women who consumed more ethanol had the greatest reduction in weight gain (P<0.001). For every unit increase of alcohol, which equates to 8g of ethanol, weight gain is reduced by 120 g. The linear regression model showed a statistically significant inverse association between ethanol intake and change in weight between baseline and phase 2 (P<0.0001). BMI at baseline and age, were negative independent predictors of weight gain.

To conclude, the results from the present investigation show that there is an inverse relationship between alcohol consumption and weight gain, and this association is dependent upon age and previous BMI.

The UKWCS was funded by the World Cancer Research Fund.


Glycaemc potency of breakfast and cognitive function in school children. By R. Micha, J. Forbes, K. Lowes, P. J. Rogers and M. Nelson, Nutritional Sciences Research Division, King’s College London, 150 Stamford Street, London, UK, SE1 9NH and 2Department of Experimental Psychology, University of Bristol, Bristol, UK, BS8 1TH

Support of the notion that breakfast is ‘the most important meal of the day’ has been provided by studies looking into the effects of breakfast omission on cognitive function (CF). Up until recently the focus has been on whether or not breakfast is important and not on the type of the breakfast that could selectively facilitate CF after an overnight fast. As such, there are inconsistencies in the findings regarding benefits relating either to the type and the timing of the breakfast and the selection of the appropriate CF tests. Furthermore, not all possible confounding factors that could affect CF besides breakfast consumption have been accounted for. At the very least the studies thus far have provided proof in support of the assumption that the brain is vulnerable to the effects of brief fasting. Recent data suggest that the reason for this is that the brain may be sensitive to short-term fluctuations of the glucose supply.

It could be argued that a low-glycaemic index (GI) breakfast would minimise glycaemic fluctuations and as such facilitate performance in the hours following consumption; the glycaemic load (GL) should also be accounted for in order to determine the glycaemic potency of the meal. The purpose of the present cross-sectional study was to investigate whether breakfast meals differing in their GI and GL produced differences in subsequent CF after an overnight fast. Our aim was to take into account other confounding factors, including Fe status, underlying physiological adaptations, socio-economic status, and individual differences in the glycogen stores; most importantly to use CF tests which have proven to be sensitive in detecting variations in the glucose supply, and time them in relation to the physiological properties of the breakfast under study. It was hypothesised that a low-GI high-GL breakfast would have the most positive effect on performance 90–120 min after breakfast.

Sixty school children (thirty-six girls and twenty-four boys) aged 11–14 years were recruited from two schools in South London. On the day of the appointment, children had their habitual breakfast, and 90–120 min after breakfast, they were tested. Glucose and Hb levels were measured in finger-prick blood samples taken immediately before and after a battery of CF tests. Mood and task demand were also assessed. The GI and the GL of the different breakfast meals were calculated using the FAO/WHO equations (Foster-Powell et al., 2002), and the international table of GI and GL values (FAO/WHO, 1998).

Participants were distributed into four GI and GL groups (see Table) below and above the median (GImedian=60.64; GLmedian=27.25). There was limited variation in the blood glucose levels before and after the tests, within ±200 mg/l for forty-nine students. This shows that the 90–120 min interval after breakfast was an appropriate model to study the effects of the glycaemic potency of breakfast on CF. The low-GI high-GL breakfast selectively enhanced performance 90–120 min after breakfast on the majority of the tests, suggesting a possible role for the glycaemic potency in CF. This effect was statistically significant for the most cognitively demanding tasks and the ones particularly sensitive to glucose fluctuations, speed of information processing test and serial sevens.

<table>
<thead>
<tr>
<th>CF tasks</th>
<th>High GI</th>
<th>Low GI</th>
<th>High GI</th>
<th>Low GI</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed of information processing correct</td>
<td>13.9</td>
<td>3.6</td>
<td>12.1</td>
<td>2.8</td>
<td>11.0</td>
</tr>
<tr>
<td>Serials correct</td>
<td>29.3</td>
<td>17.5</td>
<td>15.8</td>
<td>9.6</td>
<td>15.1</td>
</tr>
<tr>
<td>Serials correct-incorrect</td>
<td>24.6</td>
<td>21.4</td>
<td>11.8</td>
<td>10.6</td>
<td>9.9</td>
</tr>
</tbody>
</table>


Comparison of caffeine consumption in pregnancy using the Caffeine Assessment Tool and a 24 h diet recall. By T. MOORE, S.M. BOYLAN, S.F.L KIRK, J.D. THOMAS and J.E. CADE, Centre for Epidemiology and Biostatistics, University of Leeds, Leeds, UK, LS2 9LN

Studies assessing caffeine consumption during pregnancy in relation to low birth-weight show inconsistency, in part due to limited assessment of caffeine intakes. The CARE (Caffeine and Reproductive Health) study being carried out in Leeds and Leicester is studying maternal caffeine intake and birth weight outcome.

The present study is using a detailed questionnaire, known as the Caffeine Assessment Tool (CAT), to measure habitual caffeine intake at different stages of pregnancy. In addition, 24 h dietary recalls are obtained at two stages during pregnancy (14–18 weeks and 28 weeks).

The aim of the present analysis was to calculate the amount of caffeine in the 24 h diet recall and to compare this with caffeine intake from the corresponding period of pregnancy assessed using the CAT.

In order to do this, caffeine values for coffee, tea, cola, drinking chocolate and chocolate were added to DANTE, our in-house dietary analysis database used for the analysis of the recalls.

The sample assessed was 178 CARE study subjects. A detailed computer algorithm was developed to calculate caffeine intakes from the CAT. Agreement between the two methods was assessed using a weighted kappa. Agreement between the methods was moderate, weighted kappa: 0.6 at 14–18 weeks and 0.4 at 28 weeks.

Seventeen obese (mean BMI 35.1 kg/m$^2$), but otherwise healthy, men underwent a residential trial of 9 weeks, with food provided daily throughout. After a 3 d period at maintenance ($1.6 \cdot RMR$), subjects were offered two diets at ad libitum for each for a 4-week period, as either an HPLC (30% protein, 4% carbohydrate, 66% fat by energy) or an HPMC (30% protein, 35% carbohydrate, 35% fat) diet, randomised in a cross-over design. There was another 3 d maintenance period between the HPLC and HPMC treatments. All meals were provided in excess and were the same energy density (5.5 MJ/kg).

Daily intakes were recorded by weight of food eaten. Daily EE was measured by heart-rate methodology. Body weight was measured daily and subjective fatigue was assessed hourly during waking hours, using a computerised visual analogue system.

There is some controversy in the literature as to whether low-carbohydrate weight-loss diets increase subjective fatigue, relative to medium-carbohydrate diets. For example, Butki et al. (2003) reported that when physically active participants reduce carbohydrate intake, they experience increased feelings of fatigue. Recent clinical experience from physicians has indicated that obese patients who follow low carbohydrate formulas or food diets frequently complain of light-headedness, weakness, and ease of fatigue (Phinney, 2004). Conversely, advocates of low-carbohydrate diets, such as the 'Atkins diet', talk about 'enhanced energy' and feelings of euphoria that occur as a result of a ketogenic state (Atkins, 2002). Glucose is the preferred primary fuel of the brain and this may provide a mechanistic link between low carbohydrate intake and any negative physiological or psychological symptoms as the brain switches to ketone bodies as replacement fuel. Furthermore, any physiological responses may be adaptive and alter once the transition in fuel source has been completed. The present study compares weight loss, energy expenditure (EE) and feelings of fatigue in obese, sedentary men consuming a high-protein, low-carbohydrate (HPLC) ketogenic diet and a high-protein, medium-carbohydrate (HPMC) non-ketogenic diet.

The CAT consistently found a higher % of women consuming caffeine and also a higher mean caffeine intake than the recall. The recall is limited due to potential underreporting and lack of detail.

Both the CAT and 24 h diet recall can be used to assess caffeine intake. The CAT, however, is a more useful tool as it measures habitual intake and collects detailed information on caffeine consumption.
The effect of a 12-week low-glycaemic index diet on heart disease risk factors and 24 h glucose profile in healthy middle-aged volunteers at risk of heart disease: a pilot study. By E. PHILIPPOU 1, J.E. MILTON 2, A.E. BRYNES 1 and G.S. FROST 3, 1Department of Nutrition and Dietetics, Imperial College London, Hammersmith Hospital Campus, London, UK; W12 OHS, 2Department of Metabolic Medicine, Imperial College London, London, UK; W12 OHS and 3School of Biomedical and Molecular Sciences, Guildford, Surrey, UK, GU2 7XH

The glycaemic index (GI) assesses the postprandial glucose response to carbohydrate ingestion. It is expressed as the percentage of the 2 h incremental area under the curve (IAUC) after consuming a food compared to a reference food (usually white bread) (Jenkins et al. 1981). Low-GI diets reduce cardiovascular risks (Frost et al. 1998). The most accepted GI values are published in the International Glycaemic Index Tables (Foster-Powell et al. 2002) and on our website hosted by the University of Sydney (www.glycaemicindex.com). More recently a small number of UK foods have been tested (Henry et al. 2005). However, if a food has not been tested, an estimated GI has to be assigned to avoid ‘artificially’ lowering the GI of the diet. The present pilot study reports on the proportion of estimated GI values being used in the calculation of diet GI.

We used food records from a pilot parallel-design study where fourteen subjects completed two 7 d diaries: one of the habitual diet and the other on a low- or high-GI diet. A list of CHO-containing foods and the % CHO from each food item were recorded. The contribution of each food item to the total diet was calculated. Published GI values were used as a first option or if not available, an estimation was made by the researchers (E.P., J.E.M. and A.E.B.) based on the GI of foods with a similar physical and chemical make up and knowledge of how other factors such as food processing would affect the GI. Where possible, standard recipes (Food Standards Agency, 2002) were used to calculate the food’s dietary GI (relative to a glucose standard) by summing (% CHO from food item/100) x GI of food. The percentage CHO from each source was calculated by summing the (CHO contribution from each source/total CHO contribution) x 100.

Results were not normally distributed, therefore non-parametric tests were used for analysis. Median (interquartile range; IQR) are presented. One subject was excluded due to his high alcohol intake. At baseline, there were no differences between the low-GI (n = 7; four females; BMI 28.6 (IQR 28.1–29.6) kg/m²; age 54.0 (IQR 49.0–58.0) years) and high-GI (n = 6; four females; BMI 33.2 (IQR 28.2–34.2) kg/m²; age 45.0 (IQR 39.0–50.0) years) groups. By week 12, the diet GI but not the diet glycaemic load (GL) was different between the groups. Over the 12-week period both groups significantly reduced their energy intake; however, only the low-GI group lost weight and reduced their waist circumference significantly. The cholesterol profile was not different between the groups. By week 12, the low-GI group had a significantly lower 24 h and overnight (8 h) glucose profile as measured by the CGMS which might suggest an improvement in hepatic insulin sensitivity resulting in a decrease in hepatic glucose output following meals (Thorburn et al. 1993). Raised glycaemia increases cardiovascular risk even at a normal glucose-tolerance range (Cutting et al. 1999). Since none of the other parameters differed between the groups, we recognise that weight loss might have masked any effects of diet GI.

### Baseline vs Week 12

<table>
<thead>
<tr>
<th></th>
<th>High GI (n = 6)</th>
<th>Low GI (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>IQR</td>
</tr>
<tr>
<td>Diet GI</td>
<td>54.5</td>
<td>9.8–57.0</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>242.1</td>
<td>197.6–268.9</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>79.0</td>
<td>75.6–822</td>
</tr>
<tr>
<td>Δ Weight (kg)</td>
<td>96.6</td>
<td>76.2–103.5</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>101.5</td>
<td>102.5–121.3</td>
</tr>
<tr>
<td>CGMS overnight AUC</td>
<td>7806</td>
<td>792–8841</td>
</tr>
</tbody>
</table>

For the habitual diet (n = 14) the mean CHO intake was 1497.3 g (IQR 1383.4–1756.5) g over 7 d and a GI value was assigned for 97.2 (IQR 96.8–97.3) % of this. A value of 25.6 (IQR 21.0–32.6) % CHO equal to 415.2 (IQR 360.1–444.2) g CHO used in GI calculations was estimated. The low- (n = 7) and high-GI (n = 7) groups consumed 1421.0 (IQR 1148.2–1479.5) g and 1374.2 (IQR 1116.8–1477.6) g CHO over 7 d respectively. For the low-GI group, 97.7 (IQR 96.6–98.2) % and 96.9 (IQR 94.8–97.4) % of the high-GI group of this CHO was assigned a GI value. Of this, 16.0 (IQR 12.7–21.9) % was found in the published GI values between low- and high-GI diets, suggesting published GI values are not biased.

The percentage of CHO used in the GI calculation, which is based on estimated GI values, is about 20%. Examples of foods include roast potatoes, certain breads, breakfast cereals, biscuits and cakes, emphasising the importance of further testing of food products and publication of values. No difference was found in the published GI values between low- and high-GI diets, suggesting published GI values are not biased.


An exploration into the proportion of carbohydrate used in the calculation of diet glycaemic index (GI) for which GI values are estimated. By E. PHILIPPOU 1, J.E. MILTON 2, A.E. BRYNES 1 and G.S. FROST 3, 1Department of Nutrition and Dietetics, Imperial College London, Hammersmith Hospital Campus, London, UK; W12 OHS and 2School of Biomedical and Molecular Sciences, Guildford, Surrey, UK, GU2 7XH

**Note:** This text is a research article discussing the effect of a low-glycaemic index diet on heart disease risk factors and 24 h glucose profile in healthy middle-aged volunteers. It includes a pilot study comparing the effects of altering diet GI on cardiovascular risks in addition to traditional advice. The study found that a low-GI diet was associated with reduced cardiovascular risks and improved hepatic insulin sensitivity. The results suggest that weight loss might have masked any effects of diet GI.
Glycaemic properties of quinoa (Chenopodium quinoa Willd.), By V. ZEVALLOS¹, G. GRIMBLE¹ and L.I. HERENCIA².¹ Department of Health and Human Science, London Metropolitan University, 166-220 Holloway Road, London, UK, N7 8DB and ²Departamento de Fitotecnia Vegetal, Universidad Politécnica de Madrid, Ciudad Universitaria 28440, Madrid, Spain

Quinoa is an Andean crop of the Chenopodiaceae family which has been used as a basis of human diet since ancient times. In addition to its good nutritional content, it could form part of a diet for individuals with coeliac disease. A recent study reported on the glycaemic index (GI) of starch from gluten-free cereals and minor cereals and concluded that starch from quinoa was a suitable alternative to traditional starch sources (Berti et al. 2004). In view of present interest in the concept of the using GI and glycaemic load (GL) of foods as a means of controlling hyperglycaemia (Braynes et al. 2003), we have investigated the GI of two quinoa cultivars, in healthy human subjects, using a glucose meal for comparison.

The GI and GL of quinoa (brands 1 and 2) was analysed in eight healthy university students (four males and four females) aged between 21 and 35 years, with BMI of 22.9 (SD 3.7) kg/m². The procedure followed that recommended by the Food and Agriculture Organization/World Health Organization (1998). Cooked quinoa was compared with a reference food (glucose). Subjects ingested equivalent amounts (50 g) of available carbohydrate with 200 ml water (tap water) and glucose concentration was measured in finger prick blood samples at 0, 15, 30, 45, 60, 90 and 120 min thereafter, using a calibrated AutoLanced, Blood Glucose Electrode (MediSense Optium Plus). Paired sample t tests for GI and GL of quinoa 1 and quinoa 2 showed that the GI and GL did not differ between brands (P<0.05; GI 25.3 (SD 8.5) %; 23 (SD 8.7) %; GL (18.3 (SD 6.2) %; 14.7 (SD 5.6) %).

The present study is the first to examine the glycaemic effects of quinoa using the Food and Agriculture Organization/World Health Organization (1998) in vivo method. According to these results quinoa can be considered as a moderately low GI and GL food. Because of the sensory acceptability of quinoa, this might allow its use as part of a diet to control postprandial hyperglycaemia.

The introduction of semi-skimmed milk in the UK in the early 1980s saw a shift in consumer preference away from whole milk with semi-skimmed consumption overtaking whole milk by 1995 (Department for Environment, Food and Rural Affairs, 2001). Dietary data collections in the MRC National Survey of Health and Development (NSHD) (1946 British Birth Cohort) before and after the introduction of semi-skimmed milk allowed further examination of this change. At its introduction the advantage of semi-skimmed milk extended beyond being a lower-fat option; being priced at 1 p cheaper per pint (19 v. 20p). In view of present interest in the concept of the using GI and glycaemic load (GL) of foods as a means of controlling hyperglycaemia (Braynes et al. 2003), we have investigated the pattern of consumption of milk types across different social classes between 1982 and 1999 using the NSHD cohort.

From 5d food diaries collected in 1982 (n 2552), 1989 (n 2126) and 1999 (n 1649) the mean daily amount of each milk type was calculated as a percentage of the total milk consumed by each subject. Social class was also reported. Chi-squared tests were used to establish if significant associations between social class and milk type existed.

In 1982 semi-skimmed milk consumption was not reported by any subject. Skimmed milk consumption was rare (n 112, 4%). Whole milk was consumed by 95% of subjects. Results for 1989 (see Table) showed significant associations between type of milk consumed and social class (P<0.001, P<0.001 and P<0.005 for whole, semi-skimmed and skimmed milk respectively). However, more individuals in all social classes were drinking semi-skimmed milk; 78% were high- or medium-level consumers. The consumption of semi-skimmed milk decreased with descending social class. Of social class V, 75% were non-consumers. Of medium level consumers and those who only drank semi-skimmed, the reverse was true, with the highest percentage of consumers in higher social classes. For whole milk highest consumption was most common in lower social classes.

The Table shows the percentages in each social class across varying levels of milk consumption and milk types in 1989 in the NSHD.

<table>
<thead>
<tr>
<th>Social class</th>
<th>Whole milk consumption</th>
<th>Semi-skimmed milk consumption</th>
<th>Skimmed milk consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole</td>
<td>Medium</td>
<td>High</td>
</tr>
<tr>
<td>I</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>31.31</td>
<td>31.21</td>
<td>35.46</td>
</tr>
<tr>
<td>III</td>
<td>31.90</td>
<td>31.48</td>
<td>35.61</td>
</tr>
<tr>
<td>IV</td>
<td>31.90</td>
<td>31.48</td>
<td>35.61</td>
</tr>
<tr>
<td>V</td>
<td>31.90</td>
<td>31.48</td>
<td>35.61</td>
</tr>
<tr>
<td>Total</td>
<td>33.83</td>
<td>32.71</td>
<td>38.67</td>
</tr>
</tbody>
</table>

In 1999 the patterns remained similar and the associations with social class were still significant for semi-skimmed and whole milk (P<0.01 and P<0.001 respectively). However, more individuals in all social classes were drinking semi-skimmed milk; 78% were high- or medium-level consumers. These findings are consistent with previous research reporting less healthy dietary behaviours in lower social classes. The present study suggests that price was not a driving force in making a specific dietary choice, namely type of milk. An increased understanding of the motivation for change to healthier options is needed for the effective implementation of future health strategies.

Docosahexaenoic acid but not eicosapentaenoic acid mediates the anti-inflammatory effect in dendritic cells via peroxisome proliferator-activated receptor-γ-dependent pathway(s). By C. REYNOLDS1, E. DRAPER2, C.E. LOSCHER3 and H.M. ROCHE1, 1Nutrigenomics Research Group, Department of Clinical Medicine, Institute of Molecular Medicine, St James’s Hospital, Dublin 8, Republic of Ireland and 2Immunomodulation Research Group, School of Biotechnology, Dublin City University, Dublin 9, Republic of Ireland

Transcriptional regulation of splice variants of the SLC30A5 human zinc transporter in intestinal (Caco-2) and placental (JAR) cell lines. By K.A. JACKSON1, E.D. O’NEILL, J.C. MATHERS2 and D. FORD1,2

Institute for Cell and Molecular Biosciences and 2Human Nutrition Research Centre, Newcastle University, UK, NE2 4HH

Regulation of Zn transporters may contribute to maintenance of whole-body and cellular Zn homeostasis via regulated Zn influx and efflux and sequestration into organelles. The sub-cellular distribution of the Zn transporter SLC30A5, which has been detected at the apical membrane of the enterocyte and the placental syncytiotrophoblast membrane, indicates a potential role in Zn nutrition. Two splice variants of the SLC30A5 gene code for proteins of 523 amino acids (variant A) and 765 amino acids (variant B), which may potentially have distinct roles in Zn homeostasis. We sought to examine the sub-cellular distribution of these splice variants by expression as N-terminal fusions to green fluorescent protein (GFP) in mammalian cells. The splice variants have different first exons, with that of variant B being 5’ to that of variant A, so it is possible that each variant is expressed from a different promoter. We determined the effect of changes in extracellular Zn concentration in intestinal (Caco-2) and placental (JAR) cells on the activity of a reporter gene under the control of the two putative splice variant-specific promoters.

The two SLC30A5 splice variants were expressed individually in CHO cells by transient transfection of plasmid constructs produced using the vector EGFP-N (Clontech). Cells were fixed 48 h after transfection and viewed by confocal laser scanning microscopy. Variant A appeared to be localised throughout the cell and also co-localised with wheat-germ agglutinin, indicating expression at the plasma membrane. In contrast, variant B showed a pattern of distribution consistent with localisation in the Golgi apparatus.

Reporter constructs, comprising regions directly upstream of the first exons of SLC30A5 splice variants A (–2863 to +63) and B (–2145 to +50) in pBluescript SK (Invitrogen), were transiently transfected into Caco-2 and JAR cells grown under standard conditions. Reporter gene (β-galactosidase) activity was measured in cell lysates 48 h post-transfection and data were analysed by Student’s t-test. The activity of the variant B upstream region was significantly greater than negative control in both cell lines but the variant A upstream region appeared to be inactive (Caco-2, variant A 0.97 (se 0.01), negative 1.00 (se 0.02), variant B 12.30 (se 0.18), negative 1.00 (se 0.04), P<0.001; JAR, variant A 0.95 (se 0.01), negative 1.00 (se 0.05), variant B 5.52 (se 0.23), negative 1.00 (se 0.15), P<0.001) indicating expression of SLC30A5 splice variants is from a single promoter upstream of the first exon of variant B.

The effect of extracellular Zn concentration on the activity of a series of 5’ deletions of the SLC30A5 promoter region was measured in Caco-2 cells. Reporter gene (β-galactosidase) activity was measured in cell lysates 48 h post-transfection and data were analysed by Student’s t-test. The activity of the variant B upstream region was significantly greater than negative control in both cell lines but the variant A upstream region appeared to be inactive (Caco-2, variant A 0.97 (se 0.01), negative 1.00 (se 0.02), variant B 12.30 (se 0.18), negative 1.00 (se 0.04), P<0.001; JAR, variant A 0.95 (se 0.01), negative 1.00 (se 0.05), variant B 5.52 (se 0.23), negative 1.00 (se 0.15), P<0.001) indicating expression of SLC30A5 splice variants is from a single promoter upstream of the first exon of variant B.

In conclusion, the two splice variants of the SLC30A5 gene show differences in sub-cellular localisation, suggesting distinct roles in Zn homeostasis, but are controlled by a single promoter upstream of the longer transcript, which shows cell-line-specific transcriptional regulation by Zn independent of MRE in this region and involving (an)element(s) in the region –154 to +50.

The present study was funded by the MRC (studentship to K.A. J).
The cellular response of normal human colonocytes to folate deficiency in vitro: proteomic and functional analyses. By S.J. DUTHIE, C.S. BESTWICK, Y. MAVROMMATIS, M.P. MOYER and L.P. PIRIE, Nutrition and Epigenetics Group, Division of Vascular Health, Rowett Research Institute, Aberdeen, UK; AB21 9SB, Molecular Nutrition Group, Division of Gut Health, Rowett Research Institute, Aberdeen, UK; AB21 9SB and INCELL Corporation, San Antonio, CA, USA

Low folate intake is associated with an increased risk of colorectal cancer. Folate is crucial for normal cell metabolism via its ability to donate 1-C units. In the present study we used a combined proteomics and functional approach to investigate pathways affected by folate deficiency in human colonocytes. NCM460 colon cells, which retain normal mucosal characteristics including expression of villin and cytokeratins were used. Total protein from cells grown for 14 d in folate-depleted or -supplemented medium were separated by two-dimensional gel electrophoresis and analysed using PDQuest (Bio-Rad, Hemel Hempstead, UK). Proteins that differed significantly between treatments (t test) were identified by Mascot search following Nano liquid chromatography–MS–MS analysis. Intracellular folate was measured by RIA. Proliferation was determined by cell number. DNA strand breakage, misincorporated uracil and repair of oxidative DNA damage (10 μM H₂O₂) were determined by single-cell gel electrophoresis.

In excess of 100 spots, differences between treatments were identified and broadly categorised according to biochemical function. Treatment effects were seen on major metabolic pathways related to protein biosynthesis and energy metabolism and on markers of cell proliferation (for example, PCNA (down 67%)), DNA repair (for example, XRCC5 (up 1.5-fold), MSH2 (up 1.5-fold), ATP-dependent DNA helicase Q1 (down 39%)) and apoptosis (for example, BAG family chaperone protein (down 25%), DIABLO homologue (up 2-fold) and voltage-dependent anion channel protein 1 (up 1.5-fold)). The effect of folate deficiency on functional markers of folate status, genomic stability and DNA repair are shown in the Table. Intracellular folate was depleted more than 95% in cells cultured for 14 d in folate-deficient medium. Folate deficiency impaired cell proliferation, elevated uracil misincorporation and increased endogenous DNA strand breakage. Folate-depleted cells were unable to repair H₂O₂-induced oxidative DNA strand breakage over 4 h as efficiently as folate-supplemented cells. Overt markers of apoptosis such as caspase 3 and 7 activity or cell morphology were unaffected by folate status but mitochondrial membrane potential, determined by flow cytometric analysis of JC-1 monomer and aggregate fluorescence, was significantly decreased (see Table).

Table 1.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Control Mean</th>
<th>Control SEM</th>
<th>Folate deficient Mean</th>
<th>Folate deficient SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folate (ng/10² cells)</td>
<td>2.39</td>
<td>0.08</td>
<td>0.02*</td>
<td>0.02</td>
</tr>
<tr>
<td>Cell number (10⁶ cells/flask)</td>
<td>45.9</td>
<td>7.4</td>
<td>159*</td>
<td>3.3</td>
</tr>
<tr>
<td>DNA strand breaks (arbitrary units)</td>
<td>41.7</td>
<td>4.9</td>
<td>938*</td>
<td>8.0</td>
</tr>
<tr>
<td>Misincorporated uracil (arbitrary units)</td>
<td>12.4</td>
<td>3.1</td>
<td>500*</td>
<td>8.9</td>
</tr>
<tr>
<td>DNA break repair (% recovery/4h)</td>
<td>23.8</td>
<td>2.4</td>
<td>63*</td>
<td>1.6</td>
</tr>
<tr>
<td>Apo (cells)</td>
<td>23.3</td>
<td>2.1</td>
<td>330*</td>
<td>1.7</td>
</tr>
</tbody>
</table>

*P<0.001 by Student’s t test (n = 4–8).

In conclusion, proteomic and functional analyses show that folate deficiency induces changes in several pathways related to malignant transformation. Folate deficiency impaired cell proliferation and induced genomic instability, seen as increased DNA damage and altered DNA repair in normal human colon cells. In addition, there was a shift towards a more stress-sensitive or possibly pro-apoptotic state.

Oxidised low-density lipoprotein- and oxysterol-induced cell death in U937 and HL-60 blood cells. By S. LORDAN, Y.C. O’CALLAGHAN and N.M. O’BRIEN, Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland

LDL, the major carrier of plasma cholesterol, may undergo oxidation in vivo resulting in the formation of oxidised LDL (oxLDL). OxLDL plays a key role in the generation and progression of atherosclerosis and has been shown to contain a range of oxidised lipid components including oxysterols. Oxysterols may be involved in the early events of atherosclerosis observed during the development of the disease, including the induction of apoptosis in the cells of the vascular wall and in monocytes and macrophages (Bertheret et al. 2005). The objective of the present study was to investigate the cytotoxicity of oxLDL and the oxysterols, 7β-hydroxycholesterol and cholesterol-5β,6β-epoxide, in two human blood cell lines; U937 and HL-60 cells.

Cells were exposed to ox-LDL or the oxysterols, 7β-hydroxycholesterol (7β-OH) and cholesterol-5β,6β-epoxide (β-epoxide) (30 μM), for 24 h. Cell viability was assessed by the fluorescein diacetate–ethidium bromide assay and apoptotic nuclei were quantified following staining with Hoechst 33342. The induction of apoptosis was also monitored by the DNA fragmentation assay and the expression of the anti-apoptotic protein, Bcl-2, was investigated through Western blot analysis.

Table 2.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Control</th>
<th>Native LDL</th>
<th>oxLDL</th>
<th>oxLDL</th>
<th>oxLDL</th>
<th>30 μM 7β-OH</th>
<th>30 μM β-epoxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Viable cells</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td>U937</td>
<td>94.5</td>
<td>90.7</td>
<td>82.5</td>
<td>61.4</td>
<td>66.2</td>
<td>73.5</td>
<td>31</td>
</tr>
<tr>
<td>HL60</td>
<td>94.4</td>
<td>90.8</td>
<td>61</td>
<td>50.1</td>
<td>55.5</td>
<td>33.4</td>
<td>4.3</td>
</tr>
<tr>
<td>% Apoptotic cells</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td>U937</td>
<td>7.5</td>
<td>8.3</td>
<td>18.2</td>
<td>23.4</td>
<td>22.6</td>
<td>16.9</td>
<td>24.9</td>
</tr>
<tr>
<td>HL60</td>
<td>7.6</td>
<td>7.8</td>
<td>39.2</td>
<td>43.0</td>
<td>43.2</td>
<td>16.9</td>
<td>15.2</td>
</tr>
</tbody>
</table>

Values represent the mean of three independent experiments. *P<0.05, relative to the control.

Following 24 h incubation with oxLDL, there was a significant (P<0.05) increase of apoptotic nuclei in both the U937 and HL-60 cells. DNA fragmentation confirmed apoptosis in the oxLDL-treated HL-60 cells. The oxysterols 7β-OH and β-epoxide have previously been shown to induce apoptosis in U937 cells (Ryan et al. 2004). Similarly, in the present study, treatment with 30 μM oxysterols significantly (P<0.05) decreased the viability and increased apoptosis in U937 and HL-60 cells. Additionally, the DNA fragmentation assay revealed an apoptotic pattern in the two cell types. The U937 and HL-60 cells both expressed Bcl-2 in the control and native LDL-treated samples and its expression was decreased in the oxLDL- and oxysterol-treated samples. In conclusion, the HL-60 cells appear to be more sensitive to oxLDL while the individual oxysterols were more effective at inducing apoptosis in the U937 cells. These variations suggest that these compounds may activate different metabolic pathways, while the cell type also appears to play a significant role in influencing the mode and degree of cell death.

The present study was supported by the Higher Education Authority, Dublin, Republic of Ireland.


Postprandial ghrelin suppression is exaggerated following major surgery: implications for nutritional recovery. By M. NEMATY1,2, A.E. BRYNES1, P.J. HORNICK1, S.J. BRETT3 and G.S. FROST3.

1Nutrition and Dietetic Research Group, Hammersmith Hospital, Imperial College London, UK, W12 0HS. 2Faculty of Medicine, Mashhad University of Medical Sciences, Mashad, Iran. 3Cardiothoracic Surgery, NHUL, Hammersmith Hospital, Imperial College London, UK, W12 0HS. 4Division of Surgery, Anaesthetics and Intensive Care, Hammersmith Hospital, Imperial College London, UK, W12 0HS and 5School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey, UK, GU2 7XH.

Meeting patients’ nutritional requirements and preventing malnutrition is a challenge following major surgical procedures. Peptide hormones released from the gut have been reported to affect appetite (Wren et al. 2001; Frost et al. 2006) and may play a role in the altered food consumption of sick patients (Nemyt et al. 2005). The role of ghrelin in nutritional recovery after non-gastrointestinal major surgery is unknown. We used coronary artery bypass grafting (CABG) as an example of anticipated good recovery after major surgery.

Seventeen patients undergoing CABG (age 70.1 (SEM 2.2) years; BMI 29.1 (SEM 1.4) kg/m2; fifteen male) underwent fasting and postprandial (45 min after standard test breakfast) blood sampling pre-operatively (pre-op; day 0), post-operatively (post-op; day 6) and at follow-up (day 40). Changes in food intake, biochemical and anthropometric markers of nutritional status were recorded. A comparison was made to seventeen matched healthy controls (age 70.6 (SEM 2.3) years; BMI 28.4 (SEM 1.3) kg/m2).

We observed an increased post-op fasting ghrelin compared with pre-op (pre-op 402 (SEM 42) pmol/l v. post-op 642 (SEM 97) pmol/l; v. follow-up 603 (SEM 94) pmol/l; ANOVA (Fig. 1); P<0.05). Exaggerated postprandial suppression of ghrelin post-op (Δ pre-op 10 (SEM 5) pmol/l v. Δ post-op –152 (SEM 45) pmol/l; Δ follow-up –189 (SEM 65) pmol/l; P<0.05) was accompanied with a 50% reduction in food intake (post-op 5.0 (SEM 0.5) MJ/day v. controls 2.5 (SEM 0.3) MJ/day; P=0.001), leading to a 4% weight loss and a 5% reduction in muscle arm circumference loss over the length of follow-up. Using a visual analogue scale, patients post-op reported a better hunger rating than controls (post-op 23 (SEM 3) mm v. follow-up 45 (SEM 8) mm; controls 23 (SEM 3) mm; P=0.02 and P=0.008, respectively).

The present data support the hypothesis that prolonged changes in fasting and postprandial plasma ghrelin concentrations are associated with impaired nutritional recovery after CABG. Those findings are likely to be applicable to similar patient groups undergoing major surgery.


Bioavailability of carotenoids determined by a Caco-2 cell model. By L.P. O’SULLIVAN, L. RYAN and N.M. O’BRIEN, Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland.

Carotenoids are a group of fat-soluble pigments that are widespread in nature. Recent research has shown that diets rich in carotenoids may reduce the risk of CVD, certain cancers and age-related macular degeneration. The xanthophyll carotenoids, lutein and zeaxanthin, are proposed to play an important role in screening high-energy blue light and acting as potent antioxidants in the macula of the eye (Alvez-Rodrigues & Shao, 2004). The main objective of the present study was to establish a cost-effective in vitro model to determine the bioavailability of carotenoids using Caco-2 (human colon adenocarcinoma) cells. Caco-2 cells were adjusted to a density of 1.25·105 cells/ml on transwell plates (six-well plate; 24 mm diameter; 0.4 µm pore size membrane) and grown for 21–25 days until an intact differentiated monolayer was obtained. Carotenoid concentrations were determined spectrophotometrically and were delivered to cells using the ‘Tween 40’ method (During et al. 1998). Media was supplemented with oleic acid, glycerol and taurocholate to stimulate the cells to produce chylomicrons. Plates were incubated for 16 h after which apical media, basolateral media and cell monolayers were harvested and extracted twice with hexane–ethanol–acetone (50:25:25, by vol.). The carotenoid content of the media and cell extracts were quantified using HPLC (Hart & Scott, 1995).

Fig. 1. Percentage transfer of lutein and zeaxanthin, through an intact Caco-2 cell monolayer, into the basolateral chamber after a 6 h incubation. Values are means, with their standard errors represented by vertical bars (n=3).

The present study clearly demonstrated that Caco-2 cells were able to absorb and secrete carotenoids. The % xanthophyll that remained in the apical chamber was similar for both carotenoids. A greater amount of lutein remained in Caco-2 cells compared with zeaxanthin. Fig. 1 displays the % carotenoids that was absorbed by the monolayer, incorporated into chylomicrons and secreted into the basolateral chamber of the transwell plate. This model mimics the intestinal absorption of carotenoids in vivo, thus giving an indication of the bioavailability of each carotenoid. The % transport of lutein was 2–11% and the % transport for zeaxanthin was 5–12%. From Fig. 1, it appears that there is a decreasing % transfer with increasing concentration. However, the results are expressed as a % of the concentration that was added, and when expressed in terms of µg in the basolateral chamber, the values were similar for all concentrations. This may suggest that there is a certain degree of saturation occurring.

The present study was funded by Science Foundation Ireland.


References


Transcriptional regulation of the microsomal triacylglycerol transfer protein by members of the nuclear hormone receptor superfamily. By T. VALLIM, A. SALTER and A. BENNETT, Schools of Biomedical Sciences and Biosciences, Institute of Clinical Research, University of Nottingham Medical School, QMC, Nottingham, UK, NG7 2UH

Microsomal triacylglycerol transfer protein (MTP) is essential for the secretion of apo-lipoprotein B-containing lipoproteins. Previous studies have shown that elevated dietary fats (Bennett et al. 1995) and cholesterol (Bennett et al. 1996) increase MTP expression in the liver, with saturated fats showing a more pronounced effect. We have investigated transcription factors that bind to the proximal MTP promoter to identify potential targets for dietary-mediated transcriptional regulation. Promoter analysis revealed three conserved direct repeat-1 (DR-1) motifs at positions −183 to −170 (A), −170 to −157 (B) and −137 to −124 (C) relative to the translational start site, capable of binding nuclear hormone receptors such as PPAR, and hepatocyte nuclear factor 4 alpha (HNF4α). Using cell culture experiments coupled with site-directed mutagenesis and electrophoresis mobility shift assays (EMSA), two crucial DR-1 elements were identified.

The present results show that mutating the A and B DR-1 motifs had no effect on basal promoter activity, while mutating the C DR-1 motif reduced promoter activity by 90% (p < 0.002). However, the B and C DR-1 mutations reduced the effects of HNF4 overexpression by 40 and 75% respectively. Mutating both the B and C sites together completely ablates induction of transcription by HNF4. EMSA supershifts confirm affinity of HNF4 for the B and C elements, whereas mutations completely abolish any binding. Another factor also shown to be able to bind B and C DR-1 was PPARγ. However, the ability of PPARγ to regulate promoter activity requires a liver-specific context as the effect was only seen in hepatic cell line MacARH 7777. PPARγ increased promoter activity 7-fold (p < 0.002), and again both B and C mutations were required to completely abolish the increase. This data was again supported by EMSA supershifts showing both B and C DR-1 elements are able to bind PPARγ.

The present results indicate that multiple transcription factors are capable of binding to the two DR-1 elements in the proximal MTP promoter. Dietary regulation of transcription of the MTP gene may involve a change in the identity of the transcription factors occupying these DR-1 elements.

This project is supported by a studentship (for T.V.) awarded by the British Heart Foundation.


Sulforaphane as a histone deacetylase inhibitor. By K.F. CHAMBERS, M. TRAKA and R.F. MITHEN, Institute of Food Research, Norwich Research Park, Colney, Norwich, UK, NR4 7LA

Dietary protein restriction in the pregnant rat induces altered epigenetic regulation of the glucocorticoid receptor and peroxisomal proliferator-activated receptor α in the heart of the offspring which is prevented by sulforaphane (SF) treatment following SF exposure. In the present study, pregnant Sprague-Dawley rats were subjected to either protein-restricted (PR) diet with folic acid (+F) from conception to delivery, or standard chow (AIN-76A) during lactation. Litters were reduced to eight at birth and offspring were weaned onto control diet at postnatal day 28 and killed 6 d later. Methylation of the glucocorticoid receptor (GR) and peroxisomal proliferator-activated receptor α (PPARα) promoters was determined in the hearts (four rats per maternal diet, one rat per litter) by methylation-sensitive real-time PCR.

The aim of the present study was to investigate whether SF acts as an HDAC inhibitor in both mouse tissue and in human primary prostatic tissue cultures following SF treatment. In the mouse study, C57BL/6J mice (n = 5) received SF at 1 mol/g diet and the control group (n = 5) received the same diet without SF. The diet was continued for 2 weeks until the animals were killed. Small intestine, spleen and lung tissues were collected from the mice and protein was extracted for further analysis of histone H3 and H4 acetylation by Western blotting. The most prominent change was seen in the spleen tissue, where acetylated histone H3 was consistently increased (2–43-fold) in mice fed with SF. In contrast, histone H4 was not increased. Cells derived from patients with benign prostatic hyperplasia are currently underway to confirm these results.

These results support the suggestion that prenatal undernutrition may induce persistent changes in energy substrate metabolism and response to glucocorticoids in the heart, as in the liver (Lillycrop et al. 2005), by altering the epigenetic regulation of PPARα determined by nutrient availability before birth, including maternal folic acid intake during pregnancy. If true in man, the consequences for later cardiomyopathy and therapeutic implications require investigation.
Reactive oxygen species generation in mismatch repair-proficient and -deficient colorectal cancer cell lines exposed to butyrate. By J.M. COXHEAD1, W. BAL1, E.A. WILLIAMS2 and J.C. MATHERS3, 1Human Nutrition Research Centre, School of Clinical Medical Sciences, Newcastle University, UK, NE2 4HH and 2Human Nutrition Unit, Clinical Sciences (North), University of Sheffield, UK, S3 7AU

Hereditary non-polyposis colorectal cancer (HNPCC) is associated with mismatch repair (MMR) gene mutations (primarily hMLH1 and hMSH2). Loss of function of hMLH1, often due to hypermethylation of the CpG island in the promoter region of the gene, is also seen in 15–25% sporadic colorectal cancer (CRC) cases. Butyrate is an 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. Butyrate has been reported to increase reactive oxygen species (ROS) in the HT29 CRC cell line and to induce apoptosis (Giardina & Inan, 1998).

ROS generation was assessed in HCT116 (hMLH1-ve) and HCT116chr3 (hMLH1+ve) CRC cell lines at 6, 12 and 48h following butyrate exposure (0–5 mM). Both cell lines were cultured side by side on ninety-six-well plates (six wells per treatment group) with two plates per time point. Plate 1 was used to determine accumulation of viable cells using the neutral red (NR) assay (Dutt, 1980) and plate 2 to assess ROS using 2,7-dichlorodihydrofluorescein diacetate (Royall & Ischiropoulos, 1993) which, upon oxidation, yields the fluorescent compound dichlorofluorescein. Fluorescence values were divided by NR values to give ROS production relative to viable cell number. Statistical analysis was carried out using ANOVA with post hoc analysis using the Dunnett test. P≤0.05 was considered significant.

Fig. 1. ROS production in MMR-proficient and -deficient CRC cells following 48h exposure to 0.5–5 mM-butyrate. Relative ROS expressed as percentage of control (no butyrate treated) cells.

To establish if the SelP SNP is functional, we analysed the response to Se supplementation in thirty healthy volunteers by measuring several Se-related outcome measures in blood. Volunteers with the appropriate genotype were supplemented with 100µg Se/d as sodium selenite for 6 weeks and blood samples were collected before, and after, supplementation and during a 6-week wash-out period. Western blot analysis of plasma samples using polyclonal antibodies raised against human SelP peptides revealed two bands of approximately 60 and 50 kDa. The relative proportions of these two bands depended on the volunteer genotype and Se status. Moreover, females with the GG genotype had significantly higher erythrocyte thioredoxin reductase 1 concentrations compared with females with the GA genotype or with males. Overall, these results suggest that the SNP located in the SelP coding region at position 234 is functional and can influence the response to Se supplementation.

A polymorphism in the coding region of human selenoprotein P gene influences the response to selenium supplementation in healthy volunteers. By C. MEPLAN1, K. CROSLEY1, F. NICOL1, G.J. BECKETT1, J.R. ARTHUR2, J.C. MATHERS2 and J.E. HESKETH1, 1Institute of Cell and Molecular Bioscience, 2Human Nutrition Research Centre, School of Clinical Medical Sciences, Newcastle University, Framlington Place, Newcastle upon Tyne, UK, NE2 4HH, 3Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, UK, AB21 9SB and 4Clinical Biochemistry, University of Edinburgh, The Royal Infirmary of Edinburgh, St Little France Crescent, Little France, Edinburgh, UK, EH16 4SA

Se intake in the UK population is currently below recommended levels. Se status can be raised by supplementation and in some studies this has been shown to reduce the risk of cancers and mortality from HIV infection (Rayman, 2005). In eukaryotes, the biological functions of Se are exerted by selenocysteine (Sec), an amino acid constitutive of selenoproteins. These proteins have key roles in many areas of metabolism including the response to oxidative stress, immune function and thyroid metabolism. Selenoprotein synthesis depends on Se availability from the diet. One of these proteins, selenoprotein P (SelP), is unique, containing several Sec per molecule (ten Sec in the human SelP), in contrast to one Sec in all other selenoproteins. SelP is expressed in several tissues including the liver and the brain and plays a pivotal role in Se supply since it is secreted into plasma from the liver and delivers Sec to other organs to ensure selenoprotein expression (Méplan et al. 2006). To determine if genetic variations in SelP could influence the Se bioavailability, we screened its gene for novel polymorphisms (single nucleotide polymorphisms; SNP) and identified a G/A SNP in the coding region of the protein which results in an amino-acid change, at position 234. Recent work confirms the existence of this SNP (Al-Taie et al. 2004). The prevalence of the SNP in three major ethnic groups of the UK population is shown in the Table.
Influences of gender and genetic variation in the 3’ untranslated region of the glutathione peroxidise 4 gene on the response to selenium supplementation in healthy volunteers. By C. MEPLAN1, K. CROSLEY2, F. NICOL3, J.R. ARTHUR2, J.C. MATHERS2 and J.E. HESKETH1,
1Institute of Cell and Molecular Bioscience, 2Human Nutrition Research Centre, School of Clinical and Medical Sciences, Newcastle University, Framlington Place, Newcastle upon Tyne, UK, NE2 4HH and 3Rowett Institute, Greenburn Road, Bucksburn, Aberdeen, UK, AB21 9SB

The phospholipid hydroperoxide glutathione peroxidise 4 (PHGPx or GPX4) is an antioxidant selenoprotein involved in the prevention of lipid peroxidation at the cell membrane and which has been implicated in leukaemone metabolism (Briegel-Flohi, 1999). Data from animal studies showed that GPX4 is required for embryonic development and for the protection against chemically induced oxidative damage. The protein also plays a crucial role in spermogenesis.

In Caucasians, a C/T polymorphism (single nucleotide polymorphism; SNP) was originally identified in the 3’ untranslated region of the GPX4 gene, located near to the region corresponding to the selenocysteine insertion sequence (SECIS) structure (Villette et al. 2002). Since the SECIS is an RNA element required for selenocysteine incorporation during selenoprotein synthesis, genetic variation in this region could affect the synthesis of GPX4. Having observed that the SNP was present in three major ethnic groups of the UK population (Caucasian, South Asian and Chinese) (Méplan et al. 2006), we carried out an intervention trial with healthy volunteers to assess the response to Se supplementation and its modulation by gender and by the SNP in GPX4. Thirty healthy volunteers with the appropriate genotype were supplemented for 6 weeks with 100μg Se/d as sodium selenite. Blood samples were taken before, and after, supplementation and during the 6-week wash-out period to measure Se-related parameters. Lymphocytes were isolated on a Histopaque-1077 gradient for protein analysis. GPX4 protein concentrations were measured by ELISA and plasma GPX3 activity was determined by enzymatic assay.

Women had significantly higher lymphocyte GPX4 protein concentrations compared with men. This gender effect could be explained by the specific role of GPX4 in spermogenesis and the extra demand that this places on body Se in males. The response of plasma GPX3 to supplementation, and the subsequent response to wash-out, were greater in volunteers of the CC genotype compared with individuals carrying two T alleles. Thus, it appears that a polymorphism in one selenoprotein gene can affect the activity of another selenoprotein. This is consistent with the hypothesis of a hierarchy in prioritisation of Se for selenoprotein synthesis (Hesketh & Villette, 2002) so that use of limited Se supply for the synthesis of one selenoprotein is at the expense of another selenoprotein.

We thank the Food Standards Agency for financial support (N05041). J. R. A.’s laboratory is funded by the Scottish Executive Environment and Rural Affairs Department (SEERAD).

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Effect of conjugated linoleic acid isomers on plasma lipoproteins and hepatic low-density lipoprotein receptor mRNA concentration in the hamster. By E. TARLING, K. RYAN, S. STEENSON, J. LAKER, A. BENNETT and A. SALTER, Schools of Biosciences and Biomedical Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough, UK, LE12 5RD

Considerable evidence suggests that in certain species trans-10, cis-12 (10,12-CLA) conjugated linoleic acid (CLA) reduces body fat deposition. However, it has recently been reported in human subjects that 10,12-CLA increases plasma LDL concentrations, relative to the cis-9, trans-11 (c9,t11)-CLA isomer (Tricon et al. 2004). In the present study we have compared the effects of supplementing the diet with 0.25% (w/w) of rapeseed oil (control), 10,12-CLA or c9,t11 on plasma lipoprotein concentrations in hamsters. Animals were fed either normal rodent chow or chow supplemented with 17.5% fat, formulated to represent the quantity and quality of fatty acids found in a typical ‘Western’ diet (also including 0.2% cholesterol). Diets were fed for 6 weeks, after which animals were killed and plasma lipoprotein concentrations were separated by preparative ultracentrifugation and analysed for cholesterol concentration. Hepatic LDL receptor (LDLr) and β-actin mRNA were measured by quantitative PCR. LDLr mRNA concentration was then expressed relative to the β-actin mRNA, which was not affected by diet.

While no effect of CLA was seen on body-weight gain, 10,12-CLA specifically reduced the concentration of liver weight (P<0.05). LDLr mRNA was also associated with an increase in liver weight (P<0.001). The cholesterol concentration (mmol/L) of the major lipoprotein fractions and the atherogenic lipoprotein (VLDL+intermediate-density lipoprotein+LDL)anti-atherogenic HDL fraction ratio (Ather ratio) are shown in the Table. Data were analysed by two-way ANOVA with background diet (D) as one factor and CLA effects, are shown together with their standard errors of the difference.

<table>
<thead>
<tr>
<th></th>
<th>Chow Western fat</th>
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<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Control 10,12-CLA</td>
<td>Control 10,12-CLA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>0.11</td>
<td>0.06</td>
<td>0.17</td>
<td>0.64</td>
<td>0.48</td>
<td>0.06</td>
<td>0.06</td>
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<tr>
<td>LDL</td>
<td>0.23</td>
<td>0.18</td>
<td>0.18</td>
<td>0.65</td>
<td>0.45</td>
<td>0.03</td>
<td>0.10</td>
</tr>
<tr>
<td>HDL</td>
<td>2.63</td>
<td>2.54</td>
<td>2.58</td>
<td>3.36</td>
<td>3.15</td>
<td>0.06</td>
<td>0.22</td>
</tr>
<tr>
<td>LDLr mRNA</td>
<td>1.81</td>
<td>1.62</td>
<td>1.17</td>
<td>0.44</td>
<td>0.54</td>
<td>0.40</td>
<td>0.15</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.01, ***P<0.001.

There was no effect of CLA on plasma lipoproteins when animals were fed chow alone. However, in the presence of ‘Western’ fat, 10,12-CLA increased VLDL-, LDL- and HDL-cholesterol relative to both control and c9,t11-CLA diets. However, the effect on the atherogenic lipoprotein was greater on HDL such that the atherogenic ratio was increased in animals fed 10,12-CLA. The ‘Western’ fat diet clearly reduced hepatic LDL receptor mRNA concentrations and while there was no interaction between background diet and dietary CLA, 10,12-CLA tended to reduce concentrations (P=0.064).

The data confirm that while 10,12-CLA may reduce adipose tissue deposition, this is associated with potentially detrimental effects on plasma lipoproteins which could be associated with an increased risk of atherosclerosis. While there is some evidence that this may be associated with reduced hepatic LDL receptor expression, the concomitant increase in VLDL-cholesterol might also suggest an increase in hepatic VLDL production leading to an accumulation of LDL.

This project was supported by a Committee Studentship and Project Grant from the UK BBSRC.


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Blood-brain transport of leptin: influence of photoperiod and leptinaemia in sheep. By J.G.M. Adam, N.A. Findlay, L. Hinder,† J.J. Harrison and D.W. Miller.†, †Oester and Metabolic Health Division, Roslin Institute, Edinburgh, UK; ±ABE, YS and Scottish Agricultural College, Craibstone, Aberdeen, UK; ‡ABE, YS and WAH, UK.

Leptin is a characteristically anorectic hormone that acts as a signal of nutritional status to the brain. Serum leptin concentrations are commonly elevated in chronic obesity yet they fail to elicit appetite or weight loss indicative of central leptin resistance. It is hypothesised that reduced blood-brain transport of leptin in human and animal models contributes to the apparent central leptin resistance. In order to elucidate further our understanding of leptin transport from the blood into the brain, we have used sheep prepared with intracerebroventricular cannulae and managed in different physiological conditions. Sheep are seasonal animals and we tested the hypothesis that changes in the seasonal venous and cerebral leptin concentrations would alter seasonal leptin transport: from sheep kept under artificial SD (5 L:5 D) and natural LD (7 L:7 D) (both 14 h light, with 7 h dark: LD, SD), respectively, for 15 weeks in an antipodal latitude (30°N), to sheep kept under the same condition of LD (14 L:10 D; 7 h light, 7 h dark: LD), but reduced nutritional plane (increasing nutritional plane, INP) or restricted food intake (decreasing nutritional plane, DNP), respectively, for 12 weeks in natural spring daylength (seven per group) (experiment 3).

In experiment 1, mean plasma leptin concentrations increased from 5.4 (SD 1.17) ng/ml in LD and from 9.3 (SD 1.14) to 19.5 (SD 0.84) ng/ml in SD. CSF-plasma leptin concentration ratio (0.46–0.53) correlated positively with plasma leptin concentrations in INP (r² 0.39; P < 0.05) and INP sheep (0.21; P < 0.001). During experiment 2, plasma leptin concentrations increased over time in INP sheep (2.5 (SD 0.13) to 9.7 (SD 1.09) ng/ml) and decreased in DNP sheep (3.2 (SD 0.36) to 2.5 (SD 0.36) ng/ml). CSF leptin concentrations correlated positively with plasma values in both DNP (r² 0.39; P < 0.05) and INP sheep (0.21; P < 0.001). However, CSF leptin did not change proportionately to plasma values so that the CSF-plasma concentration ratio correlated negatively with plasma leptin in both INP (r² 0.06–0.33; P < 0.01) and DNP sheep (r² 0.09; P < 0.05).

The present study was financially supported by SEERAD.


Weight gain of male mice from four lines (BE, MU, ED and RO), divergently selected for high (H) or low (L) growth potential, during 28 d after infected with Heligmosomoides polygyrus or sham-infected with water (P < 0.001). These data indicate that a greater proportion of leptin enters the brain from circulating blood in LD than in DNP and is correlated with plasma leptin. Furthermore, these studies have demonstrated the value of the sheep model for exploring the dynamics of blood–brain communication selecting for increased growth performance only.

The present work was financially supported by SEERAD.
Central appetite-regulating pathways in the brain are sensitive to nutritional feedback from the periphery, mediated in part by circulating insulin. However, increasingly common conditions such as the metabolic syndrome are associated with insulin insensitivity and the present studies investigate whether central insulin insensitivity resides at the level of entry into the brain or within the brain itself. Such investigation requires a clinically relevant animal model that permits both dynamic assessment of blood–brain transport and measurement of responses to central hormone administration within the same animal. Here, we demonstrate the use of a sheep model to examine the effect of nutritional status on blood–brain transport of insulin and on appetite responses to insulin given directly into the brain by intracerebroventricular (ICV) infusion.

In experiment 1, ICV-cannulated castrated male sheep with initially low, medium or high levels of adiposity were fed a complete diet ad libitum (increasing nutritional plane; INP), in amounts calculated to maintain body weight (maintenance; static nutritional plane, SNP) or approximately half maintenance (decreasing nutritional plane; DNP), respectively (seven per group). After 12 weeks, the initially thin INP sheep had become fat and the initially fat DNP sheep had become thin, with the SNP sheep maintaining their medium level of adiposity. Concurrent samples were collected each week of cerebrospinal fluid (CSF) from the lateral cerebral ventricle and circulating blood from the jugular vein for insulin RIA (MacRae et al. 1991). Plasma insulin concentrations increased steadily over time in the INP group from about 20 to 70 μU/ml but remained at about 20 μU/ml throughout in SNP and DNP groups. CSF insulin concentration patterns in each group matched the plasma profiles but at lower magnitude, so that CSF and plasma insulin were positively correlated (r2 = 0.62; P < 0.001) with a constant ratio of 0.23.

In experiment 2, initially thin or fat ICV-cannulated castrated male sheep (nine per group) were fed for 12 weeks ad libitum or amounts restricted to half maintenance, respectively, to produce INP and DNP groups as in experiment 1. In week 0, and repeated at 4-week intervals, the sheep were infused ICV for 8 h starting at 08.00 hours with control artificial CSF on one day and with insulin (2 mU/g) on the next day. Voluntary food intake (VFI) was measured hourly by weighing and replacing food refusals, with fresh food given at 08.00 hours. As in experiment 1, plasma insulin increased steadily over the 12 weeks in INP sheep (about 30 to 100 μU/ml) but decreased rapidly (about 60 to 25 μU/ml) and thereafter remained low in the DNP group. VFI in the INP group was decreased about 25% by ICV insulin at weeks 4 and 8 when the sheep were gradually increasing in adiposity but not at week 12 when they had become fat. ICV insulin also had no effect on VFI in the DNP group when they were fat at week 0, while their food intake was restricted. There was an overall significant negative correlation between the ICV insulin-induced decrease in food intake and the level of body adiposity as measured by body condition score (after Rusell et al. 1969) (r2 = 0.43; P < 0.001).

Therefore, increased adiposity in sheep with increased circulating insulin, which is able to enter the CSF freely in proportion to wide-ranging plasma concentrations, but which is also associated with decreased sensitivity of the brain appetite-regulating pathways to insulin. The present findings indicate that central insulin insensitivity occurs at the brain receptor or post-receptor level and is not a function of reduced blood–brain transport.

The present study was funded by SEERAD and BBSRC.


Vitamin E is a major lipid-soluble antioxidant. It has been used to ameliorate diseases in which oxidant and inflammatory processes play a covert (CVD) or overt (rheumatoid arthritis) role. However, a meta-analysis on the effects of long-term, high-dose vitamin E showed a small increase in all-cause mortality (Miller et al. 2005). We examined the effects of high (600 IU/d) or moderate (75 IU/d) doses of RRR-α-tocopherol (α-toc) for 6 weeks in healthy middle-aged men. Ex-vivo IL-1β, TNF-α, IL-6, IL-8 and IL-10 production from lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells (PBMC) and a measure of the oxidative burst (fluorescence intensity (Fl)) by LPS-stimulated monocytes (mono) and phorbol myristate acetate (PMA)- or LPS-stimulated granulocytes (gran), were assessed before and after supplementation. NF-kB activation was assessed in LPS-stimulated PBMC. Subjects were genotyped for single nucleotide polymorphisms (SNP) in cytokine (-308, +252, -252, 174, 511 and -309), glutathione-S-transferase (GST) (GSTM1 NULL or active; GSTP1 NULL or active, GSTP1A A313G and C341T), Mn superoxide dismutase (C-28T) and methylethylhydrofolate reductase (MTHFR; C677T and A1298C) genes.

Irrespective of genotype, the extent and direction of the changes in cytokine and oxidant production and NF-kB activation were different according to the dose of vitamin E given.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Plasma α-toc (mg/l)</th>
<th>IL-8 (ng/ml)</th>
<th>C-reactive protein (mg/l)</th>
<th>NF-kB activity</th>
<th>FI gran (PMA)</th>
<th>FI mono (LPS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 IU</td>
<td>130*</td>
<td>3.4</td>
<td>11.5</td>
<td>2.5*</td>
<td>0.03*</td>
<td>0.25</td>
</tr>
<tr>
<td>600 IU</td>
<td>103</td>
<td>130</td>
<td>45.5</td>
<td>2.5</td>
<td>0.03</td>
<td>0.25</td>
</tr>
</tbody>
</table>

* Significantly different from 75 IU group (Mann–Whitney test; P < 0.05).

Genotype substantially modulated the effects of high-dose vitamin E on cytokine and oxidant production. In particular, a reduced ability to metabolise xenobiotics (GST SNP) prevented the pro-inflammatory influence of the vitamin, suggesting that, paradoxically, metabolism of large doses of the vitamin raises inflammatory and oxidant stress. Individuals with a genotypically determined reduced capacity to metabolise xenobiotics may escape this adverse effect.

We are grateful to the Biotechnology and Biological Sciences Research Council for funding this project.

Feeding a protein-restricted diet during pregnancy in the rat induces changes to DNA methylation in the liver of the F1 and F2 offspring after weaning. By G.C. BURDGE1, J.L. SLATER-JEFFERIES1, C. TORRENS1, M.A. HANSON1 and K.A. LILLYCROP2, 1DOHaD Centre, University of Southampton, Southampton, UK, SO16 5YA and 2Development and Cell Biology, University of Southampton, Basnett Crescent East, Southampton, UK, SO16 7PX

Phenotypic variations which are induced in offspring by variations in the intra-uterine environment can be transmitted to subsequent generations (Muskiet, 2005). Feeding a protein-restricted (PR) diet during pregnancy in the rat induces an altered metabolic phenotype in the liver of the offspring by changing the epigenetic regulation and expression of hepatic PPARα and glucocorticoid receptor (GR) (Lillycrop et al. 2005). Because such epigenetic changes may mediate transgenerational effects on phenotype, we investigated the effect of feeding a PR diet during pregnancy in F0 females on DNA methylation and expression of hepatic PPARα and GR promoters in male F1 and F2 offspring.

Wistar rats (F0) were fed a control (18% (w/w) protein) or PR (9% (w/w) protein) diet during pregnancy and chow during lactation (Langley & Jackson, 1994). Offspring were weaned onto chow. F1 males (n 6 per F0 group) were killed at day 80. F1 females were mated and fed chow throughout pregnancy and lactation, and their offspring were weaned onto chow. F2 males (n 6 per F1 group) were killed at day 80. Hepatic PPARα and GR promoter methylation was determined by methylation-sensitive real-time PCR and mRNA expression by real-time RT-PCR (Lillycrop et al. 2005).

PPARα promoter methylation was lower in the offspring of the PR group in the F1 (8.2%) and F2 (10.5%) generations (see Table). GR promoter methylation was lower in the offspring of the PR group in the F1 (10.8%) and F2 (7.9%) generations. There were no differences in PPARα or GR mRNA expression between dietary groups.

The present study was supported by the British Heart Foundation.


Genistein selectively induced growth arrest and G2-M phase cell cycle block in T47D but not MCF10A breast epithelial cells. These anti-proliferative effects were paralleled by significant differences in the association of genistein to cells and in particular its intracellular metabolism leading to the formation of both 5,7,3',4'-tetrahydroxyisoflavone (THIF) and two glutathionyl conjugates of THIF. THIF inhibited cdc2 activation via the phosphorylation of p38 MAP kinase suggesting that this species may mediate genistein's cellular actions in tumorigenic breast epithelial cells via the activation of signalling through p38.
Gene variants are associated with body mass index, weight and body fat in postmenopausal women. By S.D. ODELL1, H. SNIJDER2, X. WANG2, R. SHIRI-SVERDLOV3, J.V. van VLIET-OSTAPTCHOUK1 and T.D. SPECTOR3, 1Nutrition, Food and Health Research Centre, King’s College London, London, UK, SE1 9NH, 2Georgia Prevention Institute, Department of Pediatrics, Medical College of Georgia, Augusta, GA, USA, 3Department of Molecular Genetics, Maastricht University, the Netherlands and 4Twin Research and Genetic Epidemiology Unit, St Thomas’ Hospital, London, UK, SE1 7EH

A loss of function mutation of the tub gene results in the tubby mouse syndrome, characterised by late-onset obesity with insulin resistance and neuroendocrine defects (Coleman et al. 1990). The tub gene is predominantly expressed in the hypothalamus and tub protein phosphorylated by hypothalamic insulin receptors may mediate insulin signalling in energy homeostasis. Recently, Shiri-Sverdlov et al. (2006) reported a significant association between BMI and single nucleotide polymorphism (SNP) rs1528133, located 22 kb 3’ distal to human TUB in the flanking gene RIC3 (function unknown) in one cohort of subjects with type 2 diabetes. Two TUB SNP, rs2272382 and rs2272383, were associated with BMI in another group. We attempted a replication of these associations in a normal population approximately twice the size of the combined cohorts: 2771 women from the St Thomas’ UK Adult Twin Registry (Twins UK) (mean age 47.4 (SD 12.5) years).

Genotype and allele frequencies of the three SNP were similar to the previous study. Pairwise Linkage Disequilibrium (LD) quantified by D’/r2 was significant (P<0.05) for all SNP combinations. In the whole cohort, significant main effects of rs272382 and rs1528133 were found on waist: rs272382 (22 genotype 80.5 (SD 11.9) cm; P=0.012) and rs1528133 (11/12 genotype 78.2 (SD 10.0) cm; P=0.046). As the previous studies involved subjects of mean age approximately 70 years, we analysed pre- and postmenopausal women separately. There were no significant associations in the premenopausal group, but in postmenopausal women significant associations of rs2272382 were found with BMI, weight, waist, total fat mass and % central fat, explaining 0.22–0.52% of variances.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Premenopausal</th>
<th>Postmenopausal</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>35/34/39/4</td>
<td>35/34/39/4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>35/34/39/4</td>
<td>35/34/39/4</td>
</tr>
<tr>
<td>Total fat (%)</td>
<td>36/34/39/0</td>
<td>36/34/39/0</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>35/34/39/3</td>
<td>35/34/39/3</td>
</tr>
<tr>
<td>Central fat (%)</td>
<td>36/34/39/0</td>
<td>36/34/39/0</td>
</tr>
</tbody>
</table>

*Age-adjusted P values under recessive model (genotype 2 homozygote compared with allele 1 carriers).

Polymorphisms at IL-6–174 and TNF-α-308 and body mass index modulate the effects of fish oil supplementation on cytokine production by monocytes from healthy middle-aged men. By J. MADDEN1, A. BRUNNER1, J.J. CARRERO1, J. HADLEY1, B. TAN1, N. DASTUR1, C.P. SHEARMAN1, P.C. CALDER1, E. RAINGER2, G. NASH2, T. LUU3 and R.F. GRIMBLE1, 1School of Medicine, University of Southamptom, Southamptom, UK, S016 7PX and 2Department of Physiology, Birmingham University Medical School, Birmingham, UK, B15 2TT

Fish oil is reputed to reduce ex vivo lipopolysaccharide (LPS)-stimulated cytokine production by peripheral blood mononuclear cells (PBMC). However, many studies have failed to demonstrate this phenomenon. Cytokine gene single nucleotide polymorphisms (SNP) influence the level of cytokine production from PBMC (for example, TNF-α-308 A allele, LT-α-252 A allele, IL-6–174 G allele). In addition, adiposity may up regulate cytokine production. In the present study we examine the influence of BMI and SNP at –308, +252, –174, –511 and –1082 in the TNF-α, LT-α, IL-6 IL-1β and IL-10 genes respectively on LPS (100 ng/ml)-stimulated cytokine production (24 h) by monocytes from ninety-two healthy middle-aged men (56±8 (SD) years) before and after a 12-week period of fish oil (MaxEPA) supplementation (6 g/d). IL-1β, TNF-α, IL-6 and IL-10 were measured by cytometric bead assay. BMI was computed from weight and height. Measurements were made after an overnight fast.

<table>
<thead>
<tr>
<th>Variables</th>
<th>BMI (kg/m²)</th>
<th>SNP*</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
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<th>SD</th>
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</tr>
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<td>No</td>
<td>11/12/22</td>
<td>11/12/22</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>BMI (kg/m²)</td>
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<td>4.2</td>
<td>25.8</td>
<td>4.3</td>
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<td>0.022</td>
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<tr>
<td>Weight (kg)</td>
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<td>12.5</td>
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<tr>
<td>Total fat (%)</td>
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<td>7.4</td>
<td>36.9</td>
<td>7.2</td>
<td>37.3</td>
<td>6.4</td>
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<td>Waist (cm)</td>
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<td>35/34/39/3</td>
<td>105.0</td>
<td>10.2</td>
<td>106.6</td>
<td>11.0</td>
<td>101.1</td>
<td>8.1</td>
<td>0.004</td>
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</tr>
<tr>
<td>Central fat (%)</td>
<td>36/34/39/0</td>
<td>36/34/39/0</td>
<td>10.7</td>
<td>10.6</td>
<td>10.5</td>
<td>7.3</td>
<td>11.9</td>
<td>7.0</td>
<td>0.053</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Age-adjusted P values under recessive model (genotype 2 homozygote compared with allele 1 carriers).

Associations were confirmed by Sibling Transmission Disequilibrium Test (s-TDT). We have therefore shown association of TUB SNP rs2272382 with BMI and found additional associations with body fat measurements, but only in postmenopausal women. This finding accords with the association of the mouse homologue tub mutation with late-onset obesity.

We acknowledge the Wellcome Trust (project grant no. 073142), Arthritis Research Campaign, Chronic Disease Research Foundation and the European Union 5th Framework Programme.

References


In subjects who were not overweight the presence of an A and G allele at –308 and –174 of the TNF-α and IL-6 genes respectively resulted in a significant fall in IL-1β and IL-6 production following fish oil supplementation (see Table). Being overweight prevents this genomic influence. In a previous study in 111 healthy young men, we found that individuals in the highest tertile of TNF-α production by LPS-stimulated PBMC experienced a fall in production after 12 weeks of dietary supplementation with fish oil (Grimble et al. 2002). In the present study pre-supplementation IL-1β and IL-6 production were significantly greater in subjects with both genotypes and a BMI<25 kg/m², supporting this observation and highlighting a possible phenotypic and genotypic reason for the variability in the anti-inflammatory effects of fish oil.

We are grateful to the BBSRC for funding this project.
Influence of superoxide dismutase polymorphism and overweight status on clinical outcome and oxidant production and stress in healthy middle-aged men and rheumatoid patients receiving high-dose RRR-
tocopherol supplementation.

By H. NORMANTON 1, J.G.M. HOUDIJK 1, D.P. KNOX 2 and I. KYRIAZAKIS 1,3, 1

Parasitology Division, Moredun Southport, UK, SO16 7PX, 2School of Medicine, University of Southampton, Southampton, UK and 3Veterinary Faculty, University of Thessaly, PO Box 199, 43100 Karditsa, Greece.

Oxidant and inflammatory stress underlie the pathology of inflammatory diseases such as rheumatoid arthritis (RA) and are features of the ageing process. Obesity enhances inflammatory stress. We examined the influence of single nucleotide polymorphisms (SNP) in cytokine- and oxidant-metabolising enzyme genes and BMI on the effects of high-dose (600 IU/d) RRR-tocopherol (vitamin E), for 6 weeks, on inflammatory mediators and markers, and disease outcome in thirty-one men with early stages of RA (aged 43–70 years; 58 (± 8 years) and thirty-one age- and weight-matched healthy men.

Plasma inoprostanes, lipid peroxides and C-reactive protein were measured before (Pre-suppl) and post-supplementation. NO and prostaglandin E2 production by lipopolysaccharide-stimulated peripheral blood mononuclear cells (PBMC) were measured. In patients, disease activity score (DAS), based on number of swollen and tender joints and erythrocyte sedimentation rate, was assessed. SNP were examined at -28 in the TNF-α, lymphotixin-α, IL-6, IL-1β and IL-10 genes respectively, and in the Mn superoxide dismutase (C-28T) gene. The degree of adiposity of patients and controls was categorised by measurement of BMI subjects with a BMI >25 kg/m2 were classed as overweight. The severity of RA was assessed by a DAS.

Irrespective of BMI or genotype, vitamin E reduced DAS (P=0.031; Wilcoxon signed ranks test); however, this occurred only in overweight patients (P=0.004 < 0.074 for BMI >25 kg/m2). Overweight patients with the TT genotype for the C-28T superoxide dismutase (SOD) SNP experienced greater lipid peroxidation before supplementation. Plasma C-reactive protein concentrations (mg/l) also indicated a higher level of inflammatory stress in overweight patients with a TT rather than CT or CC genotype (10.7 (± 8.0) vs. 3.6 (± 2.7 mg/l; P=0.02) Irrespective of BMI, NO production was significantly reduced by vitamin E in patients and controls possessing a genotype which did not impair the ability to metabolise superoxide radicals (controls: P=0.753 < 0.044 and patients: P=0.176 < 0.001 for the TT v. other genotypes of the C-28T SOD SNP).

The E7-174 SNP significantly influenced inoprostane concentrations and the changes following supplementation. Patients with a GG genotype had higher pre-supplementation concentrations than patients who were GC or CC (5.1 (± 2.8) v. 1.8 (± 2.0) µmol/l; P=0.003) and showed a fall in concentration post-supplementation (~2.0 (± 2.3) v. 2.5 (± 4.5) µmol/l; P=0.005).

Effects of changes in protein supply and demand on gastrointestinal parasitism in lactating rats.

By H. NORMANTON 1, J.G.M. HOUDIJK 1, D.P. KNOX 2 and I. KYRIAZAKIS 1,3, 4

Veterinary Parasitology, SAC, Edinburgh, UK, E96 3JG, 2Parasitology Division, Moredun Research Institute, Penicuik, UK, EH26 0PZ and 4Veterinary Faculty, University of Thessaly, PO Box 199, 43100 Karditsa, Greece

The breakdown of immunity to parasites during lactation may have a nutritional basis (Coop & Kyriazakis, 1999). Reducing protein scarcity through increased protein supply or reduced protein demand would be expected to increase resistance to parasites during the periparturient period (Houdijk et al., 1999). This hypothesis is being addressed in a lactating rat model, as lactating rats show a breakdown to the intestinal nematode Nippostrongylus brasiliensis. Thirty-two rats were given a single dose of 1600 N. brasiliensis larvae before mating (primary infection), and re-infected with the same dose on day 2 of lactation. During lactation, rats received either a low-protein (LP) diet (100 g crude protein/kg DM) or a high-protein (HP) diet (300 g crude protein/kg DM). Both diets contained 18.0 MJ gross energy/kg DM and were restrictively fed at 7.5% of their parturition body weight. Litter sizes for LP groups were standardised to nine (n = 6) or three (n = 3) pups, while HP groups had nine pups (n = 6). Rats were slaughtered on day 12 to assess the concentration of nematode eggs in colon contents.

Small-intestinal mucosal biopsies were taken to quantify inflammatory cells, and mucosal scarpings were taken to assess local antibodies (IgA, IgE, IgG1 and IgG2a) and rat mast cell proteases (RMCP-II).

We are grateful to the BBSRC for funding this project.


The acute effect of triacylglycerols rich in stearic and oleic acid on vascular function. By S.E.E. BERRY1, R. BANERJII1, S. TUCKER1, S.M. CHARLES2, B. JANG2, P.J. CHOWIENZCYK2 and T.A.B. SANDERS1.1 Nutritional Sciences Research Division, King’s College London, 150 Stamford Street, London, UK, SE1 9NN and 2Cardiovascular Division, King’s College London School of Medicine, St Thomas’ Hospital, London, UK, SE1 7EH

Vascular dysfunction is widely recognised as an important factor in the development of atherosclerosis. High-fat meals have been found to impair vascular function (Steer et al. 2003), however, few studies have examined the effect of specific fatty acids. The present study was designed to investigate the postprandial effects of shea butter (rich in stearic acid) and high-oleic sunflower-seed oil on vascular function. This was determined using brachial artery flow-mediated dilatation (FMD) to measure endothelial reactivity, and by pulse-wave velocity (PWV) and pulse-wave analysis (PWA) (using the SphygmoCor system) to measure arterial stiffness. Seventeen healthy male subjects (aged 18–40 years) were fed test meals containing 50 g fat (shea butter or high-oleic sunflower-seed oil) in a randomised cross-over design. Plasma triacylglycerol (TAG) concentrations were determined fasting and at 2 h, 3 h and 4 h postprandially. FMD, PWV and PWA were measured fasting and 3 h postprandially. Results are shown below.

<table>
<thead>
<tr>
<th></th>
<th>Shea butter</th>
<th>High oleic sunflower oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma TAG (mg/dL)</td>
<td>28.3 (5.4)</td>
<td>46.3 (6.7)</td>
</tr>
<tr>
<td>FMD (% change)</td>
<td>-1 (2.7, 0.1)</td>
<td>-3 (4.4, -1.6)</td>
</tr>
<tr>
<td>PWV (m/s)</td>
<td>0.4 (0.2)</td>
<td>0.6 (0.2)</td>
</tr>
<tr>
<td>PWA (% change)</td>
<td>-38 (-43, -12)</td>
<td>-87 (-133, -40)</td>
</tr>
</tbody>
</table>

Mean (95% CI), n = 17
1 Incremental area under curve.
2 Change at 3 h from fasting levels.
3 P<0.05 compared with high oleic sunflower oil. * P<0.01 compared with fasting values, paired t-test.

Following shea butter the postprandial increase in plasma TAG was significantly lower (66% lower incremental area under curve) compared with that following the high oleic sunflower oil (ANOVA; diet×time effect P=0.001). PWV did not change postprandially, however there was a significant reduction in PWA (peripheral augmentation index) following both test fats. FMD was significantly different between meals (P=0.02).

An inverse relationship between postprandial TAG response and impairment in endothelial function following a high fat meal has been previously demonstrated (Marchesi et al. 2000). This may explain the difference in responses observed in the current study following shea butter and high oleic sunflower oil. As FMD and postprandial lipaemia are associated with increased risk of heart disease, dietary advice with regards to the intake of stearic and oleic acid should also consider their postprandial TAG and FMD responses, as well as their effect on blood cholesterol levels.

With special thanks to Karen McNell at the Cardiovascular Division, King’s College London School of Medicine, St Thomas’ Hospital, London, UK.


Effects of parasitic infection on anorexia and leptin levels in lambs of two different breeds. By K. ZARALIS1, B.J. TOLKAMP3, J.G.M. HOUDHRI1, A.R.G. WYLIE1 and I. KYRIAZAKIS1, 1Animal Nutrition and Health Department, SAC, Edinburgh, UK, E100 5TG, 2Department of Agriculture and Rural Development and Queen’s University of Belfast, Belfast, UK, BT9 5PX and 3University of Thessaly, Karditsa, Greece

Infection with nematode parasites detrimentally affects production efficiency in grazing animals, mainly through a reduction in food intake (FI) (anorexia). There is evidence that a reduction of FI in nematode-infected lambs is a direct consequence of the immune system activation (Greer et al. 2005). In addition, leptin levels increase during infection and inflammation in many models of disease and this increase has been associated with anorexia in nematode infections (Matarese et al. 2005). Whether leptin levels increase during infection in parasitised lambs is not known. Moreover, differences in nutrient partitioning between breeds of low and high production potential may affect the ability of the hosts to express immunity (Coop & Kyriazakis, 1999).

The purpose of the present study was to test the hypotheses that: (a) lambs of a high production potential breed exhibit a higher degree of anorexia than lambs of a low production potential breed during a primary infection; (b) leptin levels are higher in infected than in non-infected lambs and are positively associated with the degree of anorexia.

Ninety-six weaned lambs (12 weeks of age), half Suffolk×Greyface (S) crosses and half Scottish Blackface (B), were used in the trial. Half of each breed were either trickle infected with 21,000 infective Teladorsagia circumcincta larvae per week (INF), or not infected (NIN). NIN lambs were assigned to one of four feeding treatments: ad libitum (n 12) (CAD); 90% of ad libitum (n 4) (C90); 80% of ad libitum (n 4) (C80); 70% of ad libitum (n 4) (C70). All infected lambs were fed ad libitum. Lamb body weight, FI and faecal egg count (FEC) were recorded weekly. Blood samples were taken weekly and analysed for leptin levels using an ovine-specific RIA. FI and leptin data were analysed using REML. FEC were analysed using ANOVA with infection level interaction was not significant (P>0.05). PWV did not change postprandially, however there was a significant decrease in FMD in S lambs as a result of infection, leptin levels were not significantly affected by infection, which is against the expectations of hypothesis (a). There were significant differences in leptin levels between lambs fed ad libitum and those fed at 80 and 70% of ad libitum, indicating that leptin levels correlate positively with FI in growing lambs. Leptin levels did not differ between the two breeds (P=0.439) and the breed×infection level interaction was not significant (P=0.488). Despite a significant decrease in FI in S lambs as a result of infection, leptin levels were not significantly affected by infection, which is against the expectations of hypothesis (b). However, these results must be interpreted with care because there may have been differences between breeds in parasite exposure before the start of the experiment. Further research with parasite naïve lambs is required to identify whether leptin levels increase during the aquisition phase of immunity.


Table 1. Average food intake: S-CAL (●), S-INF (●●), B-CAL (●), B-INF (●●). The mean daily FI for both INF and CAL lambs in the two breeds are shown in Fig. 1. Anorexia was observed in the INF S lambs as there was a significant treatment×time×breed interaction (P<0.05), attributable to the reduction in FI in the INF lambs of the S breed but not of the B breed (P=0.677). FEC analysis indicated that the lambs were not entirely parasite naïve before the start of the trial. However, 9 lambs had significantly higher FEC (P<0.05) than B lambs. The results suggest that these breeds have significant differences in their ability to control infection, which is in agreement with hypothesis (a). There were significant differences in leptin levels between lamb fed ad libitum and those fed at 80 and 70% of ad libitum, indicating that leptin levels correlate positively with FI in growing lambs. Leptin levels did not differ between the two breeds (P=0.439) and the breed×infection level interaction was not significant (P=0.488). Despite a significant decrease in FI in S lambs as a result of infection, leptin levels were not significantly affected by infection, which is against the expectations of hypothesis (b). However, these results must be interpreted with care because there may have been differences between breeds in parasite exposure before the start of the experiment. Further research with parasite naïve lambs is required to identify whether leptin levels increase during the aquisition phase of immunity.
Fatty acids differentially affect endothelial cell inflammatory gene expression. By D.I. SHAW, W.L. HALL and C.M. WILLIAMS, Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, University of Reading, UK, RG6 6AP

Vascular dysfunction is an abnormality associated with the metabolic syndrome, cardiovascular disease (CVD) and type 2 diabetes. Prospective studies have suggested dietary fatty acid composition is associated with endothelial function, but the underlying mechanism is unclear. In vitro studies have shown certain fatty acids affect markers of endothelial function, however findings are inconsistent. The comparison of fatty acids is often limited and only a small number of endpoints have been investigated. Consequently, findings to date do not allow comparison of the effects of different fatty acids.

The aim of the present study was to carry out a systematic investigation to assess the effect of various fatty acids (100 μM) on the expression of a broad spectrum of genes associated with endothelial dysfunction (VCAM-1, E-selectin, MCP-1, eNOS) using human umbilical vein endothelial cells (HUVEC). Cells were maintained at 37°C and experiments carried out when cells were 90% confluent. Real time reverse transcriptase polymerase chain reaction (RT-PCR) was used to measure gene expression. The gene expression ratios, relative to control, were calculated using the Pfaffl equation (Pfaffl, 2001) which normalises for control treatment and the house keeping gene β-actin.

<table>
<thead>
<tr>
<th>Fatty Acid (100 μM)</th>
<th>VCAM-1</th>
<th>E-selectin</th>
<th>MCP-1</th>
<th>E-NOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td>Arachidonic acid (AA)</td>
<td>0.65</td>
<td>0.29</td>
<td>0.52</td>
<td>0.16</td>
</tr>
<tr>
<td>DHA</td>
<td>0.52</td>
<td>0.23</td>
<td>0.73</td>
<td>0.36</td>
</tr>
<tr>
<td>EPA</td>
<td>1.14</td>
<td>0.28</td>
<td>1.21</td>
<td>0.25</td>
</tr>
<tr>
<td>Linoleic acid (LA)</td>
<td>1.77</td>
<td>0.75</td>
<td>1.45</td>
<td>0.57</td>
</tr>
<tr>
<td>Oleic acid (OA)</td>
<td>1.67</td>
<td>0.37</td>
<td>1.05</td>
<td>0.33</td>
</tr>
<tr>
<td>Palmitic acid (PA)</td>
<td>1.13</td>
<td>0.39</td>
<td>2.67</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Values expressed as means (n=4). 
1 Significantly different to arachidonic acid **, DHA **, EPA *, LA*, OA**, 2 significantly different to EPA **, LA **, PA**, 3 significantly different to PA **, *P<0.05, **P<0.01, ***P<0.001.

Fatty acids, of various subclasses, cause up- and down-regulation of inflammatory gene expression in unstimulated HUVEC, relative to control (RTC). Docosahexaenoic acid (DHA) and AA reduced the expression of genes encoding for pro-inflammatory proteins. RTC. In comparison to DHA, eicosapentaenoic acid (EPA) resulted in increased up-regulation of eNOS (P<0.05) and MCP-1 (P<0.006) relative gene expression; similar trends were noted for VCAM-1 and E-selectin. LA, PA and OA resulted in gene specific increases in gene up-regulation RTC. In contrast to some current reports, OA caused increased up-regulation of inflammatory gene expression. In the case of MCP-1, OA resulted in an increased up-regulation compared with AA, DHA and PA with differences nearly reaching levels of significance (P=0.06). The mechanisms involved are likely to be multifactorial as the present findings suggest the response depends on the particular fatty acid and the gene investigated.

This work was supported by funding from the European Commission, Framework Programme 6 as part of the LIPOGENE project (FOOD-CT-2001–509944).

Glycaemic response to a high-fibre, low-glycaemic index novel food product in human volunteers: possible implications for dietary management of type 2 diabetes mellitus. By V. TROWSE1, P. AMUNA1, F.B. ZOTOR1 and N.L. HILL2, 1Medway School of Science, University of Greenwich, UK and 2Centre for Human Nutrition, University of Sheffield, Sheffield, UK

Low-glycaemic index (GI) diets have been shown to improve glycaemic control and lipid metabolism in type 2 diabetes (Jenkins et al. 1987; Wolever & Mehling, 2002) and improve fibrinolytic activity (Järvi et al. 1999). The objective of the present study was to test the clinical efficacy of a novel low-GI, high-fibre recipe by determining the effect of a composite meal on the glycaemic responses and lipid profiles in non-diabetic healthy adults.

Ten healthy Caucasian volunteers (one male and nine females) were recruited in a cross-over design. Three separate two-hour glucose tolerance and lipid profile tests involving consumption of isoenergetic amounts of white bread (control) and muffins (test meal) (a blend of kidney beans, pearl barley, wholegrain wheat, almonds and dried potato flour) followed by a 2-week feeding trial substituting a known isoenergetic quantity of the test meal at breakfast whilst maintaining their habitual diet throughout the 3-week study. Finger-prick capillary samples were taken at baseline and at 30, 45, 60, 80, 100 and 120 min intervals, for glucose (GM7 analyser, Analox Instruments Ltd, London, UK) and total cholesterol, HDL and triacylglycerol measurements (Reflotron II spectrophotometer, Boehringer Mannheim GmbH, Germany) after an overnight fast.

Mean fasting plasma glucose, triacylglycerol and total cholesterol were 4.13±0.14, 1.13±0.07 and 4.39±0.27 mmol/l respectively. Predicted GI of the test meal was 46.61 units (Foster-Powell et al. 2002). The actual measured GI was 45.49. The area under the glucose curve (AUC) was significantly lower after the test meal in both the pre- (–53%; P<0.01) and post-intervention period (–56%; P<0.001). The AUC after the 2-week consumption of the test meal was not significantly different from that at baseline (P>0.05). There were no significant differences in the AUC for serum triacylglycerol and LDL-cholesterol in control and test meals (P>0.05). The AUC for cholesterol was significantly higher after the test meal in both the pre- (P=0.006) and post-intervention period (P=0.01) compared with control. The AUC for HDL-cholesterol was significantly lower after the test meal in both the pre- (P=0.008) and post-dietary period (P=0.007) compared with control but fasting serum HDL-cholesterol increased by 20% after intervention (P<0.05). No significant differences were seen in fasting triacylglycerols, cholesterol or LDL-cholesterol (P>0.05).

The GI of 45.49 in the present study confirms this novel product to be a very-low-GI food product. The impact of the test meal on lipid profiles is also encouraging. These findings suggest a possible role for this product in the dietary management of type 2 diabetes in the UK.


Insulin sensitivity from an oral labelled glucose test. By M.E. PENNANT, L.J.C. BLUCK and W.A. COWARD, MRC Human Nutrition Research, Elsie Widdowson Laboratory, Felsham Road, Cambridge, UK, CB1 9NL

To develop an understanding of the astrology of diabetes and the metabolic syndrome an easy method giving a quantitative measure of insulin sensitivity is crucial. The most common method currently used to measure insulin sensitivity, the intravenous glucose tolerance test (IVGTT) (Bergman, 1979), has been improved by the addition of a stable isotope glucose tracer (Avogaro, 1989; Horovick, 1998). Stable isotopes are not produced in large quantities by the body so the contribution of body glucose production to changes in glucose concentration need not be considered. Because the number of unknown variables are reduced, stable isotopes give a more reliable estimation of insulin sensitivity.

Although improved using stable isotope glucose tracers, the IVGTT is far from ideal since it operates under non-physiological conditions, involving intravenous administration of large doses of glucose and insulin. Subsequently, there have been attempts to develop more physiological tests for insulin sensitivity involving oral glucose administration (Casno et al. 2000; Soonthrumpun et al. 2003).

An obvious improvement to an oral glucose test is the addition of a stable isotope glucose tracer to the oral glucose load. We have developed a labelled glucose test, incorporating a stable isotope glucose tracer, to more accurately determine insulin action.

Eighteen healthy, glucose-tolerant individuals participated in an isotope-labelled, frequently sampled, oral glucose tolerance test. Subjects were given a drink containing 68 g glucose and 7.5 g [3H]-glucose and blood samples were taken for glucose, insulin and isotope ratio measurements. To validate the test, an intravenous injection of 250 mg [13C]glucose was given 45 min after the glucose drink to determine insulin sensitivity by an established method, the orally stimulated intravenous glucose tolerance test (OSIVGTT) (Black et al. 2006). Insulin sensitivity from the unlabelled method was derived from areas under the insulin and glucose curves whilst those for the new labelled test were derived from areas under the insulin and labelled glucose curves.

Once adjustments had been made for hypoglycaemia, insulin sensitivity assessed by the unlabelled method was significantly correlated with insulin sensitivity determined by the OSIVGTT (r 0.62; P=0.00075); however, for the labelled test the correlation improved (r 0.72; P=0.00097).

An oral glucose test is more physiological than an intravenous test and incorporation of a stable isotope into a glucose drink allows more accurate quantification of insulin sensitivity compared with an unlabelled glucose test.

Numerous studies have been published describing consumers' use and understanding of nutrition labels and nutrition overall (Higginson et al. 2000a,b). However, only a limited number of studies exist comparing the differences in nutritional knowledge between physically active and non-active subjects (Barr, 1987). Therefore, the present study aimed to examine whether there are any differences in the use and understanding of nutrition labels in gym users (GU) and non-gym users (NGU). Subjects were recruited over a 5-week period in February and March 2005 in the Greater London area. GU were classified as those who go to the gym and/or participate in fitness classes at least twice per week. One hundred and eighty-six subjects participated in the present questionnaire-based survey (128 GU and 58 NGU). Although there was a spread of subjects, there was a predominance of females and the age distribution was skewed towards the 25–34 age group.

58% of subjects reported to always or mostly read nutrition labels, with total and saturated fats and total energy being the most often sought information. All subjects were asked to state the current Guideline Daily Amounts for total fat intake for both males and females, but only 4 subjects could state the amount for males (95 g), and 23 subjects could state the guidelines for females (70 g), with all the correct responses given by females. There was no significant difference between the responses of GU, NGU or subjects currently wanting to reduce their weight or fat intake. Approximately three quarters (75% and 72% for males and females recommendations respectively) answered they did not know.

When asked to identify which macronutrient contained the most calories per gram, only 37% gave the correct answer (fat), with 30% of respondents believing that sugars contained the most calories per gram (Figure 1). Furthermore, 53% thought saturated fats contained the most calories per gram as compared to other types of fats, whilst only 12% answered correctly (Figure 2).

The results of this study suggest that although the majority of consumers in the present study tended to read nutrition labels, with significantly higher reading rates amongst females (P=0.05), there was no significant difference in reading levels and understanding between gym and non-gym users. Although the reading level of labels is high, it does not necessarily mean consumers are able to translate the information into healthy eating. Indeed, the overall low knowledge of the calorie content of macronutrients and especially fats indicates consumers need to be further educated on this issue if they are to make informed healthier food choices.
Effect of consuming cooked broccoli along with a high- or low-protein meal on the uptake of sulforaphane in healthy human subjects. By V. RUNGAPAMESTRY1, A.J. DUNCAN2, Z. FULLER2 and B. RATCLIFFE1,1 The Robert Gordon University, Saint Andrew Street, Aberdeen, UK, AB25 1HG and 2The Macaulay Institute, Craigiebuckler, Aberdeen, UK, AB15 2QH

In broccoli, sulforaphane, the isothiocyanate metabolite of the glucosinolate glucoraphanin, has been implicated in the cancer-protective effects of brassica vegetables (Verhoeven et al. 1997). When broccoli is consumed, sulforaphane is released in vivo from the hydrolysis of glucoraphanin by residual plant myrosinase and/or colonic microflora. Recent work indicates that the highest yield of isothiocyanates, on hydrolysis of glucosinolates in cooked brassica in vivo, was from lightly cooked compared with raw or fully cooked brassica (Rungapamestry et al. 2005). Alkyl isothiocyanates have been shown to interact with proteins in vitro (Björkman, 1973). To study meal matrix effects on glucosinolate hydrolysis and absorption, volunteers (n=12) were each offered a low- or high-protein meal along with 150g lightly cooked broccoli (microwaved for 2min) or fully cooked broccoli (microwaved for 5.5min) following a Latin square design. Volunteers received mustard containing 17.25 (SEM 0.27)μmol pre-formed allyl isothiocyanate (AITC)/g with each meal to control for intra- and inter-individual variation in the absorption of isothiocyanates. Each meal was separated by a wash-out period of at least 48h. Urine was collected for 24h following each meal and analysed for excretion of allyl mercapturic acid (AMA) and sulforaphane mercapturic acid (SFMA), the biomarkers of AITC and sulforaphane absorption respectively, by HPLC (Mennincke et al. 1987).

![Graph showing excretion of AITC and AMA](image)

Although glucoraphanin intake was not markedly different between broccoli treatments, consumption of lightly cooked broccoli produced a higher yield of sulforaphane from hydrolysis of its glucoraphanin precursor in vivo (P<0.001) (Fig. 1 (a)). Protein content of the meal significantly increased the absorption and excretion of AITC from mustard (P<0.001) (Fig. 1 (b)) but not the hydrolysis of glucoraphanin and its excretion as SFMA from broccoli (Fig. 1 (a)). This difference may relate to the different origins of isothiocyanates arising in the gut; urinary AMA arose from ingestion of pre-formed isothiocyanates, while SFMA was produced following hydrolysis of glucoraphanin to sulforaphane in the gut. Isothiocyanates may be more likely to interact with the meal matrix if they are ingested pre-formed rather than after their production from hydrolysis of glucosinolates in the alimentary tract.

The present study was supported by the Food Standards Agency (project code T01027).

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Inter-individual variation in the absorption of salicylic acid from food. By A. WOOD3, G. BAXTER2, F. THIES1 and G. DUTHIE1, 1Rowett Research Institute, Aberdeen, UK, AB10 6UX, 2Dumfries and Galloway Royal Infirmary, Dumfries, UK, DG1 4AP and 3College of Medicine and Life Sciences, University of Aberdeen, Aberdeen, UK, AB25 2ZD

The anti-inflammatory and anti-neoepithelial effects of aspirin are ascribed to its major metabolite, salicylic acid (SA). The presence of this natural phenolic acid in fruit, vegetables, herbs and spices may explain in part why plant-based diets lower disease risk (Paterson & Lawrence, 2001). However, it is not clear whether SA is absorbed from the diet, a prerequisite to exerting a protective effect. Consequently, the objectives of the present study were to determine the SA content of a range of spices, a food group reported to be a particularly rich dietary source (Swain & Dutton, 1985), and then to assess the absorption of SA from a salicylate-rich spice preparation. Three fasted volunteers (one female and two males, aged 32±16 years, [mean±SD]) ingested a yoghurt flavoured with spice (cumin) containing 134 μg SA and urine was collected at intervals over 12h. SA contents of food and urine and the concentration of the major urinary metabolite salicyluric acid (SU) were measured in duplicate by HPLC with electrochemical detection (Baxter et al. 2001), peak identities being confirmed by GC-MS. The Table shows the SA content of several spices. Cumin was used in the intervention trial.

<table>
<thead>
<tr>
<th>Food</th>
<th>SA (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamon</td>
<td>1.26</td>
</tr>
<tr>
<td>Cumin</td>
<td>2.98</td>
</tr>
<tr>
<td>Garam masala</td>
<td>1.30</td>
</tr>
<tr>
<td>Paprika</td>
<td>0.48</td>
</tr>
<tr>
<td>Turmeric</td>
<td>2.09</td>
</tr>
<tr>
<td>Nutmeg</td>
<td>2.82</td>
</tr>
<tr>
<td>Natural yoghurt</td>
<td>0</td>
</tr>
</tbody>
</table>

SA was present in all spices, cumin being the richest source (see Table). However, consumption of cumin-flavoured yoghurt was associated with large inter-individual variation, marked increases in SA and SU in urine being observed in only one of the male volunteers (see Figure), with approximately 75% of the ingested SA being bioavailable.

![Graph showing excretion of SA](image)

As approximately 70–80% of SA is converted to SU in vivo (Needs & Brookes, 1985), the lack of increase in the water-soluble conjugate in urine of two of the volunteers suggests that SA may not be absorbed from the food matrix in all subjects. Such inter-individual variation may indicate that diet may not be a potential source of this recognised chemopreventative phenolic acid in all individuals.

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

Role of eating frequency and macronutrient content of in-between-meal snacks in body weight control in overweight men aged 25–50 years – preliminary results. By S. ZAVERI and S. DRUMMOND, Department of Dietetics, Nutrition and Biological Sciences, Queen Margaret University College, Edinburgh, EH12 8TS

The role of eating frequency has been increasingly studied in relation to body weight control. Contrary to popular opinion, studies (Fabry et al. 1964; Metzner et al. 1977; Kant et al. 1995; Drummond et al. 1998) have shown an inverse relationship between body weight status (BMI) and eating frequency. Therefore, a high eating frequency may be beneficial in appetite control. In addition, there is collecting evidence that protein is more satiating than carbohydrate and fat (Teff et al. 1989; Barkeling et al. 1990; Golay et al. 1997). Presently, the study aimed to assess the impact of increasing daily eating frequency (EF) with either high-carbohydrate (HC), high-protein (HP) or high-fat (HF) snacks on body weight control in overweight men.

Fifty nine men aged 25–50 yrs with BMI 25–35 kg/m² were randomly assigned to Control (C) (n 13), HC (n 14), HP (n 18) or HF (n 14) group. All volunteers were provided with advice to reduce fat in the diet. Commonly eaten ready available snacks were chosen for this study. Two snacks consisting of either cereal bars (494kJ), almonds (143kJ) or crisps (109kJ) were introduced to three groups respectively for 12 weeks. Therefore, the snacks were not isocaloric. In addition, the HP snack (almonds) were also high in total fat but high in MUFA (65% of total fat). Dietary intake was recorded in a 4-day unweighed diet diary and hunger ratings were recorded on a 100 mm visual analogue scale (VAS) at baseline, 6 and 12 weeks. Differences across time and between groups were analyzed using repeated measures ANOVA.

Although, there was an increase in mean EF in HP group compared to HC group at 12 weeks, there was no corresponding increase in mean energy intake. The hunger rating significantly increased in C group from 4.1 (so 1.4) at baseline to 4.5 (so 1.6) at 12 weeks (P<0.05). The hunger rating decreased in HP group (4.5 (so 1.2) v. 4.1 (so 0.9)) although this failed to reach statistical significance (P=0.09).

HP snack compared to HC and HF snack promoted a higher frequency of eating, which may be more satiating and lead to energy compensation in subsequent meals. Snacks such as almonds, with higher protein content than more traditional snacks, may play a role in appetite control in the long term and may help in decreasing the risk of obesity.

This study was supported by Kellogg Group. Almonds were supplied by Almond Board of California.

Exposure to a maternal low-protein diet in rat pregnancy programmes metabolic syndrome-like phenotype in 18-month-old offspring. By A.M. EHRUMA 1, S.C. LANGLEY-EVANS 2 and A.J. BENNETT 3, School of Biomedical Sciences, Queen’s Medical Centre and Division of Nutritional Sciences, University of Nottingham, Nottingham, UK. NG7 2UH

Epidemiological studies have demonstrated the association between prenatal and postnatal growth and CVD, hypertension, impaired glucose tolerance, non-insulin-dependent (type 2) diabetes, insulin resistance, and obesity in adult life (Barker et al. 1993; Osmond et al. 1990, Kermack et al. 2001). These are the main components of the metabolic syndrome (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001). Evidence from experimental studies in animals strongly supports the hypothesis that components of the metabolic syndrome are programmed in utero. However, there is a gap in the literature describing lipid and insulin profiles from studies using a low-protein diet model. The aim of the present study was to determine plasma and hepatic lipid, as well as plasma glucose and insulin, concentrations in offspring of protein-restricted mothers at 18 months of age.

Pregnant Wistar rats were randomly allocated to five treatment groups. A control group was fed 180 g casein/kg diet throughout pregnancy and four other experimental groups were fed a 90 g casein/kg, low-protein diet (LP) during the first (LP early; day 0–7 gestation), second (LP mid; day 8–14 gestation) and third week (LP late; day 15–22 gestation), or HP (n 14) group. All volunteers were provided with advice to reduce fat in the diet. Either cereal bars (949 kJ), almonds (1434 kJ) or crisps (1099 kJ) were introduced to three groups respectively for 12 weeks. Therefore, the snacks were not isocaloric. In addition, the HP snack (almonds) were also high in total fat but high in MUFA (65% of total fat). Dietary intake was recorded in a 4-day unweighed diet diary and hunger ratings were recorded on a 100 mm visual analogue scale (VAS) at baseline, 6 and 12 weeks. Differences across time and between groups were analyzed using repeated measures ANOVA.

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Data are mean values, with their standard errors represented by vertical bars (four to ten observations per group).

* P<0.05, ** P<0.01, *** P<0.001 relative to control of same sex (analyzed by two-way ANOVA).

Fetal exposure to a low-protein diet significantly elevated plasma triacylglycerols in all animals except for males exposed over the whole of gestation. Increased hepatic triacylglycerols were noted in all LP-exposed groups, which showed histological evidence of hepatic steatosis. Significant hyperglycaemia and hyperinsulinaemia were noted in all LP-exposed offspring, with the exception of the LP mid group.

These data strongly indicate that maternal protein restriction in fetal life promotes elevated plasma lipid, glucose and insulin and also elevation of hepatic triacylglycerol deposition at 18 months. These findings suggest insulin resistance and increased hepatic lipogenesis develop with ageing in such animals. A striking finding of the present study was that the offspring appeared to develop the metabolic syndrome as they aged, but without any apparent obesity.
Nutritional and hormonal influences on hepatic sterol regulatory element-binding protein-1c expression in low-metabolism-regulated genes.

School of Biomedical Sciences, Queen's Medical Centre and Division of Nutritional Sciences, School of Biomedical Sciences, Queen's Medical Centre and MCMULLEN2 and A.J. BENNETT 1.

(1) Langley-Evans, 2006). However, the mechanistic basis of this association is not fully understood.

Sterol regulatory element-binding protein-1c (SREBP-1c) is a transcription factor that responds to nutritional status and metabolic programming has not yet been established. The aim of the present study was to investigate whether SREBP-1c regulates metabolic gene expression in liver, adipose and muscle tissue. We have previously demonstrated that hepatic SREBP-1c mRNA level in offspring was decreased from 4 weeks up to 9 months of age, following fetal exposure to a low-protein diet (Erhuma et al., 2005). In the present study LP-exposed offspring were subjected to nutritional and hormonal manipulations in order to investigate programmed responses. Pregnant rats were fed either a control (180 g casein/kg diet) or LP (90 g casein/kg diet) diet during pregnancy. LP feeding was targeted at early (day 0–7), mid (day 8–14) or late (day 15–22) gestation. A standard laboratory chow diet was fed to all rats at littering and was used to wean the offspring at the age of 4 weeks. At 13 weeks of age the offspring were either fed a high-fat diet (400 g lard/kg diet) or chow (29 g fat/kg diet) for 10 weeks. Liver tissue was collected at the end of the feeding period. Expression of SREBP-1c mRNA was determined by RT-PCR (see Figure). In offspring of the control animals, high-fat feeding increased expression by 4-fold. In LP-exposed offspring, basal SREBP-1c mRNA expression was suppressed and the high-fat induction was greatly blunted.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Treatment</th>
<th>Hepatic SREBP-1c mRNA</th>
<th>Hepatic PPAR-gamma mRNA</th>
<th>Hepatic PPAR-alpha mRNA</th>
<th>Hepatic MCAD mRNA mRNA/18S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Chow Diet</td>
<td>Control LP early LP mid LP late</td>
<td>Control LP early LP mid LP late</td>
<td>Control LP early LP mid LP late</td>
<td>Control LP early LP mid LP late</td>
</tr>
<tr>
<td>Female</td>
<td>Chow Diet</td>
<td>Control LP early LP mid LP late</td>
<td>Control LP early LP mid LP late</td>
<td>Control LP early LP mid LP late</td>
<td>Control LP early LP mid LP late</td>
</tr>
</tbody>
</table>

Data are means, with their standard errors represented by vertical bars (four to six observations per group). * Significant effect at P < 0.05, ** Significant effect at P < 0.01, *** Significant effect at P < 0.001 relative to control group fed chow (P < 0.05). § Significantly different from control group fed high-fat diet (P < 0.05). ¶ Significantly different from control LP all LP early LP mid LP late.

Expression of hepatic FAS (Fig. A) and PPAR-gamma (Fig. B) were significantly upregulated by exposure to a low-protein diet in utero (95–107). Hepatic SREBP-1c mRNA expression at 18 months was significantly lower in the LP group (Fig. C). Hepatic PPAR-alpha mRNA expression at 18 months was also significantly lower in the LP group (Fig. D) and the downstream target of this transcription factor, MCAD, were significantly suppressed (Fig. E). Taken together, these findings suggest that intra-uterine protein restriction programmes both lipogenic and fat breakdown pathways. Increased hepatic lipogenesis and decreased hepatic fat oxidation associated with an impaired insulin signalling system may explain metabolic dysregulation in animals subject to prenatal undernutrition.

Diet-mediated suppression of hepatic SREBP-1c expression was removed by prenatal exposure to a low-protein diet. Over-exposure of fetal tissues to maternal glucocorticoids may be the mechanism mediating such programming.
Folate-deficient diets fed to pregnant rats cause changes in the mRNA associated with lipid metabolism in the fetus. By W.D. REES, S.M. HAY, C.J. MCNEIL and C.A. MALONEY, The Rowett Research Institute, Greenbush Road, Backsham, Aberdeen, UK, AB2 9SB

Folic acid, methionine and choline participate in a series of cyclical metabolic reactions to produce S-adenosyl methionine (SAM). Phospholipid methylation in the liver, a major consumer of SAM, is involved in regulating the flux of lipid between liver, plasma and peripheral tissues via the liver-derived lipoproteins (Watkins et al. 2004). Folate deficiency during gestation may perturb this process. Subsequent changes in lipid metabolism may result in metabolic programming of the offspring. These experiments were undertaken to investigate the effect of maternal diets deficient in folate, methionine and choline on the expression of mRNA coding for proteins involved in the synthesis of fatty acids (acetyl CoA carboxylase; ACC-1) and fatty acid oxidation (carnitine palmitoyl transferase; L-CPT-1) in the pregnant rat.

Female Rowett Hooded Lister rats were fed one of three semi-synthetic diets; a control containing the equivalent of 180 g casein/kg, the same diet deficient in folate (−F) and one deficient in folate with lower concentrations of methionine and choline (−F LM LC). After 2 weeks the animals were mated. The dams continued to be fed the experimental diets until they were killed on day 21 of gestation. Triacylglycerols were estimated using a kit from ThermoElectron (Labmedics, Manchester, UK). Total RNA was extracted with TriZol reagent (Sigma, Poole, Dorset, UK). The relative expression of mRNA in samples from the maternal and fetal tissues was assessed using a kit from Applied Biosystems.

Significantly lower fetal weights were recorded in the Zn-restricted group (0.64 (±0.02) g; n 6) compared with both Zn-adequate (0.69 (±0.02) g; n 6; P<0.05) and Zn-supplemented groups (0.75 (±0.02) g; n 6; P<0.001). Placentas from dams fed the Zn-restricted diet had significantly lower weights (0.112 (±0.004) g; n 6; P<0.01) than those from dams fed the Zn-supplemented diet (0.127 (±0.004) g; n 6), but did not differ in weight compared with those from dams fed the Zn-adequate diet (0.110 (±0.003) g; n 6). Although there was a trend suggestive of a reduction in placental Igf2 mRNA with restricted Zn and increased placental Igf2 mRNA with Zn supplementation (See Fig. 1), no significant differences were observed between groups when analysed by one-way ANOVA or linear regression.

These data indicate that differences observed in placental and fetal development in the present study were not due to Zn-dependent changes in placental Igf2 transcription or mRNA stability. The findings do not exclude the possibility that Zn nutrition during pregnancy may alter placental Igf2 expression at the level of mRNA expression in fetal tissues, and thus affect fetal and/or placental weight. Further experiments are needed to determine the extent to which placental expression of Igf2 is influenced by Zn nutrition during pregnancy.

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Folate deficiency causes changes in lipid metabolism in the dam and fetus. Folate status may modulate the effects of fat in the maternal diet. The resulting changes in fetal metabolism may influence the developing insulin axis and lead to metabolic programming of the offspring.

The present study was supported by the Scottish Executive, Environment and Rural Affairs Department as part of the core funding to the Rowett Research Institute and by the European Union Sixth Framework Programme EARNEST (CT-2005–00306).


The effect of increasing the eicosapentaenoic acid and docosahexaenoic acid content of a test meal on the postprandial changes in blood triacylglycerol concentration in men and post-menopausal women aged 50 to 65 years consuming their habitual diets. By G.C. Burdge1, J. Powell2 and P.C. Cakler1, 1Institute of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton, UK, SO16 7PX and 2Unilever Research Colworth, Bedfordshire, UK, MK44 1LQ

The magnitude of the change in blood triacylglycerol (TAG) concentration following a meal is an independent risk factor for CVD and is associated with increased risk of myocardial infarction (Karpe, 1997). The magnitude of the postprandial lipaemia (PPL) is greater in men compared with premenopausal women, although this advantage is lost in post-menopausal women (Cohen & Schall, 1988). Increasing dietary EPA and DHA consumption reduces PPL (for a review, see Williams, 1997). Some studies, but not all, have shown that increasing the EPA+DHA content of a test meal reduced the magnitude of PPL (Williams, 1997). Importantly, these studies investigated the effect of meal fatty acid composition on PPL in young men. We have investigated the effect of consuming an EPA- and DHA-enriched meal on PPL in middle-aged men and women, the main target age group for interventions to reduce risk of CVD.

Subjects were healthy men (n 11) aged 58 (so 5) years and post-menopausal women (n 11) who were not using hormone-replacement therapy (56 (so 4) years) with BMI<25 kg/m2 and fasting TAG<2.0 mmol/l. After an overnight fast, subjects consumed either a reference (REF) meal (55 g total fat, 130 g total carbohydrate; 4.3 MJ total energy; EPA+DHA 0.6% total fatty acids) or an EPA+DHA (ED)-enriched meal (56 g total fat; 130 g total carbohydrate; 4.3 MJ total energy; EPA+DHA 4.1% total fatty acids). The meals were consumed at least 14 d apart. Blood was collected from an indwelling venous cannula at baseline and at intervals up to 6 h. Plasma TAG concentration was measured by standard automated colorimetric assay. The fatty acid composition of plasma TAG at peak TAG concentration was measured by GC (Burdge et al. 2000).

Peak EPA+DHA concentration was 5.4-fold greater (P<0.0001) in men and women after the ED meal v. the REF meal. There were no significant differences by Student’s unpaired t test between men and women after the REF or ED meals (see Table). There was no significant difference between meals by Student’s paired t test for men or women (see Table).

Together these results suggest that increasing the EPA+DHA content of a meal has no significant effect on PPL in middle-aged individuals. One implication is that long-term intake of EPA+DHA in the background diet is of greater importance for achieving health benefits associated with EPA and DHA than the level of acute consumption in a meal.

Supplied by Unilever Corporate Research.


Impact of dietary changes in n-6:n-3 polyunsaturated fatty acid ratio on serum triacylglycerols in healthy men and women. By C.S. Moore, S.P. Bryant, G.D. Mishra, J.D. Krebs, L.M. Browning and S.A. Jebb, Medical Research Council Human Nutrition Research, Elscot Walden Laboratory, Fulbourn Road, Cambridge, UK CB1 9NL

Previous studies have shown reductions in serum triacylglycerols (TAG) with high-dose long-chain (LC) n-3 PUFA supplements. Effects of increased dietary n-3 PUFA are less clear (Lindgren et al. 1991; Tidwell et al. 1993) and may be modulated by habitual n-6:n-3 PUFA. The present study examines the impact on TAG of reducing dietary n-6:n-3 PUFA by changes in linoleic acid:α-linolenic acid (LA:ALA) and/or increasing LC n-3 PUFA.

Healthy, overweight men and women (age 35–65 years; n 142) were assigned to a control group (habitual diet) or one of four interventions for a 24-week period. Intervention groups received either two portions of oily fish (OF; 4.5 g EPA+DHA) or white fish (WF; 0.7 g EPA+DHA)/week, and replaced habitual household fats with ones high in either sunflower-seed (SF; high LA:LNA) or rapeseed (RS; low LA:ALA) oil.

There was a significant effect of dietary treatment on TAG (Group×Time P=0.05); at 12 weeks the OF–RS group showed lower TAG than the WF–SF group (P=0.05) and at 24 weeks the control, OF–RS and OF–SF groups showed significantly lower TAG concentrations than the WF–SF group (P=0.05). The decrease in TAG in the OF groups combined was 6.6%. The effect was greatest in those with lower dietary LA:ALA (OF–RS, 10.4% v. OF–SF, 2.8%) although not significant. The table shows TAG (geometric means and standard deviations; mmol/l).
Postprandial hepatic glycogen and lipid synthesis of rats maintained on a high-protein diet with varied fat and vitamin B6 levels. By M. MARJI, N. HWALLA and O.A. OBEID, Department of Nutrition and Food Science, American University of Beirut, Beirut, Lebanon

High-protein diets are used to reduce weight and concerns still exist about the possible adverse health effects of such a diet. Vitamin B6 is known to be involved in protein and fat metabolism and its requirement increases when protein intake increases (Okada et al. 1998). We have recently found an increase in liver weight under high-protein high-fat conditions (Obeid et al. 2006).

The present experiment was conducted to study the effects of a high-protein diet with varied vitamin B6 and fat levels on the liver status of rats. Forty-five male Sprague–Dawley rats were randomly divided into six groups to receive one of the high-protein (45% of energy) diets: low fat (23% of energy)—low vitamin B6 (0.25 mg/100 g diet) (LF-LB6); low-fat (23% of energy)—subnormal vitamin B6 (0.5 mg/100 g diet) (LF-SNB6); low-fat (23% of energy)—normal vitamin B6 (1.75 mg/100 g diet) (LF-NB6); high-fat (41% of energy)—low vitamin B6 (0.25 mg/100 g diet) (HF-LB6); high-fat (41% of energy)—subnormal vitamin B6 (0.5 mg/100 g diet) (HF-SNB6); high-fat (41% of energy)—normal vitamin B6 (1.75 mg/100 g diet) (HF-NB6). Rats were maintained on their respective diet for 6 weeks. At the end of the experiment, overnight fasted rats were tube-fed 4 ml water (containing 1.25 g of their respective diet), immediately injected intraperitoneally with 147.98 MBq 3H2O, and killed by decapitation 1 h later. Blood and liver were taken for analysis as described by Obeid et al. (2006).

Plasma glucose concentrations as well as liver weights were similar among all groups. Rats maintained on the high-fat diets had significantly higher fat percentages in their livers than rats fed low-fat diets. Rats fed diets low in fat and hence higher in carbohydrates had significantly higher rates of liver glycogenesis, but liver glycogen content was similar between the groups. The rate of liver lipogenesis was not affected by the level of vitamin B6 nor by the fat intake. Vitamin B6 level did not have an effect on fat and glycogen content of the liver nor on the rates of glycogenesis and lipogenesis in the liver.

In conclusion, a high-protein diet that is slightly deficient in vitamin B6 does not affect liver weight or fat content after 6 weeks. Increased fat intake seems to be the major determinant for increased fat content in the liver.

Lack of effect of moderate intakes of docosahexaenoic acid on in vivo lipid indices of lipid peroxidation. By T.A.B. SANDERS1, D.C.S. TALBOT2 and H.E. THEOBALD1, 1Nutritional Sciences Research Division, King’s College London, 150 Stamford Street, London, UK, SE1 9NH and 2Unilever Corporate Research, Colworth Park, Sharnbrook, Bedford, UK, MK44 1LQ

DHA (22: 6-3) is extremely susceptible to oxidation and animal studies have found that high intakes increase liver oxidation in vivo. Theobald et al. (2004) reported that a moderate intake of 0.7 g DHA increased plasma LDL-cholesterol concentrations in middle-aged healthy subjects who were moderately hypercholesterolaemic, but found no evidence to suggest that plasma antioxidant concentrations were altered. It was suggested that this may have been a consequence of activating the LXR receptor which has oxidised lipids as ligands. F2-isoprostanes are a family of metabolites arising from the oxidation of arachidonic acid by reactive oxygen species (ROS). 8-Isoprostane F2α is currently regarded as the most reliable marker of in vivo ROS production and non-enzymic lipid peroxidation (Morrow, 2005). The concentration of this metabolite can be determined in urine along with its stable urinary metabolite 2,3-dinor-isoprostane F2α using ‘dissociation enhanced lanthanide fluoro immunoassay’ (DELFIA) technology (Perkin Elmer Life Sciences, Boston, MA, USA) and consequently may act as a biomarker of whole-body lipid oxidation. We report the urinary isoprostane excretion in subjects described by Theobald et al. (2004) before and after 3 months’ treatment with DHA or placebo. The subjects had made a 24 h urine collection and samples of urine had been stored at −20°C for 5 years.

Table 1. Effect of a high-protein diet with varied fat and vitamin B6 levels on liver glucose and fat synthesis in rats maintained on their respective diet

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma glucose (mg/dl)</th>
<th>Liver weight (g)</th>
<th>Liver fat (% dry wt)</th>
<th>Liver glucose (mg/g liver)</th>
<th>Liver glycogenesis*</th>
<th>Liver lipogenesis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF-LB6</td>
<td>8 1397.5 126 11.64 0.4</td>
<td>29.49 2.6 3.97 0.6</td>
<td>34.23 6.7 3.99 0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF-LB6</td>
<td>8 1490.6 103 12.61 0.5</td>
<td>35.88 2.1 3.55 0.4</td>
<td>23.69 4.0 3.45 0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LF-SNB6</td>
<td>7 1247.1 17 10.86 0.5</td>
<td>30.76 1.3 4.48 0.5</td>
<td>45.01 4.6 3.82 0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF-SNB6</td>
<td>7 1343.8 48 11.86 0.5</td>
<td>37.96 2.1 4.94 0.7</td>
<td>26.39 2.1 3.49 0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LF-NB6</td>
<td>7 1377.1 16 12.05 0.4</td>
<td>32.47 1.5 4.70 0.7</td>
<td>35.24 5.1 4.55 0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF-NB6</td>
<td>7 1265.7 19 12.27 0.5</td>
<td>34.7 2.0 4.27 0.5</td>
<td>27.20 2.4 4.02 0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05 according to fat using two-way ANOVA (vitamin B6 and fat x vitamin B6).

Plasma glucose concentrations as well as liver weights were similar among all groups. Rats maintained on the high-fat diets had significantly higher fat percentages in their livers than rats fed low-fat diets. Rats fed diets low in fat and hence higher in carbohydrates had significantly higher rates of liver glycogenesis, but liver glycogen content was similar between the groups. The rate of liver lipogenesis was not affected by the level of vitamin B6 nor by the fat intake. Vitamin B6 level did not have an effect on fat and glycogen content of the liver nor on the rates of glycogenesis and lipogenesis in the liver.

The 2,3 diol metabolite occurred in five-fold higher concentrations than 8-isoprostane F2α. The immunoassay for the 2,3 diol metabolite gives much higher values (approximately 10–30-fold) than the GC-MS assay (Il’yasova et al. 2004). However, the values reported were similar to those reported in other studies in the literature using immunoassays. We were unable to find any influence of DHA on these measures of isoprostane excretion.

Diurnal variation in glycaemic index: comparison of a low- and high-glycaemic index mixed meal. By M. GIBBS, A. JETHA, D. MELLOR and S. HAMPTON. Biomathematics and Statistics Scotland, Rowett Research Institute, Bucksburn, Aberdeen, UK. AB21 9SB.

Research into the glycaemic index (GI) of foods and improved metabolic parameters, in areas such as diabetes, CHD, obesity and renal disease, has led to an increased awareness of low-GI (LGI) diets. Diurnal variation in the postprandial hormone and metabolic responses has been well documented (Hampton et al. 1996; Ribeiro et al. 1998); however, no study to date has measured the diurnal variation of postprandial glycaemia following LGI and high-GI (HGI) mixed meals. The present study investigates diurnal changes in the GI of an HGI and an LGI meal; the study also compares the glycaemic responses of the two meals. It was hypothesised that the glycaemic responses of HGI and LGI meals would be greater in the evening compared with the morning, and the glycaemic response prolonged after the evening meal. The HGI meal, when consumed in the evening, would give a significantly greater glycaemic response compared with the LGI meal, which may have implications for dietary choice at evening meals.

In a randomised controlled, single-blind, cross-over study, nine healthy subjects (eight females, one male), mean age 23.8 (sd 5.14) years with BMI of 21.13 (sd 2.63) kg/m², were given an HGI or LGI meal or glucose control (GC) in the morning (08.00 hours) or the evening on six separate occasions. The HGI meal consisted of French bread and macaroni cheese and the LGI meal consisted of Burgen bread and baked beans. Subjects fasted (10 h) following a controlled pre-meal, before each leg of the study. Blood samples were taken using a fingerprick method, at baseline (0), and at 15, 30, 45, 60, 90 and 120 min postprandially. Samples were analysed for glucose using the YSI 2300 STAT plus glucose and lactate analyser (YSI). Comparison between meals and diurnal comparisons were made by paired t tests.

The GI of each meal was initially calculated by the method of Frost & Dornhorst (2000), from the published GI of the ingredients (HGI=77 and LGI=38). The observed GI of the meals was calculated from the incremental area under the curve of the glucose responses as 64 for the HGI and 53 for the LGI. This difference in calculated v. observed GI may reflect a change in GI when consumed as a mixed meal.

There was a significant increase in glycaemic response in the evening compared with the morning following both meals and GC (HGI P=0.01; LGI P=0.01; GC P=0.003) and postprandial glucose levels remained elevated for longer in the evening. The percentage increase in observed GI from morning to evening was similar for both meals (HGI 167 (sd 53)%; LGI 163 (sd 61)%; P=0.954), representing a 2.3-fold increase for both responses.

There is a clear diurnal variation in postprandial glycaemic response following consumption of a mixed meal. Although no significant difference was found in postprandial glucose responses between the meals in the morning or the evening, the diurnal difference in glycaemic response suggests that consuming a main meal late in the day has an undesirable metabolic impact irrespective of the GI of the meal. The lack of statistical support for a difference between meals may have arisen due to difference in calculated and observed GI, and the choice of meal composition for the HGI meal, and suggests that the concept of variability in GI being exacerbated diurnally should not be dismissed. Further studies investigating a wider range of GI meals consumed late at night could elucidate differences in glycaemic response.

Funding support was from the University of Surrey GI testing service.


Fat and carbohydrate do not displace each other in the diet. By G.W. HORGAN, S. WHYBROW and J. STUBBS. Biomathematics and Statistics Scotland, Rowett Research Institute, Bucksburn, Aberdeen, UK. AB21 9SB.

It has been claimed that individuals eating a normal diet tend to choose either fat or carbohydrate (CHO) as an energy source, with one displacing the other. This is true when fat or CHO are expressed as a percentage of total energy intake, but this is no more than a consequence of macronutrient compositions adding to 100%, with protein typically contributing a smaller amount. If absolute amounts are examined, then the fat–CHO correlation becomes positive. Again, this says nothing about choice: larger individuals eat more of both than smaller individuals.
Antioxidant imbalance in ulcerative colitis. By P. RANA¹, A.S. ANDERSON¹ and J.H. CUMMINGS², ¹Centre for Public Health Nutrition Research, Ninewells Medical School, Dundee, DD1 9SY and ²Division of Pathology and Neuroscience, Ninewells Medical School, Dundee, DD1 9SY

Ulcerative colitis (UC) is a diffuse inflammatory disease of the bowel with a remitting and relapsing course in which there is an increased production of free radicals. Trace elements are an important part of the antioxidant defence against oxidative stress. A study of 24 UC patients found that the patients with moderately active disease had significantly lower plasma iron, selenium and glutathione peroxidase levels (Sturkolos et al. 1998). Another study carried out in 75 well-nourished cases of UC found high serum levels of copper and zinc which correlated with haematological parameters of relapse of disease (Dalekos et al. 1998). A randomised controlled trial in which subjects were given an oral supplement enriched with fructose, fructose, vitamin E, vitamin C and selenium found clinical improvement in supplemented subjects with a decreased requirement for steroids (Seidler et al. 2005).

It has been hypothesised that the abnormalities of trace elements may be due to inadequate intake, reduced absorption and increased losses as a result of the inflammatory process and that deficiency of trace elements may contribute to the continued inflammatory process of IBD (Ojuawo & Keith, 2002). This study was a secondary analysis of data from a study on 82 patients with UC (Magee et al. 2005). All the subjects had completed a 7-day estimated diet diary. Intakes were compared to the UK Dietary Reference Values (DRVs) (Department of Health, 1991). Sigmoidoscopy score was used as a measure of disease activity in UC and associations with nutrient intakes were determined using Spearman’s correlation analysis (higher score indicates a stronger correlation). Statistical analysis was carried out using SPSS version 11.5.

Comparison of trace element intakes of males and females with Dietary Reference Values

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Intake</th>
<th>RNI</th>
<th>Intake</th>
<th>RNI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper (mg)</td>
<td>1.3</td>
<td>1.2</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Selenium (µg)</td>
<td>54</td>
<td>75</td>
<td>60</td>
<td>87</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>13</td>
<td>8.7</td>
<td>11.6 (95%)</td>
<td>14.8 (95%)</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>8.3</td>
<td>&gt;4mg/d (safe intake)</td>
<td>7.4</td>
<td>&gt;3mg/d (safe intake)</td>
</tr>
</tbody>
</table>

It is shown in the table that the selenium intake was lower than the Reference Nutrient Intake (RNI) (P<0.001) for males and females. Iron intake in males was significantly higher than the RNI (P<0.01) while intakes were lower than RNI in females 19–50 years (P<0.01).

Copper intake was significantly correlated with sigmoidoscopy score (r=0.344, P<0.01). In males, iron (r=0.457, P<0.01), copper (r=0.556, P<0.001) and zinc (r=0.348, P<0.05) were positively correlated with sigmoidoscopy score. In females, iron correlated negatively with sigmoidoscopy score (r=0.54, P<0.05).

A regression analysis for the whole group revealed that 9% (r²=0.09) of the variation in the sigmoidoscopy score was explained by copper intake. In females (n=39), copper, iron and vitamin E explained 38.9% (r²=0.389) of the variation in sigmoidoscopy score.

Copper intake was positively correlated with the disease activity thus indicating a possible relation with heightened inflammation. Low selenium intake may also be a contributory factor to the continued inflammation.

The overall results showed that the different kinds of beverages have varying antioxidant capacities in vitro. However, it is important to point out that FRAP values give an overall holistic measure of antioxidant capacity in an isolated system, and consideration also needs to be given to the potential effects in vivo. For example moderate consumption of alcoholic beverages has been reported to be cardioprotective, but excessive consumption is very damaging. In conclusion, the microprocessor-controlled robotic ELISA reader combination is suitable for high-throughput analysis of alcoholic and non-alcoholic solutions or beverages. It also has the advantage of minimising labour time, cost and experimental error.

References


The application of a high-throughput robotic ELISA-reader system to measure total antioxidant capacity in alcoholic and non-alcoholic beverages and wine-related compounds. By M.C.Y. WONG, R. WEST, A. SPARR, C. LLOYD, M.J. ARNO, H. WISEMAN and V.R. PREEDY, Nutritional Sciences Research Division, King’s College London, Franklin-Wilkins Building, 150 Stamford Street, London, UK, SE1 9NH

It has been suggested that measurement of total antioxidant capacity can be more meaningful than assessing concentrations of individual components. As a consequence, a number of assays have been developed to determine antioxidant potential in diverse biological samples, such as food substances, tissue extracts and blood. In particular, the ferric-reducing antioxidant power (FRAP) assay has been applied extensively to clinical and nutritional biochemistry as well as food science. However, the experimental procedures of the original FRAP assay are prone to artificial data (i.e. operate error) and the assay is also labour intensive. We therefore investigated the usage of a high-throughput technique for measuring FRAP using a microprocessor-controlled robot-ELISA reader combination. This method allowed us to assay ninety-six samples (and potentially 486) in 1 h, compared with only five samples in the original method. The results demonstrated that there was a highly significant correlation between data between the manual and robotic methods (r=0.95; P<0.001). The robotic method was then applied to the analysis of variety of alcoholic and non-alcoholic beverages including red wine, white wine, red wine capsules and tablets, grape juice and orange juices.

Comparison of trace element intakes of males and females with Dietary Reference Values

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Intake</th>
<th>RNI</th>
<th>Intake</th>
<th>RNI</th>
</tr>
</thead>
<tbody>
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<td>&gt;3mg/d (safe intake)</td>
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</table>

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The overall results showed that the different kinds of beverages have varying antioxidant capacities in vitro. However, it is important to point out that FRAP values give an overall holistic measure in an isolated system, and consideration also needs to be given to the potential effects in vivo. For example moderate consumption of alcoholic beverages has been reported to be cardioprotective, but excessive consumption is very damaging. In conclusion, the microprocessor-controlled robotic-ELISA reader combination is suitable for high-throughput analysis of alcoholic and non-alcoholic solutions or beverages. It also has the advantage of minimising labour time, cost and experimental error.

In many countries, beers are consumed in preference to wine. In the UK, beers contribute to about half of the total alcoholic beverage market. Beer has an appreciable antioxidant content to the extent that its consumption in moderate amounts significantly increases plasma antioxidant capacity. However, there is little information on the antioxidant capacities of UK beers and whether this is influenced by the brewing or bottling process. We hypothesised that (1) UK beers have a high total antioxidant capacity; (2) draught beers contain a greater antioxidant capacity in comparison with bottled beers; (3) organic and fruit beers have higher antioxidant capacities than traditional or ‘normal’ beers. To test this, we used the ferric-reducing antioxidant power (FRAP) assay to determine the in vitro antioxidant capacity in approximately forty different beers. Beers were classified according to their country of origin (i.e. UK, Europe, Asia and Australia) and storage conditions (bottled or draught).

The results demonstrated that organic and non-organic UK beers have a high total antioxidant capacity. The total antioxidant capacities of UK draught beer from local pubs were significantly higher than corresponding bottled beers. Surprisingly, ‘fruit beers’ were shown to have the highest antioxidant capacity, though it could be argued that their classification as a ‘beer’ is a misnomer. In conclusion, the results indicate that UK beers contain appreciable quantities of antioxidants though further work is needed to ascertain whether other antioxidant assays provide similar conclusions.

Differences in typical food portion sizes eaten by institutionalised older adults compared with free-living. By W.L. Wrieden, K.L. Barton, A.J. ADAMSON and L. COCHRANE, 1 School of Medicine, University of Dundee, Ninewells Medical School, Dundee, UK, DD1 9SY and 2 Human Nutrition Research Centre, University of Newcastle upon Tyne, Newcastle upon Tyne, UK, NE2 4HH

The energy and nutrient intake of groups of individuals can be estimated using published food portion sizes (Food Standards Agency, 2002). These were calculated using the data from adults aged 16–64 years in the 1986–7 Dietary and Nutritional Survey of British Adults (Gregory et al. 1990) but there are no UK portion sizes for older individuals. Given the reduced energy needs of the older population (Department of Health, 1991) it would be expected that portion sizes may be smaller.

As part of a study aimed to produce a set of typical food portion weights for younger and older adults, food portion information was extracted from the National Diet and Nutrition Survey (NDNS) of people aged 65 years and over, carried out in 1994–5 (Finch et al. 1998). This used a 4 d weighed intake methodology and recorded portion weights for free-living older adults (n 1275) and those living in institutions (n 412). As such it provides the most recent comprehensive information available on the foods and portion sizes eaten by this group.

Eighty different kinds of foods and drinks were recorded at least 100 times by individuals living in institutions and sixty-eight were found to have significantly different portion sizes compared with those calculated for free-living individuals.

Smallers portions were recorded for breads, porridge, biscuits and cakes, custard and puddings, and vegetables but not white sugar. This was despite the fact that higher energy intakes were found for the institutionalised adults compared with the free-living (Finch et al. 1998). Tea, milk in tea, water and squash or fruit drink were the most frequently recorded drinks and white sugar the most frequently recorded food. Median weights (g) and interquartile ranges (IQR) are given for the fourteen most frequently recorded foods.

<table>
<thead>
<tr>
<th>Food</th>
<th>Institutionalised</th>
<th>Free-living</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (g)</td>
<td>IQR (g)</td>
</tr>
<tr>
<td>White sugar</td>
<td>8</td>
<td>5–10</td>
</tr>
<tr>
<td>Red cup of fruit</td>
<td>7</td>
<td>5–12</td>
</tr>
<tr>
<td>Bread, white or self-sliced</td>
<td>38</td>
<td>30–64</td>
</tr>
<tr>
<td>Butter</td>
<td>10</td>
<td>6–14</td>
</tr>
<tr>
<td>Bread, white or self-sliced, toasted</td>
<td>37</td>
<td>28–54</td>
</tr>
<tr>
<td>Bread, wholemeal</td>
<td>36</td>
<td>28–60</td>
</tr>
<tr>
<td>Gravy</td>
<td>43</td>
<td>26–66</td>
</tr>
<tr>
<td>Semi-sweet biscuit</td>
<td>14</td>
<td>7–15</td>
</tr>
<tr>
<td>Currant or sweet white sauce</td>
<td>100</td>
<td>63–122</td>
</tr>
<tr>
<td>Marmalade</td>
<td>15</td>
<td>9–22</td>
</tr>
<tr>
<td>Carrots, cooked</td>
<td>40</td>
<td>25–70</td>
</tr>
<tr>
<td>Porridge</td>
<td>77</td>
<td>13–226</td>
</tr>
<tr>
<td>Cottage-type cereal</td>
<td>30</td>
<td>24–34</td>
</tr>
<tr>
<td>Chicken</td>
<td>73</td>
<td>54–113</td>
</tr>
</tbody>
</table>

Total antioxidant capacity: * Mann–Whitney test for two independent samples.

Includes brown, granary and oatmeal.

*The reasons for the differences are unclear and could reflect smaller appetites of those in care. or a reflection of the slightly different methodology used for the institutionalised surveys (where only one main meal per day was weighed and other portions were estimated). However, the higher energy intakes of the institutionalised adults could be due to the types of foods eaten and an increased frequency of eating due to the routine imposed by the institution.

Work to test the use of typical portion sizes in males and females aged 75+ years and institutionalised individuals aged over 65 years is recommended but will be dependent on weighed food diaries being available from institutionalised adults. These typical portion weights will enable researchers to apply more relevant portion weights to surveys and should be particularly useful for the proposed rolling programme of NDNS using the multiple-pass 24 h recall method.

Funding provided by Food Standards Agency is gratefully acknowledged (project no. N08025).


Vitamin E supplementation and mechanical texture measurement of cooked poultry meat.

By O.B. KENNEDY1, B.J. STEWART-KNOX2, P.C. MITCHELL2 and D.I. THURNHAM2.

1Hugh Sinclair Human Nutrition Unit, School of Food Biosciences, University of Reading, PO Box 226, Reading, UK, RG6 6AP and 2Northern Ireland Centre for Food and Health, School of Biomedical Sciences, University of Ulster, Cromore Road, Coleraine, UK, BT52 1SA

Texture is one of the most important quality attributes of meat. Lipid oxidation leads to meat spoilage and has been reported to cause adverse changes in the flavour, colour and texture of poultry meat.

Vitamin E has been found to be effective in reducing lipid oxidation and drip loss. The present study investigates the effect of vitamin E supplementation upon drip loss and cook loss in poultry and instrumental measures of poultry texture. Broiler chickens (either corn-fed or wheat-fed) were supplemented with one of three levels of vitamin E (75, 250 and 500 mg/kg). Drip and cook losses from raw and cooked carcasses respectively were measured. Instrumental texture as shear force (force) was measured using a TA.XT2 texture analyser equipped with a Warner Bratzler attachment (Stable Micro System, Godalming, Surrey, UK). Measurements were carried out on breast meat following a standardised cooking procedure.

<table>
<thead>
<tr>
<th>Vitamin E level</th>
<th>Cook loss (%)</th>
<th>Force (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Corn-fed</td>
<td>Wheat-fed</td>
</tr>
<tr>
<td>75 mg/kg</td>
<td>22.37±2.10</td>
<td>23.78±2.60</td>
</tr>
<tr>
<td>250 mg/kg</td>
<td>22.57±3.43</td>
<td>23.18±3.19</td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>21.96±3.37</td>
<td>22.64±3.54</td>
</tr>
</tbody>
</table>

Vitamin E did not appear to affect drip, cook and overall loss. Meat from chickens supplemented with the 500 mg/kg level of vitamin E had higher resistance to shear force than meat from chickens supplemented with 75 and 250 mg/kg, indicating greater toughness. Corn-fed chicken breast meat required significantly less force to shear than wheat-fed chicken, indicating greater tenderness. Supranutritional levels of vitamin E in broiler feeds did not lead to any improvement in the texture of cooked poultry meat and resulted in significantly tougher meat than meat from broilers that were supplemented with either the 75 or 250 mg/kg levels.

Diet, lifestyle and osteoporotic fracture risk in post-menopausal South Asian women living in Blackburn, UK.

By S. MITRA1, J.F. McCANN2, I. BHOJANI3, P.C. FOSTER1, P.A. JUDD2, B. ELLAH1 and N.M. LOWE1, 1Department of Biological Sciences, University of Central Lancashire, Preston, UK, PR1 2HE, 2Medical Rehabilitation Centre, Royal Preston Hospital, Preston, UK, PR2 9HT, 3Royal Preston Hospital, Blackburn, UK, BBI 4DX, 4Lancashire School of Health and Postgraduate Medicine, University of Central Lancashire, UK, PR1 2HE and 5Department of Biomedical Sciences, University of Chester, Chester, UK, CH1 4BJ

Sedentary lifestyle, early onset of menopause (<45 years of age), family history and low dietary Ca and vitamin D intakes are among the key risk factors for osteoporotic fracture (Buist et al. 2002). It has been suggested that South Asian diets coupled with reduced exposure to sunlight may compromise Ca and vitamin D status (Alfham et al. 1995). However, there is a paucity of detailed information regarding osteoporotic fracture risk in this population. The purpose of the present study was to investigate dietary and lifestyle risk factors for osteoporotic fracture in post-menopausal South Asian women living in Blackburn (Lancs, UK).

Medical history and relevant lifestyle factors, including activity level and age of menopause, were assessed in seventy South Asian women using a structured questionnaire. Dietary intake was assessed by interviewer-administered food-frequency questionnaire and analysed using a food composition database (WinDiets Research; Robert Gordon University, Aberdeen, UK). Broadband ultrasound attenuation (BUA) of the calcaneus was determined by contact ultrasound somometry (McCue CUBA Clinical Ultrasonometer; McCue Plc., Southampton, UK).

Of the women, 38% were found to be under-reporting energy intake (EI) (EI:BMR ratio <1.1) and therefore their data were omitted from further analysis. The mean age of the forty-three participants included in the analysis was 56 (SD 4.4) years; with a mean BMI of 30.2 (SD 4.96) kg/m2. The mean age of menopause was 48 (SD 5) years. A summary of the activity levels in this group of women compared shown in the Table.

<table>
<thead>
<tr>
<th>Day of week</th>
<th>Number of d</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–2 d</td>
<td>5</td>
<td>243.45 ± 2.56</td>
<td>284.516 ± 4.97</td>
</tr>
<tr>
<td>3–4 d</td>
<td>9</td>
<td>269.40 ± 3.87</td>
<td>291.48 ± 3.87</td>
</tr>
<tr>
<td>5 d</td>
<td>20</td>
<td>284.47 ± 2.15</td>
<td>287.51 ± 3.30</td>
</tr>
</tbody>
</table>

The mean energy intake was 7920 (SD 1448) kJ/d. Ca intake was 751 (SD 240) mg/d, 107% of the reference nutrient intake. Vitamin D intake was 1.6 (SD 1.16) µg/d, significantly (P<0.001) lower than the national average for this age group of 3.5 µg/d (Office for National Statistics, 2004). BUA measurements were 66.7 (SD 16.5) dB/MHz, 95.9 (SD 24.3)% of the normative value for this age range.

The present study indicates that despite a sedentary lifestyle and low dietary vitamin D, bone densitometry measures do not suggest that bone quality is significantly impaired. High BMI may be a protective factor against osteoporosis in this population.

A proteomic analysis of aortic proteins in zinc and metallothionein deficiency. By J.H. Beattie 1, M.-J. Gordon 1, G.J. Rucklidge 1, M.D. Reid 1 and I.S. Kwun 2, 1 Rowett Research Institute, Aberdeen, UK, AB21 9SB and 2 Department of Food Science and Nutrition, Andong National University, Andong, South Korea

Atherosclerosis develops over a lifetime and may be influenced by genetic, lifestyle and nutritional factors. Epidemiological studies suggest that an adequate intake of dietary Zn may help to protect against heart disease. In addition, Zn may protect against pro-inflammatory stress in vascular endothelial cells and metallothionein (MT) may modulate NO signalling and participate in the antioxidant response in vascular cells.

The objective of the present study was to identify proteins and protein interactions that are modified by Zn and MT deficiency in rodent aorta, using two-dimensional gel proteomics. In one set of studies, 3-week-old male rats were given either acutely (<1 mg Zn/kg) or marginally (6 mg Zn/kg) Zn-deficient semi-synthetic diets for 5–6 weeks. Controls rats consumed a Zn-adequate diet (35 mg Zn/kg) and animals pair-fed with acutely deficient rats also consumed the adequate diet. Protein expression profiles in aorta were determined using two-dimensional gel proteomics. In a second set of studies, aortic protein expression in adult male mice with a targeted deletion of the MT-1 and MT-2 genes (MTKO mice) and appropriate controls (WT; both genotypes on a 129Sv genetic background) was studied using two-dimensional gel proteomics.

In the rat studies, both marginal and acute Zn deficiency decreased the level of aortic proteins associated with carbohydrate metabolism and lipid biosynthesis. Acute Zn deficiency also suppressed proteins related to the cytoskeleton. Although MT deficiency also decreased cytoskeleton-related protein levels, it increased levels of some enzymes relating to carbohydrate and energy metabolism. Principal component analysis followed by correlation analysis revealed key proteins affected by MT deficiency. These included small GTP-binding and related proteins.

We conclude that a prominent effect of both Zn and MT deficiency in aorta is on carbohydrate metabolism. Since Zn modulates insulin receptor phosphorylation, we propose that the influences on glucose metabolism which we observed may relate to the effects of Zn on insulin signalling. Zn and MT deficiency may have opposite effects on cellular levels of labile Zn and therefore opposite effects on insulin signalling and carbohydrate metabolism.

J. H. B., M. J. G., G. R. and M. D. R. were funded by the Scottish Executive Environment and Rural Affairs Department. I. S. K. was funded by the Korean Ministry of Health and Welfare, grant no. 03-PJ-PG3-22000-0044.

Does dietary fibre intake protect against the risk of developing breast cancer? Evidence from the UK Women’s Cohort Study. By J.E. CADE, V.J. BURLEY and D.C. GREENWOOD, Nutritional Epidemiology Group, Centre for Epidemiology and Biostatistics, University of Leeds, Leeds, UK, LS2 9LN

Whether dietary fibre intake is associated with the risk of breast cancer is unclear. Previous cohort studies have been limited by a narrow range of fibre intakes. However, a high dietary fibre intake may be protective against breast cancer. The UK Women’s Cohort Study (UKWCS) is well placed to explore the risks of breast cancer associated with dietary fibre since the study was designed to have a wide range of relevant exposures through inclusion of large numbers of vegetarians.

The UKWCS (Cade et al. 2004) has 35 792 subjects with approximately one-third in each of three main groups: vegetarian, fish eaters (not meat) and meat eaters, ensuring a wide range of dietary fibre consumption. This analysis includes 350 postmenopausal and 257 premenopausal women who developed invasive breast cancer during 240 959 person-years of follow up. Fibre and breast cancer relationships were explored using Cox regression modelling adjusted for measurement error and potential confounders.

The mean age of the cohort was 52 (SD 9) years at baseline. The majority of the women were white, married with children, well educated (27% had a degree) and middle class (63% (National Statistics) NS-socio-economic class 1). The mean BMI of the women was 24.5 (SD 4.3) kg/m². Only 11% of the cohort were current smokers.

In premenopausal women a statistically significant inverse relationship was found between total fibre intake and risk of breast cancer ($P$ for trend=0.01). Being in the top quintile of total fibre intake was associated with a hazard ratio of 0.50 (95% CI 0.25, 1.00) compared with the lowest quintile. This was not seen in the postmenopausal women. In the premenopausal women, fibre from cereals and fruit were inversely associated with risk of breast cancer (fibre from cereals, $P$ for trend=0.03; fibre from fruit, $P$ for trend=0.09). Fibre from vegetables was not significantly associated with risk of breast cancer in the present study.

These data suggest that in this cohort, premenopausal (but not postmenopausal) women with a high dietary fibre intake are at lower risk of breast cancer than low fibre consumers. Fibre from cereals and possibly fruit may be particularly important in this relationship.

The UK Women’s Cohort Study was funded by the World Cancer Research Fund.

The effect of dietary protein restriction in pregnant rats on the expression of DNA methyltransferases and methyl CpG binding protein 2 in the liver after weaning. By K.A. Lillycrop1, A.A. Jackson 2, M.A. Hanson 3 and G.C. Burdge 3, 1 Institute of Human Nutrition, University of Southampton, Tremona Road, Southampton, UK, SO16 6YD and 2 Department of Development and Cell Biology, Rowett Research Institute, Aberdeen, UK, AB21 9SB 3 Wageningen Centre for Food Sciences and Wageningen University, Wageningen, The Netherlands.

The present results suggest that reduced dietary protein in pregnancy induces hypomethylation of gene promoters in offspring by reducing the capacity of DNMT 1 to methylate hemi-methylated DNA during DNA replication, rather than by impaired methylation of CpG dinucleotides.

In a proteomics study we established that the cholesterol- and triacylglycerol-lowering properties of fish oil could be explained by differential expression of long-chain acyl-CoA thioester hydrolase protein (as an indicator of n-3 oxidation) and adipophilin (as an indicator of liver lipid content). There was no significant effect of prenatal undernutrition on the expression of DNMT 3a or 3b promoters may be achieved by impaired DNA methylation, loss of CpG methylation during de novo methylation or active demethylation. Lower expression of MeCP 2, together with hypomethylation of CpG, would tend to facilitate acetylation of histones leading to increased transcription. These findings suggest that altered epigenetic regulation of gene expression as a result of prenatal nutritional exposure is primarily the result of impaired DNMT 1 activity and involves altered 1-carbon metabolism.

n-3 Fatty acids, present in fish and fish oil, may lower the risk of CVD but mechanisms are not well understood. In a proteomics study we established that the cholesterol- and triacylglycerol-lowering properties of fish oil could be explained by differential expression of long-chain acyl-CoA thioester hydrolase protein (as an indicator of n-3 oxidation) and adipophilin (as an indicator of liver lipid content). There was no significant effect of prenatal undernutrition on the expression of DNMT 3a or 3b promoters may be achieved by impaired DNA methylation, loss of CpG methylation during de novo methylation or active demethylation. Lower expression of MeCP 2, together with hypomethylation of CpG, would tend to facilitate acetylation of histones leading to increased transcription. These findings suggest that altered epigenetic regulation of gene expression as a result of prenatal nutritional exposure is primarily the result of impaired DNMT 1 activity and involves altered 1-carbon metabolism.

**Table 1:**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Expression Relative to Control Group (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td><strong>PR</strong></td>
</tr>
<tr>
<td>Mean SE</td>
<td>Mean SE</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>DNMT 1</td>
<td>100.0 ± 4.5</td>
</tr>
<tr>
<td>DNMT 3a</td>
<td>100.0 ± 3.5</td>
</tr>
<tr>
<td>DNMT 3b</td>
<td>100.0 ± 2.6</td>
</tr>
<tr>
<td>MBD 2</td>
<td>100.0 ± 9.2</td>
</tr>
<tr>
<td>MeCP 2</td>
<td>100.0 ± 9.3</td>
</tr>
</tbody>
</table>

*Values significantly different from the control group are indicated by * 1-Way ANOVA with Bonferroni’s post hoc analysis; # t-test. ND, not determined.*
Fish oil has shown beneficial effects in reducing mortality from CVD. Atherosclerosis, which underlies CVD, is a strong inflammatory component involving the development of atheroma, an accumulation of lipids and inflammatory cells, which are released by an imbalance in arterial wall metabolism. The effects of fish oil and other dietary fats on these processes are of considerable interest. Fish oil, rich in n-3 long-chain polyunsaturated fatty acids (PUFA), has been shown to reduce inflammation, platelet aggregation, and the risk of cardiovascular disease (CVD) in many studies. However, the benefits of fish oil supplementation vary depending on genetic factors, such as single nucleotide polymorphisms (SNPs) in genes involved in fatty acid metabolism. The influence of the CD36 gene polymorphisms on the response of cardiovascular risk factors to fish oil supplementation in middle-aged men was investigated in the OPTILIP study.

The OPTILIP study was a randomised parallel dietary intervention, designed to examine the effects of fish oil on cardiovascular risk factors and the modulatory influence of CD36 SNP upon them. Healthy middle-aged men and male patients with peripheral vascular disease were genotyped for CD36 SNPs in the CD36 gene which are part of a haplotype associated with raised plasma NEFA and insulin resistance. The study compared four diets providing 6% energy as n-6:n-3 ratios of between 5:1 and 3:1 with a control diet (ratio 10:1). Diets were 6 months in duration and enriched in either n-3 long-chain PUFA, does not influence insulin sensitivity or post-heparin plasma lipase activities in older men and women. However, increasing the dietary intake of n-3 long-chain PUFA from 0.2% to 0.7% energy (1 g/L of fish oil) increases the bioactivity of fish oil (data not shown), only individuals with a CD36-A31118 G-A homozygous genotype showed a reduced sdLDL by 4.5-6.9% and increased HDL by 2.0-4.1% in the control group (0.05 to 0.5)% and increased HDL in both groups (Wilcoxon signed rank test; P=0.03). Fish oil supplementation caused a significant fall in plasma TG (–0.14 mmol/l) and a rise in HDL (0.03 mmol/l) in both control groups but not in the healthy middle-aged subjects shown in the Table.

<table>
<thead>
<tr>
<th>CD36 SNP</th>
<th>Mean ± SD</th>
<th>Change in TG</th>
<th>Change in HDL</th>
<th>Change in LDL</th>
<th>Change in LDL:HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD36-31118 AA</td>
<td>0.32 ± 0.09</td>
<td>0.05 ± 0.09</td>
<td>0.71 ± 0.05</td>
<td>28 ± 0.05</td>
<td>68 ± 474</td>
</tr>
<tr>
<td>CD36-31118 AG</td>
<td>0.08 ± 0.52</td>
<td>0.03 ± 0.60</td>
<td>0.16 ± 0.13</td>
<td>48 ± 9</td>
<td>414 ± 77</td>
</tr>
<tr>
<td>CD36-31118 GG</td>
<td>0.15 ± 0.56</td>
<td>0.06 ± 0.64</td>
<td>0.04 ± 0.14</td>
<td>57 ± 18</td>
<td>307 ± 40</td>
</tr>
</tbody>
</table>

In conclusion, dietary advice to decrease the n-6:n-3 fatty acid ratio on insulin sensitivity, lipoprotein size and postprandial lipemia in older men and women. The OPTILIP study, by M.D. Griffiths, T.A.H. Sanders, I.G. Davies, F.E. Lewis, S. Sluiter, T.A.B. Sanders, I.G. Davies, F. Lewis, S. Slaughter, D.J. Millward, and B.A. Griffin, Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey, UK, is currently ongoing. The OPTILIP study was supported by the Food Standards Agency (project no. N2014/5). We are grateful to the BBSRC for funding this project.
Genetic determinants of plasma non-esterified fatty acid composition: a twin study. By P. HAGGARTY, C. TUYA, G. HOAD, D.M. CAMPBELL, G. HORGAN, L. MASSON and G. McNEILL, 1Rowett Research Institute, Aberdeen, UK, 2Biological Research Unit, NHS Grampian, Aberdeen, UK, 3Department of Obstetrics and Gynaecology, Aberdeen University, Aberdeen, UK, 4Aberdeen, UK, 5Biostatistics and Statistics Scotland, Aberdeen, UK, 6Aberdeen University, Aberdeen, UK, AB2 2ZD

A key aim of public health policy in many countries is to reduce the proportion of saturated fats in the diet to avoid chronic diseases such as CVD. Many risk factors for CVD, such as circulating lipoprotein, and HDL- and LDL-cholesterol concentrations, have heritabilities in excess of 60%. It has not been established to what extent the response to dietary fatty acid intake may also be genetically determined. The aim of the present study was to quantify the genetic contribution to circulating plasma NEFA using classical twin analysis in sixty monozygotic and seventy-one dizygotic twin pairs; fifty-three male and seventy-eight female; mean age 33.2 (SEM 0.80) years.

Weight, height and percentage body fat (by bioelectrical impedance) was measured in each twin. Physical activity level and smoking habits were assessed by questionnaire and dietary fatty acid intake determined using the Scottish Collaborative Group food-frequency questionnaire. The fatty acid composition of the NEFA in a fasting blood sample from each twin was determined on a DB23 column (J&W Scientific, Folsom, CA, USA) on a Hewlett-Packard 5890 series II gas chromatograph with Chemstation ( Hewlett-Packard Ltd, Cheshire, UK) using flame ionisation detection. The additive genetic effect on plasma NEFA composition was calculated in an ACE twin model using an implementation of Mx (Neale et al. 2003) for twin data from the variance-covariance matrix for each of the parameters.

There was no evidence for a genetic effect on total or individual MUFA or total n-3 or n-6 long-chain PUFA (LCPUFA) but 50% of the variability in total saturated fatty acids (SFA) was genetically determined. Furthermore, most of the individual SFA were also substantially genetically determined (31% for C14: 0; 57% for C15: 0; 64% for C17: 0; 62% for C18: 0). The only exception was C16: 0 which had no significant genetic component. About 30% of the variability in the NEFA essential fatty acid concentration was genetic (32% for C18; 2n-6 and 27% for C18: 3n-3). The only other PUFA which appeared to be under some degree of genetic control was C20: 3n-6 (dihomo γ-linolenic); 47% genetically determined. For those fatty acids with a significant genetic component, further adjustment for sex, age, percentage body fat, physical activity level, smoking and the fatty acid composition of the diet made no substantive difference to the magnitude of the genetic effect. This suggests that the genetic control of fatty acid composition, the SFA in particular, is largely metabolically determined and is not mediated by genetic influences on body fatness or behaviour such as physical activity, smoking or dietary choices.

It is interesting that about 30% of the plasma variation in the essential fatty acids was genetically determined but the most striking finding was the large genetic effect on both total and individual SFA and the virtual absence of any genetic influence on the LCPUFA composition. The only exception to the SFA genetic effect was for C16: 0 which is thought to be the fatty acid most readily synthesised in man. A better understanding of the relative importance of genetics and diet in modulating fatty acid metabolism will help improve public health advice on dietary fat.

IL-1R1-/- mice are resistant to obesity-induced insulin resistance following a high-fat diet. By S. Toomey, J. Browne and H.M. Roche, Nutrigenomics Research Group, Department of Clinical Medicine, St James’s Hospital, Dublin 8, Republic of Ireland

Obesity is associated with a complex systemic pro-inflammatory state that has been implicated in the development of medically important conditions, including atherosclerosis and insulin resistance. Characteristics of obesity-induced inflammation include elevated expression of pro-inflammatory molecules by adipose tissue, liver and skeletal muscle and increased pro-inflammatory protein concentrations in the circulation. Recent research showed the infiltration of macrophages into obese adipose tissue which suggests that adipose tissue macrophages may be an important source of the chronic inflammatory response associated with the development of insulin resistance. The present study addressed the hypothesis that a dysregulated macrophage response may protect against obesity-induced insulin resistance (Weisberg et al. 2003).

The present study was carried out to investigate the possible links between obesity and the pro-inflammatory response using IL-1R1-/- mice, which have a compromised macrophage response. Eight C57BL/6 controls and eight IL-1R1-/- were fed a high-fat diet (60% energy from fat) for 18 weeks. At the end of the study serum glucose, insulin and TAG concentrations were determined and adipose tissue gene expression analysis by RT-PCR and cDNA microarray was completed.

Both groups gained similar weight after the high-fat diet; however, the IL-1R1-/- mice were protected against insulin resistance. IL-1R1-/- animals had significantly reduced serum glucose (P < 0.05) and insulin (P < 0.05) concentrations compared with control. HOMA levels, an index of insulin sensitivity, were improved in the IL-1R1-/- group (P < 0.05). Serum TAG concentrations were also reduced in the IL-1R1-/- mice; however, this did not reach significance. Microarray gene expression analysis showed that 462 genes were up regulated in the IL-1R1-/- mice, the majority of which were involved in metabolism, signalling, transcription and translation, and transport. In contrast, 318 genes most involved in inflammation were down regulated. RT-PCR confirmed that whole adipose tissue expression of GLUT4 and insulin receptor substrate (IRS)-1 mRNA, important biomarkers of insulin sensitivity, were significantly increased in IL-1R1-/- animals (P < 0.05). Additionally, TNFα, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1a and IL-10 were significantly reduced (P < 0.05). Adipocyte-specific gene expression analysis also showed significant up regulation of GLUT4 and IRS-1 and down regulation of TNFα, MCP-1, MIP-1a, and IL-6 in the IL-1R1-/- group. The Table shows gene expression analysis.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control Mean</th>
<th>IL-1R1-/- Mean</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT4</td>
<td>1.00</td>
<td>1.00</td>
<td>0.030</td>
</tr>
<tr>
<td>IRS-1</td>
<td>1.472</td>
<td>1.472</td>
<td>NS</td>
</tr>
<tr>
<td>TNFα</td>
<td>1.00</td>
<td>1.00</td>
<td>0.052</td>
</tr>
<tr>
<td>MIP-1a</td>
<td>0.0527</td>
<td>0.0440</td>
<td>0.0389</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.097</td>
<td>0.0289</td>
<td></td>
</tr>
</tbody>
</table>

The present study suggests that disrupting components of the IL-1-1-mediated inflammatory response results in significant protection from obesity-induced insulin resistance.

Hunger and appetite response to a high-protein ketogenic diet in obese men feeding *ad libitum*. By A.M. Johnston1, S. Murison1, D.M. Bremer1, G. Horgan2 and G.E. Lobley1, 1Rowett Research Institute, Bucksburn, Aberdeen, UK, AB21 9SB and 2Biometrics and Statistics Scotland, Rowett Research Institute, Bucksburn, Aberdeen, UK, AB21 9SB

It is now generally accepted that altering the macronutrient composition of dietary intake can influence hunger and satiety. High-protein weight-loss diets, therefore, have come under scrutiny as a potential tool to aid weight loss (Halton & Hu, 2004), because of the observation that energy intake is less and satiety is higher on such diets (Nichols-Richardson et al. 2005). Certain popular low-carbohydrate (ketogenic) diets, such as the ‘Atkins diet’, also involve high protein intakes but there have been relatively few studies that directly compare high-protein, low-carbohydrate (HPLC; ketogenic) v. high-protein, medium-carbohydrate (HPMC; non-ketogenic) diets. Although a ketogenic state is not absolutely essential for increased satiety on high-protein diets, voluntary intakes appear to be greater for such diets when they include moderate (35–45% of energy; Skov et al. 1999) as opposed to low (<10% energy, Studler et al. 2003) carbohydrate content. These comparisons suggest an involvement of ketone body metabolism in regulation of appetite.

We studied seventeen obese (mean BMI 35.1 kg/m²), but otherwise healthy, men in a residential trial of 9 weeks, with food provided daily throughout. Subjects consumed a maintenance diet fed to energy balance (1.6 × RMR) for 3 d and then were offered two *ad libitum* diets, each for a 4-week period, involving either an HPLC (30% protein, 4% carbohydrate, 66% fat by energy) or an HPMC (30% protein, 35% carbohydrate, 35% fat) diet, randomised in a cross-over design. All meals were provided in excess and were the same energy density (5.5 MJ/d). Daily intakes were recorded by weight of food eaten. Body weight was measured daily and motivation to eat was assessed hourly during waking hours, using a computerised visual analogue system. Average *ad libitum* energy intake was significantly lower on the HPLC (ketogenic) diet, in comparison with the HPMC (non-ketogenic) diet (*P* = 0.025), with average intakes of 7.25 and 7.95 MJ/d, respectively. Weight loss was significantly greater on the HPLC (ketogenic) diet, compared with the HPMC (non-ketogenic) diet, with average losses of 6.34 and 4.35 kg, respectively (*P* = 0.006). Fat loss, determined from four-compartment model analysis, also tended to be greater on HPLC than HPMC (5.2 v. 4.1 kg; *P* = 0.070). Over the 4 weeks, hunger was lower (*P* = 0.020) on the HPLC diet. Subjects had no overall preferences for either diet (*P* = 0.198), as assessed by post-meal questionnaires.

In conclusion, subjects on the ketogenic diet ate slightly less and yet were less hungry. Therefore, this diet appeared, in the short term at least, to promote satiety in obese men, suggesting that the combination of high protein and low carbohydrate supply influences perceived appetite and motivation to eat. Further work is in progress which attempts to unravel the mechanisms involved and the impact on metabolic health.

Hunger and appetite response to a high-protein ketogenic diet in obese men feeding *ad libitum*. By A.M. Johnston1, S. Murison1, D.M. Bremer1, G. Horgan2 and G.E. Lobley1, 1Rowett Research Institute, Bucksburn, Aberdeen, UK, AB21 9SB and 2Biometrics and Statistics Scotland, Rowett Research Institute, Bucksburn, Aberdeen, UK, AB21 9SB

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An in vitro method to determine the micellarisation percentage of carotenoids in a variety of vegetables. By L. Ryan, O.F. O’Connell and N.M. O’Brien, Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland

Carotenoid absorption involves several steps from the breakdown of the food matrix and release of carotenoids into the lumen of the gastrointestinal tract through to their incorporation into lymphatic lipoproteins. The transfer of carotenoids into bile salt micelles is essential for carotenoid absorption. Many studies have documented the carotenoid content of various vegetables but little is known about the availability of these carotenoids for absorption by the human body. The objective of the present study was to determine the percentage micellarisation of a range of carotenoids, known to be prominent in the human body, using an in vitro digestion procedure. The micellarisation percentage was calculated by measuring the transfer of carotenoids from the in vitro digestate into the micellar fraction. The vegetables selected included spinach, broccoli, red pepper and sweet potato. Raw vegetables were homogenised and subjected to an in vitro digestion procedure as described by Garrett et al. (1999). Digesta were ultracentrifuged to isolate the aqueous, micellar fraction. Samples from whole vegetable, homogenate, digestate and micelles were extracted twice under amber light with 1 ml hexane:ethanol–acetone (50:25:25, by vol.). The carotenoid content of the samples was quantified by HPLC (Hart & Scott, 1995). The transfer of carotenoids from the in vitro digestate to the micelles (% micellarisation) was determined.

<table>
<thead>
<tr>
<th>Vegetable</th>
<th>Lutein</th>
<th>Zeaxanthin</th>
<th>b-Cryptoxanthin</th>
<th>b-Carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinach</td>
<td>19.0</td>
<td>3.7</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Broccoli</td>
<td>38.4</td>
<td>0.8</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Red pepper</td>
<td>97.7</td>
<td>15.9</td>
<td>71.1</td>
<td>13.1</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>97.0</td>
<td>6.2</td>
<td>92.2</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Mean ± SE

Three independent experiments.

Replacing breakfast and snacks with ready-to-eat cereals contributes to weight and fat loss in overweight individuals. By S.A. Clemes, V.J. Burley, S.F.L. Kirk and R.H. Hooper, Department of Human Sciences, Loughborough University, Leicester, UK; LE11 3TU; and Nutritional Epidemiology Group, Centre for Epidemiology and Biostatistics, University of Leeds, Leeds, UK; LS2 9LN

With the growing problem of obesity, simple yet effective dietary interventions are required to realign the balance between energy intake and energy expenditure. The objective of the present study was to test the effectiveness of a modest dietary intervention, one which may be sustainable over the long term. The primary aim was to investigate whether the replacement of snacks and/or desserts with low-fat ready-to-eat (RTE) cereals, a simple and convenient high-carbohydrate food, would lead to favourable changes in body weight, fat and body shape over a period of 4 weeks. A secondary aim was to determine whether any changes brought about as a result of the dietary intervention were enhanced by modest increases in daily exercise.

Participants (BMI ≥ 25 kg/m²) were assigned to one of three study groups, consisting of: (i) a control group (n = 79; twenty-four male; fifty-five female; age 39.7 (sd 13.3) years; BMI 29.7 (sd 3.3) kg/m²) who received no dietary instruction; (ii) a cereal group (n = 86; thirty male; fifty-six female; age 38.7 (sd 12.2) years; BMI 29.9 (sd 4.4) kg/m²) who were instructed to eat a 45 g portion of RTE cereal (wheat and rice flakes with <1.5% fat) for breakfast with 125 ml semi-skimmed milk, and to replace either a snack or dessert later in the day with either a second portion of cereal or with two cereal bars plus a glass of milk or low-energy yoghurt; (iii) a cereal exercise group (n = 87; thirty-two male; fifty-five female; age 39.1 (sd 11.7) years; BMI 30.2 (sd 3.8) kg/m²) who followed the same dietary intervention as the cereal group and, in addition, were instructed to increase their exercise by walking briskly for 30 min/day on top of any exercise that they habitually undertook. All participants used a pedometer (SW-200; New Lifestyles Inc., Lee’s Summit, MO, USA) to record steps throughout the study. Participants completed two 3 d dietary records, completed on one weekend day and two weekdays. The first diary was completed at baseline and the second was completed on the same days of the week during week 4. Measurements of weight, body fat, and waist circumference were taken at baseline and at 2 and 4 weeks.

The three groups did not differ significantly at baseline in terms of their age, weight, height, BMI, percentage body fat, waist circumference, or in their reported daily energy, macronutrient and RTE cereal intake. All three groups reported significant reductions in energy intake relative to baseline (all P < 0.01) (control) = 740 kJ/d, cereal = 1418 kJ/d, cereal+exercise = 1092 kJ/d). Significant reductions in reported fat intake were observed in the two intervention groups (about 27 g/d, both P < 0.001), resulting in significant reductions in the percentage food energy derived from fat (about 7.5%; both P < 0.001). No changes in fat intake were observed in the control group. The two intervention groups increased their RTE cereal intake by about 56 g/d (both P < 0.001). Significant reductions in weight (P < 0.001) were observed in the two intervention groups relative to baseline (cereal = 0.82 kg, cereal + exercise = 0.7 kg), and in comparison with the control group (0.01 kg). The weight losses observed in the two intervention groups were primarily of fat mass. Changes in weight, body fat and waist circumference did not differ significantly between the two intervention groups. No significant changes in these variables were seen in the control group. Mean daily step counts did not differ significantly between groups, indicating that the exercise group did not comply with the additional exercise required.

For these overweight individuals, the beneficial changes seen over the study in the two treatment groups were the result of the dietary intervention. This modest intervention therefore holds promise as a simple weight-management strategy.

This study was funded by the Kellogg Company, Manchester, UK.


The present research was funded by Science Foundation Ireland.
Preliminary study to investigate what characteristics underlie successful weight loss? By C.J. BYE1,2, J.H. LAVIN2, S. WHYBROW1 and R.J. STUBBS1, 1Leeds Metropolitan University, Civic Quarter, Leeds, UK, LS1 3HE, 2Slimming World, Clover Nook Road, Alfreton, UK, DE55 4RF and 3Rowett Research Institute, Greenburn Road, Aberdeen, UK, AB21 9SB

Obesity is an ever-increasing problem and it is vital that means of preventing and treating the present epidemic are identified and employed. Why some individuals are successful at weight loss and others are unsuccessful, remains largely unknown.

The present study explored differences between individuals who fail to lose weight or regain lost weight and those who are more successful. Motivations for weight loss, preoccupying cognitions, dietary intake and physical activity levels were investigated in terms of their effect on weight-loss success. The present study also piloted measures that are being used in an European study (Dygenex; project contract no. FOOD-CT-2005-513946) aiming to identify psychological and behavioural predictors of weight control.

Females aged 219 years who were current or previous members of a weight-management organisation (Slimming World) for 26 months were included. ‘Success’ was defined as those who had lost >5% of body weight whilst on the weight-loss programme. ‘Less successful’ was defined as those who had lost <5% whilst on the programme or those who had previously lost >5% of initial body weight, regained weight in the previous 6 months and were currently struggling to lose regained weight. Participants were recruited from three Slimming World groups and the company’s head office in the Derbyshire and Nottinghamshire area of the UK. Fifty consented to take part, with thirty-six actually taking part (72%) (twenty-two successful and fourteen less successful). The average age of participants was 46 years, with an average amount of weight loss in successful respondents of 16.7% and 1.7% in less successful.

Motivations for weight loss were measured using a validated questionnaire (Ogden, 2000) using statements related to health, attractiveness, confidence, symptom relief and external pressure motivations for weight loss. None of the motivations differed significantly between the groups. Pre-occupying cognitions were measured using a validated questionnaire (Vreugdenburg et al. 2005), using statements related to food: body shape and dietary preoccupations, e.g. awareness of the energy and macronutrient content of food. Diet preoccupation was significantly higher in successful respondents compared with less successful (P = 0.032).

Dietary intake and activity levels were measured over 3d using a weighed food diary and an Intelligent Device for Energy Expenditure and Activity (Zhang et al. 2006) respectively in a sub-sample of participants (n = 9). Reported daily energy intake was lower in successful (6.6 MJ) than less successful (7.6 MJ) respondents, achieved through lower percentage intakes of fat. Differences were not statistically significant. Differences observed in the activity data were also not significant, possibly due to low subjects numbers.

Antibiotic-enhancing effects of *Ganoderma lucidum* (Lingzhi) against methicillin-resistant *Staphylococcus aureus*. By S. WACHTEL-GALOR, M.V. BOOST and I.F.F. BENZIE, Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong SAR, China

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of hospital-acquired infection of surgical wounds, bedsores, and ulcers, which can lead to septicemia and death (Schito, 2006). The lack of effective drugs to combat the increasing prevalence and antibiotic resistance of MRSA is worrying, and there is a need for new approaches and agents that alone, or in combination with existing antibiotics, can improve efficacy of treatment.

In the present study, *Ganoderma lucidum*, a woody mushroom with a strong reputation in Asia for health benefits, typically taken in soups and teas or as a food supplement, (Wachtel-Galor et al. 1998) was examined for antimicrobial effects, alone and in combination with antibiotics, against *S. aureus*. MRSA (five clinical strains) and methicillin-sensitive *S. aureus* (MSSA; three strains) were tested. Three extracts of *G. lucidum* were prepared: a hot water extract (MHW), a commercially available hot water extract (CHW), and a polysaccharide-rich extract (MPR). Antibacterial susceptibility testing by broth microdilution was performed in microtitre plates. Minimal inhibitory concentration (MIC) was determined according to Clinical Laboratory Standards Institute guidelines. Effects were determined by use of a checkerboard pattern of dilution and comparing endpoints with those of the drug alone. The results are shown in the Table: MIC for *S. aureus* with penicillin with or without *G. lucidum*.

Results showed that *G. lucidum* has a synergistic or additive effect with penicillin against both MRSA and MSSA, as evidenced by an up to 32-fold lowering of the MIC of penicillin. No direct antimicrobial effect of *G. lucidum* was seen (results not shown).

Development of bacterial resistance to antibiotics is one of the most serious healthcare problems today. Introducing new agents that may have direct antibacterial effects or that work in combination with known antibiotics and show low toxicity is important. In the present study we have found evidence that extracts of *G. lucidum* can enhance the antimicrobial activity of antibiotics against MRSA. These interesting findings have important potential for the development of more effective treatment of antibiotic-resistant infections, and formed the basis of a US patent application that was filed by us on 13 September 2005 (application no. 11/224,291).

Nutrition and health status of African-Caribbeans living in Staffordshire: By J.EIRLAND and A.SRIVASTAVA, Faculty of Health and Life Sciences, Coventry University, Coventry, UK, CV1 5FB


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Faculty of Health and Life Sciences, Coventry University, Coventry, UK, CV1 5FB

Vascular 3

Tayside Institute of Child Health, Ninewells Hospital, Dundee, UK, DD1 9SY and

Research Unit, University of Aberdeen, Aberdeen, UK, AB25 2ZD

Population living in the UK. In the most recent National Diet and Nutrition Survey for adults the results have not been analyzed according to ethnic group and anthropometric data are not collected in the survey. Despite this, cardiovascular disease (CVD) is the main cause of death in the UK, the origins of which are thought to begin early in life. The Health of Ethnic Minority Groups (HENDON et al. 2001) showed that 40% of the subjects were overweight (BMI >25 kg/m²), with only 18% of adults being classified as a normal weight (BMI <20 kg/m²). Slightly higher proportions of women were overweight with only 15% of women being classified as overweight. The sample comprised thirty-nine adults aged 19–65 years (mean 46.7 years) of which 15% were male. It was found that 85% of subjects were obese (BMI >30 kg/m²), with only 18% of adults being classified as normal weight (BMI <20 kg/m²). Slightly higher proportions of women were overweight with only 15% of women being classified as overweight. The mean waist circumference (WC) of the subjects was calculated from weight and height (kg/m²) and waist circumference (WC) was also measured. BMI was calculated from weight and height (kg/m²) and waist circumference (WC) was also measured. The mean waist circumference (WC) of the subjects was calculated from weight and height (kg/m²) and waist circumference (WC) was also measured.

(1) Children reported some degree of control over their food choices and perceived the presentation of food to be positive aspects of the diet into account when developing health education strategies for this group.

(2) Teachers used the resources to promote healthy eating and physical activity. (3) Children generally considered themselves quite active. The amount of time they spent watching television or playing on the computer varied, but children were keen to try a challenge to reduce it; and (4) Teachers raised the issue of trying to fit extra health lessons into an already busy curriculum and did not have literature regarding the encouragement of appropriate physical activity and inactivity.

The present study aimed to develop an intervention that would bring about changes in diet, physical activity, body size, and biochemical and physiological indicators of early CVD risk. The present methodological paper describes the development of this intervention and summarizes the findings of a pilot dietary and anthropometric study of African-Caribbeans living in Staffordshire, in collaboration with Staffordshire County Council. Fundings on the health status of the subjects. BMI was calculated from weight and height (kg/m²) and waist circumference (WC) was also measured.

The sample comprised thirty-nine adults aged 19–65 years (mean 46.7 years) of which 15% were male. It was found that 85% of subjects were obese (BMI >30 kg/m²), with only 18% of adults being classified as normal weight (BMI <20 kg/m²). Slightly higher proportions of women were overweight with only 15% of women being classified as overweight. The mean waist circumference (WC) of the subjects was calculated from weight and height (kg/m²) and waist circumference (WC) was also measured. BMI was calculated from weight and height (kg/m²) and waist circumference (WC) was also measured.

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Development of a computer-based tool for measuring schoolchildren's diets. By M.S. TAYLOR1, C. SUMMERBELL1, A. ADAMSON2, B. LANG2 and S. CROOKS1, 1Food And Nutrition Group, University of Teesside, Middlesbrough, UK, TSI 3BA and 2Human Nutrition Research Centre, School of Clinical Medical Sciences, William Leech Building, University of Newcastle, Newcastle upon Tyne, UK, NE2 4HH

Children's lifestyles, particularly their diets and levels of physical activity, are currently a cause of concern. Partly this concern is for the increasing levels of obesity in children, that have serious health implications in both the short and long term. (Parliamentary Office of Science and Technology, 2003). The most frequently used method for assessing eating patterns in children is the food diary. However, this can be inaccurate (particularly in overweight children) and difficult (particularly in children with poorer literacy skills) for the children to complete (Rockett & Colditz, 1997). In addition, analysis of data from 5d recording requires considerable amounts of researchers' time.

As part of a PhD project, a website has been designed and programmed, allowing schoolchildren to report their usual diets in a fun and interactive way, and saving many hours of researchers' time. Children choose from a pictorial list of commonly-eaten foods, thus improving speed of assessment and removing the need for higher levels of literacy. The list was derived from key references including NDNS, and adapted after pilot work. The website also asks about physical activity, facilitating more complete analyses of children's lifestyles with respect to energy balance.

Data obtained using the website shows over-reporting of energy intake, but nonetheless correlates significantly with data obtained using the 5d diary, R=0.210; P<0.01. Bland Altman plots were used to examine differences between methods of measurement (Altman & Bland, 1983).

Although comparisons between reported energy intake and calculated resting energy expenditure derived from age, sex, height and weight (Schofield, 1985) showed non-significant correlation with either method, there is a slightly higher degree of correlation with the new website than the diary.

To avoid contamination of the data collected for this and ongoing research, the website is not currently available for general use.

Perceived persuasiveness of nutrition education messages with different levels of technical language. By N. ASANTE-AMPADUH and A. WISE, The Robert Gordon University, St Andrew Street, Aberdeen, UK, AB25 1HG

It has been suggested that the use of technical language in nutritional education messages for the public is not as persuasive (Wise et al. 1996). In the present study, questionnaires contained eight nutrition education messages about eight different foods. Each statement consisted of two sections: (1) an instruction to change a dietary practice, and this was followed on the next line by the word "Why?"; (2) a reason for the action given in a statement comprised of three components – a nutritional concept, the physiological significance and a consequent health benefit. For each message, the command was the same, but the three components of reason were varied in non-technical (N) or technical (T) terms using every possible combination of N and T components from N-N-N to T-T-T. There were two messages each about obesity, dental caries, diverticular disease, and coronary artery disease. There were in total sixty-four different messages, which were arranged in a Graeco Latin square in eight different questionnaires. After each message, subjects were asked to rate the message for persuasiveness and technicality on an eight-point scale and frequency of current compliance (never, sometimes, usually and always). Subjects were selected from those seated in a cafe of a city-centre shopping mall. The mean scores for the persuasiveness and technicality of each message type were calculated separately for each sex, age group, social class, frequency of compliance and for each food. Using this information, the scores were adjusted to remove the contributions related to each of these factors so that the average scores for each message type, unbiased by the other factors, could be derived.

The response rate was high (97%) and the numbers of participants were sixty-six males and 134 females. The Table shows the mean perceived technicality and persuasiveness (adjusted for age, sex, social class, food, and perceived compliance) for different intended levels of technicality. One-way ANOVA for adjusted technicality showed that it was influenced as expected by the level of intended technicality (P<0.001). Perceived persuasiveness did not differ significantly with level of intended technicality, but adjusted scores for perceived technicality and persuasiveness were significantly correlated (r 0.23, P<0.001). This suggests that individuals tended to be a little more persuaded when they perceived messages as more technical, which is the opposite conclusion to that of the previous research. For example, according to the prediction that the inclusion of technical words can add credibility to the author and thus increase the perceived validity and persuasiveness of the information (Pettig & Cacioppo, 1986). More research is required to elucidate further the potential importance of how the public can best be persuaded by different types of wording in nutrition education messages.

<table>
<thead>
<tr>
<th>Technicality</th>
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<tr>
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</tr>
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<tr>
<td>T-N-N</td>
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<tr>
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</tr>
<tr>
<td>T-N-T</td>
<td>5.04</td>
</tr>
<tr>
<td>T-T-N</td>
<td>5.39</td>
</tr>
<tr>
<td>T-T-T</td>
<td>5.68</td>
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</tbody>
</table>


n-3 Long-chain polyunsaturated fatty acid intake from fish and depressed mood: non-linear or confounded association? By K.M. APPLETON1, T.J. PETRIS2, R.C. HAYWARD3, S.V. HEATHERLEY3, S.A. MCAUGHTON4, P.J. ROGERS3, D. GUNNELL5, A.R. NESS6 and D. KESSLER2, 1School of Psychology, Queen’s University, Belfast, 18–30 Malone Road, Belfast, UK; Blyth SBF; 2Unit of Primary Health Care, Department of Community Based Medicine, University of Bristol; 1Woolwood Road, Bristol, UK; BSS 1IAU; 3Department of Experimental Psychology, University of Bristol; 8 Woodland Road, Bristol, UK; BSS ITN; 4MRC Human Nutrition Research, Elford, Woodbridge Laboratory, Bablour Road, Cambridge, UK; 5TEE 930; 6Faculty of Science, University of Kuwait, Kuwait

Various biochemical, epidemiological and clinical evidence suggests an association between low dietary intakes of n-3 long-chain PUFA (n-3 LCPUFA) and higher depressed mood. This association is further supported by cross-sectional studies in non-clinical populations (Tamskanen et al. 2001; Silvers & Scott, 2002). In these cross-sectional studies, however, n-3 LCPUFA intake is often considered as some high/low consumption, yet analysis of categories of n-3 LCPUFA intake may not accurately describe the nature of the association. This analysis investigated n-3 LCPUFA intake and depressed mood in a sample of the general UK population.

n-3 LCPUFA intake, depressed mood and demographic variables (sex, age, index of multiple deprivation based on postal code and date of questionnaire completion) were measured simultaneously by self-report questionnaire. n-3 LCPUFA intake was measured using a short food-frequency questionnaire, and subsequently calculated using intake from fish and intake from fish plus supplements. Depressed mood was assessed using the short form of the Depression, Anxiety and Stress Scales (Lovibond & Lovibond, 1995).

Complete data were available for 2674 individuals from throughout Bristol, UK. Levels of n-3 LCPUFA intake (from fish: mean (sd) = 0.1 (1) portions fatty fish/week; from fish plus supplements: mean (sd) = 1.6 (2.4) portions of fatty fish/week or equivalent) and depressed mood scores (mean (sd) = 7.8 (9.0)) were comparable with those of the general UK population (Gregory et al. 1990; Lovibond & Lovibond, 1995; Finch et al. 1998). Using polynomial regression, a statistically significant non-linear relationship between fish intake and depressed mood was found (linear β = 1.29, P < 0.01; non-linear β = 0.33, P = 0.01). This relationship was predominantly negative (greater n-3 LCPUFA intake was associated with lower depressed mood scores). The incremental decrease in depressed mood score diminishes as n-3 LCPUFA intake increases. This non-linear relationship, however, was attenuated when adjusting for demographic variables. Using an adjusted regression model, n-3 LCPUFA intake from fish was not associated with depressed mood. No relationships between n-3 LCPUFA intake from fish plus supplements and depressed mood were found.

These findings provide some evidence that higher levels of fish-derived n-3 LCPUFA intake, as assessed by the questionnaire used here, are associated with lower levels of depressed mood, but the decrease in depressed mood diminishes as n-3 LCPUFA intake increases. The association is, however, confounded by age and index of multiple deprivation. Associations between age and depression, and the dietary intake of fish and depressed mood have been demonstrated previously (Gregory et al. 1990; Finch et al. 1998; Weich & Lewis, 1998). The observed attenuation of the effect suggests that apparent associations between fish intake and depressed mood may be part of a more fundamental relationship between diet or lifestyle and health. There were also no effects in n-3 LCPUFA intake when we measured using fish plus supplements. The relationship between n-3 LCPUFA intake and depressed mood is thus only apparent when considering n-3 LCPUFA intake from fish. This suggests that any relationship between fish intake and depressed mood is more related to fish intake than to n-3 LCPUFA intake. This again raises the possibility that the consumption of n-3 LCPUFA from fish may be a proxy for a diet or lifestyle that is associated with lower levels of depressed mood.

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

Eid N, Al-Hooti S, Boursly N & Khalafawi M (1986) 320
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Changing trends in physical characteristics and obesity risk in 6–13-year-old Kuwaiti school children, evidence of a nutritional and epidemiological transition? By H. AL-SHAMARI1, P. AMUNA1, I. TEWFIK1, A. BUMEJDA1 and F. ZOTOR2, 1School of Biosciences, University of Westminster, New Cavendish Street, London, UK; W.W. SIS, 2Medway School of Science, University of Greenwich, Chatham Maritime, UK; ME4 4TB and 3Faculty of Science, University of Kuwait, Kuwait

Increasing levels of obesity have recently been reported in Kuwaiti children (Moussa et al. 1999). The objective of the present study was to examine changes in physical characteristics in school-age children over a 20-year period in this genetically homogeneous population and to identify the impact of environmental risk factors associated with the nutritional and epidemiological transition.

A total of 1536 children aged 6–13 years (768 male; 768 female) were recruited by a two-stage stratified sampling procedure of which anthropometric variables of a subsample of 94 (ninety-nine male; ninety-five female) were measured over a 12-week period between 2003 and 2004 and also using a 3-day dietary diary. Results were compared with data of a similar cohort of children reported in Kuwait 20 years earlier (Eid et al. 1986).

All subjects were above the 50th percentile curve for BMI with 32.9% classified as overweight (85th percentile, i.e. BMI 25kg/m2) and 31.96% as clinically obese (90th percentile, i.e. BMI >30kg/m2) (Frisancho, 1990; Magbool, 1994; Cole et al. 2000; National Center for Health Statistics, 2000). Eid et al. (1986) reported that age- and sex-matched Kuwaiti children were shorter than American children. In the present study, Kuwaiti schoolchildren were heavier than American children and tended to have comparable height when compared with the National Center for Health Statistics and Centers for Disease Control and Prevention’s reference population except for girls at age 11 to 13 years. There was a distinct and significant upward trend in weight, height and BMI in 2004 compared with 1984, thus indicating that for such a genetically homogeneous population, hereditary predisposition alone cannot explain these observations (Figures 1 & 2).

Our findings support earlier evidence that environmental risk factors including lifestyle changes, physical inactivity and poor food choices related to rising household income are contributory, and that this population is in nutritional transition and may be at increased risk of non-communicable diseases unless appropriate interventions are implemented to reverse the trend.

Fig. 1. A comparison of mean BMI of 6-13 year-old Kuwaiti schoolchildren in 2004— compared to a similar age and sex matched cohort (Eid et al., 1986) 20 years earlier (—).
Indirect estimates of net acid excretion and net rate of endogenous non-carbonic acid production in the young British population: analysis of the National Diet and Nutrition Survey aged 4–18 years. By L. VOKES1, R. H.T. GANNON2, D.J. MILLWARD3, D.P. LOVELL1, H.M. MACDONALD1, L.A. Frassetto4, T. Reme5 and S.A. Lanham-New1, 1Department of Nutrition and Dietetics, Queen Alexandra Hospital, Cosham, Portsmouth, UK, 2PO6 3LY, 2Centre for Nutrition and Food Safety and 3Post Graduate Medical School, University of Surrey, Guildford, UK, GU2 7XH, 4Department of Medicine and Theophanics, University of Aberdeen, Aberdeen, UK, AB25 2ZD, 5Department of Medicine and General Clinical Research Center, University of California, San Francisco, CA, USA and 6Department of Nutrition and Health, Research Institute of Child Nutrition, Dortmund, Germany

Adults ingest approximately 50–100 mEq H+ ions/d from sulfur-containing amino acids and other dietary components in the Western diet, resulting in mild but chronic metabolic acidosis. Increased dietary H+ ingestion consequently increases renal net acid excretion (RNAE) due to homeostatic urinary H+ excretion via urinary buffers. Two models exist for the indirect estimation of RNAE using dietary intake data: net renal non-carbonic acid excretion (NEAP); a total dietary protein/dietary K ratio (Frassetto et al. 1998) and indirectly estimated net acid excretion (NAEindirect): mEq of SO4 + P – K – Mg (Remer et al. 2003). The aims of the present study were to: (1) estimate dietary acid-generating potential by two RNAE prediction models using National Diet and Nutrition Survey data; (2) compare these estimates and assess the nutrient and food consumption profile of the diet at increasing estimated dietary acidity; (3) identify the major whole food contributors to dietary acidity. NEAP (n 1701) and NAEindirect (n 1684) were used to estimate the RNAE of young British individuals as shown in the Table below.

<table>
<thead>
<tr>
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<th>Range</th>
<th>Spearman’s ρ correlation</th>
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<tr>
<td>NEAP (g/mEq per d)</td>
<td>1701</td>
<td>45.7</td>
<td>10.0</td>
<td>45.1</td>
<td>6.42–103.10</td>
</tr>
<tr>
<td>NAEindirect (mEq/d)</td>
<td>1684</td>
<td>44.9</td>
<td>14.3</td>
<td>43.3</td>
<td>2.14–102.84</td>
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Comparison of dietary composition identified significant positive associations between NEAP and dietary protein, P and Ca intake and meat and fish consumption (P<0.0037). Protein, P, K, Mg and Ca intake and meat, fish and notably vegetable consumption increased with an increase in NAEindirect (P<0.001). Vegetable intake was inversely associated with NEAP (NS) and fruit consumption and K and Mg intakes were inversely associated with NAEindirect (P<0.001). Potato and fruit were the major whole food contributors. The difference in the major whole food contributors identified by the two models reflects important but subtle differences in dietary nutrient sensitivity. These differences and previous work (Pynne et al. 2004) have led to the suggestion perhaps vegetable consumption increased with NAEindirect due to sensitivity to dietary P of NAEindirect. Unlike the NEAP model, overall the diet of young British individuals has been shown to be acid generating.

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


Recruitment to a dose–response study of the effects of increased fruit and vegetable intake on vascular function: study design and subject characteristics. By S.E.E. BERRY1, U. MULLA1, P.J. CHOWIENZYK2 and T.A.B. SANDERS1, 1Nutritional Sciences Research Division, King’s College London, 150 Stamford Street, London, UK, SE1 9NN and 2Cardiovascular Division, King’s College London School of Medicine, St Thomas’s Hospital, London, UK, SE1 7EH.

Fruit and vegetable (F&V) consumption is associated with decreased risk of CVD, but the dose–response relationship with established risk factors is uncertain. It has been hypothesised that F&V lowers blood pressure (BP) by increasing K intake. DRFRUITNVEG is a randomised dose–response cross-over trial (ISRCTN50011192; www.controlled-trials.com) designed to test the hypothesis that an increased intake of F&V lowers BP and improves arterial compliance and endothelial function in subjects with moderately elevated BP (120/80 and 160/100 mmHg) and that this effect is attributable to an increased intake of K. Four treatments were compared containing low, medium and high intakes of F&V. Following a 3-week run-in on the low intake (three portions F&V per d) subjects were allocated one of four orthogonal treatment sequences. Each intervention lasted 6 weeks and was separated by a 3-week wash-out period. Measurement of urinary Na and K excretion, 24 h ambulatory BP, arterial stiffness and endothelial function were made and fasting blood samples obtained at the end of each treatment period. The target was to recruit forty-eight subjects onto the study with a minimum of thirty-two subjects completing. We report our experience in recruiting subjects so as to inform for the planning of future studies.

A recruitment email was sent to all staff and students of King’s College, London, and a leaflet was sent to educational establishments in South London. Subjects were offered a BP check and details of the study. A total of 421 attended for the BP check, and ninety-one potentially suitable subjects attended for a clinic visit for anthropometry, BP measurement and a blood test at least 1 week later. Seventy-three of these subjects had BP that met the inclusion criteria. A total of fifty-seven subjects were randomised to one of four orthogonal treatment sequences; fifty-two completed the run-in phase; twenty-seven subjects have completed the whole study, twenty-three are due to complete in September 2006. Their details are shown in the Table.

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Our experience suggests that when recruiting for a trial to enlist pre- or mildly hypertensive subjects who are not on lipid- or BP-lowering medication, it is necessary to screen eight subjects to recruit one subject.

The present study (N02030) was funded by the Food Standards Agency.
Typical food portion sizes are a valuable tool which can be used to assist in the estimation of dietary intakes for groups of individuals where weighed intakes are not available. Current published food portion sizes (Food Standards Agency, 2002) were calculated using the data from the 1986–7 Dietary and Nutritional Survey of British Adults (Gregory et al. 1990). However, changes in lifestyle (for example, increasing experiences with a wider food culture and less time for food preparation) since the 1986–7 survey has led to a greater variety of foods being available in the UK. There is also evidence from the UK that portion sizes for specific foods, for example, soft drinks, hamburgers, and French fries have increased over the last three decades (Nilsen & Popkin, 2003). The aim of the present study was to produce a set of typical food portion weights for adults by age.

Data from the latest National Diet and Nutrition Surveys (NDNS) of adults aged 19–64 years (2000–1) and people aged 65 years and over (1994–5) were obtained from the UK Data Archive (www.data-archive.ac.uk). Food portion data from 3411 individuals were extracted and similar foods were grouped and recoded in order to facilitate processing. Age was grouped by the following categories (19–34, 35–49, 50–64, 65–74 and 75+ years). A comprehensive analysis of the factors affecting portion size was carried out. The data were found to be highly variable, with a significant number of extreme values. Thus, median portion sizes (with 25th and 75th percentiles) were calculated and non-parametric methods were used to identify associations with portion-weight variability. Where these differences occurred (and numbers were sufficient), individual medians were calculated for food groupings of age and/or sex. To test the use of the calculated portion sizes, weighed food diaries of Scottish women aged 25–46 years (n = 55) collected in 1996–7 and Scottish men and women aged 40–74 years (n = 64) collected in 1999–2002 were reanalysed for energy and nutrients using the actual and calculated weights (medians) for each food. The methods of Bland & Altman (1986) were used to investigate the agreement between nutrient intakes calculated from actual and calculated portion sizes by age group.

Similar foods were grouped into 751 food categories. Some of the food groupings with larger numbers were broken down according to mode of consumption, for example, milk in tea or coffee, in cereal, as a drink, etc. No cut-off points for inclusion were used and all similar foods regardless of the number of times they had been consumed were included in the food groupings. Portion weights were calculated for those food groupings with ten or more consumers (in either of the 19–64 or 65+ NDNS datasets). Overall, 184 food groupings had age- and sex-specific median weights calculated; 121 age-specific weights and fifty-five sex-specific weights. A total of 391 foods had weights calculated with no age or sex split (for example, because there were no statistical differences between subgroups or because there were less than fifty records available). Mean daily energy and nutrient intakes were calculated from the food diaries using the actual and calculated weights (medians) for each food (using an age- or sex-specific portion weight where available). Differences between the energy and nutrient intakes from the actual and calculated portion weights were small for females but considerably larger for males (for example, for energy and protein) although most individual values lay within two standard deviations of the mean difference. For more information please see www.food.gov.uk.

In conclusion, this project has enabled typical portion weights for adults to be updated, and for age- and sex-specific portion weights to be calculated for an extensive range of food items. These details should enable more accurate assessments of nutrient intake to be undertaken and underline the limitations of applying typical portion sizes for dietary assessment and other purposes (for example, food labelling). It is envisaged that these data will form part of the fourth edition of the Food Portion Sizes publication.

Funding provided by the Food Standards Agency is gratefully acknowledged (project no. N08026).


Working towards a web-based pan-European access to on-line nutrient databases: UK nutrition researchers' needs and expectations. By A. FRAODT and M.M. RAATS. Food, Consumer Behaviour and Health Research Centre, University of Surrey, Guildford, UK, GU2 7XH

Food composition tables or nutrient databases are designed to provide information on the composition of foods in a particular country, giving values for energy and major essential nutrients and other important food components. In January 2005, the importance of European cooperation in the domain of food composition data (FCD) was recognised by the funding the European Food Information Resource Network (EurofIR, www.eurofir.net) that aims to advance previous collaborative efforts to improve FCD quality, availability, comparability and accessibility by linking on-line nutrient databases through an Internet-based pan-European access system. Although it is recognised that user input is regarded as important in developing and sustaining databases, relatively little published data exist with regard to FCD user needs, requirements and expectations (Rand et al. 1985; Greenfield & Southgate, 2003). The present research aims to identify how the key user group of nutrition researchers use FCD, cope with needs not being met by the currently used tools to access FCD and future requirements of a pan-European resource.

Data were collected at an interactive workshop held at the Nutrition Society Summer Meeting 2005, consisting of introductory presentations and small group discussions attended by nutrition researchers (n = 20). Discussions were recorded. Facilitators evaluated the group discussions and the outcomes based on a predefined set of evaluation criteria (i.e. independence, trustworthiness, clarity, access to resources, group dynamics, efficacy of the process, fairness, transformation, satisfaction, and task-related outcomes). Analysis was based on transcripts, group discussion summary sheets, facilitator observations and participant event evaluation questionnaires. Qualitative data analysis software was used to structure and summarise the data.

The primary FCD sources used by participants were UK based. Others mentioned were US and Italian databases. Data are being accessed via hard copy, electronic format, self-constructed databases, commercial software packages, and the Internet. FCD are being used for calculating food portion sizes, analysing dietary information, foods, and recipes, checking own data generated through calculations, and determining flavonoids and bioactive compounds in foods.

Shortcomings of currently used tools include insufficient historical records of data, information on analytical methods, coverage of commonly consumed (for example, filo pastry), composite or take-away foods, missing nutrients, no timely reflection of new products and recipes, and inflexibility of software products. The shortcomings of currently available FCD are addressed by use of equivalent foods, ingredients and recipes, alternative databases, manufacturers and retailer information, and scientific literature. Formalised user feedback mechanisms with software developers, food manufacturers and retailers and authoritative organisations were suggested.

Participants viewed the linking of various national databases through an on-line access system that can also function as an interactive information bank as useful for their work. They acknowledged that a European collaboration will result in harmonised database structures, a wider range of available food components and secondary data (for example, historical data, analytical methods, recipe information), and new data being made available (for example, bioactive compounds, information on cooking methods). Participants suggested providing free-of-charge access to the pan-European information source, special training to different user target groups, varying access levels related to the depth of information needed by different user target groups, a link to general dietary patterns and expanding the scope of EurofIR beyond European foods and into additives and plant extracts causing allergies.

The present study is a first step to obtain views from nutrient database users regarding future requirements and expectations of a pan-European nutrient information resource. A standardised workshop model that was developed based on the workshop outcomes and evaluations will be used in other European countries and, in addition, with other user groups to collect further data.

Project number (IPRE 51944). The present study was completed on behalf of the EuroFIR Consortium and funded under the European Union 6th Framework Food Quality and Safety Programme.

Increasing antioxidant intake from fruits and vegetables – practical possibilities in the Scottish population. By M.A. HALEEM1, K.L. BARTON1, S. RAY1, A.S. ANDERSON1, G. BORGES1 and A. CROZIER1, 1Centre for Public Health Nutrition Research, Ninewells Hospital, University of Dundee, Dundee, UK, DD1 9SY and 2Institute of Biological and Life Sciences, University of Glasgow, Glasgow, UK, G12 8QQ

Fruit and vegetables contain a wide variety of antioxidants, which are likely to reduce oxidative damage and assist in the prevention of chronic diseases. In vitro antioxidant capacity (AOC) of fruits and vegetables provides information on the antioxidant potential in the diet and can be used as one indicator to help guide dietary choices. The aim of the present study is to assess the current AOC intake from fruits and vegetables in the UK population (and subgroups) and to examine different consumption models in order to identify a practical maximum dietary AOC intake from fruit and vegetables.

AOC intake from fruit and vegetables was estimated using (a) AOC of individual fruit and vegetables determined by the ferric-reducing antioxidant power (FRAP) assay and (b) data on quantity and frequency of consumption of fruit and vegetables determined from the National Diet and Nutrition Survey (NDNS) 2000–1 data (obtained from the UK Data Archive; www.data-archive.ac.uk/). The AOC of eighty different fruits and vegetables were measured in the present study by the FRAP assay. A significant difference was found in AOC intake between London and South East England (730 μmol/d) and Northern England (610 μmol/d) (P < 0.05). A similar difference was also found between Central and South West England (660 μmol/d) and Northern England (P < 0.05). From the NDNS survey 2000–1, it was found that of the 123 subjects in Scotland, 113 subjects consumed less than 400 g fruit and vegetables per day. The mean AOC for this Scottish sample was 685 μmol/d and the mean AOC for individuals consuming more than 400 g fruit and vegetables was 2120 μmol/d. Data from consumption of different fruit and vegetables showed that strawberries, apples, clementines, purple broccoli and cauliflower were the top five sources of AOC in the Scottish diet. The Table shows average daily intake of AOC from the five top fruit and vegetables in the Scottish NDNS survey.

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Mean intake (μmol/d)</th>
<th>Average portion weight (g)</th>
<th>Mean AOC (μmol/d) Vegetable</th>
<th>Mean intake (μmol/d)</th>
<th>Average portion weight (g)</th>
<th>Mean AOC (μmol/d) Vegetable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strawberries</td>
<td>23.0 (n=15)</td>
<td>86</td>
<td>1570</td>
<td>32 (n=8)</td>
<td>103</td>
<td>1520</td>
</tr>
<tr>
<td>Apples</td>
<td>56 (n=35)</td>
<td>150</td>
<td>1200</td>
<td>89 (n=22)</td>
<td>105</td>
<td>960</td>
</tr>
<tr>
<td>Oranges</td>
<td>50.0 (n=48)</td>
<td>105</td>
<td>900</td>
<td>8 (n=8)</td>
<td>33</td>
<td>250</td>
</tr>
<tr>
<td>Pears</td>
<td>48.0 (n=22)</td>
<td>157</td>
<td>430</td>
<td>13 (n=30)</td>
<td>64</td>
<td>180</td>
</tr>
<tr>
<td>Banana</td>
<td>44.0 (n=69)</td>
<td>97</td>
<td>200</td>
<td>32 (n=26)</td>
<td>61</td>
<td>70</td>
</tr>
</tbody>
</table>

The combination of the top two fruits plus one vegetable or the top fruit plus two vegetables of average portion weight had a much higher antioxidant capacity than the average (mean) antioxidant capacity achieved with 2400 g of non-specific fruit and vegetables. Certain fruit and vegetables have a very high AOC. With the current low levels of antioxidant capacity in the Scottish population, selection of these fruit and vegetables would help them to achieve a higher AOC intake, potentially offering beneficial health effects.

Plasma adiponectin levels after a high-fat meal and a standard mixed meal in lean and obese, young and older men. By F. TSOFLOU1, C.L. FYFE1, I. MATHESON1, A.A. SNEDDON1, D. JACKSON2, K.W.J. WAHLE1 and L.M. WILLIAMS1, 1Obesity and Metabolic Health Division, Rowett Research Institute, Aberdeen, UK, AB21 9SB and 2Robert Gordon’s University, Schoolhill, Aberdeen, UK, AB10 1FE

Adiponectin is an adipocyte hormone involved in the regulation of glucose and lipid metabolism. Adiponectin levels decrease with increasing obesity but other factors may also play a role in the regulation of plasma adiponectin. For example, adiponectin levels have been reported to increase, decrease and remain unchanged with increasing age. The acute increase of dietary composition on circulating adiponectin also remains contentious. To clarify how age and dietary composition may influence plasma adiponectin, first, the effect of age was investigated on baseline plasma adiponectin levels in lean and obese young and older healthy men. Second, the postprandial response of plasma adiponectin to a standard and a high-fat meal in these subjects was also investigated.

Lean (BMI 23.6 (± 0.4) kg/m²; n = 14) and obese (BMI 32.5 (± 0.5) kg/m²; n = 13), young men (age 31 (± 0.8) years) and lean (BMI 23.6 (± 0.3) kg/m²; n = 22) and obese (BMI 31.9 (± 0.4) kg/m²; n = 16) older men (age 57 (± 0.8) years) were fasted overnight and received either a high-fat meal or a standard mixed meal separated by a 2d interval. To replicate times of peak adiponectin secretion previously published in the literature, plasma adiponectin was measured at baseline and at 4h after ingestion of the high-fat meal and at 1h and 3h after ingestion of the standard meal. Fasting plasma adiponectin concentrations did not change significantly after consumption of the high-fat meal in any group.

** Significantly different from baseline (P < 0.004; two-way ANOVA followed by post hoc test).

The present findings are consistent with previous studies where no postprandial changes in adiponectin were found after a high-fat meal and indicate a temporary decrease in plasma adiponectin acutely after a standard mixed meal in young lean men.
Red grape juice protects against exercise-induced DNA damage. By A.McE. JENKINSON1, S.J. DUTHIE2, J.A.M. KYLE3, and G.G. DUTHIE1, 1School of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, UK, AB25 2ZD, 2Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, UK, AB21 9SB and 3Department of Environmental and Occupational Medicine, University of Aberdeen, Foresterhill, Aberdeen, UK, AB25 2ZP.

Muscle damage and soreness can arise following exercise and may limit exercise participation, particularly in untrained individuals. Adverse effects on muscle structure occur when exercise is unaccustomed or excessive and may be due in part to production of reactive oxygen species which can disrupt cell membranes, damage DNA and consequently impair cell function. Consumption of antioxidants, such as phenolic compounds found in fruit juice, may protect against free radical-induced damage during exercise. The aim of the present study was to assess whether a phenolic-rich drink (red grape juice) could alter circulating indices of DNA damage following muscle-damaging exercise in human volunteers.

Sixteen healthy volunteers, aged 24±4 years participated in a double-blind, placebo-controlled, exercise trial. Volunteers were requested to maintain normal dietary habits and to abstain from red wine and phenolic-rich fruit juices throughout the study. Before exercise, subjects were randomly assigned to consume either 400 ml red grape juice (142.9 mg total phenols/l) or 400 ml placebo solution (15.6 % (w/v) sugars, similar to the fruit juice, total phenols 13.1 mg/l). After 30 min following juice consumption, subjects completed a single bout of seventy maximal eccentric contractions of the forearm flexor muscles of the non-dominant arm. Venous blood samples were collected 1 week before exercise (baseline), immediately before consumption of the drink (baseline 2), +30 mins (immediately before exercise), and +24 h, +48 h and +5 d. Lymphocyte DNA damage was assessed using single-cell gel electrophoresis. Plasma creatine kinase (EC 2.7.3.2) activity was assessed at baseline, and at +24 h, +48 h and +5 d.

Plasma CK, a marker of muscle damage, was significantly elevated above baseline levels at +24 h (P<0.01) and +48 h (P<0.05) in the placebo group but not in the supplemented group (see Table). Endogenous strand breakage was also significantly increased from baseline values in the placebo group at +24 h (P<0.05), +48 h (P<0.01) and 5 d (P<0.05) after muscle-damaging exercise (see Table). However, there was no change in the red grape juice group.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Baseline 2</th>
<th>+30 min</th>
<th>+24 h</th>
<th>+48 h</th>
<th>+5 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma CK activity (U/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red grape juice</td>
<td>49.5 ± 8.4</td>
<td>49.5 ± 8.4</td>
<td>72.0 ± 13.2</td>
<td>72.0 ± 13.2</td>
<td>72.0 ± 13.2</td>
<td>407.6 ± 2470</td>
</tr>
<tr>
<td>Placebo</td>
<td>51.0 ± 9.0</td>
<td>51.0 ± 9.0</td>
<td>11.2 ± 18.8</td>
<td>13.2 ± 178.9</td>
<td>87.1 ± 407.6</td>
<td>4176 ± 2470</td>
</tr>
<tr>
<td>Endogenous strand breakage (arbitrary units)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red grape juice</td>
<td>32.6 ± 3.3</td>
<td>32.6 ± 3.3</td>
<td>37.0 ± 2.8</td>
<td>38.1 ± 1.7</td>
<td>35.1 ± 3.7</td>
<td>40.9 ± 4.8</td>
</tr>
<tr>
<td>Placebo</td>
<td>35.9 ± 3.4</td>
<td>35.9 ± 3.4</td>
<td>45.0 ± 6.1</td>
<td>49.1 ± 5.2</td>
<td>61.2 ± 4.5</td>
<td>64.7 ± 7.9</td>
</tr>
</tbody>
</table>

While several studies suggest that exhaustive exercise increases DNA damage and that increased antioxidant consumption may reduce this effect, there is little evidence demonstrating this in response to specific muscular exercise. The present study demonstrates that DNA damage is increased following eccentric muscle-damaging exercise but is ameliorated by consumption of a phenolic-rich fruit juice before the exercise bout.

Funded by the University of Aberdeen and the Scottish Executive Environment and Rural Affairs Department (SEERAD).

Lower maternal vitamin E and zinc intakes during pregnancy are associated with an increased risk of asthma in 5-year-old children. By L.C.A. CRAIG1, G. DEVEREUX1, S.W. TURNER2, G. McNELLY3, S. MARTINDALE4, P.J. HELMS5 and A. SEATON4, 1Department of Environmental and Occupational Medicine, University of Aberdeen, Aberdeen, UK, AB25 2ZP and 2Department of Child Health, University of Aberdeen, Aberdeen, UK, AB25 2ZG.

In the UK there has been a dramatic increase in the prevalence of asthma, with 5.2 million individuals now being treated for asthma (Asthma UK, 2004). In children, asthma is one of the commonest causes of hospital admission and long-term medication use. To investigate the hypothesis that decreasing dietary intake of foods rich in antioxidants by mothers during pregnancy has contributed to the recent dramatic increases in asthma and allergic disease, 2000 women were recruited during pregnancy and their diets characterised at 34 weeks gestation using food-frequency questionnaires (FFQ) (Scottish Collaborative Group in Child Health, 2004). Their children have been followed up for 5 years. A FFQ completed by 1751 mothers during pregnancy, a respiratory questionnaire completed for 1253 children at 5 years of age and a food-frequency questionnaire (SCG version C1) to characterise the children’s own diet was collected for 1156 children. Dietary and supplement intakes were summed to give total nutrient intake, energy adjusted and divided into fifths. Logistic regression analysis was carried out to determine the relationship between nutrient intakes and childhood respiratory symptoms with adjustment for covariates and the Table shows the odds ratios (OR) for the lowest v. the highest quintile of maternal nutrient intake.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal vitamin E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheeze in last 12 months</td>
<td>0.557</td>
<td>0.330, 0.938</td>
</tr>
<tr>
<td>Wheeze without cold in last 12 months</td>
<td>0.489</td>
<td>0.236, 1.012</td>
</tr>
<tr>
<td>Seen doctor with wheeze in last 12 months</td>
<td>0.493</td>
<td>0.268, 0.905</td>
</tr>
<tr>
<td>Ever asthma</td>
<td>0.571</td>
<td>0.356, 0.957</td>
</tr>
<tr>
<td>Doctor confirmed asthma</td>
<td>0.589</td>
<td>0.346, 1.004</td>
</tr>
<tr>
<td>Asthma and wheeze in last 12 months</td>
<td>0.458</td>
<td>0.239, 0.878</td>
</tr>
<tr>
<td>Maternal Zn</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short of breath in absence of a cold in last 12 months</td>
<td>0.377</td>
<td>0.088, 0.731</td>
</tr>
<tr>
<td>Ever asthma</td>
<td>0.612</td>
<td>0.361, 1.039</td>
</tr>
<tr>
<td>Asthma in current year in last 12 months</td>
<td>0.444</td>
<td>0.232, 0.846</td>
</tr>
</tbody>
</table>

* Adjusted for maternal age, maternal smoking, maternal vitamin C intake, father’s social class, maternal age of leaving full-time education, deprivation index, birth weight, birth head circumference, birth crown–heel length, child’s sex, birth order, breast-feeding, use of antibiotics by child in first year of life, maternal vitamin E or Zn intake.

In 5-year-old children, maternal vitamin E intake during pregnancy was inversely associated with wheeze in the previous year, wheeze in the absence of a cold, asthma ever, doctor confirmed asthma and asthma with wheeze in the previous year. Maternal Zn intake during pregnancy was inversely associated with breathlessness in the absence of a cold, asthma ever and asthma with wheeze in the previous year. Longitudinal analysis showed that children born to mothers from the lowest quintile of vitamin E intake were 3.5 (95% CI 1.4, 8.7) (P=0.008) times more likely to be of the persistent wheezing phenotype (wheezing 0–2 years and 5 years) and 5.1 (95% CI 1.5, 17.7) (P=0.009) times more likely to be of the early persistent asthma phenotype (onset before the age of 2 and present at 5 years) than children born to mothers from the highest quintile of vitamin E intake. The associations between maternal nutrient intakes and childhood symptoms and asthma were stronger in children who were breast-fed. No associations were found with other antioxidant vitamins (vitamin C or β-carotene). No associations were found with the children’s own diet for these nutrients. Previous results from the study have shown that lower maternal intake of vitamin E during pregnancy is associated with increased neonatal immune responses to allergens, suggesting one possible mechanism for the observed findings.

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Do functional selenoprotein SNPs predict the risk of prostate cancer? By M.P. RAYMANN, M.L. COOPER1, I. VISHNUBHATLA1, H.O. ADAM2, H. GRÖNHORST, K. BALTER3 and F.R. GREEN1,
1School of Biomedical and Molecular Sciences, University of Surrey, Guildford GU2 7XH
2Department of Medical Epidemiology, Karolinska Institute, Sweden and 3Department of Oncology, Umeå University, Sweden.

A considerable body of evidence suggests that an inadequate intake of selenium (Se), is a risk factor for prostate cancer. Se, specified in the genetic code as the amino acid selenocysteine (Sec) by the UGA codon, is incorporated into selenoproteins that carry out important protective functions of Se. SNPs in selenoprotein genes have been associated with the risk of cancers such as those of lung and bladder (Rayman, 2005). This may be explained by the fact that such SNPs can affect selenoprotein functionality or efficiency of synthesis and therefore the amounts of selenoproteins formed.

Our hypothesis was that in a low Se environment, men who have SNP alleles that affect their ability to make selenoproteins will have a higher risk of prostate cancer or a greater risk of advanced disease than men who do not have these alleles.

We chose to investigate the association between specific SNPs in selenoprotein genes (Sep15 C817T; Gpx1 Pro198Leu, and Gpx4 C718T) and risk of prostate cancer in a study population of men living in a known low selenium environment (Sweden). All these SNPs are functional in that they have been shown to affect selenoprotein synthesis in response to Se availability or to be associated with known cancer risk factors or with risks of specific cancers.

We obtained a unique set of DNA samples from 1500 prostate cancer cases and 800 cancer-free male controls collected as part of the CAPS (Cancer of the Prostate in Sweden) study. Using standard molecular biology techniques, DNA from study participants was genotyped (TaqMan™ assay) and the results analysed with respect to clinical and other data. Measurement of plasma Se concentrations in a subset confirmed the relatively low Se status of the CAPS study participants.

We found no case-control association for any of the SNPs investigated with the risk of prostate cancer or advanced prostate cancer, even after adjustment for age and geographical location. However, sub-group analysis showed that possession of Sep15 T811A125 (rare) genotype confers a significantly higher risk of prostate cancer in men with serum prostate-specific antigen (PSA) >100ng/ml, while possession of the Gpx4 718T allele appears to be associated with higher risk in men with BMI >28.

We also looked at a alanine to valine polymorphism at codon 16 in 1636 selenoprotein superoxide dismutase manganese (MnSOD), as this polymorphism is known to modify the risk of bladder cancer associated with the Gpx4 Pro198Leu polymorphism (Ichimura et al. 2004). Though neither prostate cancer nor disease severity per se was affected by MnSOD Ala16Val genotype, when the study population was stratified by geographical region, cases from SE Sweden and Stockholm possessing a valine allele had a lower risk of prostate cancer if they were “Ever-smokers” (P=0.03) or if their BMI was >28 (P=0.07). There is a suggestion that this may be an effect of lower Se status in that region, as this polymorphism is known to interact with Se status (Li et al. 2005). We found no interaction between the MnSOD Ala16Val and Gpx4 Pro198Leu polymorphisms and prostate cancer risk.

We conclude that environmental and lifestyle factors could influence the effect of genetic variation in selenoenzymes and MnSOD on prostate cancer risk.

Supported by the US National Cancer Institute, the UK Prostate Cancer Charitable Trust and the Daphne Jackson Fellowships Trust.


Impact of high-protein, low- and moderate-carbohydrate diets on the microbial community and on metabolite concentration in faeces: possible implications for gut health. By G.E. LOBLEY1, A. BELENGUER1, H-O. ADAMI 2, H. GRO¨ NBERG2, K. B A¨ LTER3 and F.R. GREEN1, 1Department of Medical Epidemiology, Karolinska Institute, Sweden and 2Department of Oncology, Umeå University, Sweden.

Low-carbohydrate diets have proved popular with the public as a weight-loss strategy. Limited carbohydrate delivery may, however, impact on supply to both body tissues and the large bowel, with possible resultant changes in the fermentation products, including butyrate, a metabolite considered to have beneficial effects on colonic health (Topping & Clifton, 2001). High protein intake may also result in product formation within the colon that is deleterious to health. Responses to dietary interventions can be assessed using faeces as a surrogate for distal colon samples (Hold et al. 2002).

Obese male volunteers (n 17; BMI range 30–42 kg/m²), resident in the Human Nutrition Unit at the Rowett Research Institute, were fed a maintenance (M) diet for 3d and then offered two diets ad libitum, either a high-protein low-carbohydrate (HPLC; 30% protein, 4% carbohydrate, 66% fat by energy) or a high-protein moderate-carbohydrate (HPMC; 30% protein, 35% carbohydrate, 35% fat) diet, each supplied for 4 weeks in a randomised cross-over design (Johnstone et al. 2006). All meals were the same energy density (5.5 MJ/kg) and daily intakes were recorded by weight. Faecal samples were taken on three occasions, at the end of the M period and after 4 weeks on each of the diets. The faeces were then analysed for metabolite concentrations and for the main bacterial groups and sub-groups by fluorescent in situ hybridisation (FISH) using ten riboprobes.

Total carbohydrate intake (MJ/d) differed between diets (6.4, 2.6 and 0.4 for M, HPMC and HPLC respectively; P<0.001), while protein intake (MJ/d) was lower for M than for either of the high-protein diets (16, 2.2, 2.1; P<0.001). Faecal ammonia concentrations (µmol) were greatest for M and lowest for HPLC (52, 43 and 33; P=0.003); this pattern was similar to carbohydrate rather than protein intake and did not match faecal-N concentrations. Faecal total SCFA concentrations also decreased with reduced carbohydrate intake (114, 74, 56 mM; P<0.001) as did the proportion of butyrate (0.157, 0.115, 0.075; P<0.001). Bacterial numbers (log count per g faeces) differed between diets (10.70, 10.55, 10.58; P<0.001) and 80%-90% of the bacteria were accounted as species detected by FISH. Both the absolute and proportional contribution (0.114, 0.078, 0.033; P<0.001) of the butyrate-producing Roseburia and Eubacterium rectale (Rrec) group decreased as both carbohydrate and NSP intake reduced (R=0.78 and R 0.73, respectively for log bacterial count; both P<0.001). The abundance of the Rrec group (per g faeces) showed good correlation with butyrate concentration (R 0.68; P<0.001).

The sensitivity of the Rrec group to carbohydrate supply and effects on butyrate production within the colon may have consequences for the long-term use of low-carbohydrate diets. In addition, based on ammonia concentrations, low carbohydrate supply may reduce colonic fermentation of protein or degradation of urea entering the large bowel.

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The apo B signal peptide insertion/deletion (I/D) polymorphism has been shown to influence plasma levels of total and LDL-cholesterol, such that individuals with the D/D genotype have higher levels of total and LDL-cholesterol than I/I individuals (Boekholdt et al. 2003). Genetic variation may affect lipid levels by directly determining levels (‘level genes’), or by influencing the response to environmental factors such as diet (‘variability genes’) (Berg, 1989). The present study aimed to detect variability genes, by assessing differences between genotype groups in within-pair differences in lipid levels in twins.

The subjects included 140 same-sex, monozygotic (MZ) and dizygotic (DZ) twin pairs aged 18–75 years who were recruited for a study of CHD risk factors in twins between September 1998 and February 2002, and in June 2005. A fasting blood sample was taken for analysis of lipids and DNA extraction. Genotype was determined by PCR. For the first 110 twin pairs, the polymorphic alleles were visualised under UV light after electrophoresis of the PCR products on an agarose gel stained with ethidium bromide. For the remaining thirty twin pairs, the polymorphic alleles were identified using the Wave system (Transgenomics Ltd).

The absolute difference in lipid levels within each twin pair was calculated, and the Table shows the mean and 95% CI within-pair difference for each genotype group for the sixty-four MZ twin pairs and for the MZ pairs combined with the fifty-five concordant DZ pairs, i.e. those in which both twins had the same I/D genotype. Since MZ pairs are genetically identical, all within-pair differences are due to environmental factors, but for concordant DZ twins, within-pair differences are due to both environmental factors and other genes which are not shared. Combining MZ and DZ pairs therefore dilutes the effect of environmental variation on within-pair differences but increases the power due to the larger sample size.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total cholesterol (mmol/l)</th>
<th>LDL-cholesterol (mmol/l)</th>
<th>Total cholesterol (mmol/l)</th>
<th>LDL-cholesterol (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/I</td>
<td>0.62</td>
<td>0.48</td>
<td>0.30</td>
<td>0.19</td>
</tr>
<tr>
<td>I/D and D/D</td>
<td>0.40</td>
<td>0.30</td>
<td>0.18</td>
<td>0.16</td>
</tr>
</tbody>
</table>

* Number of twin pairs.

There were borderline significant differences between genotype groups in within-pair differences in total and LDL-cholesterol in MZ twin pairs, such that I/I individuals had greater within-pair differences in total and LDL-cholesterol levels compared with carriers of the D allele (I/D and D/D). Similar trends were seen in the combined group of MZ and DZ pairs.

The results suggest that individuals with the apo B I/I genotype are more sensitive to the influence of environmental factors on total and LDL-cholesterol levels than carriers of the D allele. Further work to assess whether the effect of dietary fat intake on lipid levels is greater in the I/I subjects than in carriers of the D allele is in progress.
Taste sensitivity phenotype and preference for vegetables in the UK Women’s Cohort Study. By J.E. COCKROFT and J.E. CADE, Nutritional Epidemiology Group, Centre for Epidemiology and Biostatistics, The University of Leeds, 30/2 Hyde Terrace, Leeds, UK, LS2 9LN

Genetic sensitivity to the bitter taste of phenylthiocarbamide (PTC) and 6-s-propylthiouracil (PROP) has been linked to a number of sensory dislikes to foods such as bitter-tasting vegetables, coffee, green tea and certain sharp cheeses. More recently a number of claims have been made about the health benefits of genetic taste sensitivity. However, published results are conflicting (Kaminski et al., 2002; Yackinous & Guinard, 2002) and few large-scale epidemiological studies have been conducted in this area. The present study investigated the relationship between taste sensitivity phenotype and preferences for vegetables in a sub-sample of middle-aged women taking part in the UK Women’s Cohort Study (Cade et al. 2004) (n=3401).

The data were obtained from postal questionnaires including a 229-item food preference checklist (Meiselman et al., 1994). Subjects were asked to indicate how much they liked or disliked each food item using the nine-point hedonic preference scale. Participants were classified as non-tasters, tasters and supertasters of PTC using a filter paper screening procedure (Drewnowski, 2001) and a labelled magnitude scale. All participants were aged 41-80 years. Mean food preference scores were compared by taster status using one-way ANOVA. The results Table displays food preference scores (possible range 0 to 9, where 9 = extremely like) by taster status for selected vegetables only.

<table>
<thead>
<tr>
<th>Food</th>
<th>Non-tasters</th>
<th>Tasters</th>
<th>Supertasters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean 95% CI</td>
<td>Mean 95% CI</td>
<td>Mean 95% CI</td>
</tr>
<tr>
<td>Radicchio</td>
<td>6.58 6.45, 6.71</td>
<td>7.15 6.41, 6.51</td>
<td>5.66 5.96, 6.35</td>
</tr>
<tr>
<td>Red cabbage</td>
<td>6.58 6.45, 6.71</td>
<td>7.15 6.41, 6.51</td>
<td>5.66 5.96, 6.35</td>
</tr>
<tr>
<td>Rocket</td>
<td>6.58 6.45, 6.71</td>
<td>7.15 6.41, 6.51</td>
<td>5.66 5.96, 6.35</td>
</tr>
<tr>
<td>Broccoli</td>
<td>6.58 6.45, 6.71</td>
<td>7.15 6.41, 6.51</td>
<td>5.66 5.96, 6.35</td>
</tr>
<tr>
<td>Raw sprouts</td>
<td>5.00 4.80, 5.20</td>
<td>7.11 4.90, 7.43, 5.43</td>
<td>4.47 4.19, 4.75</td>
</tr>
<tr>
<td>Watercress</td>
<td>7.02 5.70, 6.36</td>
<td>5.44 7.25, 7.00, 7.41</td>
<td></td>
</tr>
<tr>
<td>Garlic</td>
<td>7.03 6.70, 7.29</td>
<td>5.69 6.51, 7.11</td>
<td>6.89 6.69, 7.08</td>
</tr>
<tr>
<td>Chilli pepper</td>
<td>6.84 6.08, 7.51</td>
<td>7.17 6.47, 7.43</td>
<td>6.48 6.25, 7.72</td>
</tr>
</tbody>
</table>
| Leeks          | 7.04 6.70, 7.39 | 7.17 6.47, 7.43 | 6.48 6.25, 7.72 | 0.02

The results show that genetic taste blindness to PTC is associated with increased hedonic preferences for a range of vegetables in this cohort of middle-aged women, particularly raw crucifers and raw or cooked allium vegetables. However, although statistically significant differences were found between the groups the actual size of the effect was small. For example, tasters and supertasters did have significantly lower mean preference ratings for watercress than did non-tasters (see Table), the mean ratings for all groups fell between 7 and 8 indicating a moderate overall liking for watercress amongst all groups. In order for genetic taste status to impact on disease risk it must be shown that PTC taste response not only influences food preferences but also impacts on patterns of food consumption. Future work should focus on food consumption data using rigorous epidemiological methods.

The present study was funded by the Medical Research Council as part of a Special Training Fellowship in Health Services and Health of the Public Research.


Enhancing the population of *Bifidobacteria* in the colon has no effect on the metabolism of glucosinolates from cabbage. By Z. FULLER 1, V. RUNGAPAMESTRY 2, B. RATCLIFFE 3, P. LOUIS 1 and AJ. DUNCAN 1, 1The Macaulay Institute, Craigiebuckler, Aberdeen, UK, AB15 8QH, 2The Robert Gordon University, Aberdeen, UK, AB25 8TB and 3Rowett Research Institute, Aberdeen, UK, AB21 9SB

Brassicas contain glucosinolates such as sinigrin, which, on consumption, break down into compounds, including isothiocyanates, which have been linked to cancer prevention. Consumption of raw or partially cooked vegetables, containing active plant myrosinase, results in extensive glucosinolate hydrolysis which leads to absorption of large quantities of isothiocyanates in the upper digestive tract. These are excreted as mercapturic acid conjugates in the urine. Following normal cooking, plant myrosinase is denatured (Kral et al. 2002) and isothiocyanates are formed in the colon due to the myrosinase-like activity of some of the microflora, for example, *Bifidobacteria* (Nugon-Baudon et al. 1990). Stimulation of colonic bifidobacterial populations may increase production of isothiocyanates in the colon.

Six healthy human volunteers received 5 g of a pre-biotic (inulin) twice per d for 21 d (period 1) and a further six volunteers received no supplementation (control). Treatment allocations were reversed for period 2 which also lasted 21 d. Faecal samples were obtained before, and 16 d after, the start of the supplementation during each period. At the end of each period each volunteer consumed two meals, separated by 48 h, containing 150 g partially cooked (microwaved for 2 min) or fully cooked (microwaved for 5.5 min) cabbage. Urine was collected for 24 h after the meals to determine excretion of allyl mercapturic acid (AMA), a marker of isothiocyanate uptake.

Faecal bifidobacterial populations increased (P<0.001) following pre-biotic supplementation (Fig. 1), but this did not influence uptake of AMA after the consumption of fully cooked cabbage (Fig. 2). Excretion of AMA was greater following the consumption of partially cooked cabbage (Fig. 2).

![Fig. 1. Bifidobacterial population before, during the control period and after pre-biotic supplementation.](https://example.com/bifidobacteria.png)

![Fig. 2. Excretion of AMA following consumption of partially (2 min) and fully cooked (5.5 min) cabbage.](https://example.com/ama_excretion.png)
Does ascorbic acid protect type 2 diabetes subjects from hyperglycaemia-induced endothelial dysfunction? By S.W. CHOI,1,2 L.F.F. BENZIE3, C.S.Y. LAM4, S.W.S. CHAT,1 J. LAM1, C.H. YU1, J.J. KWAN5, Y.H. TANG6, G.S.P. YEUNG6, V.T.F. YEUNG6, G.C. WOO6, J.J. STRAIN7 and B.M. HANNIGAN1,1 Faculty of Health and Social Sciences, The Hong Kong Polytechnic University, Kowloon, Hong Kong; 2Centre for Diabetes Education and Management, Our Lady of Maryknoll Hospital, Kowloon, Hong Kong and 3Faculty of Life and Health Sciences, University of Ulster, UK.

Type 2 diabetes mellitus (DM) leads to vascular dysfunction and CHD. Ascorbic acid (AA) is an important dietary derived antioxidant that is inversely correlated with CHD risk and associated with endothelial function (Benzie, 2005). Intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are endothelial adhesion molecules. Their expression is up regulated if the endothelium is activated, as occurs with inflammatory lesions that underlie arterogenesis. Increased ICAM-1 is believed to pre-date lesion formation. Plasma levels of cleared soluble fractions of these molecules are reported to be increased in DM subjects, even without overt vascular complications (Fasching et al. 1996). The present study investigated relationships between AA, hyperglycaemia and ICAM-1 and VCAM-1 in type 2 DM patients and was part of a biomarker profiling study to identify patients at high risk of vascular disease.

Fasting blood samples were collected from 234 consenting type 2 DM patients with no advanced complications, age 59.5 (± 10.6) years. HbA1c, plasma glucose (FPG), AA, soluble ICAM-1 and VCAM-1 were measured (see Table). Pearson’s correlational analysis was performed.

Subjects were grouped by sex and sub-grouped according to degree of hyperglycaemia and to AA level. Group 1A had FPG of 6.1–9.6 mmol/l (‘acceptable control’) and AA > 47.5 µM (sample mean); group 1B had FPG in the same range but AA > 47.5 µM. Groups 2A and 2B had FPG > 9.6 mmol/l (‘poor control’) and AA below and above 47.5 µM, respectively. The results showed that in poorly controlled men, but not women, with lower AA, sICAM-1 directly correlated with FPG (r = 0.482; P < 0.001) and HbA1c (r = 0.482; P =< 0.001). No such relationship was seen in men with acceptable glycaemic control, or with higher AA even if glycaemic control was poor. No correlation of FPG or AA with VCAM-1 was seen.

The observed association between poor glycaemic control and a sensitive biomarker of endothelial dysfunction in type 2 DM men with poor AA status indicates that these men may have higher risk of vascular disease than men whose glycaemic control and/or AA status is better. Good glycaemic control is a known modulator of vascular risk in DM. The role of AA is less clear. The data suggest that the finding of low AA in combination with poor glycaemic control may help identify type 2 DM men at high risk of vascular complications. Long-term follow up is underway.


Supported by the Biotechnology and Biological Sciences Research Council.
Iron absorption is normal in subjects with inflammatory bowel disease but haematins do not predict iron requirements. By W.B. COOK1, J.J. POWELL1 and M.C.E. LOMER2. 1MRC Collaborative Centre for Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge, UK; CBI 9NL and 2Gastrointestinal Laboratory, The Rayne Institute, St Thomas’s Hospital, London, UK. 

Anaemia of chronic disease (ACD), whereby Fe absorption, mobilisation and incorporation into Hb is restricted, is a well-recognised response in inflammatory conditions. This, probably, is driven by the hormone hepcidin. However, diseases such as inflammatory bowel disease (IBD) are relapsing-remitting disorders and curiously, even during long-term remission, patients have a higher than expected prevalence of Fe deficiency and anaemia. This may point to underlying sub-clinical disease leading to aspects of ACD. It also leads to confusion over the interpretation of the haematins which may be skewed, again, even in the absence of clear clinical inflammation.

One of the best measures of Fe requirements is the assessment of Fe absorption. Here we used serum Fe curves following a single oral dose of ferrous sulfate (200 mg; 60 mg Fe) because unlike erythrocyte incorporation methods, this does not rely upon effective erythropoiesis for measurement. We studied thirty quiescent IBD subjects and thirty-three matched controls. Haematins were also assessed at baseline. Serum Fe curves were identical between the two groups, peaking at 180 min post-ingestion with an increase of 17.8 (SD 10.5) μmol/l for control subjects and 17.5 (SD 12.2) μmol/l for IBD subjects. As expected the non-anaemic Fe absorbers (n 18) and non-absorbers (n 10) could be easily separated in the control group based on baseline haematins being, respectively: ferritin 38.3 (SD 45.9) and 88.9 (SD 50.8) μg/l, serum transferrin saturation 21.7 (SD 8.0) and 36.7 (SD 8.8) %, serum Fe 18.4 (SD 6.4) and 20.6 (SD 6.3) μmol/l and soluble transferrin receptor 4.3 (SD 1.8) and 3.7 (SD 0.5) mg/l. In contrast, for the non-anaemic IBD subjects (n 19 absorbers, n 7 non-absorbers) no such separation was observed, haematins being, respectively: ferritin 48.4 (SD 25.3) and 39.5 (SD 15.2) μg/l, serum transferrin saturation 25.5 (SD 7.5) and 25.8 (SD 12.2) %, serum Fe 15.0 (SD 4.6) and 14.7 (SD 7.2) μmol/l and soluble transferrin receptor 4.0 (SD 0.8) and 4.4 (SD 0.4) mg/l. In both groups, Fe-deficiency anaemia (n 5 controls, n 4 IBD) was associated with a marked increase in Fe absorption.

We conclude that Fe absorption is normal in subjects with IBD but that Fe deficiency, in the absence of anaemia, cannot be detected in this group using standard clinical haematins. This could lead to patients being supplemented who do not require Fe (with the associated side effects) and others missing supplementation when they do require Fe. Whether hepcidin, pro-hepcidin and IL-6 analyses of these samples can throw further light on the issue will be investigated in subsequent work. Interestingly, it appears that once anaemia is present, in quiescent patients, then the drive for Fe absorption is high enough to overcome any ‘block’ during the Fe-deficient, non-anaemic state.

Changes in human copper metabolism after dietary intervention: a metabolomic study highlighting the use of genetic algorithms in interpreting 1H nuclear magnetic resonance spectra from urine samples. By J.R. DAINTY, L.J. HARVEY, G. LEGALL, E.K. KEMSLEY, I.J. COLQUHOUN and S.J. FAIRWEATHER-TAITE. Institute of Food Research, Colney Lane, Norwich, UK. 

Determining the role of diet in metabolic regulation is one of the key objectives of nutrition research. Although many of the dietary related metabolic changes are subtle and minor, post-genomic tools for quantifying them are beginning to emerge. These tools (transcriptomics, proteomics and metabolomics) have the capacity to take a holistic (global) rather than reductionist view of metabolism but it is arguable that only metabolomics has the true potential in human nutrition to quantify whole-body effects in vivo. Although the application of metabolomics is quite widespread amongst microbiologists and plant scientists, its use in nutrition is still novel and untested.

In the present study, our aim was to assess whether an increase in dietary Cu intake could be detected by significant changes in urinary metabolite concentration and, if so, to identify these metabolites and assess if any of them could be used as novel biomarkers of Cu status. The present study was performed with no dietary control (except for Cu supplements); all subjects consumed their habitual diet over the entire study period but filled in food diaries to provide a snapshot of their eating habits. The rationale for providing no dietary control was to assess whether the hypothesised subtle changes in Cu-related metabolite concentrations could be significantly quantified against a large background ‘noise’ of changes in metabolite concentrations caused by other (unknown) dietary components. If a consistent set of metabolites could be identified in such a study then we would have confidence in declaring it a robust biomarker for Cu status.

Six healthy male volunteers (age 34–57 years) were recruited from the Norwich region and given a daily Cu supplement of 6 mg/d for 6 weeks. They provided 24 h urine samples for eight consecutive days in two separate periods before the supplement intervention and in one period immediately after. Urine samples were sub-sampled into 500 μl portions and a phosphate buffer added before spectral acquisition on a 600 MHz Bruker Avance-600 NMR spectrometer (Rheinstetten, Germany). The 1H spectra that were produced were analysed with MATLAB software using a collection of multivariate methods: principal components analysis, partial least squares, canonical variate analysis and a genetic algorithm for feature selection in linear discriminant analysis (GA-LDA). Given the enormous size of the dataset and the very large number of distinct features in the high-resolution NMR spectra, it was concluded that the GA-LDA was the most useful technique for interpreting the results. Care was taken to exclude instrumental and chemical noise in the analysis and to avoid over-fitting of the data.

The results from the GA-LDA indicate that a few, small metabolite peaks in the NMR spectrum of the individuals’ urine samples may be able to discriminate the before and after Cu-supplemented periods. The identification of these metabolites is on-going and will be reported at the meeting.

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Maintenance of genome stability is the consequence of a balance between the generation of DNA lesions and their removal via DNA repair. DNA repair is often compromised in cancer development. For example, heritable defects in mismatch repair genes are associated with hereditary non-polyposis colorectal cancer and mutations in nucleotide excision repair genes underly syndromes such as xeroderma pigmentosum with its associated increased risk of skin cancer. Folate has a critical role in normal DNA synthesis and repair through its ability to donate 1-C units for nucleotide metabolism. Folate deficiency increases DNA damage in vitro and in vivo and low dietary folate intake is associated with an increased risk of epithelial cell malignancies including colorectal cancer. In the present study we investigated whether folate insufficiency, in addition to increasing DNA damage, induces genomic instability by negatively affecting DNA repair.

Blood, liver and colon were harvested from Rowett strain male hooded Lister rats fed a control or folate-free diet for 24 weeks. Plasma, whole-blood and tissue folate were measured by RIA. Repair activities of 8-oxoguanine-DNA glycosylase (OGG1), which catalyses the removal of mutagenic 8-oxo-7,8-dihydroguanine from DNA, and O6-alkylguanine-DNA alkyltransferase (MGMT), which repairs mutagenic and toxic O6-guanine damage were determined by [3H]labelled oligonucleotides cleavage and [3H]methyl group transfer respectively. Both of these substrate lesions are implicated in the development of human cancers.

Blood and tissue folate was decreased in rats fed a folate-depleted diet for 24 weeks (see Table). Folate deficiency increased OGG1 and MGMT activity in rat liver by 27 and 25% respectively. DNA repair activity in colon was unaffected by folate deficiency (data not shown).

In conclusion, a moderate but prolonged folate deficiency increases DNA repair activity in rat liver but not colon. This may reflect the ability of the liver to up regulate DNA repair enzymes in response to elevated DNA damage or possible imbalances in the nucleotide precursor pool.

The Scottish Executive Environment and Rural Affairs Department (SEERAD) and CR-UK funded the present study.

Macular pigment response to a xanthophyll supplement of lutein, zeaxanthin and meso-zeaxanthin By R.A. BONE1, J.T. LANDRUM2, Y. CAO3, A.N. HOWARD4 and D.I. THURNHAM2, 1Florida Intl University, 11200 SW 8th Street, Miami, FL 33199, 2University of Ulster, Coleraine, BT52 1SA

Age-related macular degeneration (AMD) is a disease with multiple underlying risk factors, many of which appear to involve oxidative stress. Therefore it is not surprising that macular pigment, with its antioxidant and actinic blue light-screening properties, has emerged as a potentially important line of defence against the disease. Recently, a supplement has appeared on the market containing lutein (L), zeaxanthin (Z) and the third major carotenoid of the macular pigment, meso-zeaxanthin (MZ). Here we report the results of a study in which 8 male and 2 female subjects (mean, ±SD; 30, 10.9 y) were given one gelatin capsule/day containing non-esterified MZ, L and Z (14.9, 5.5 & 1.4 mg resp) as a suspension in soyabean oil, with a meal for 120 days. Macular pigment optical density (MPOD) was measured in each eye by flicker photometry at least four times prior to supplementation, then twice weekly during the 120 day supplementation period and for the 4 week period following supplementation. MPOD rose at a mean (±SD) rate of 0.59±0.79 mAU/day (mAU=milli-absorbance unit) in the 10 subjects who participated, a rate that is very similar, on a per milligram of supplement basis, to that observed in an earlier study involving supplementation with lutein (Landrum et al. 1997). The mean (±SD) increase in lutein and the combined Z isomers were 0.08 (0.09) and 0.17 (0.05) resp and neither correlated with change in MPOD but the percentage changes in the plasma concentrations at 120 days were inversely related to their respective baseline concentrations (L, R<0.04, Z, R<0.05).

Two additional subjects were recruited for a detailed carotenoids analysis at 42 days. Baseline plasma, whole-blood and tissue folate were measured by RIA. Repair activities of 8-oxoguanine-DNA glycosylase (OGG1), which catalyses the removal of mutagenic 8-oxo-7,8-dihydroguanine from DNA, and O6 alkylguanine-DNA alkyltransferase (MGMT) (A) and OGG1 (B) activity in liver. Means and SEM for twelve F+ (F+) and eight F– (F–) group * P<0.003 (Student’s t test).

In conclusion, a moderate but prolonged folate deficiency increases DNA repair activity in rat liver but not colon. This may reflect the ability of the liver to up regulate DNA repair enzymes in response to elevated DNA damage or possible imbalances in the nucleotide precursor pool.

The Scottish Executive Environment and Rural Affairs Department (SEERAD) and CR-UK funded the present study.

Acknowledgements: The studies were supported by The Howard Foundation, Cambridge.


Assessment of skin colour pigmentation in patients undergoing ultraviolet B treatment. By A. MAVROEIDI1,2, F. ONEILL1,2, C. THIND3, A. ORMEROD3, D.M. REID1,2 and H.M. MACDONALD1,2, 1Osteoporosis Research Unit, University of Aberdeen, Woolmanhill Hospital, Aberdeen, UK, AB25 2ZD, 2School of Medicine, University of Aberdeen, Aberdeen, UK, AB25 2ZD and 3Dermatology Department, Aberdeen Royal Infirmary, Aberdeen, UK, AB25 2ZN

Vitamin D has long been considered an anomaly in the nutrition world since cutaneous synthesis through sunlight exposure (wavelengths 290–315 nm) is the major source of this nutrient for most healthy individuals. There is currently no dietary recommendation for vitamin D intakes for adults under the age of 65 years, although there is a reference nutrient intake (RNI) of 10 μg for the elderly (over 65 years) and those who cover up and are at risk of vitamin D deficiency (Department of Health, 1991). Most assessments of sunlight exposure use questionnaires, which are subjective and rely on recall. The aim of the present study was to investigate an objective method of measuring sunlit exposure so that an individual’s exposure to the wavelengths required for cutaneous synthesis of vitamin D can be assessed.

Eighteen female patients (age 17–69 years) undergoing phototherapy treatment (wavelength 311 nm) as part of their routine care at the Dermatology Department at Aberdeen Royal Infirmary were recruited. A single operator used the commercially available light reflectance spectrophotometer (CM-2600d Spectrophotometer, Konica Minolta Photo Imaging, UK) to assess skin pigmentation, which occurs at the same wavelengths as required for the synthesis of vitamin D. Measurements were carried out in triplicate on the forehead and checkbones after calibration against standard white before each measurement series. The repeatability for spectral reflectance of the spectrophotometer has a standard deviation of 0.2% for 360 to 380 nm (manufacturers’ data). A course of UVB treatment usually involves between eighteen and twenty visits to the phototherapy unit. Measurements of skin pigmentation were taken on four to seven occasions throughout the course of treatment.

The following are preliminary results. We recorded measurements for L* (black–white), A* (green–red) and B* (blue–yellow) axes of colour according to the CIE L*A*B system, however only L* results are presented here. The measurement scale for L* extends from 100 for white to 0 for black.

We noted a trend in decreasing L* measurements with increasing number of visits (Fig. A). Fig. B and Fig. C for right cheek, left cheek and forehead respectively.

Changes in L* data were also related to the actual dose of UVB light throughout the course of treatment (data not shown).

Although the data appear promising, further assessments are required to confirm the validity of these results. Future analysis of the data will involve using statistical techniques to combine the colour axes into one variable and assess if that changed throughout treatment. If this method proves successful, it could be used as a non-invasive method for estimating vitamin D status from sunlight exposure.

The present study was funded by the Food Standards Agency. Any views expressed are the authors’ own.


Predictors of fruit, vegetable and juice intake in an elderly Northern Irish population. By M.C. McKINLEY, S.E.C.M. GILCHRIST, J.V. WOODSIDE, U. CHAKRVARTHY and I.S. YOUNG, On Behalf Of The European Eye Study (EUREYE) Investigators, School of Medicine and Dentistry, Queen’s University Belfast, Belfast, UK, BT12 6BJ

Frequent consumption of fruit and vegetables is likely to play a role in disease prevention. Given the many problems associated with the accuracy and validation of dietary intake assessments, a reliable plasma biomarker of fruit and vegetable intakes would be an invaluable tool in epidemiological research. Carotenoids and vitamin C status may be possible biomarkers. Few studies have been carried out to investigate the relationship between dietary intake of fruits, vegetables and juices and concentrations of vitamin C and carotenoids in elderly subjects. Therefore the aim of the present study was to examine the relationship between plasma vitamin C, serum carotenoids (lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene and lycopene), and fruit, vegetable and juice intake in the Belfast cohort of the European Eye (EUREYE) study.

The EUREYE study is a multi-centre, population-based, cross-sectional study aimed at evaluating the prevalence of age-related macular degeneration (AMD) in elderly European populations, and to investigate risk factors for AMD. Belfast was one of the centres for the EUREYE study, and 675 subjects aged >65 years (n 335 male; n 340 female) were recruited from this location. Subjects completed lifestyle questionnaires, including a detailed food-frequency questionnaire that included questions on forty-eight different fruits and vegetables. Frequencies (consumption frequency/week) of individual fruit, vegetable and juice items on the food-frequency questionnaire were converted to total fruit, vegetable, total juice and total fruit, vegetable and juice score in portions per d. A non-fasting blood sample was collected (following consumption of a standardised breakfast), and concentrations of lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene and lycopene were assessed in serum by HPLC according to Craft et al. (1992), while vitamin C was assessed in plasma by automated fluorescence assay according to Vuilkemper & Kock (1989).

When linear regression was used to examine the independent predictors of fruit, vegetable and juice scores, with adjustment for all other carotenoids, vitamin C, age, BMI, smoking, alcohol, and vitamin supplementation use, it was shown that only plasma vitamin C predicted overall total fruit, vegetable and juice score in both males and females (males β = 0.21, P = 0.002; females β = 0.19, P = 0.006). Plasma vitamin C also independently predicted total juice score in males and females (males β = 0.18, P = 0.011; females β = 0.15, P = 0.035), total vegetable score in females only (β = 0.15, P = 0.035), and total fruit score in males only (β = 0.24; P < 0.001). Apart from serum β-cryptoxanthin, no other carotenoids were significantly associated with fruit, vegetable and juice intake. Serum β-cryptoxanthin was a significant independent predictor of total fruit score in both males and females (males β = 0.33, P = 0.002; females β = 0.46; P = 0.006).

The present study has shown that plasma vitamin C and serum β-cryptoxanthin concentrations are consistently associated with total fruit, vegetable and juice intake and total fruit intake respectively, and that this association persists after adjustment for a variety of potential confounders. Plasma vitamin C and serum β-cryptoxanthin therefore have to potential to be used as biomarkers of intake in this elderly population.


Several dietary compounds from plant sources modulate inflammatory pathways implicated in the development of colorectal cancer (Surh, 1999). For example, cinnamic acids abundant in fruits, vegetables and cereals (see Fig. 1) inhibit the formation of inflammatory and potentially neoplastic prostanoids in colonocytes (Russell et al. 2006). However, the extent to which the parent compounds are metabolised in the colon and the nature of the products formed, could affect the potential health benefits of cinnamic acid-rich foods.

![Fig. 1. Predominant cinnamic acids present in fruits, vegetables and cereals.](https://example.com/fig1.png)

Consequently, to assess the effects of gut microflora on cinnamic acids in foods, the concentrations of the predominant cinnamic acids were determined in strawberries by HPLC-UV and GC-MS. Following consumption of strawberries by three fasted volunteers, it was observed that the cinnamic acids were not detected in the plasma and, therefore, were likely to be available for transformation by the gut microflora. Freeze-dried strawberries were incubated (72 h; 37°C) with faecal inoculants (0.2% (v/v) in yeast extract casitone fatty acid (YCFA) medium) from two volunteers consuming a Western-style diet (see Fig. 2). Concentrations of sinapic, ferulic and p-coumaric acids significantly declined (P<0.05) and significant inter-individual variation was observed for sinapic and p-coumaric acid (P<0.05).

![Fig. 2. Availability and metabolism of cinnamic acids from strawberries.](https://example.com/fig2.png)

Individual differences in the composition and activities of the colonic microflora may alter the metabolic fate of potentially anti-inflammatory cinnamic acids with associated implications for gut health. The ability of the resultant metabolites to modulate prostanoid production requires investigation.

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Safety and tolerance of calcium pantothenate in healthy adults. By M. WARNOCK and D.E. McBEAN, School of Health Sciences, Queen Margaret University College, Clerwood Terrace, Edinburgh, UK, EH12 8TS

Calcium pantothenate or vitamin B₅ is important in the production of the adrenal hormones and the formation of antibodies. It aids in vitamin utilisation, and helps to convert fats, carbohydrates, and proteins into energy. It is required for normal functioning of the gastrointestinal tract, necessary metabolic functions and in the prevention of certain forms of anaemia. Pantethine, an active stable form of vitamin B₅, has been gaining attention in recent years as a possible treatment for high cholesterol.

Individuals with rheumatoid arthritis (RA) have been found to have blood levels of pantothenic acid that are lower in than those without this condition (Barton-Wright & Elliot, 1963). Pantothenic acid may have antioxidant and radioprotective activities. It has putative anti-inflammatory, wound-healing and antiviral activities. A study conducted in 1980 concluded that 2000 mg calcium pantothenate improved symptoms of RA including morning stiffness and pain (General Practitioner Research Group, 1980). As a preliminary to a double-blind placebo-controlled trial of RA patients, a study was undertaken to investigate the safety of ingesting 2000 mg calcium pantothenate daily.

The present study was a randomised, blind, controlled study, where the effects of the ingestion of 2000 mg daily of calcium pantothenate (n = 18) were compared with control (800 mg daily of ascorbic acid, vitamin C) (n = 8) in healthy adults. Subjects underwent twelve consecutive weeks of treatment, taking two tablets, twice daily with food. At 4-weekly intervals data were collected from the subjects to examine the safety and tolerance of calcium pantothenate. Safety data were obtained from subject self-reporting of compliance, side effects and tolerance to the treatments. This information was recorded continuously by the subjects and reported to the researchers at three 4-weekly intervals.

No adverse events were reported in either the control or the calcium pantothenate treatment groups, indicating that the large doses are extremely well tolerated in a healthy population. Further work is required to identify both the safety profile and efficacy of high-dose calcium pantothenate in RA patients.


1Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong and 2Division of Clinical Pharmacology, The Chinese University of Hong Kong, Hong Kong

Diabetes mellitus (DM) is associated with increased oxidative stress (King & Loeken, 2004). However, transient increases in plasma glucose induced by oral glucose loading were reported to deplete antioxidants and increase antioxidants directly in both healthy and type 2 DM subjects (Ceriello et al., 1998). This is worrying, and has important implications for long-term health effects of frequent intake of sugar-rich foods in non-DM subjects. Further, if direct, hyperglycaemia-induced changes are confirmed in type 2 DM patients, enrichment of the diet with antioxidant-rich foods is advisable. The primary aim of the present study was to study the acute effect of ingestion of 75 g glucose on biomarkers of oxidative stress and antioxidant status in healthy subjects and in type 2 DM patients in a controlled intervention trial. Their fasting biomarker profiles were also compared.

Ten healthy (age 37.1 (± 12.3) years) and twenty type 2 DM subjects (age 51.5 (± 8.5) years) were recruited with their informed consent. Healthy subjects took 75 g glucose (n = 5) in 300 ml water or 300 ml water alone (n = 5), and venous blood was taken at 0 (fasting), 30, 60 and 120 min post-ingestion. The procedure was repeated within 2 weeks with the other treatment. Type 2 DM patients also underwent a standard oral glucose tolerance test. In all plasma samples malondialdehyde and allantoin (markers of oxidative stress), total antioxidant capacity (ferric-reducing ability of plasma (FRAP) value), ascorbate, urate and lipid-standardised α-tocopherol (markers of antioxidant status), high-sensitivity C-reactive protein (hsCRP, a marker of inflammation) and glucose were determined. hsCRP data were log-transformed for analysis.

Significant post-ingestion increases (P<0.05) (ANOVA for repeated measures) in glucose were seen in healthy and type 2 DM subjects as expected; fasting, peak and 2h glucose levels were 5.4 (± 0.2), 8.8 (± 1.6) and 5.8 (± 0.9) mmol, respectively, for healthy subjects and 8.3 (± 2.9), 17.4 (± 5.5) and 16.1 (± 2.7) mmol, respectively, for DM subjects. At all time points, type 2 DM subjects had significantly (P<0.05; unpaired t test) lower plasma ascorbate (43 (± 17) v. 64 (± 16) μmol), higher allantoin (31.0 (± 22.1) v. 7.7 (± 3.0) μmol) and higher hsCRP (0.7 (interquartile range 0.3–1.3) v. 2.1 (interquartile range 0.9–3.1) mg/l) than healthy subjects. However, no significant post-ingestion changes were seen in any markers of oxidative stress, antioxidant status or inflammation in either group.

Data from the present controlled trial confirm that antioxidant status is lower and that inflammatory and oxidative stress status higher in type 2 DM patients. However, in contrast to a previously published uncontrolled study (Ceriello et al., 1998), these new data do not support an increase in plasma glucose per se as having a direct pro-oxidant effect in vivo.


Dietary protein restriction in the pregnant rat induces altered covalent modifications to histones at the glucocorticoid receptor promoter in the liver of the offspring after weaning. By K.A. LILLYCROP1, A.A. JACKSON2, M.A. HANSON2 and G.C. BURDGE1, 1Development and Cell Biology, University of Southampton, Bassett Crescent East, Southamp ton, UK; SO16 7PX; 2Institute of Human Nutrition, University of Southampton, Tremona Road, Southampton, UK; SO16 6YD and 3DOHaD Centre, University of Southampton, Southampton, UK; SO16 5YA

Feeding a protein-restricted (PR) diet during pregnancy in the rat induces a phenotype in the offspring characterised by hypertension, insulin resistance and dyslipidaemia (Bertram & Hanson, 2001). We have shown that varying the protein content of the maternal diet modified the expression of the hepatic glucocorticoid receptor (GR) in the offspring by altering the methylation of CpG dinucleotides in the exon 10 promoter (Lillycrop et al. 2005). Such epigenetic changes to the regulation of gene expression provide a causal mechanism to explain persistent phenotypic modifications in the offspring. Long-term changes to the regulation of gene expression also involve covalent modifications to the structure of histones, primarily acetylation and methylation of specific lysine residues (Bird, 2002). We have investigated the effect of feeding a PR diet during pregnancy in the rat on histone acetylation and methylation at the hepatic GR exon 10 promoter in their offspring.

Rats were fed either a control (18% (w/w) casein) or PR (9% (w/w) casein) diet from conception to delivery, then standard chow (AIN-76A) during lactation (Lillycrop et al. 2005). Litters were reduced to eight at birth, and offspring were weaned onto chow at postnatal day 28 and killed 6d later. Hepatic GR promoter methylation was determined (six per group) by methylation-sensitive real-time PCR and GR mRNA expression was measured by real-time RT-PCR (Lillycrop et al. 2005). Histone modifications were assessed by chromatin immunoprecipitation assays.

Feeding the PR diet during pregnancy decreased GR promoter methylation (33%) and increased GR expression (84%). Histone H3 and H4 acetylation were increased (174 and 302%, respectively) as was H3K4 methylation (92%) (see Table). H3K9 di-methylation was decreased by 81%, while tri-methylation of H3K9 did not differ significantly between groups (see Table).

Histone modifications

\[ \text{Relative to control group (\%)} \]

| Parameter                  | Control | PR  \\n|----------------------------|---------|------|
| GR10 mRNA expression       | 100     | 67   |
| GR10 promoter methylation  | 100     | 184  |
| Histone H3, K9 acetylation | 100     | 274  |
| Histone H4, K9 acetylation | 100     | 402  |
| Histone H3, K4 methylation | 100     | 1025 |
| Histone H3, K4 di-methylation | 100 | 19   |
| Histone H3, K20 methylation | 100    | 103  |

* Student’s unpaired t test.

These findings show that for the offspring, altered maternal diet in pregnancy is associated with an increase in histone modifications that facilitate transcription, while di- and tri-methylation of lysine 9 on histone H3 which suppress transcription were reduced or did not differ. These observations suggest that induction by prenatal nutrition of changes to gene expression that lead to a modified phenotype involves altered DNA methylation and specific modifications to the histone structure.

Supported by the British Heart Foundation.

Bertram CE & Hanson MA (2008) British Medical Bulletin 60, 103–121.


Regulation of protein kinase C alpha and iota expression in MCF-7 breast cancer cells by conjugated linoleic acids. By R.H. CRABB-WYKE1, M. GOU1, P. KONG1, S.D. HEYS2 and K.W.J. WAHL1, 1The Robert Gordon University, School of Life Sciences, St Andrews Street, Aberdeen, UK, AB10 1FR and 2University of Aberdeen, Department of Surgery, Aberdeen, UK

Breast tumours make up 15% of the total cancer burden in the UK and about 8% of the total number of cancer-related deaths. Protein kinase C (PKC) isoforms have been shown to be strongly modulated in many androgen-sensitive tumour cell lines. Increased expression of some PKC isoforms has also been linked to immortality in many cancer cell lines. Conjugated linoleic acids (CLA) are naturally occurring positional and geometric isomers of the fatty acid linoleic acid. CLA have been shown to reduce growth and increase apoptosis in prostate cancer cells, and to alter their PKC expression both in animal models and in human cell lines. CLA, especially the isoforms cis-9, trans-11 and trans-10, cis-12, also appear to inhibit the growth of breast cancer cells, although the role which the various PKC isoforms play in this mechanism has yet to be established.

We investigated the effect of CLA on the oestrogen-sensitive MCF-7 cell line for 24 h at 24µM of CLA (optimum concentration, previously shown in our laboratory) (Song et al. 2004) on different PKC isoforms. We measured the variations of two PKC isoforms (α, and i) both known to be anti-apoptotic in the cytosol and the membrane. The expression of PKC was measured by Western blot analysis, using PKC isoform-specific antibodies and β-actin as the housekeeping protein.

Results indicate that the CLA isoforms used (cis-9, trans-11, trans-10, cis-12, and a 50:50 mix of the two) cause a decrease of PKC-α expression in the membrane-bound form of the protein compared with the control, significantly the CLA 10:12 isomer causes the greatest decrease of around 75% of the control and a smaller, but still significant decrease in the amount of PKC-α in the cytosolic protein fraction (80% of the control). This effect is also seen in prostate cancer cell lines (LNCaP and PC3), which has been previously reported by our laboratory (Song et al. 2004). Expression of PKCU was confirmed and a significant decrease (5% of the control) observed in the membrane bound protein fraction of cells incubated with the 50:50 isomer mix. A very small decrease in PKCU expression was observed in the membrane bound fraction of the cells incubated with the CLA 9:11 and CLA 10:12 isomers separately.


Pharmacological suppression of gastric acid as a novel strategy for reducing non- haem iron absorption and limiting storage iron in hereditary haemochromatosis. By C. HUTCHINSON1, C. GEISSLER1, J. POWELL2 and A. BOMFORD1, 1The Iron Metabolism Interdisciplinary Research Group, King’s College London, Franklin-Wilkins Building, 150 Stamford Street, London, UK, SE1 9HN and 2MRC Human Nutrition Research, Fulbourn Road, Cambridge, UK, CB1 9NL

Treatment of hereditary haemochromatosis (HH) consists of repeated phlebotomy to initially remove excess Fe and thereafter maintain Fe stores within the normal range. Gastric acid plays an important role in the absorption of non- haem Fe and suppression of gastric acid, for instance using proton-pump inhibitors (PPI), could limit dietary Fe absorption in patients with HH where Fe absorption is up regulated. These studies aimed to: (i) compare the quantity of Fe removed by phlebotomy needed to maintain Fe stores within normal limits before and during PPI; (ii) compare the absorption of Fe from a test meal in patients before and during PPI.

First, the quantity of blood removed to maintain Fe stores within the normal range was compared in six patients during two periods, namely (a) before prescription of PPI and (b) while taking PPI; the paired Student’s t test was used for a within-group comparison of parameters before and during PPI.

Second, fourteen fully treated patients participated in an investigation of the effect of a PPI on postprandial Fe absorption; average serum ferritin and Hb were 87.8 (± 14.1) µg/l and 138 (± 4) g/l, respectively. These patients consumed a vegetarian meal containing 14.5 mg non-haem Fe before and after PPI (either 30 mg lansoprazole or 20 mg omeprazole) daily for 7d. Serum Fe was determined in blood collected before and at 30 min intervals up to 4h after each meal. Both absorption tests were performed after an overnight fast. A repeated-measures ANOVA was used to compare postprandial increase in total serum Fe before and during PPI.

Results indicate that the CLA isoforms used (cis-9, trans-11, trans-10, cis-12, and a 50:50 mix of the two) cause a decrease of PKC-α expression in the membrane-bound form of the protein compared with the control, significantly the CLA 10:12 isomer causes the greatest decrease of around 75% of the control and a smaller, but still significant decrease in the amount of PKC-α in the cytosolic protein fraction (80% of the control). This effect is also seen in prostate cancer cell lines (LNCaP and PC3), which has been previously reported by our laboratory (Song et al. 2004). Expression of PKCU was confirmed and a significant decrease (5% of the control) observed in the membrane bound protein fraction of cells incubated with the 50:50 isomer mix. A very small decrease in PKCU expression was observed in the membrane bound fraction of the cells incubated with the CLA 9:11 and CLA 10:12 isomers separately.

Oral ferrous sulfate is associated with an increase in serum non-transferrin-bound iron and a decrease in plasma ascorbate. By C. HUTCHINSON1, J. POWELL2, I. MUDWAY3 and C. GEISSLER1. The Iron Metabolism Interdisciplinary Research Group, King’s College London, Franklin-Wilkins Building, 150 Stamford Street, London, UK, SE1 9HN and 2MRC Human Nutrition Research, Fulbourn Road, Cambridge, UK, CB1 9NL, 3Lang Biology, Pharmaceutical Sciences Research Division, Franklin-Wilkins Building, 150 Stamford Street, London, UK, SE1 9HN.

We previously found serum non-transferrin-bound Fe (NTBI) after the ingestion of 200 mg FeSO4 (65 mg Fe) in subjects with Fe-deficiency anaemia (IDA) (Hutchinson et al. 2004). NTBI is a potential catalyst for the formation of reactive oxygen and N species, the removal of which is dependent on the antioxidant defence system. Ascorbate is a major contributor to plasma antioxidant capacity. In the present study we aimed to (i) confirm the occurrence of NTBI after oral Fe, (ii) investigate the change in plasma ascorbate following 200 mg FeSO4 and (iii) determine whether there is diurnal variation in plasma ascorbate.

Twelve women with IDA completed a study of NTBI and plasma ascorbate after FeSO4 and sixteen women completed a study of diurnal variation in plasma ascorbate: in the latter study, two subjects had IDA, three were Fe deficient and eleven Fe replete. The study protocols were identical except that the subjects in the first ingested 65 mg Fe as FeSO4 (Alpharma, Barnstable, Devon, UK) with the meal; both groups consumed two slices of white bread with honey and a dilute cordial drink between 08.30 and 09.30 hours after an overnight fast, and blood samples were collected once before and at intervals up to 7 h after ingestion of the meal. Blood samples from the first group were analysed for serum NTBI and from both groups plasma ascorbate. The significance of changes in serum NTBI and plasma ascorbate concentration were evaluated using a repeated-measures ANOVA. The relationship between serum NTBI and plasma ascorbate after ingestion of FeSO4 was determined using a simple linear fit.

Serum NTBI was increased for up to 7 h after 65 mg Fe as FeSO4 (P<0.001; see Fig. 1). The decrease in plasma ascorbate after ingestion of FeSO4 was significantly greater than the decrease in plasma ascorbate over the same period in the diurnal variation study where subjects did not receive Fe (P<0.001; see Fig. 2), suggesting that ingestion of oral Fe induced generation of O2•–. However, the decline in plasma ascorbate concentration was not correlated with the increase in serum NTBI (r=0.05; P=0.87). An indicator of overall antioxidant capacity and oxidative damage may provide more information about the catalytic potential of NTBI after a dose of oral Fe.

Fasting blood samples were obtained from ten healthy subjects supplemented with 200 mg FeSO4 twice daily for 1 week. Plasma samples for AA analysis were then either treated with DTPA (217 mmol/l) or left untreated with water before storage at −80°C. AA analysis was performed using reverse-phase HPLC system with electrochemical detection (400 mV, 0.2 mA) as described by Esteve et al. (1997). Serum Fe and ferritin were measured using routine biochemistry tests. AA concentrations were significantly higher (P<0.05) in the DTPA-treated samples (70.9±7.53 (SE) μmol/l) than untreated samples (65.35±7.53 (μmol/l)). Serum Fe and ferritin ranged from 11 to 44 μmol/l and 21 to 165 μmol/l, respectively. NTBI only present in two subjects serum. This experiment demonstrates that DTPA prevents metal-catalysed losses of AA from plasma during storage and sample processing. We conclude that plasma pre-treatment with DTPA protects AA and improves the determination of this antioxidant in Fe-supplemented subjects. The possible benefit of DTPA treatment in plasma samples from subjects with a high level of NTBI should be investigated.

H.J.M is supported by The Public Service Department of Malaysia.


Gene expression in rat liver exposed to acute alcohol and daidzein. By J.Ci. LIN, M.J. ARNO, M.C.Y. WONG, V.R. FREEDY and H. WISEMAN, Nutritional Sciences Research Division, King’s College London, Franklin-Wilkins Building, 150 Stamford Street, London, UK, SE1 9NH

Alcohol dosage results in oxidative stress, which may cause alteration in gene expression, modulation of signal-transduction cascades and induction of several different cellular responses. The objective of the present study was to identify and characterise differentially expressed genes after acute alcohol dosage. We also hypothesised that daidzein pre-treatment ameliorates alcohol-induced perturbations in gene expression.

To address this, rats (0.1 kg body weight (BW)) were treated with either ethanol (EtOH; 75 mmol/kg BW) or daidzein (100 mg/BW) in an experimental design that entailed a pre-treatment stage of 2 d followed by a treatment period of 1 d. Male Wistar rats were divided into four groups (pre-treatment + treatment) as follows: (A) carrier + saline (control); (B) daidzein + saline; (C) carrier + EtOH and (D) daidzein + EtOH. Controls were treated with either carrier (10% (v/v) fat emulsion) or saline (0.15 mol NaCl/l). At the end of the study rats were killed and liver samples were dissected in order to isolate total RNA and measure mRNA using the Affymetrix GeneChip Rat230A oligonucleotide arrays (three chips from each group).

The study revealed that 9373 genes (59%) of the 15 923 probe sets or genes represented on the array were expressed in rat livers. Cut-offs of minimum 150% increase or 50% decrease were selected for further bioinformatic processing. Thus, 139, 54 and 284 genes were deemed to be significantly altered by alcohol, daidzein, and a combination of daidzein and alcohol, respectively. Expression data mining, using the GeneSpring® 7.3 platform (Agilent Technologies, Inc., Palo Alto, CA, USA), was used to ascertain the functionality of affected genes or their role in specific pathways. For example, alcohol altered genes linked to cell growth and/or maintenance, lipid metabolism, cholesterol biosynthesis, energy pathway, catabolism and protein folding. Genes which were altered in response to daidzein are linked to apoptosis and regulation of growth. Furthermore, additional pathways involved in cell proliferation, coenzymes and prosthetic group metabolism and electron transport were altered by a combination of daidzein and alcohol. However, genes related to oxidative stress (such as superoxide dismutase, catalase and glutathione peroxidase) were not overtly affected by daidzein or alcohol or their combination. This indicates that the perturbation due to alcohol and the effects of daidzein may occur at numerous sites. Moreover, due to the complexity of the bioinformatic processing, the influence of daidzein in ameliorating the effect of alcohol is still being examined. Further studies, such as using quantitative RT-PCR, are needed to validate these observations and to ascertain whether daidzein is protective in terms of gene expression.

The effects of conjugated linoleic acid on the surface expression of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 on TNF-α-stimulated human umbilical vein endothelial cells measured by flow cytometry. By S. MULKREW1, M. GOUA1, A.A. SNEDDON2 and K.W.J. WAHLE1, 1The Robert Gordon University, St Andrew Street, Aberdeen, UK, AB25 1HG and 2The Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, UK, AB21 9SB

In the early stages of atherosclerosis both vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) are involved in the recruitment of leucocytes onto the endothelium. Studies have demonstrated that adhesion molecule expression can be down regulated by n-3 PUFA. Conjugated linoleic acids (CLA) are a family of positional and geometric isomers of linoleic acid, preferentially found in dairy products, beef fat, vegetable oils and spreads. CLA is recognised to have anti-carcinogenic properties. It has been shown that CLA affects the transcription factor NF-κB, present in the VCAM-1 and ICAM-1 gene promoter. However, the effects of CLA on adhesion molecule expression in human umbilical vein endothelial cells (HUVEC) have not been studied. The purpose of the present study was to analyse the variations of VCAM-1 and ICAM-1 surface expression on HUVEC under inflammatory conditions (i.e. TNF-α stimulation).

It was found that VCAM-1 and ICAM-1 were both reduced by cis-9, trans-11-CLA, trans-10, cis-12-CLA and a CLA mix at 25μM. However, there was little change in VCAM-1 or ICAM-1 expression with 50μM-CLA.
The potential effects of plant extracts in protecting against oxidant-induced injury to Caco-2 cells. By S.A. AHERNE, J.P. KERRY and N.M. O’BRIEN, Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland

Experimental evidence suggests that most herbs and spices, especially those of the Lamiaceae family, possess a wide range of biological and pharmacological activities (Bozin et al. 2006). Three commonly used herbs belonging to the Lamiaceae family are rosemary (Rosmarinus officinalis), oregano (Origanum vulgare) and sage (Salvia officinalis). In the present study the effects of rosemary, oregano, sage and echinacea (Echinacea purpurea) extracts on oxidant-induced cell injury were investigated in human colonic carcinoma Caco-2 cells. In addition, effects of the plant extracts on the antioxidant status of the cells were assessed by determining catalase (EC 1.11.1.6), superoxide dismutase (EC 1.15.1.1) and glutathione (GSH) content.

Caco-2 cells were cultured in Dulbecco’s modified Eagle’s medium and maintained in a humidified atmosphere of 37°C. Initially, Caco-2 cells were supplemented with increasing concentrations (5–1000 μg/ml) of rosemary, oregano, sage and echinacea extracts for 24 h. EC50 values were determined and concentrations corresponding to greater than 90% cell viability were selected for subsequent experiments. Cells were supplemented with extracts of rosemary (15 μg/ml), oregano (60 μg/ml), sage (60 μg/ml) or echinacea (250 μg/ml) for 24 h. Antioxidant status was determined by measuring catalase activity, superoxide dismutase activity and GSH content. Following pre-treatments with the plant extracts, cells were exposed to increasing concentrations of H2O2 for 2 h at 37°C. Cell viability was assessed using the neutral red uptake assay (Babich & Borenfreund, 1992), where results are expressed as a percentage of the control.

### Table 1: % Cell viability (control=100%)

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<tr>
<th>Extract</th>
<th>H2O2 treatment</th>
<th>Mean ± SE</th>
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* n = 6; Statistical analysis by one-way ANOVA, followed by Dunnett’s test.
  * P < 0.01 when compared with control; † P < 0.05 when compared with H2O2-treated cells.

Cell viability significantly decreased with increasing concentrations of each extract, except for echinacea which was toxic only at the highest concentration. Of the plant extracts tested, rosemary was the most toxic (concentration of compound that resulted in 50% cell deaths (EC50) 122.7 μg/ml) and echinacea the least toxic (EC50 1421 μg/ml). Rosemary, oregano, sage and echinacea had no significant effect on catalase and superoxide dismutase activities. Sage was the only plant extract to significantly increase GSH content (P < 0.01). Treatment with H2O2 at 100 μg/ml for 2 h did not significantly affect cell viability. Exposure to 250 or 500 μg/ml H2O2 for 2 h significantly decreased cell viability when compared with control (P < 0.01). Sage was the only plant extract to significantly protect against H2O2-induced cell injury (P < 0.05). The present findings suggest that sage may protect against H2O2-induced cell damage via GSH modulation.

The present study was funded under the National Development Plan 2000–2006 by the Department of Agriculture and Food.

The effect of maternal nutrient restriction between 30 and 80d gestation followed by juvenile obesity on glucocorticoid receptor messenger RNA abundance and kidney function in young adult sheep. By P.J. WILLIAMS, L. KURLAK, H. BUDGE, T. STEPHENSON, A. PERKINS, M.E. SYMONDS and D.S. GARDNER, Centre for Reproduction and Early Life, School of Human Development University Hospital and Veterinary Medicine and Science, Sutton Bonnington, University of Nottingham, UK, NG7 2UH

Maternal nutrient restriction between 30 and 80d gestation results in offspring with a disproportionately larger placenta and increased tissue glucocorticoid sensitivity in a range of tissues including the kidney and adipose tissue (Whorwood et al. 2001). When these offspring are raised under optimal conditions only modest changes in their cardiovascular control and metabolic regulation are observed (Gopalakrishnan et al. 2005). The aim of the present study was therefore to examine what effect raising previously nutrient-restricted (NR) offspring within a restricted physical environment would have on glucocorticoid receptor (GR) mRNA abundance and kidney function.

At 30 d gestation, eighteen sheep were randomly allocated to receive either a control (C; 7 MJ/d; 7); NR 88 (SE 1) kg (n 7) or an NR diet (50% of C; 5); NR 88 (SE 1) kg (n 7) and concentrations corresponding to greater than 90% cell viability were selected for subsequent experiments. Cells were supplemented with extracts of rosemary (15 μg/ml), oregano (60 μg/ml), sage (60 μg/ml) or echinacea (250 μg/ml) for 24 h. Antioxidant status was determined by measuring catalase activity, superoxide dismutase activity and GSH content. Following pre-treatments with the plant extracts, cells were exposed to increasing concentrations of H2O2 for 2 h at 37°C. Cell viability was assessed using the neutral red uptake assay (Babich & Borenfreund, 1992), where results are expressed as a percentage of the control.

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<td>Rosemary + H2O2</td>
<td>6.0%</td>
<td>6.0%</td>
<td>6.0%</td>
<td></td>
</tr>
<tr>
<td>Oregano + H2O2</td>
<td>7.0%</td>
<td>7.0%</td>
<td>7.0%</td>
<td></td>
</tr>
<tr>
<td>Sage + H2O2</td>
<td>8.0%</td>
<td>8.0%</td>
<td>8.0%</td>
<td></td>
</tr>
<tr>
<td>Echinacea + H2O2</td>
<td>9.0%</td>
<td>9.0%</td>
<td>9.0%</td>
<td></td>
</tr>
</tbody>
</table>

* n = 6; Statistical analysis by one-way ANOVA, followed by Dunnett’s test.
  * P < 0.01 when compared with control; † P < 0.05 when compared with H2O2-treated cells.

Cell viability significantly decreased with increasing concentrations of each extract, except for echinacea which was toxic only at the highest concentration. Of the plant extracts tested, rosemary was the most toxic (concentration of compound that resulted in 50% cell deaths (EC50) 122.7 μg/ml) and echinacea the least toxic (EC50 1421 μg/ml). Rosemary, oregano, sage and echinacea had no significant effect on catalase and superoxide dismutase activities. Sage was the only plant extract to significantly increase GSH content (P < 0.01). Treatment with H2O2 at 100 μg/ml for 2 h did not significantly affect cell viability. Exposure to 250 or 500 μg/ml H2O2 for 2 h significantly decreased cell viability when compared with control (P < 0.01). Sage was the only plant extract to significantly protect against H2O2-induced cell injury (P < 0.05). The present findings suggest that sage may protect against H2O2-induced cell damage via GSH modulation.

The present study was funded under the National Development Plan 2000–2006 by the Department of Agriculture and Food.


Supported by the British Heart Foundation and the University of Nottingham.
Dietary intake of fish and depressed mood: genuine association or just a mirage? By K.M. Appleton1, J.V. Woods2, J.W.G. Yarnell3, D. Arveiler4, B. Haas5, P. Amouyel6, M. Montaye7, J. FERRERIES8, J.H. RAIDVETS9, P. DUCMETIERE10, A. BINGHAM11 and A. EVANS11, 1School of Psychology, Queen’s University Belfast, 18–30 Malone Road, Belfast, UK, BT9 5BP, 2School of Medicine and Dentistry, Queen’s University Belfast, UK, BT12 6BJ, 3The Strasbourg MONICA Project, Strasbourg, France, 4The Lille MONICA Project, INSERM U58, Lille, France, 5The Toulouse MONICA Project, INSERM U58, Toulouse, France and 6The Coordinating Center, INSERM U258, Hôpital Paul Brousse, Villejuif, France

Recent studies demonstrate an association between dietary fish intake and depressed mood (Silvers & Scott, 2002). This research ties in with clinical evidence suggesting an association between dietary intakes of n-3 PUFA and depression. Recent work, however, suggests that associations between depressed mood and fish consumption may not be directly related to n-3 PUFA intake, but may be related to general diet or lifestyle (Appleton et al., 2006). Associations between depression and lifestyle are well known. The present analysis investigates associations between depressed mood and general diet.

The analysis was conducted on data collected as part of The Prospective Epidemiological Study of Myocardial Infarction (PRIME) – a 5-year cohort study investigating the predictors of myocardial infarction in 9758 men aged 50–59 years, from Northern Ireland and France (PRIME Study Group, 1998). At the start of this study (1991–4), data on diet was collected using a food-frequency questionnaire measuring frequency of consumption of eleven food groups, including fish, and data on depressed mood was collected using a self-report questionnaire based on the Welsh Pure Depression sub-scale of the Minnesota Multiphasic Personality Inventory (Rodda et al., 1971). Data on demographics, socio-economic status based on type of work (SES) and highest level of education were also collected.

Using an unadjusted regression model, depressed mood was negatively associated with fish intake (linear β = −0.22, P < 0.01; non-linear β = 0.18, P < 0.01). On addition of all other food groups, the association between depressed mood and fish intake weakened, yet remained significant, but independent associations were also found between depressed mood and intakes of cake (β = −0.03, P < 0.01), eggs (β = −0.05, P < 0.01), offal (β = 0.05, P < 0.01), fried potatoes (β = −0.05, P < 0.01), raw vegetables (β = −0.06, P < 0.01), and fruit (β = −0.03, P < 0.01). On addition of the demographic variables, the associations between depressed mood and fish intake, and also with other food groups were attenuated, but associations between depressed mood and demographic variables were also significant (age β = −0.02, P < 0.02; SES β = −0.03, P < 0.01; education β = −0.11, P < 0.01).

First, these analyses provide evidence for an association between dietary fish intake and depressed mood (Silven & Scott, 2002; Appleton et al., 2006). Second, however, these analyses also provide evidence for associations between depressed mood and intake of a number of other food groups. Greater depressed mood is not only associated with lower intakes of fish, but is also associated with lower intakes of cake, raw vegetables and fruit, and higher intakes of fried potatoes, eggs and offal. These associations suggest that depressed mood may be related to a number of factors, not just n-3 PUFA. The pattern of these associations, however, also suggests that associations between depressed mood and dietary intake may be unlikely to result solely from individual nutrients. Fish, cake, and fresh fruit and vegetables are expensive commodities, typically consumed by those of a higher affluence; potatoes, eggs and offal by comparison are cheaper items, typically consumed by those more deprived (Gregory et al., 1990). The pattern of associations, thus, may suggest that associations between depressed mood and dietary intake demonstrate wider associations between depressed mood and lifestyle.

In summary, these analyses provide evidence for an association between fish intake and depressed mood. This association, however, may not only represent a direct association, but may also reflect wider associations between depressed mood and lifestyle.

The dietary phyto-oestrogen daidzein protects against the formation of hepatic malondialdehyde–protein adducts induced by oxidative stress. By M.C.Y. WONG1, O. NIEMELÄ1, S. PARIKKA2, H. KOIVISTO2, K. TRICK3, M.R. LABBE4, V.R. PREDY5 and H. WISEMAN1, 1Nutritional Sciences Research Division, King’s College London, Franklin-Wilkins Building, 150 Stamford Street, London, SE1 9NN, 2EP Central Hospital Laboratory, Seinajoki, Finland and 3Bureau of Nutritional Sciences, Health Canada, Ottawa, Canada KIA 0L2

The cytoxic agent α-galactosamine (GalN) has previously been shown to induce oxidative stress in the rat which can perturb metabolic function. GalN studies also provide a framework for investigating the therapeutic effectiveness of cytoprotective agents. We hypothesised that α-tocopherol (ATC) and the phyto-oestrogen daidzein will be protective against GalN-induced oxidative stress. To test this, male Wistar rats (0.102–0.113 kg body weight (BW)) were either pre-treated with ATC (30 mg/kg BW) or daidzein (100 mg/kg BW) for 1 h then treated with either saline or GalN (1 g/kg BW) for 23 h before being killed and livers extracted. There were six groups (six rats per group): (A) carrier-saline; (B) carrier+GalN; (C) ATC+GalN; (D) ATC+GalN; (E) daidzein+saline; (F) daidzein+GalN. Immunohistochemical staining techniques were used to determine levels of malondialdehyde (MDA)–protein adducts in the liver, as an index of oxidative stress. Also spectrophotometry was used to measure the activities of hepatic superoxide dismutase (SOD) (total and Cu-Zn), total glutathione peroxidase and Se-dependent glutathione peroxidase, as indices of antioxidant potential.

The results of the present study showed that GalN administration significantly increased the MDA staining scores in the liver, from a mean of 0.58 (group A) to 2.17 (group B; P < 0.01). Total and cytosolic SOD activities were also increased significantly (total SOD from a mean of 37.5 U/mg protein in group A to 45.4 U/mg protein in group B; P < 0.01), education (β = −0.03, P < 0.01), and also with other food groups were attenuated, but associations between depressed mood and demographic variables were also significant (age β = −0.02, P < 0.02; SES β = −0.03, P < 0.01; education β = −0.11, P < 0.01).

First, these analyses provide evidence for an association between dietary fish intake and depressed mood (Silven & Scott, 2002; Appleton et al., 2006). Second, however, these analyses also provide evidence for associations between depressed mood and intake of a number of other food groups. Greater depressed mood is not only associated with lower intakes of fish, but is also associated with lower intakes of cake, raw vegetables and fruit, and higher intakes of fried potatoes, eggs and offal. These associations suggest that depressed mood may be related to a number of factors, not just n-3 PUFA. The pattern of these associations, however, also suggests that associations between depressed mood and dietary intake may be unlikely to result solely from individual nutrients. Fish, cake, and fresh fruit and vegetables are expensive commodities, typically consumed by those of a higher affluence; potatoes, eggs and offal by comparison are cheaper items, typically consumed by those more deprived (Gregory et al., 1990). The pattern of associations, thus, may suggest that associations between depressed mood and dietary intake demonstrate wider associations between depressed mood and lifestyle.

In summary, these analyses provide evidence for an association between fish intake and depressed mood. This association, however, may not only represent a direct association, but may also reflect wider associations between depressed mood and lifestyle.
Reduced maternal folate supply decreases genomic DNA methylation in the offspring of C57Bl/6J mice. By K.J. WALTHAM, E.A. WILLIAMS and J.C. MATHERS, Human Nutrition Research Centre, School of Clinical Medical Sciences, Newcastle University, Newcastle upon Tyne, UK, NE2 4HH and 2Human Nutrition Unit, Division of Clinical Sciences, University of Sheffield, Sheffield, UK, S7 7AU

Epidemiological studies suggest an inverse association between folate intake and risk of colorectal cancer. Putative mechanisms for this association include chromosomal instability, impaired DNA synthesis and repair, and aberrant DNA methylation when folate status is low. According to the Barker hypothesis, compromised nutritional status in utero increases the risk of disease in adult life (Barker, 1997). The present project was designed to test the hypothesis that reduced folate supply in utero affects genomic DNA methylation in the adult offspring.

Female C57Bl/6J mice were randomised to semi-purified folate-depleted (Low) or folate-supplemented (High) diets (containing 0.4 and 8 mg folate/kg diet respectively) for 5 weeks before mating. Mice remained on the test diets throughout pregnancy and lactation. At weaning the offspring were randomised to either depleted (Low) or supplemented (High) diets (containing 0 or 8 mg folate/kg diet respectively) resulting in four dietary regimens, i.e. High–High, High–Low, Low–Low and Low–High (maternal–postweaning folate supply). At 10 weeks post-weaning, mice were killed for the assessment of genomic DNA methylation via the cytosine extension assay (Pogribny et al. 1999) in samples of small-intestinal (SI) mucosa. In this assay the incorporation of [3H]dCTP (expressed as degradations per min [dpm/μg DNA in the Table] is directly proportional to the number of unmethylated cytosines in the genome. Effects of dietary exposure were tested by ANOVA using a 2×2 design.

<table>
<thead>
<tr>
<th>Material diet*</th>
<th>Weaning diet*</th>
<th>Mean incorporation (dpm/μg)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>High (n=15)</td>
<td>13.105</td>
<td>8.061, 19.855</td>
</tr>
<tr>
<td>High</td>
<td>Low (n=14)</td>
<td>12.590</td>
<td>7.568, 19.203</td>
</tr>
<tr>
<td>Low</td>
<td>High (n=16)</td>
<td>18.107</td>
<td>12.170, 25.692</td>
</tr>
<tr>
<td>Low</td>
<td>Low (n=12)</td>
<td>30.091</td>
<td>21.306, 40.391</td>
</tr>
</tbody>
</table>

*Probability of effect: maternal diet P<0.003, weaning diet P=0.034, maternal diet×weaning diet P=0.124

There was a highly significant (P<0.003) effect of maternal diet on genomic DNA methylation, with mice derived from folate-depleted mothers having a much greater proportion of unmethylated cytosine residues in DNA from the SI mucosa. In contrast, no effect of the folate content of the weaning diet (fed to the offspring) and no maternal×weaning diet interaction on genomic DNA methylation were detected.

These data suggest that compromised maternal folate supply results in offspring with hypomethylated DNA and that this effect is not attenuated by feeding a folate-replete diet from weaning. Conversely, significant restriction of post-weaning folate supply for up to 10 weeks has no detectable effect on genomic DNA methylation in mice born to mothers with adequate folate supply through pregnancy and lactation. The effects of this aberrant epigenetic marking on long-term health of mice remain to be investigated. These observations may be of mechanistic importance in understanding the biological basis of programming induced by poor maternal nutrition.

The present study was funded by the World Cancer Research Fund (project grant 2001/37).


Effects of age, body mass index and genotype on nucleotide excision repair in healthy adults. By J. TYSON, A. SPIERS, E. CAPLE, J.E. HESKETH and J.C. MATHERS, Human Nutrition Research Centre, School of Clinical Medical Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, UK, NE2 4HH and 2Biological and Food Sciences, Northumbria University, Newcastle upon Tyne, UK, NE1 8ST

Nucleotide excision repair (NER) is responsible for the repair of bulky DNA lesions caused by UV light, food-derived 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, as little as 11% of the population mean, have been associated with an increased risk of cancer at several sites (Lockett et al. 2005). Polymorphisms in DNA repair genes and environmental factors, including dietary exposure, are possible determinants of NER capacity.

NER capacity was quantified in lymphocytes from forty-eight young healthy adults (mean 22 (range 18–30) years). Recruits took a multivitamin supplement (containing Se and vitamins A, C and E) for 6 weeks and information on diet and lifestyle was collected. Baseline (pre-supplementation) NER capacity was measured using the plasmid-based host cell reactivation assay which is specific for NER (Athas et al. 1991). In addition, subjects were genotyped for four polymorphisms in three key NER genes: ERCC5 Lys939Gln and ERCC2 Lys751Gln and XPC poly A/T insertion/deletion.

NER capacity varied 10.5-fold within this population (2.3–25%; mean 10 (SD 5%) ). There was no effect of sex on NER capacity but age and BMI were both significantly inversely associated with NER capacity (P<0.05) which decreased by about 5% per year of age and by 3.5% for every 1 unit increase in BMI. No single polymorphism had a significant effect on NER capacity. However, significant interactions between the XPC Lys939Gln and both ERCC2 Lys751Gln and ERCC5 Asp1104His polymorphisms were observed when subjects were categorised based on the presence or absence of the uncommon (a) allele (see Figure). These interactions remained significant (P<0.05; testing for two-way interactions using ANOVA) after correcting for age and BMI.

The high inter-individual variation in NER capacity can be explained in part by effects of age and BMI which both appeared to reduce NER capacity. In addition, multiple rather than single genotypes appear to be important determinants of NER capacity. Individual NER capacity, although partially genetically determined, may be modifiable through dietary and lifestyle interventions.

The study was funded by BBBSRC grant 13/D05721. J.T. is funded by a BBBSRC studentship. We thank J. Matta (Puerto Rico) for helpful advice on the NER assay.


An *in vitro* method to determine the percentage micellarisation of carotenoids in a variety of fruits. By O.F. O’CONNELL, L. RYAN and N.M. O’BRIEN, Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland

Carotenoids are responsible for the red, yellow and orange pigments in some fruits and vegetables. Approximately 600 carotenoids have been identified and they can be divided into the carotenoids, for example, lycopene and β-carotene which contain only carbon and hydrogen groups and the xanthophylls, for example, lutein, zeaxanthin and β-cryptoxanthin which are their oxygenated derivatives. The absorption of carotenoids by the intestine is dependent on these fat-soluble pigments being packaged into micelles. The objective of the present study was to determine the percentage micellarisation of carotenoids from fruit using an *in vitro* digestion procedure as described by Garrett et al. (1999). The fruits selected were orange, kiwi, red grapefruit and honeydew melon. Raw fruit was homogenised before the simulated gastric and intestinal digestion procedure. Digesta were ultracentrifuged to isolate the aqueous micellar fraction. The carotenoids from whole fruit, homogenate, digestate and micelles were extracted twice using a solvent mixture of hexane, acetone and ethanol (Olives Barba et al. 2006). The carotenoid content was quantified using HPLC (Hart & Scott, 1995). The percentage micellarisation of each carotenoid was determined by calculating the transfer from the digestate to the micelles.

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Lutein (%)</th>
<th>Zeaxanthin (%)</th>
<th>β-Cryptoxanthin (%)</th>
<th>Lycopene (%)</th>
<th>β-Carotene (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange</td>
<td>44.0</td>
<td>26.9</td>
<td>16.8</td>
<td>74.4</td>
<td>26.9</td>
</tr>
<tr>
<td>Kiwi fruit</td>
<td>29.7</td>
<td>17.2</td>
<td>11.6</td>
<td>16.4</td>
<td>46.3</td>
</tr>
<tr>
<td>Red grapefruit</td>
<td>44.4</td>
<td>25.6</td>
<td>4.3</td>
<td>97.1</td>
<td>24.7</td>
</tr>
<tr>
<td>Honeydew melon</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

More than two independent experiments.

Digested kiwi contained the highest content of lutein (26.1 µg/100 g). *In vitro*-digested red grapefruit had the highest content of zeaxanthin, containing 16.8 µg/100 g. Digested oranges had the greatest level of β-cryptoxanthin (48.4 µg/100 g). Finally, digested red grapefruit had the highest level of lycopene and β-carotene, containing 405.0 and 263.5 µg/100 g, respectively. There was high transfer of the carotenoids from the digestate to the micelles, particularly for zeaxanthin, β-carotoxanthin and β-carotene. Garrett et al. (1999) reported that lycopene from tomatoes had low levels of micellarisation; however, in the present study we found 66.8% of lycopene was micellarised from red grapefruit. The carotenoid content of vegetables is generally higher than fruit (Ryan et al. 2006). However, we found there is more efficient transfer of carotenoids from digestate to micelles in fruit. The difference could be due to the location of carotenoids in the food matrix. In fruit, carotenoids are found in chromoplasts dissolved in oil droplets and it is through an oil emulsion that carotenoids are transferred to the bile salt micelles (Farr & Clark, 1997).

The present research was funded by Science Foundation Ireland.


Are low-income consumers disadvantaged by higher-priced and lower-nutritional quality fruit and vegetable availability? A preliminary study. By E. WAKEFORD and R.M. FAIRCHILD, School of Health Sciences, University of Wales Institute, Cardiff, Western Avenue, Cardiff, UK, CF5 2YB

The perception that healthy foods are expensive is a barrier to low-income consumers meeting the UK Government’s healthy eating guidelines, including the ‘5-a-day’ initiative (Food Standards Agency Wales & Welsh Assembly Government, 2003). The present study aimed to compare the price and nutritional quality of fruit and vegetables available in an area of multiple deprivation in Cardiff. Two stores were selected based on proximity to the area (a discount food store (B), and an independent food store (C)), representing the stores that could be easily reached on foot. The remaining two stores (a leading supermarket (A), and a department store selling food (D)) required public or private transport to access them. Samples of apple, tomato, cauliflower, carrot and orange were chosen based on popularity amongst UK consumers (British Broadcasting Corporation, 2003) and availability in all four stores during the research period (November, 2003). Items were purchased by the researcher based on aesthetics (visual appearance and texture) and stored overnight in a refrigerator (cauliflower, carrots and tomato) or at room temperature (apple and orange), imitating normal consumer storage patterns. Samples were analysed, in duplicate, the next morning using standard methods for vitamins A and C (Kirk & Sawyer, 1991). Meeting the UK 5-a-day recommendation cost the consumer 35–65% more than those from the supermarket (B). In addition, the fresh fruit and vegetables purchased from stores B (discount food store) and C (independent food store) resulted in a higher vitamin C and carotene content (see table). This provides some encouraging, if limited, evidence that low income is no more than a perceived barrier to eating a healthy diet and that nutritional quality is not dependent upon price.

<table>
<thead>
<tr>
<th>Carotenoids (µg)</th>
<th>Vitamin C (mg)</th>
<th>Store</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Orange</td>
<td>341</td>
<td>30.8</td>
</tr>
<tr>
<td>Tomato</td>
<td>52.0</td>
<td>52.9</td>
</tr>
</tbody>
</table>

Elevated urinary \( \text{F}_{2}\)-isoprostane concentrations are associated with congestive heart failure. By G.C. Mckeeman, C.M. Hughes, P.P. McKeown and I.S. Young, 1Nutrition and Metabolism Research Group, CCPS, Queen’s University Belfast, Belfast, UK, BT12 6BJ and 2Epidemiology and Public Health Research Group, CCPS, Queen’s University Belfast, Belfast, UK, BT12 6BJ

Congestive heart failure (CHF) is a major health problem and carries a poor prognosis. Patients with CHF typically develop multiple nutritional deficiencies associated with loss of appetite and development of cachexia. Studies have shown that oxidative stress mediated by reactive oxygen species may play a significant role in disease pathogenesis (Nomura-Sakurawa et al., 2003). \( \text{F}_{2}\)-isoprostanes, isomers of prostaglandin \( \text{F}_{2\alpha} \), are stable endproducts of lipid peroxidation derived from arachidonic acid. They are released from the site of free radical injury and have emerged as the best indicators of oxidative stress (Cracowski & Ormezzano, 2004). Urine measurement of isoprostanes is preferred due to the non-invasive approach and the lack of sample auto-oxidation. GC-MS and similar chromatographic techniques are regarded as superior to immunoassays (Young, 2005).

Urine samples were obtained from twenty-two patients with moderately severe CHF (New York Heart Association class II or III) and twenty-five controls. Most cases of heart failure were caused by ischaemic disease; three patients had dilated cardiomyopathy and two valvular heart disease. A new method for extracting \( \text{F}_{2}\)-isoprostanes from urine using anion-exchange solid-phase extraction (Lee et al., 2004) was used before a double derivatisation procedure involving pentafluorobenzyl bromide followed by bis-(trimethylsilyl)trifluoroacetamide. \( \text{F}_{2}\)-isoprostanes were analysed by GC-MS-NCI (negative chemical ionization) using a method adapted from Mori et al. (1999) on a Thermo Trace GC Ultra coupled to a Thermo DSQ mass spectrometer and an AS3000 auto sampler. \( \text{F}_{2}\)-isoprostane concentrations were expressed per mg of urinary creatinine.

Median urinary \( \text{F}_{2}\)-isoprostane concentrations were 61.9 (interquartile range (IQR) 40.1, 85.5) pg/mg creatinine in the control group and 76.8 (IQR 60.5, 112.5) pg/mg creatinine in the heart failure patients. Statistical analysis using the Mann-Whitney U test revealed that this increase was significant \( (P=0.037) \). When patients were grouped by severity of heart failure, urinary \( \text{F}_{2}\)-isoprostane levels were 76.8 (IQR 67.4, 112.0) and 82.7 (IQR 50.8, 125.4) pg/mg creatinine in heart failure grades II (n = 12) and III (n = 10) respectively.

The present small study has used a newly developed GC-MS method to demonstrate that urinary \( \text{F}_{2}\)-isoprostanes are increased in patients with heart failure compared with controls, indicating that there is increased lipid peroxidation and oxidative stress in this condition. Urinary \( \text{F}_{2}\)-isoprostane concentrations are associated with severity of heart failure, although more analysis is required to elucidate if this is a significant correlation. Elevated urinary \( \text{F}_{2}\)-isoprostanes could be a useful marker of morbidity in heart failure and further study may highlight a potential benefit of antioxidant therapy in CHF.


Methods used for collecting and handling multidisciplinary qualitative data in the Food in Later Life Project. By M.M. Raats, M. Lumbergs and THE FOOD IN LATER LIFE PROJECT TEAM, Food, Consumer Behaviour and Health Research Centre, 1Department of Psychology and 2School of Management, University of Surrey, Guildford, UK, GU2 7XH

The practical barriers to successful cross-national research have been identified as problems of coordination among different languages, expertise and team compositions (Mangen, 1999). The implications of interpretation and translation of spoken discourse in social research where the interviewer or researcher does not share the respondent’s mother tongue have received attention in the literature (Jentsch, 1998). The ‘Food in Later Life’ project (www.foodinlaterlife.org) brought together a multidisciplinary team from nine research centres in eight European countries (UK, Denmark, Germany, Poland, Portugal, Spain, Sweden and Italy). Using a range of both quantitative and qualitative methods, the same data were collected in all eight countries and comparisons were made between men and women, those aged 65–74 years and over 75 years, and between those living alone and living with others. In four of the six studies carried out within the project, substantive datasets based on semi-structured, in-depth qualitative interviews were collected (study 2: n = 400, study 3: n = 400, studies 4 and 5: n = 640). In contrast to some other cross-cultural studies where the primary dataset was translated before analysis (for example, Tsai et al., 2004), the project team carried out a two-phase analysis procedure where the first phase of analysis was carried out on original-language material using a common-language (English) coding structure. The second phase of analysis was carried out in English.


The present study has been carried out with financial support from the Commission of the European Communities, specific RTD programme ‘Quality of Life and Management of Living Resources’, QLKI-2002-02447. ‘Choosing foods, eating meals: sustaining independence and quality of life in old age’: it does not necessarily reflect its views and no way anticipates the Commission’s future policy in this area.
High prevalence of CHD has been reported in Sri Lanka, which cannot be accounted for by the traditional risk factors. Identification of new risk factors may be helpful in the treatment and prevention of CHD. It has been shown that free radicals are involved in the formation of atheromatous plaque and thrombosis (Halliwell, 1994).

The present case–control study was conducted to investigate the relationship between CHD and serum ‘antioxidant power’ measured as the ferric reducing ability of plasma (FRAP) (Benzie & Strain, 1996). Thirty CHD patients (twenty-five males and five females) with acute myocardial infarction (MI) or unstable angina admitted to the hospital after the onset of symptoms for the first time, and thirty sex- and age-matched population-based healthy controls free of CHD were studied. Cases were free of diabetes, hypertension and not taking hyperlipidaemic drugs.

In addition to the determination of FRAP, the fasting plasma concentrations of total cholesterol (TC), HDL-cholesterol (HDL-C), triacylglycerol (TAG) and glucose were measured using enzymic methods and LDL-cholesterol (LDL-C) was calculated using the formula. Paired t tests and χ² tests were used to compare mean values and prevalence of risk factors, respectively. Odds ratios (OR) were used to estimate the relative risks.

<table>
<thead>
<tr>
<th>MI patients (n = 30)</th>
<th>Controls (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± sd</td>
<td>Mean ± sd</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.5 ± 10.5</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>46.7</td>
</tr>
<tr>
<td>Ex-smoker (%)</td>
<td>26.7</td>
</tr>
<tr>
<td>Non-smoker (%)</td>
<td>26.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.5 ± 4.1</td>
</tr>
<tr>
<td>FRAP value (μmol/l)</td>
<td>1236.6 ± 226.4</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>4.5 ± 1.2</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>2.9 ± 1.1</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>0.9* ± 0.2</td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td>1.8* ± 0.6</td>
</tr>
<tr>
<td>TC:HDL-C</td>
<td>5.7* ± 2.1</td>
</tr>
<tr>
<td>LDL-C:HDL-C</td>
<td>3.7 ± 1.6</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.0 ± 0.9</td>
</tr>
</tbody>
</table>

* Significantly different from controls (P < 0.05); † χ² test (P = 0.05)

The FRAP value and HDL-C were significantly lower in MI patients than in the controls (see Table; P = 0.05). Fasting TAG, TC:HDL-C and prevalence of smoking were significantly higher in MI patients than in the controls (P < 0.05). The OR for the second and first tertiles of FRAP were 1.2 (95% CI 0.35, 4.31) and 2.8 (95% CI 0.77, 10.0), respectively, but were not significant. Among the other coronary risk factors, smoking (OR 3.1 (95% CI 1.07, 9.3)), HDL-C (OR 4.8 (95% CI 1.38, 14.5)) and TC:HDL-C (OR 4.4 (95% CI 1.3, 14.5)) were significantly associated with MI. The findings of the present study indicate that patients with MI have a lesser antioxidant defence, suggesting that serum antioxidant potential may have a protective effect against oxidative stresses.

In conclusion, MI patients have more oxidative stress compared with controls. Smoking, low HDL-C and high TC:HDL-C were found as the independent risk factors for CHD. The present study demonstrated an association of antioxidant power measured as FRAP with the atherosclerosis progression; however, it did not confirm antioxidants as an independent risk factor for CHD.

Obesity is a widespread disease both in the UK and worldwide and the prevalence is increasing in epidemic proportions. The increased occurrence of obesity has been accompanied by extra pressure to find novel and efficient treatments resulting in the development of different dietary weight-loss approaches.

An ongoing 1-year randomised controlled trial is comparing a healthy-eating 2510 kJ (600 kcal) deficit high-carbohydrate (HC) diet with two high-protein (HP) diets: (a) a protein-sparing modified fast (PSMF) using conventional food and (b) a nutritionally complete formula very-low-energy diet (VLCD; LighterLife Programme).

The HC diet was a standard healthy-eating, low-fat (<30% total energy intake) approach where the patient's energy requirements were determined and 2510 kJ (600 kcal) were removed to result in daily energy deficiency. The PSMF was a low-fat, HP diet where the patient ate conventional food while restricting their carbohydrate intake to 40 g/d. The LighterLife Programme used a VLCD in parallel with weekly group sessions of cognitive behaviour therapy (CBT) to determine the underlying causes of the patient's eating behaviour. The programme consisted of three stages:

Stage 1 – 100 d of weight loss and small-group counselling;
Stage 2 – 4-week blocks of weight loss and small-group counselling for clients who desire further weight loss;
Stage 3 – a 12-week weight-management module where conventional food is reintroduced with ongoing group counselling.

Ninety-nine patients (fifteen males, eighty-four females) with a BMI ≥25 kg/m² were recruited (aged 18–68 years (41.4 (SD 11.6) years); BMI 35–66 kg/m² (44.7 (SD 7) kg/m²); starting weight 85–175 kg (119.6 (SD 20.8) kg)). Patients were excluded if they were under 18 years of age, on weight-reducing medication or anti-depressants, history of renal disease, evidence of active malignant disease, pregnant or lactating.

Patients entered a 3–12-month phase of the HC diet with those who failed to achieve 5% weight loss at 3 months and 10% at 6 months were randomised to HP diet a or b.

An ANOVA with a post hoc Tukey test was carried out to compare the changes in weight between baseline and 6 months for the three dietary approaches.

At 6 months there was a significant difference in weight loss (F 15.9; P < 0.001) with greatest weight loss achieved on LighterLife followed by the HC diet and then the PSMF (mean weight loss = 21.8, 13.8 and 3.3 kg respectively).

These initial results suggest a potential role of VLCD and CBT in the effective treatment of obesity. However, further research is needed to examine the efficacy of VLCD and CBT in achieving long-term weight maintenance in the British population.
Menopause-related android pattern of body fat distribution may partially explain the greater risk of cardiovascular and metabolic disease during the menopausal years (Rexrode et al. 1998). To date, however, the association of body fat distribution and CVD remains unclear.

The objectives of the present study were to compare the adiposity, body composition and a range of risk markers of CHD in postmenopausal and premenopausal women and to evaluate the relationship between body fat distribution and risk markers of CHD. Thirty-six healthy premenopausal, and thirty-six postmenopausal women were the subjects. Weight, height, waist circumference (WC) and hip circumference were measured. Bioelectrical impedance analysis was used to assess body composition. Blood pressure, fasting levels of glucose and lipid risk markers of CHD were determined.

Results from the new GEM2 system were compared against those from the original GEM (GEM Nutrition Limited). The original GEM is a classic, ventilated hood indirect calorimeter with robust and accurate oxygen, carbon dioxide and flow sensors (Nicholson, 1996). Using original GEM the mean REE was 8.948 MJ/d, ranging from 7.991 to 10.498 MJ/d. Using the GEM2 the mean REE was 8.994 MJ/d, ranging from 6.941 to 10.724 MJ/d. Expressing these differences as percentage yielded an average error of 0.25%.

Graph 1 indicates that the two systems follow a similar pattern over the duration of the experiment, tracking up and down together. This reflects the oscillations in the daily RMR measurements to be expected from one person on a day to day basis. The original GEM results show that the daily RMR for a single subject has a variation of 23.9% across the duration of the study.

In conclusion, increased total and abdominal adiposity of postmenopausal women compared with premenopausal women may adversely affect CHD risk markers, especially TAG, glucose and blood pressure.
Reduction of growth rate of infants after the age of 6 months due to parents’ smoking. By A. DIAZAYERY1, M. AHMADI2, A. RAHIMI3, H. EFTEKHAR1 and R. SHEIKH-OLESLAM1, 1School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, Tehran, Iran, 2Damaghan University of Medical Sciences, Damaghan, Iran and 3Department of Nutrition, Ministry of Health and Medical Education, Tehran, Iran.

Growth of exclusive breast feed infants after the age of 6 months, may slow down when supplementary feeding starts. It is not known whether smoking by the parents affects this check in growth rate. This retrospective case-control study was conducted to determine the effect of parents’ smoking on growth rate after the age of 6 months in the Damaghan rural areas of north-central Iran.

The 226 randomly selected infants included in the study had grown normally until the age of 6 months. The case children (n 116; 51.2 % girls) were ones whose growth rate had decreased afterwards, and controls (n 110; 50.0 % girls) were ones who continued to grow normally, until the age of 9 months.

Information was collected by interviewing mothers (mean ± s = 27.1 ± 5.3 years) and from the infants’ health files. The birth weight of the cases (mean ± s = 3211.2 ± 201.0 g) and controls (mean ± s = 3280.9 ± 221.0 g) were the same (P = 0.197); as were the corresponding weights at 6 months of age (mean ± s = 7765.6 ± 408.0 and 7946.7 ± 433.1 g, P = 0.138).

At the age of 9 months, however, the body weight of the case group (mean ± s = 8427 ± 601.0 g) was lower than that of controls (mean ± s = 9261 ± 623.9 g; P < 0.001). The odds ratio (OR) for smoker fathers was 1.895 (95% CI 1.007, 3.564) and for smoker mothers 5.981 (95% CI 1.323, 27.036). Also, an infant of a mother over 35 years old was more likely to have a slower growth rate (OR 3.048 (95% CI 1.076, 8.260)).

Inappropriate preparation of supplementary food, too, increased the probability of a slower growth rate (OR 2.546 (95% CI 1.077, 6.023)). Based on logistic regression analysis (see Table), the age group of mothers had no effect on the growth rate of the infants per se; it was probably the higher rate of smoking at higher ages – e.g., more exposure of the infant to smoke that resulted in a slower growth rate of an infant after the age of 6 months. The Table also shows that correction for fathers’ smoking, and procedure of preparing supplementary food – as two other confounding factors – still leaves mothers’ smoking as a statistically significant independent factor with a negative effect on the growth of an infant after 6 months of age.

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>se</th>
<th>Wald statistic</th>
<th>df</th>
<th>P</th>
<th>OR</th>
<th>95% CI</th>
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<tr>
<td>Mothers’ age group</td>
<td>0.8017</td>
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<td>2.497</td>
<td>1</td>
<td>0.1139</td>
<td>2.293</td>
<td>0.826, 6.023</td>
</tr>
<tr>
<td>Mothers’ smoking</td>
<td>1.7342</td>
<td>0.7758</td>
<td>4.963</td>
<td>1</td>
<td>0.0254</td>
<td>5.664</td>
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<tr>
<td>Procedure of supplementary food preparation</td>
<td>0.7484</td>
<td>0.4792</td>
<td>2.497</td>
<td>1</td>
<td>0.1183</td>
<td>1.0173</td>
<td>0.8266, 5.496</td>
</tr>
<tr>
<td>Mothers’ smoking</td>
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<td>0.7999</td>
<td>4.603</td>
<td>1</td>
<td>0.0309</td>
<td>5.3852</td>
<td>1.6677, 24.3853</td>
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<tr>
<td>Fathers’ smoking</td>
<td>0.3607</td>
<td>0.3806</td>
<td>1.057</td>
<td>1</td>
<td>0.3033</td>
<td>1.4472</td>
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<tr>
<td>Procedure of supplementary food preparation</td>
<td>0.7619</td>
<td>0.6791</td>
<td>2.529</td>
<td>1</td>
<td>0.1117</td>
<td>1.2424</td>
<td>0.8377, 5.4790</td>
</tr>
</tbody>
</table>

It is concluded that smoking of the mothers had a truly negative effect on the growth rate of infants after the age of 6 months.

Evidence for the development of specialised bacterial communities on resistant starch in the human colon that promote butyrate formation. By S.H. DUNCAN, E.C. McWILLIAM Leitch, A. WALKER and H.J. FLINT, Microbial Ecology Group, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, UK, AB21 9SB

Resistant starch that escapes digestion in the upper gastrointestinal tract provides an important substrate for the dense population of bacteria that inhabit the large intestine. It is estimated that more than 500 different bacterial species inhabit the large intestine (Eckburg et al. 2005). Currently it remains unclear which groups of the colon microbiota colonise resistant starch, or how inter-individual variation influences the attached bacterial population composition. Fermentation of dietary substrates in the colon results in the formation of SCFA, mainly acetate, propionate and butyrate and gases. In particular, butyrate is important in the maintenance of colonic health and is the major fuel for the colonocytes.

In the present study we developed a single-stage fermentor system to examine the colonisation of insoluble resistant starch (Hylon VII). Following incubation of starch in fermentors that were inoculated separately with faecal samples from four different donors, the insoluble fraction was recovered and washed extensively. The samples were then fixed for fluorescent in situ hybridisation (FISH) analysis, using a panel of ten group-specific probes, to visualise bacterial colonisation. Separately, 16S rRNA clone library analysis was performed to estimate the major colonising bacteria. The attached bacteria varied with faecal donor. Overall, Bifidobacterium (mainly B. adolescentis and B. breve), Ruminococcus bromii and Eubacterium rectale sequences accounted for 41, 25 and 18% of starch-attached sequences respectively. FISH analysis was largely consistent with these findings with the exception of the estimate of the abundance of the E. rectale group colonising starch (by the inoculum from one donor). Although resistant starch is widely reported to be butyrogenic, among the primary colonisers found here only E. rectale is a butyrate-producer. It is probable therefore that metabolic cross-feeding, in particular of lactate and acetate (Duncan et al. 2004, Belenguer et al. 2006) to other non-adherent species, contributes to butyrate formation from starch in the mixed gut ecosystem.

In conclusion, the present study suggests that the colonisers of insoluble resistant starch found in the gut are restricted to relatively few specialised groups of bacteria, most of which have been cultivated. Apparently the primary colonisers of starch present in the colon microbiota can vary between individuals, and this may have important consequences for the impact of dietary starch upon metabolism in the colon, in particular with respect to the formation of butyrate and of hydrogen.

References:


Effect of wholegrain food on markers of cardiovascular risk in middle-aged healthy volunteers.

By P. TIGHE1, N. VAUGHAN1, J. BRITTENDEN1, G. HORGAN2, W.G. SIMPSON1, W. MUTC1, G. DUTHIE1 and F. THIES1, 1College of Life Science and Medicine, University of Aberdeen, Aberdeen, UK, AB25 2ZD, 2BIOSS, Rowett Research Institute, Aberdeen, UK, AB9 2UX and 3Rowett Research Institute, Aberdeen, UK, AB9 2UX

CVD is a major cause of premature mortality in the UK. Epidemiological studies suggest that consumption of wholegrain foods (WGF) may lower CVD risk (Liu et al. 1999). However, current recommendations that consumption of three servings of WGF daily may be cardioprotective have not been validated (Anderson et al. 2000). The aim of the present on-going study is to assess the effects of increased consumption of WGF on markers of CVD risk in relatively high-risk individuals.

To date, thirty volunteers (age 40–65 years; sixteen males and fourteen females) were randomised into one of three dietary intervention groups and asked to consume three portions per d of either refined, wheat-based or oat+wheat-based foods for 12 weeks. Blood samples were collected at baseline, mid-way and post-intervention and analysed for lipid profiles, lipoproteins and high-sensitivity C-reactive protein (hsCRP; a marker of inflammation). Insulin sensitivity was determined using the QUICKI method (Katz et al. 2000). Arterial stiffness was assessed by pulse contour analysis (Pulsed Trace PCA; Micromedical Ltd). Blood pressure, BMI and waist circumference were also measured at each time point.

BMI was positively associated with insulin concentrations (r = 0.420; P = 0.041) and hsCRP (r = 0.518; P = 0.003) and negatively associated with HDL-cholesterol (r = -0.373; P = 0.039). Age was positively associated with stiffness index (r = 0.580; P = 0.001).

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SE</th>
<th>Mean</th>
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<td>hsCRP (ng/ml)</td>
<td></td>
<td></td>
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<tr>
<td>Pre-intervention</td>
<td>0.74</td>
<td>0.20</td>
<td>2.55</td>
<td>0.92</td>
<td>1.73</td>
<td>0.58</td>
</tr>
<tr>
<td>Post-intervention</td>
<td>1.17</td>
<td>0.36</td>
<td>1.90</td>
<td>0.66</td>
<td>1.15</td>
<td>0.40</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pre-intervention</td>
<td>5.37</td>
<td>0.21</td>
<td>5.83</td>
<td>0.26</td>
<td>6.1</td>
<td>0.35</td>
</tr>
<tr>
<td>Post-intervention</td>
<td>5.46</td>
<td>0.24</td>
<td>6.08</td>
<td>0.20</td>
<td>5.8</td>
<td>0.31</td>
</tr>
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<td>HDL-cholesterol (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-intervention</td>
<td>1.36</td>
<td>0.16</td>
<td>1.45</td>
<td>0.09</td>
<td>1.70</td>
<td>0.11</td>
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<tr>
<td>Post-intervention</td>
<td>1.37</td>
<td>0.18</td>
<td>1.53</td>
<td>0.09</td>
<td>1.71</td>
<td>0.11</td>
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<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-intervention</td>
<td>3.34</td>
<td>0.15</td>
<td>3.87</td>
<td>0.19</td>
<td>3.83</td>
<td>0.32</td>
</tr>
<tr>
<td>Post-intervention</td>
<td>3.28</td>
<td>0.15</td>
<td>3.97</td>
<td>0.15</td>
<td>3.52</td>
<td>0.23</td>
</tr>
</tbody>
</table>

None of the biomarkers in blood (see Table) differed significantly after dietary intervention. However, hsCRP increased by 60 (SEM 33) % in the refined group while decreasing by 26 (SEM 12) and 39 (SEM 12) % in the wheat- and oats+wheat-based groups respectively. Serum total cholesterol concentration remained unchanged in the refined and wheat-based groups while decreasing by 5 % in the oats group. This was associated with a decrease in LDL-cholesterol (−7 %) and apoB concentrations (−5 %). However, arterial stiffness, insulin sensitivity and blood pressure were unaffected by dietary treatment.

Although the interpretation of these results may be limited due to the small sample size, the initial data is suggestive that the daily consumption of three portions of oat+wheat-based WGF may protect against CVD although further investigations are necessary.

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Influence of breast-feeding and birth weight on body mass index and waist:hip ratio in Greek women.

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Obesity can be defined as the accumulation and storage of excess fat in the adipose tissue which results in physical and psychological health impairment (Garrow et al. 2000). The prevalence of obesity in the European Union is expected to rise by 2.4% in women and 2.2% in men by the year 2010 (WHO, 2005). In some countries such as Greece, Finland and Germany the prevalence is expected to rise even more than the above figure as people have more sedentary lives (WHO, 2005). The health benefits of breast-feeding are widely acknowledged. Recent studies in the area of breast-feeding indicate that breast-feeding may reduce the prevalence of obesity in later life (Owen et al. 2005). However, approximately one-third of women in the UK still choose to formula-feed from birth and 75% mothers are using formula by 4 months (Wall, 2006). This is in contrast to the prevalence of breast-feeding in Greece, which has increased since the 1970s (WHO, 1999; Antoniou et al. 2005).

The objective of the present study was to investigate the impact of breast-feeding and birth weight on anthropometric measurements in adult women. The present study was carried out in Greece where fifty women, of similar socio-economic and educational status, gave written informed consent. Questionnaires were utilised to gather information about birth weight and duration of breast-feeding of the participants as infants. The following anthropometric measurements were carried out: height (m), weight (kg), BMI (kg/m²), body fat (%), waist circumference (cm), hip circumference (cm) and waist:hip ratio (W:H). The average was calculated as well as the sest (standard error). The average age of the participants was 30 (± 5.51) years old, the mean BMI of the first group was 21.8 (± 1.91) whereas the second’s group was 35.5 (± 3.1). The duration of breastfeeding when infants for the first group was 7.8 (± 2.1) months and for the second group was 3.7 (± 1.9) months.

Pearson’s correlation coefficient was calculated for breast-feeding v. BMI, breast-feeding v. W:H and breast-feeding v. waist circumference. The results show a significant negative correlation between duration of breast-feeding and current BMI (P<0.05). Similar negative correlations were obtained for duration of breast-feeding v. waist circumference and W:H. In addition, there appears to be a significant positive correlation between the duration of formula-milk feeding and current BMI (P<0.05). However, the results with regards to birth weight show no significant relationship for birth weight v. BMI, birth weight v. waist circumference and birth weight v. W:H.

The results from the present study suggest that breast-feeding may be a protective factor against adult obesity.


