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.
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 folate acid/kg; n 6), control with folate (CPF; 180 g casein/kg diet with 5 mg folate acid/kg; n 6), MLP (90 g casein/kg diet with 1 mg folate acid/kg; n 6) or low protein with folate (MLPF; 90 g casein/kg diet with 5 mg folate 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 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).

![Graph](https://example.com/graph.png)

**Effect of Folate on Gonadal Fat Deposition in 4 Week Old Animals**

+ A - control
+ B - control + folate
+ C - low protein
+ D - low protein + folate
+ Male
+ Female

**Effect of Folate on Perirenal Fat Deposition in 4 Week Old Animals**

+ A - control
+ B - control + folate
+ C - low protein
+ D - low protein + folate
+ Male
+ Female

**Graph 1 and 2.** Data are mean±SEM for 11–19 rats per group. ANOVA indicated effect of maternal diet on gonadal fat pad ($P<0.000$) and on perirenal fat ($P<0.000$).

Supplementation of folate during pregnancy reduces abdominal fat deposition in the offspring in the rat model, with sex-specific effects in the offspring of rats fed a protein-replete diet. The mechanisms are as yet unknown, but the data have interesting implications for the possible relationship between childhood obesity and maternal diet in pregnancy.

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.

Healthcare professionals’ knowledge of infant-feeding recommendations in selected clinics in Singapore. By W.M. Han 1, J.B. Morgan 1 and Y.H. Chan 2, 1 School of Biomedical & Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH and 2 Biostatistics Unit, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

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 & Pimmington, 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 70% (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.

<table>
<thead>
<tr>
<th>Profession</th>
<th>Doctor (n = 41)</th>
<th>Pharmacy (n = 220)</th>
<th>Nurse (n = 159)</th>
<th>Total (n = 220)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score (%)</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>WHO</td>
<td>22</td>
<td>54</td>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>Singapore</td>
<td>16</td>
<td>39</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>Age of introduction of complementary foods</td>
<td>18</td>
<td>44</td>
<td>70</td>
<td>123</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></td>
<td>1.0</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 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 well documented as a primary health concern in recent times. Results of studies examining gastric emptying (GE) in overweight individuals have shown conflicting results. Gastric emptying is an important factor in the regulation of food intake and may be altered in obesity.\(^{1}\)

Rapid GE may result in a shortened satiety period, resulting in overeating and obesity.\(^{2}\) Results of studies examining GE in obesity have shown inconsistent results.\(^{3}\) It is hypothesized that GE may be altered in obesity.

The aim of the present study was to investigate the effect of consumption of five portions of fruit and vegetables, in the form of juice shots, on mouth to caecum transit time (MCTT), macronutrient diet, and nutritional status of obese and lean men. The study was designed to examine the effect of macronutrient composition of the meal on GE.

Sixteen healthy males consented to participate in the present study, approved by the University of Limerick Research Ethics Committee; eight obese (31.0 ± 8.7 years, 113.3 ± 14.2 kg, 179.4 ± 4.5 cm, BMI 35.2 ± 3.6 kg/m\(^2\)) and eight lean controls, age and height matched (30 ± 7.4 years, 77.6 ± 6.3 kg, 182.5 ± 4.5 cm, BMI 23.3 ± 1.6 kg/m\(^2\)). Dietary intake was recorded for 1 week before the first test session using a weighed food diary. MCTT, satiety and fasted and 60 min postprandial blood samples were assessed. Breath H\(_2\) was tested every 5 min using a H\(_2\) meter (Micro Medical, Chatham, UK). MCTT was defined as an increase in breath H\(_2\) of at least 3 parts per million for three consecutive readings. The test procedure was replicated using the alternative test meal.

Statistical significance \((P < 0.05)\) 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 ± 24.0 min, high-fat 75.7 ± 8.1 min) and the lean group (low-fat 103.1 ± 39.7 min, high-fat 82.1 ± 17.3 min). 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 than lean males for both high- and low-fat meals.\(^{4}\)

The authors acknowledge funding from Unilever Bestfoods.
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. AUCOTT2, 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 sex-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.

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 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 is always accurately reflect the amount which is available for absorption and utilisation.

In vitro digestion procedure

The present study was funded by the Department of Agriculture and Food, Dublin, under the Food Institutional Research Measure.

Reference

The effect of increased wholegrain food consumption on daily intake of nutrients. By A.R. JONES, S. KUZNESOF, D.P. RICHARDSON and C.J. SEAL. Human Nutrition Research Centre, School of Agriculture, Food and Rural Development, University of Newcastle, Newcastle upon Tyne, UK, NE1 7RU and DPR Nutrition Limited, 34 Grimwade Avenue, Croydon, Surrey, UK, CR0 5DG

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/day were recruited into a 16-week intervention study during which they were asked to consume three servings of WGF/day for the first 8 weeks and six servings of WGF/day for the final 8 weeks. Prescribed quantities of WGF were provided to all participants. 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). Daily whole-grain intake was calculated using ingredient data for foods containing ≥10% whole grain, expressed on a DM basis. Nutrient intakes were calculated using WindFit (Univation Ltd, Aberdon, 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>Mean ± SEM</th>
<th>Mean ± SEM</th>
<th>Mean ± SEM</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-grain intake (g)</td>
<td>27 ± 4</td>
<td>50 ± 7</td>
<td>59 ± 7</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Energy (MJ)</td>
<td>10 ± 0.5</td>
<td>10 ± 0.6</td>
<td>10 ± 0.7</td>
<td>0.116</td>
<td></td>
</tr>
<tr>
<td>% Energy from fat</td>
<td>31 ± 0.4</td>
<td>31 ± 0.5</td>
<td>32 ± 0.5</td>
<td>0.117</td>
<td></td>
</tr>
<tr>
<td>% Energy from protein</td>
<td>15 ± 0.4</td>
<td>15 ± 0.5</td>
<td>16 ± 0.5</td>
<td>0.450</td>
<td></td>
</tr>
<tr>
<td>% Energy from CHO</td>
<td>48 ± 0.5</td>
<td>48 ± 0.6</td>
<td>49 ± 0.6</td>
<td>0.471</td>
<td></td>
</tr>
<tr>
<td>% Energy from alcohol</td>
<td>6 ± 0.9</td>
<td>6 ± 0.9</td>
<td>6 ± 0.9</td>
<td>0.734</td>
<td></td>
</tr>
<tr>
<td>NSP (g)</td>
<td>16 ± 1.0</td>
<td>18 ± 1.0</td>
<td>20 ± 1.0</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>Mg (mg)</td>
<td>340 ± 20.0</td>
<td>371 ± 16.0</td>
<td>389 ± 18.1</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>Mn (mg)</td>
<td>4 ± 0.3</td>
<td>5 ± 0.5</td>
<td>5 ± 0.5</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Na (mg)</td>
<td>328 ± 236.4</td>
<td>309 ± 166.8</td>
<td>307 ± 171.4</td>
<td>0.176</td>
<td></td>
</tr>
<tr>
<td>Ca (mg)</td>
<td>973 ± 62.0</td>
<td>980 ± 51.7</td>
<td>983 ± 45.9</td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td>Fe (mg)</td>
<td>15 ± 1.0</td>
<td>15 ± 0.7</td>
<td>15 ± 0.7</td>
<td>0.688</td>
<td></td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>0.949</td>
<td></td>
</tr>
<tr>
<td>Thiamin (mg)</td>
<td>2 ± 0.1</td>
<td>2 ± 0.1</td>
<td>2 ± 0.1</td>
<td>0.118</td>
<td></td>
</tr>
<tr>
<td>Total folate (µg)</td>
<td>3.06 ± 20.7</td>
<td>280 ± 14.1</td>
<td>312 ± 20.1</td>
<td>0.358</td>
<td></td>
</tr>
<tr>
<td>Vitamin B6 (µg)</td>
<td>124 ± 13.4</td>
<td>123 ± 9.8</td>
<td>127 ± 11.5</td>
<td>0.792</td>
<td></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 day 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. These results also suggest that increased WGF consumption does not significantly affect intakes of those micronutrients readily fortified in refined products such as breakfast cereals.

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


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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 exercise intervention 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

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>End</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>All</td>
<td>Women</td>
</tr>
<tr>
<td>Men</td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>HEP</td>
<td>3075*</td>
<td>1280</td>
</tr>
<tr>
<td>LEP</td>
<td>3015*</td>
<td>1210</td>
</tr>
</tbody>
</table>

Mean values were significantly different between conditions: * P<0.05, *** P<0.0001

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 1887) vs. 1218 (so 2171); P<0.05). This approached significance in men (–439 (so 2456) vs. 1452 (so 2732); P=0.076), but not in women (318 (so 1318) vs. 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, 25% 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 weighted 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.

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.


Exercise induced anorexia: a role for PYY, GLP-1 and PP?

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Abstracts of Original Communications 2006

Supplement use and comparative differences in nutritional status amongst patients with multiple sclerosis.

By H. BENNEWITH, J.W. WELLS, C.O. LEARY, D.O’REILLY, A. DUNCAN, I. WOOD, L. PAUL and A. PAYNE, 1, 2

1 Institute of Neurological Sciences, Southern General Hospital, Glasgow, UK, G51 4TF and 2 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 it with nutritional and non-nutritional recommendations. Patients attending MS outpatient clinics were asked for information on current usage of nutritional and non-nutritional 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. 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: Adults Aged 19 to 64 Years Summary Report. Vol. 5: Report of the Diet and Nutrition Survey 2004). The preparations used ranged from one to thirteen per day, an intake of 3.20 (SD 3.28) 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: Adults Aged 19 to 64 Years Summary Report. Vol. 5: Report of the 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 Mann Whitney tests identified significant differences at \( P < 0.02.\)

Blood nutrient levels of a range of vitamins and minerals and other nutritional indicators were measured. Twenty-nine male and seventy-six female patients (\( n = 105 \)) were recruited. Patients were asked to refrain from taking any nutritional supplements throughout the study using visual analogue scales and subsequent food intake at a buffet meal was measured at the end (3h post-breakfast). Exercise significantly increased mean PYY, GLP-1 and PP levels. Blood nutrient levels of all nutrients measured, two independent sample Mann Whitney tests identified significantly higher levels for the nutrients shown in the Table.

<table>
<thead>
<tr>
<th>Vitamin/Dietary Supplement</th>
<th>SU Mean (nmol/l or ng/ml)</th>
<th>Reference ranges (nmol/l or ng/ml)</th>
<th>Value Reference ranges</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>3.96</td>
<td>8.70-24.00</td>
<td>1.32</td>
<td>0.01</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>3.70</td>
<td>2.05-9.10</td>
<td>1.73</td>
<td>0.01</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>1.50</td>
<td>0.40-3.50</td>
<td>0.94</td>
<td>0.01</td>
</tr>
<tr>
<td>Calcium</td>
<td>3.40</td>
<td>0.90-1.50</td>
<td>0.74</td>
<td>0.01</td>
</tr>
<tr>
<td>Iron</td>
<td>10.70</td>
<td>8.00-12.00</td>
<td>10.70</td>
<td>0.01</td>
</tr>
<tr>
<td>Folate</td>
<td>10.70</td>
<td>2.70-17.00</td>
<td>10.70</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Significant differences at \( P < 0.02.\)

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.

Conclusions Exercise, a self-selected therapy to promote well-being in MS, whilst not being able to produce a short-term negative energy balance, may be able to produce significant changes in appetite and energy homeostasis which 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, 21-26 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 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 T01033. Thanks to the Caffeine And Reproductive Health (cARE) study team: V.A. Dolby, S.M. Chell, B.E. Longshaw, D. Cambridge, K. Cussen, T. Moon, 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. CHEN, A.M. STEPHEN, C.J. PRYNNE and M.E.J. WADSWORTH, MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge, UK, CB1 9NL and MRC 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 Generalised 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. PARRETT, 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 (Ultra sensitive ELISA, Mercodia, Sweden) and ghrelin (Phoenix, Belmont, CA, USA) concentrations were analysed using commercially available methods.

As expected, the area under concentration v. 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.

Flavonoids are bioactive polyphenol compounds that are an integral part of the human diet. Little is known about their role on bone health in man although flavonoids reduce osteoclast action in cellular models and citrus juice has been shown to positively affect bone strength in orchidectomised rats (Deyhim et al. 2006). 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). The aim of the 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 XR26s DXA) and completed a food-frequency questionnaire (FFQ) at both visits. They provided second early morning fasting 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 women using 218 4-d food diaries. Pearson correlations for energy-adjusted total flavonoids, flavonols, catechins and flavanones were 0.76, 0.75, 0.66 and 0.60 respectively (P<0.001).

The mean flavonoid intake of the diets was 306.8 (SD 198.9)mg/day at follow-up (n 2929). The specific flavonones naringin and hesperidin accounted for 15% of the total dietary flavonoid intakes (8 and 6.9% respectively). Flavonoids, and in particular flavanones, have been shown to positively affect bone strength in orchidectomised rats (Deyhim et al. 2006). 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.

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 has been associated with reduced bone turnover in this cohort (Macdonald et al. 2005). In addition we now show that flavonoid intake is associated with peak bone mass and bone turnover, supporting the evidence from cellular and animal studies. Different components of fruit and vegetables may affect bone health in different ways and further work is required to elucidate this mechanism.

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


Implementing lifestyle interventions to reduce chronic disease risk: lessons from diabetes prevention trials. By A.S. ANDERSON1, C.S. FERGUSON 1, K.L. BARTON 1 and R.J.C. STEELE 2, 1Centre for Public Health Nutrition Research and 2Department of Surgery and Molecular Oncology, University of Sheffield, Herries Road, UK, S5 7AU

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 in an overall 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 Xecondo study (Torgersen et al. 2004) and the Oslo Diet and Exercise Study (Torgesen 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 though 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 health professionals, though it is not clear if lay 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 aid 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.


Development of high-fibre, low-glycaemic index novel food products for the management of type 2 diabetes mellitus employing the food multimix concept. By P. AMUNA1, N.L. HILL 2, F.B. ZOTOR 1 and V. TROUSEL1, 1Medway School of Science, University of Greenwich, Chatham Maritime, UK, ME4 4TB and 2Centre for Human Nutrition, University of Sheffield, Herries Road, UK, S5 7AU

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 (Finn et al. 2004). In the present study, the Food Multimix (FMM) concept was employed in order to produce food recipes of high fibre, low total sugar, low total fat and moderate protein content using commonly-consumed plant-based food ingredients in the UK.

FMM have the potential to aid glycaemic control in human subjects and may benefit type 2 diabetes. 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 commodity level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starches selected</td>
<td>Polenta, potatoes, brown rice, pearled barley, wholewheat bread (high-fibre, high-complex-carbohydrate sources)</td>
</tr>
<tr>
<td>Proteins selected</td>
<td>Soybeans, kidney beans, chick peas, lentils dried peas (avoidance of high-fat animal protein sources)</td>
</tr>
<tr>
<td>Vitamins and minerals selected</td>
<td>Spinach, tomatoes, carrots, apples, dates</td>
</tr>
<tr>
<td>Natural fat 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;4kJ/g (1 kcal/g)</td>
<td>To ensure low-energy density to aid glycaemic control and weight loss in obese subjects</td>
</tr>
<tr>
<td>&lt;25% energy from fat</td>
<td>To keep GL low to aid glycaemic control</td>
</tr>
<tr>
<td>≥25% energy from fat</td>
<td>To maintain low energy density</td>
</tr>
<tr>
<td>≤5% energy from fibre</td>
<td>To maintain low content, energy density and minimise net weight gain</td>
</tr>
<tr>
<td>Fiber content ≥2.5 g/100g FMM</td>
<td>To provide ≥50% recommended fibre intake/300 g and aid glycaemic control without inhibiting digestion and absorption of other key nutrients</td>
</tr>
<tr>
<td>Sugar content &lt;8 g/100g FMM</td>
<td>To maintain low GI</td>
</tr>
<tr>
<td>Vitamins and minerals providing &gt;80% RNI/100g</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. LAW1, M.A. GOSNEY2 and O.B. KENNEDY3, 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;b&lt;/sup&gt;</td>
<td>4.32&lt;sup&gt;b&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;b&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;b&lt;/sup&gt;</td>
<td>5.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.48&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;b&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 were 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. LOCK1 and J.E. COCKROFT2, 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 inductory 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 from the results of the PFFQ (n = 57). Out of the 7 schools that where visited, 173 pupils were seen by the research team and a total of 57 Phunky Foods Frequency questionnaires were filled out and returned (53% 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’.

<table>
<thead>
<tr>
<th>Food group</th>
<th>Number of eating occasions foods from each group were consumed on a daily basis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Bread, other cereals and potatoes</td>
<td>3.4</td>
</tr>
<tr>
<td>Milk and dairy</td>
<td>2.9</td>
</tr>
<tr>
<td>Food containing fats, and food and</td>
<td>5.1</td>
</tr>
<tr>
<td>drinks containing sugar</td>
<td></td>
</tr>
<tr>
<td>Meat, fish and alternatives</td>
<td>2.1</td>
</tr>
<tr>
<td>Fruit and vegetables</td>
<td>5.9</td>
</tr>
</tbody>
</table>

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 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. Caude, Centre for Epidemiology and Biostatistics, University of Leeds, UK. IS6 9LX

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 4 d 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 \(P<0.0001\). 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.


Glycaemic potency of breakfast and cognitive function in school children. By R. Micha, J. Forbes, K. Lowes, P. J. Rogers and M. Nelson, 1Nutritional 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 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 glycoen 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 London. On the day of the appointment, children had their habitual breakfast, and 90–120 min later 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, with a range of 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 the 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>Low GI</th>
<th>Low GL</th>
<th>High GL</th>
<th>High GI</th>
<th>ANOVA P</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>13.0</td>
</tr>
<tr>
<td>Serials correct</td>
<td>29.3</td>
<td>17.3</td>
<td>25.6</td>
<td>9.6</td>
<td>15.1</td>
</tr>
<tr>
<td>Serials sevens correct-incorrect</td>
<td>24.6</td>
<td>21.4</td>
<td>11.8</td>
<td>10.6</td>
<td>6.9</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.

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.

Seventeen obese (mean BMI 35.1 kg/m²), on average 35.7 years old, 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×RMR), subjects were offered two diets ad libitum 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.

EE was similar on the HPLC and HPMC diets (11.3 and 11.9 MJ/d; P=0.167), with subjects maintaining usual sedentary levels of activity. Average energy intake was lower on the HPLC than the HPMC diet (7.25 v. 7.95 MJ/d; P=0.02) resulting in greater weight loss (6.34 and 4.35 kg, respectively; P=0.006). Despite this, subjectively rated fatigue was similar between diets (P=0.176), although a ‘days into dieting’ effect was noted (P<0.01), with subjects feeling more fatigued the first 10 d into the protocol. This may reflect a period of adaptation in subjects to become accustomed to either reduced intake and/or the study demands. In conclusion, a very low carbohydrate intake (20 g/d) for sedentary obese men during weight loss did not appear to influence feelings of either fatigue or euphoria at any stage compared with moderate carbohydrate intake (170 g/d). Active individuals may respond differently.

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.

<table>
<thead>
<tr>
<th>Source of caffeine</th>
<th>% consuming</th>
<th>Mean caffeine intake (mg/day) (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tea</td>
<td>70</td>
<td>65</td>
</tr>
<tr>
<td>Coffee</td>
<td>58</td>
<td>34</td>
</tr>
<tr>
<td>Soda</td>
<td>55</td>
<td>23</td>
</tr>
<tr>
<td>Drinking chocolate</td>
<td>29</td>
<td>6</td>
</tr>
<tr>
<td>Chocolate</td>
<td>83</td>
<td>51</td>
</tr>
</tbody>
</table>

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.

This study is funded by the Food Standards Agency (FSA).


An explanation of the 12-week low-glucemic index diet on heart disease risk factors and 24-hour glucose profile in healthy middle-aged volunteers at risk of heart disease. A pilot study. By E. PHILIPPO 1, J.E. MILTON 1, A.E. BRYNES 1 and G.S. FROST 2, 1

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The low-glycaemic index (GI) diet is a measure of the postprandial glucose response to ingested carbohydrate. The glycemic index of a food represents the glycemic impact of a food compared with a reference food. GI is expressed as a percentage of the glycemic index of the reference food. GI is described as 100. GI values of ≤70 are considered low, and GI values of >70 are considered high. GI values of foods of a similar content are estimated using a mathematical formula based on published values. The GI of a food is calculated as the relative increase in glucose levels compared to a reference food (i.e. white bread). GI values of foods of a similar content are estimated using a mathematical formula based on published values. The GI of a food is calculated as the relative increase in glucose levels compared to a reference food (i.e. white bread).

The present pilot study suggests that a low-GI diet might be more beneficial in heart disease prevention as it significantly reduces weight, waist circumference and lowers blood pressure in healthy middle-aged volunteers. The results were not normally distributed, therefore non-parametric tests were used for analysis. Medians and interquartile ranges (IQR) are reported.

For the habitual diet (%), the median total CHO intake was 1497.3 (IQR 1383.4–1756.5) g over 7 d. For the low-GI group, 97.7 (IQR 96.6–98.2) % and 96.9 (IQR 94.8–97.4) % CHO were used in the diet GI calculations based on the published GI values of foods. For the habitual diet, in the low-GI group and 243.0 (IQR 203.8–291.5) g CHO in the low-GI group and 16.8 (IQR 16.0–21.4) % CHO were used in the GI calculations.

Comparison was made between the low- and high-GI groups of the proportion of CHO used in the diet GI calculations based on the published GI values of foods. More evidence is needed to determine the impact of diet GI on heart disease prevention.

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Comparison was made between the low- and high-GI groups of the proportion of CHO used in the diet GI calculations based on the published GI values of foods. More evidence is needed to determine the impact of diet GI on heart disease prevention.
Glycaemic properties of quinoa (*Chenopodium quinoa* Willd.). By V. ZEVALLOS1, G. GRIMBLE1 and L.I. HERENCIA2. 1Department 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 (Brynes *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 fingertip 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 a part of a diet to control postprandial hyperglycaemia.


Milk choice across social class in the MRC National Survey of Health and Development. By A.P. RICKARD1, A.M. STEPHEN1, C.J. PRYNNE1, A.P. MANDER1 and M.E.J. WADSWORTH2. 1MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge, UK, CB1 9NL and 2MRC National Survey of Health and Development, University College London Medical School, Department of Epidemiology and Public Health, 1–19 Torrington Place, London, UK, WC1E 6BT

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) than whole milk there was also an economic benefit. The aim of the present study was to identify the pattern of consumption of milk types across different social classes between 1982 and 1999 using the NSHD cohort.

From 5 d food diaries collected in 1982 (n 2552), 1989 (n 1649) and 1999 (n 1649) 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. In 1989 semi-skimmed milk consumption was 10% (n 151, 9 %); whole milk was consumed by 95% of subjects. In 1999 the patterns remained similar and the associations with social class were still significant (P<0.005). Tests showed there was no significant association between social classes and consumption of whole milk or skimmed milk.

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 a part of a diet to control postprandial hyperglycaemia.


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. DRAPER1, C.E. LOSCHER1 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

Dendritic cells (DC) play a key role in initiation of the inflammatory response, directing the adaptive immune response and determining the nature of the T cell response to inflammatory stimuli. The long-chain n-3 PUFA EPA and DHA have long been recognised as having the potential to modulate the immune response. However, little has been determined in terms of the differential effects of the long-chain n-3 PUFA in DC.

The effects of 25 μM EPA and -DHA on dendritic cells isolated from BALB/c mice 10–14 weeks of age were investigated. Bone marrow-derived immature DC were obtained by culturing bone marrow from femurs and tibia of mice in RPMI 1640 medium supplemented with fetal calf serum and granulocyte macrophage colony-stimulating factor for 7 d. Dimethyl sulfoxide, EPA or DHA were added to the cells on day 5 and on day 7 of culture. DC were stimulated with lipopolysaccharide (LPS; 100 ng/ml) or medium alone for 24 h in the presence of the PPARγ inhibitor, GW9662 (10 μM), or the prostaglandin E2 inhibitor, indomethacin (10 μM). After 24 h supernatant fractions were removed and IL-12p70 concentrations were measured (DuoSet ELISA kits; R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions.

Treatment of DC with EPA and DHA suppressed LPS-induced IL-12 production. This anti-inflammatory effect was associated with significant down regulation of NF-kB p65 expression. Interestingly experiments specifically designed to determine if PPARγ mediated this effect demonstrated that the anti-inflammatory effect of DHA, but not EPA, was negated by the presence of GW9662, a specific PPARγ inhibitor.

This observation requires further investigation to determine the full nature of the PPARγ mediated effect(s).

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. O’NEILL, J.C. MATHERS2 and D. FORD1,2, 1Institute 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 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 determine 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 pBlueTOPO (Invitrogen), were transiently transfected into Caco-2 and JAR cells grown under standard conditions. Reporter gene activity 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 5.52 (SE 0.23), negative 1.00 (SE 0.15), 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 that 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 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 that 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 and JAR cells. SLC30A5 promoter activity was significantly reduced in Caco-2 cells above 100 μM-Zn but no effect was seen in the JAR cell line. This response was retained for all constructs, indicating that the Zn-responsive element is within the −154 to +50 region (corresponding to the shortest construct) and that the response to Zn does not involve sequences matching the metal-responsive element (MRE) consensus found in the promoter upstream of this region. Similarly, site-directed mutagenesis of the single MRE at position −416 to +50 had no effect on the transcriptional response to Zn in Caco-2 cells.

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-type-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. DUTHIE¹, C.S. BESTWICK², Y. MAVROMMATIS³, M.P. MOYER³ and L.P. PIRIE¹, ¹Nutrition and Epigenetics Group, Division of Vascular Health, Rowett Research Institute, Aberdeen, UK; AB21 9SB, ²Molecular Nutrition Group, Division of Gut Health, Rowett Research Institute, Aberdeen, UK; AB21 9SB and ³INCELL 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 (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. Overlap of 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).

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.

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


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 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 (Berthier 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 Cu-oxDL 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.
Postprandial ghrelin suppression is exaggerated following major surgery; implications for nutritional recovery. By L. O’SULLIVAN, L. Bioavailability of carotenoids determined by a Caco-2 cell model. By L. O’SULLIVAN, L. BIO

Postprandial ghrelin suppression is exaggerated following major surgery; implications for nutritional recovery. By L. O’SULLIVAN, L. Bioavailability of carotenoids determined by a Caco-2 cell model. By L. O’SULLIVAN, L. BIO

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 (Nemy 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 51) pmol/l v. Δ post-op 152 (SEM 43) pmol/l; Δ follow-up 159 (SEM 65) pmol/l; P < 0.05) was accompanied with a 50% reduction in food intake (post-op 4.5 (SEM 0.8) meals/day v. follow-up 2.5 (SEM 0.5) meals/day; P < 0.05), 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 3.8 (SEM 0.5) mm v. follow-up 4.5 (SEM 0.8) mm v. controls 2.3 (SEM 0.3) 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 patients groups undergoing major surgery.


Bioavailability of carotenoids determined by a Caco-2 cell model. By L. O’SULLIVAN, L. Bioavailability of carotenoids determined by a Caco-2 cell model. By L. O’SULLIVAN, L. BIO

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 (Alves-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 colonic adenocarcinoma) cells. Caco-2 cells were adjusted to a density of 1.25x10⁵ cells/ml on transwell plates (six-well plate; 24 mm diameter; 0.4 mm pore size membrane) and grown for 21–25 d until an intact monolayer was obtained. The % xanthophyll that remained in the apical chamber was similar for both carotenoids. A % transport 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 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 system 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.

The association of single nucleotide polymorphisms with β-glucosidase activity in oral epithelium. By L.A. WAKELING, E.F. McGARR and D. FORD. Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle upon Tyne, UK, NE2 4HH

Consumption of soybean isoflavones may have a number of health benefits, including reduced risk of cancer. Isoflavones occur in unfermented plant sources as β-glucosides. The glucose moiety is cleaved by β-glucosidases (lactase-phlorizin hydrolase (LPH) and cytosolic β-glucosidase (CBG)) to release the aglycone during absorption in the small intestine. Hydrolysis of the isoflavone β-glucoside genistein to the corresponding aglycone genistein was recently demonstrated in shedded oral epithelial cells (Walle et al. 2005). Direct release of the isoflavone aglycone by β-glucosidase activity in the oral cavity may be particularly important with respect to protection against oral cancer.

The aim of the study was to establish if genotype with respect to non-synonymous single nucleotide polymorphisms (SNP) in the open reading frame of LPH and/or CBG, identified through database searches, influences β-glucosidase activity in shedded oral epithelial cells.

The genotype of 100 healthy, premenopausal, female participants, of between 18 and 50 years of age, with respect to SNP in LPH (G666A (Val219Ile), A4926G (Asn1639Ser) and CBG (T1417A (Tyr456STOP)) was determined using assays based on the analysis of real-time PCR hybridisation probe melting temperature. The same subjects provided a saliva sample after rinsing for 2×30s with 5 ml 0.2% chlorhexidine mouthwash to eliminate bacteria and brushing the oral epithelial surfaces to slough off cells. Samples of saliva produced by three subjects before and after the chlorhexidine mouthwash were plated on nutrient- and blood-based agar to determine the efficacy of the procedure to eliminate oral bacteria, whose endogenous β-glucosidase activity may have contributed to measured values. β-Glucosidase activity was measured in lysates prepared from oral epithelial cells, following centrifugation and washing, using the substrate p-nitrophenyl β-D-glucopyranoside.

SNP were present at the following frequencies, all in Hardy-Weinberg equilibrium: LPH position 666: 82 G/G, 17 G/A, 1 A/A; LPH position 4926: 75 G/G, 27 G/A, 1 A/A; CBG position 1417: 68 T/T, 27 T/A, 5 A/A. The range of β-glucosidase activities measured in oral epithelial cell lysates was 0–10.6 U/mg. Growth of oral bacteria was detected in all three subjects tested before the chlorhexidine mouthwash only, and β-glucosidase activity was detectable in all three corresponding samples of oral epithelial cell lysate, indicating the mouthwashing procedure effectively eliminated contaminating bacterial β-glucosidase from oral epithelial cell lysates. When data were grouped by presence or absence of the minor allele, univariate ANOVA indicated a significant interaction between the major alleles at position 666 in LPH (G and position 1417 in CBG (T) (P = 0.024; parameter estimate = -4.2 U/mg) and between the major alleles at position 666 in LPH (G and position 4926 LPH (A) (P = 0.022; parameter estimate = 4.6 U/mg).

In conclusion, the present study indicates complex effects of genotype with respect to SNP in LPH and CBG on oral β-glucosidase activity. Such effects may modulate the potential protective effect of isoflavone consumption against oral cancer.

The present study was funded by BBSRC (studentship to L. A. W.) and approval for the study was granted by the Newcastle and North Tyneside Local Research Ethics Committee.


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), over-expression of HNF4 increased wild-type promoter activity up to 25-fold (p < 0.002) in the McARH 7777 cell line and while mutation to the A DR-1 element showed no change in activation of the promoter by HNF4α, B and C DR-1 mutations reduced the effects of HNF4α 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 MacARD 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.
Dietary protein restriction in the pregnant rat induces altered epigenetic regulation of the glucocorticoid receptor and peroxisomal proliferator-activated receptor α. By K.A. LILLYCROP, M.A. HANSON and G.C. MITHEN, Institute of Food Research, Norwich Research Park, Colney, Norwich, UK, NR4 7UA

In healthy individuals, glucose and fatty acids are substrates for ATP generation in the heart. There is emerging evidence from patients with type 2 diabetes mellitus that preferential use of fatty acid β-oxidation for energy production may be linked to cardiomyopathy (Fink, 2004). PPARα activity is important for regulating fatty acid β-oxidation in the heart and is increased in hearts of rats with experimentally induced diabetes (Fink, 2004). Prenatal undernutrition is related inversely to risk of type 2 diabetes mellitus in man (Poole & Byrne, 2005) and insulin resistance in rats (Bertram & Hanson, 2000). We have shown that maternal dietary protein restriction induces persistent alterations to hepatic and carbohydrate metabolism in the offspring by altering the epigenetic regulation of PPARα and the glucocorticoid receptor (GR) (Lillycrop et al., 2005). Here we have tested the hypothesis that prenatal protein restriction induces hypomethylation of the GR and PPARα promoters in the heart, and that this is prevented by supplementation of the protein-restricted (PR) diet with folic acid.

Wistar rats were fed a control diet (15% (w/w) casein; 1 mg/kg folate acid), a PR diet (9% (w/w) casein; 1 mg/kg folate acid) or the PR diet supplemented with 5 mg/kg folate acid (PR+F) 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 6 d later. Methylation of the GR and PPARα promoters was determined in the hearts of the offspring (four rats per maternal diet, one rat per litter) by methylation-sensitive real-time PCR (Lillycrop et al., 2005).

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 normal prostate and benign prostatic hyperplasia, PNT1A and BPH-1 respectively, showed no increase in acetylation of histone H3 or H4. Studies in primary epithelial and fibroblast cells derived from patients with benign prostatic hyperplasia are currently underway.

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 mg/kg 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 by Western blotting.

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 7UA

The consumption of broccoli is associated with a reduced risk of cancer (van Poppel et al., 1999). The main isothiocyanate responsible for this activity is thought to be sulforaphane (4-methylsulfonylbutyl isothiocyanate; SF). SF confers its anti-carcinogenic activity through three widely known mechanisms: the induction of phase II enzymes (Brooks et al., 2001); the induction of apoptosis (Wang et al., 2004); and the control of the cell cycle (Gamet-Payrastre et al., 2000). A more recent mechanism includes the role of SF in gene control through epigenetic modification of histones. Histones are involved in DNA structuring and are modified through acetylation, predominantly of histones H3 and H4, which promotes transcription of associated genes. Cell-cycle and apoptotic gene promoters have been shown to be associated with acetylated histones (Myzak et al., 2006). Acetylation is prevented by histone deacetylases (HDAC), and SF has been demonstrated as an inhibitor of HDAC using in vitro models (Myzak et al., 2004).

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 normal prostate and benign prostatic hyperplasia, PNT1A and BPH-1 respectively, showed no increase in acetylation of histone H3 or H4. Studies in primary epithelial and fibroblast cells derived from patients with benign prostatic hyperplasia are currently underway.
Reactive oxygen species generation in mismatch repair-proficient and -deficient colorectal cancer cell lines exposed to butyrate. By J.M. COXHEAD, W. BAL, E.A. WILLIAMS and J.C. MATHERS,
Human Nutrition Research Centre, School of Clinical Medical Sciences, Newcastle University, UK, NE2 4HH and Human Nutrition Unit, Clinical Sciences (North), University of Sheffield, UK, S3 7AU

Hereditary non-polyposis colorectal cancer (HNPPC) 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 a SCFA endproduct of bacterial fermentation of carbohydrates in the colon and is an important energy substrate for normal colonocytes. Butyrate is also a potent anti-neoplastic agent associated with suppression of proliferation, induction of differentiation and increased apoptosis. 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 (0−5mM). 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 by measuring 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 0.5−5mM butyrate. Relative ROS expressed as percentage of control (no butyrate treated) cells.

Exposure to butyrate increased ROS in both cell lines and at all concentrations. At 6, 12 and 48h time points there was a significant dose-dependent increase in relative ROS compared with the control for both cell lines. After 48h butyrate exposure, there was a significant overall increase in the level of relative ROS in the HCT116 cell line compared with HCT116chr3 (see Fig. 1). These results suggest that MMR-deficient cells may be more susceptible to damage by ROS following butyrate exposure. Such damage could trigger apoptosis and so contribute to the anti-neoplastic action of butyrate.

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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), compared to one Sec in all other selenoproteins. SelP is expressed in several tissues, including the liver and 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 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.

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 50kDa. 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.

We thank the Food Standards Agency for financial support (N0304I). J.R. A’s laboratory is funded by the Scottish Executive Environment and Rural Affairs Department (SEERAD).

Influences of gender and genetic variation in the 3′ untranslated region of the glutathione peroxidase 4 gene on the response to selenium supplementation in healthy volunteers. 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

The phospholipid hydroperoxide glutathione peroxidase 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 leukotriene metabolism (Brigelius-Flohe, 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 spermatogenesis.

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 structure 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) (Méplán 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 spermatogenesis 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 (N05081). J.R.A.’s laboratory is funded by the Scottish Executive Environment and Rural Affairs Department (SEERAD).


Considerable evidence suggests that in certain species trans-10, cis-12 (t10,c12)-conjugated linoleic acid (CLA) reduces body fat deposition. However, it has recently been reported in human subjects that t10,c12-CLA increases plasma LDL concentrations, relative to the trans-9, cis-11 (c9,t11)-CLA isomer (Trixon et al. 2004). In the present study we have compared the effects of supplementing the diet with 0.25% (w/w) of rapped oil (control), t10,c12-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 lipoproteins 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, t10,c12-CLA specifically reduced the size of the perirenal adipose tissue depot, irrespective of the background diet (P<0.05). Consumption of the t10,c12 isomer 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) ison-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 (C) as a second factor. Significance levels for an interaction between the factors (D×C), or individual effects, are shown together with their standard errors of the difference.

<table>
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<th>Lipoprotein</th>
<th>Chow</th>
<th>Western fat</th>
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<td>VLDL</td>
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<td>LDLr mRNA</td>
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* P<0.05, ** P<0.01, *** P<0.001.

There was little effect of CLA on plasma lipoproteins when animals were fed chow alone. However, in the presence of ‘Western’ fat, t10,c12-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 than on HDL such that the atherogenic ratio was increased in animals fed t10,c12-CLA. The ‘Western’ fat diet clearly reduced hepatic LDL receptor mRNA concentrations and while there was no interaction between background diet and dietary CLA, t10,c12-CLA tended to reduce concentrations (P=0.064).

The data confirm that while t10,c12-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.

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Selection for growth increases the penalty of parasitism on growth performance in mice. By J.G.M. Houdijk1 and L. Bunger2,1Animal Health and Nutrition Team, SAC, Edinburgh, EH9 3JG and 2Animal Breeding and Development Team, SAC, Edinburgh, UK, EH9 3JG

The consequence of sub-clinical gastrointestinal parasitism in farm animals, i.e. reduced food intake and growth, is often more pronounced in breeds with a high production potential compared with breeds with a low one (for example, Houdijk et al. 2001). Since within-breed selection lines with sufficiently different production potentials are not readily available in farm animal species, it cannot be excluded that these differences in disease resistance may arise from between-breed differences in genetic resistance to parasites rather than production potential per se. Therefore, the objective of the present experiment was to assess the consequence of parasitism on growth performance in mice which had been selected within breed for divergent growth potentials. Eight groups of twenty weaned male mice were derived from four mouse lines (BE, MU, ED and RO), where each had been divergently selected for high (H) or low (L) growth potential over at least twenty generations (Bunger et al. 2001). At day 35 of age, each of these eight genotypes was either doosed with 250 infective larvae of the intestinal nematode parasite Heligmosomoides polygyrus or sham-infected with water (n = 10). All mice were individually housed and had ad libitum access to a low-protein food (5% crude protein). Food intake and body weight were assessed twice weekly until 28 d post-infection, and feces were collected on day 24 post-infection to assess fecal egg counts (FEC). FEC were log-transformed before statistical analysis, and observed gain, intake and FEC were analyzed (ANOVA; 4×2×2 factorial design).

The figure shows mice body-weight gain. Line, potential and infection interacted for absolute weight gain (P < 0.05). Infected mice grew slower than non-infected mice (P < 0.001) but this effect was more pronounced in H lines than in L lines (P < 0.001), with the largest effect of infection present in ROH mice (P < 0.05). Main effects did not interact for intake and FEC (P > 0.20). Intake of infected mice was on average 6% lower than that of non-infected mice in the first week post-infection (P < 0.05) but this reduced to 3% over the total 28 d period (P < 0.10). ED mice had higher FEC than MU mice (P < 0.05) whilst that of RO and BE mice were intermediate. L mice had about 80% lower FEC than H-mice (P < 0.01).

Leptin is a characteristically anorectic hormone that acts as a signal of nutritional status to the brain. Circulating concentrations are high in clinical obesity yet the failure to elicit appetite or weight loss, indicative of central leptin insensitivity. From the few published measurements of leptin in human cerebrospinal fluid (CSF), it is hypothesised that reduced blood–brain transport of leptin contributes to this apparent central leptin resistance. In order to advance further our understanding of this phenomenon, we have developed an animal model for dynamic, longitudinal assessment of leptin transport in vivo. Leptin concentrations are measured in repeated samples of blood and CSF from sheep prepared with intracerebroventricular cannulae and managed in different physiological conditions. Sheep are seasonal animals and we tested the hypothesis that changes in photoperiod or leptinemia would alter leptin blood–brain transport. Blood and CSF from the jugular vein and lateral cerebral ventricle, respectively, were taken for leptin RIA (Marie et al. 2001) repeatedly from sheep kept 15 weeks in artificially long daylength (LD; 16 h light/8 h dark) or short days (SD; 8 h light/16 h dark) with ad libitum food (six per group) (experiment 1), or thin or fat sheep given sufficient food to maintain their body weight in natural summer LD in June and after 5 weeks in artificial SD (six per group) (experiment 2), and initially thin or fat sheep given ad libitum food (increasing nutritional plane, INP) or restricted food (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 (SE 1.77) ng/ml in LD and from 9.3 (SE 1.14) to 19.5 (SE 0.84) ng/ml in SD. CSF leptin concentrations correlated positively with concurrent plasma values in LD (r2 = 0.57; P < 0.001), with CSF:plasma concentration ratio 0.16, but remained a lower proportion of plasma values (about 0.02) and did not correlate in SD (r2 = 0.05). Plasma leptin concentrations were lower in thin fat sheep in natural summer LD (4.0 (SE 0.42) v. 11.1 (SE 2.16) ng/ml) and in artificial SD (5.0 (SE 0.59) v. 7.2 (SE 0.73) ng/ml) (P < 0.05). However, CSF leptin concentrations were not different between thin vs fat sheep but were higher in both groups in natural summer LD (0.85 (SE 0.22) v. 1.04 (SE 0.10) ng/ml) than in artificial SD (0.23 (SE 0.05) v. 0.46 (SE 0.07) ng/ml) (P < 0.01); thus, CSF leptin correlated with plasma concentrations in LD (r2 = 0.39; P < 0.05) but not in SD, and the CSF:plasma concentration ratio (about 0.06–0.33) correlated negatively with plasma concentrations in LD (r2 = 0.33; P < 0.05). During experiment 3, plasma leptin concentrations increased over time in INP sheep (2.5 (SE 0.13) to 9.7 (SE 1.99) ng/ml) and decreased in DNP sheep (3.2 (SE 0.36) to 2.5 (SE 0.36) ng/ml). CSF leptin concentrations correlated positively with plasma values in both INP (r2 = 0.07; P = 0.01) and DNP sheep (r2 = 0.09; 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 = 0.43; P < 0.001) and DNP (r2 = 0.21; P = 0.001) groups.

These data indicate that a greater proportion of leptin enters the brain CSF from circulating blood in LD than in SD and with decreased leptinemia. Furthermore, these studies have demonstrated the value of the sheep model for exploring the dynamics of blood–brain communication in vivo. Elucidating the mechanisms of reduced efficiency of leptin uptake by the brain in sheep in SD and in conditions of increased leptinemia may shed new light on mechanisms underlying the similar phenomenon seen in clinically obese subjects.

The present work was funded by Scottish Executive Environment and Rural Affairs Department (SEERAD) and BBSRC.

Insulin–brain–brain transport and appetite responses to central insulin infusion in sheep. By E.J. BENNETT1,2, J.L. HARRISON2, P.A. FINDLAY1, D.W. MILLER1 and C.L. ADAM1, 1Obesity and Metabolic Health Division, Rowett Research Institute, Aberdeen Centre for Energy Regulation and Obesity, Bucksburn, Aberdeen, UK, AB21 9SB and 2Scottish Agricultural College, Craibstone, Aberdeen, UK, AB21 9YA

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 insulinRIA (MacRae et al. 1991). Plasma insulin concentrations increased steadily over time in the INP group from about 20 to 70 mU/l but remained at about 20 mU/l 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 h with control artificial CSF on one day and with insulin (2ng/h) on the next day. Voluntary food intake (VFI) was measured hourly by weighing and replacing food refusals, with fresh food given at 08.00 h. As in experiment 1, plasma insulin increased steadily over the 12 weeks in INP sheep (about 30 to 100 mU/l) but decreased rapidly (about 60 to 25 mU/l) 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, 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 is associated with increased circulating insulin, which is able to enter the CSF freely in proportion to wide-ranging plasma concentrations, but it 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 SEIRAD and BBBSR.


RRR-α-tocoopherol supplementation has pro- and anti-inflammatory and pro- and antioxidant effects in peripheral blood mononuclear cells in healthy middle-aged men depending upon dosage and genotype for xenobiotic metabolising enzymes. By A. ENGLAND1, J. SLATER-JEFFERIES2, E.A. MILES1, L. BROWNING3, K. GRIMALDI4, R. GILL-GARRISON2, P.C. CALDER1, W.M. HOWELL1 and R.F. GRIMBLE1, 1Institute of Human Nutrition and School of Medicine, University of Southampton, Southampton, UK, SO16 7PX and 2Sciona Ltd, Boulder, CO, USA

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-tocoopherol (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 (FI)) by LPS-stimulated monocytes (mono) and peripheral blood granulocytes (gran) were assessed before and after supplementation. NF-κB activation was assessed in LPS-stimulated PBMC. Subjects were genotyped for single nucleotide polymorphisms (SNP) in cytokine (LT-α b, TNF-α a, IL-1 b, IL-6, IL-8 and IL-10 genes respectively), glutathione-S-transferase (GST) (GSTM1, GSTT1 or active, GSTP1 A313G and C341T), Mn superoxide dismutase (C-28T) and methyltetrahydrofolate reductase (MTHFR; C677T and A1298C) genes.

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

| 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.

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**Table 1.**

<table>
<thead>
<tr>
<th>Dose</th>
<th>Change in B-6</th>
<th>Change in R-1β</th>
<th>NF-κB activity</th>
<th>FI gran (PMCA)</th>
<th>FI mono (LPS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2.3 ± 0.5</td>
<td>22 ± 42</td>
<td>8 ± 22</td>
<td>108 ± 209</td>
<td>22 ± 47 ± 69</td>
</tr>
<tr>
<td>One</td>
<td>3.5 ± 0.6</td>
<td>12 ± 24</td>
<td>7 ± 22</td>
<td>58 ± 91</td>
<td>12 ± 74</td>
</tr>
<tr>
<td>Two</td>
<td>4.0 ± 0.7</td>
<td>14 ± 26</td>
<td>16 ± 22</td>
<td>37 ± 48</td>
<td>35 ± 43</td>
</tr>
<tr>
<td>Three or Four</td>
<td>4.0 ± 0.8</td>
<td>16 ± 28</td>
<td>20 ± 22</td>
<td>14 ± 43</td>
<td>17 ± 43</td>
</tr>
</tbody>
</table>

* Significant effect of supplement (Wilcoxon signed ranks test; P < 0.05).

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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. LILLYCROP1, 1DOHaD Centre, University of Southampton, Southampton, UK, SO16 5YA and 2Development and Cell Biology, University of Southampton, Bassett 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 F0 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.

<table>
<thead>
<tr>
<th></th>
<th>F1 18% protein diet group</th>
<th>F1 9% protein diet group</th>
<th>F2 18% protein diet group</th>
<th>F2 9% protein diet group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>DNA methylation (%)</td>
<td>100.0 ± 0.6</td>
<td>100.0 ± 2.1</td>
<td>100.0 ± 0.6</td>
<td>100.0 ± 2.1</td>
</tr>
<tr>
<td>PPARα</td>
<td>91.8 ± 1.3</td>
<td>89.5 ± 2.8</td>
<td>93.2 ± 2.9</td>
<td>92.1 ± 1.8</td>
</tr>
<tr>
<td>GR</td>
<td>100.0 ± 2.0</td>
<td>100.0 ± 2.0</td>
<td>100.0 ± 2.0</td>
<td>100.0 ± 2.0</td>
</tr>
<tr>
<td>mRNA expression (%)</td>
<td>100.0 ± 0.6</td>
<td>100.0 ± 2.8</td>
<td>100.0 ± 15.0</td>
<td>100.0 ± 24.3</td>
</tr>
<tr>
<td>PPARα</td>
<td>129.4 ± 15.8</td>
<td>117.8 ± 14.1</td>
<td>115.0 ± 18.2</td>
<td>112.6 ± 10.5</td>
</tr>
<tr>
<td>GR</td>
<td>113.0 ± 24.8</td>
<td>112.6 ± 10.5</td>
<td>115.0 ± 18.2</td>
<td>112.6 ± 10.5</td>
</tr>
</tbody>
</table>
*Significantly different (P<0.05) between maternal diets within a generation (Student’s unpaired t test).

These results show that induction of hypomethylation of hepatic PPARα and GR promoters in the offspring by maternal PR diet persists in the F2 generation. In contrast to post-weaning rats (Lillycrop et al. 2005), there was no effect on PPARα or GR transcription at day 80. One possible explanation is energy-rich nutrient supply during lactation induced transcription of these genes, which returned to basal levels after weaning (Panadero et al. 2005). Nevertheless, the PR F1 and F2 offspring may retain capacity for an exaggerated metabolic response to nutrient challenge. These findings suggest an epigenetic mechanism for the transgenerational transmission phenotypic variations induced by prenatal nutrition.

The present study was supported by the British Heart Foundation.


The intracellular genistein metabolite 5,7,3',4'-tetrahydroxyisoflavone mediates G2-M cell cycle arrest in cancer cells via modulation of the p38 signalling pathway. By J.P.E. SPENCER1, D.T. NGUYEN2, E. HERNANDEZ-MONTES3, D. VAUZOUR1, A.H. SCHO¨ NTHAL2 and E. CADENAS3, 1Molecular Nutrition Group, School of Food Biosciences, University of Reading, PO Box 226, Reading, UK, RG2 6AP and 2Department of Molecular Pharmacology and Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA 90089, USA

The cellular actions of genistein are believed to mediate the decreased risk of breast cancer associated with high soya consumption. We have investigated the intracellular metabolism of genistein in T47D tumorigenic and MCF-10A non-tumorigenic cells and assessed the cellular actions of resultant metabolites (see Figure).

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. Genistein was selectively taken up into T47D cells and was subject to metabolism by CYP450 enzymes 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. THIF exposure activated p38 and caused subsequent inhibition of cyclin B1 (Ser 147) and cdc2 (Thr 161) phosphorylation, two events critical for the correct functioning of the cdc2-cyclin B1 complex. We suggest that the formation of THIF may mediate the cellular actions of genistein in tumorigenic breast epithelial cells via the activation of signaling through p38.
**TUB** gene variants are associated with body mass index, weight and body fat in postmenopausal women. By S.D. ODELL1, H. SNIEDER2, X. WANG3, R. SHIRI-SVERDLOV3, J.V. van VLIET-OSTAPTCHOUR1 and T.D. SPECTOR4, 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 mouse **Tub** gene results in the **tubby** mouse syndrome, characterised by late-onset obesity with insulin resistance and neurosensory 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, are 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).

Gene type 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 rs2772382 and rs1528133 were found on waist: rs2272382 (22 genotype 80.5 (SD 11.1) vs. 11k/12 79.0 (SD 10.4) cm; P=0.012) and rs1528133 (11k/12 genotype 78.2 (SD 10.0) vs. 22 79.4 (SD 7.9) 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></td>
<td>No</td>
<td>11/12</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>358/319/94</td>
<td>24.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>358/319/94</td>
<td>64.6</td>
</tr>
<tr>
<td>Total fat (kg)</td>
<td>361/319/91</td>
<td>20.7</td>
</tr>
<tr>
<td>Total fat (%)</td>
<td>356/319/90</td>
<td>32.1</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>353/319/93</td>
<td>86.0</td>
</tr>
<tr>
<td>Central fat (kg)</td>
<td>361/319/90</td>
<td>10.7</td>
</tr>
<tr>
<td>Central fat (%)</td>
<td>356/319/90</td>
<td>26.1</td>
</tr>
</tbody>
</table>

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

Associations were confirmed by Sibbling 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.

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. CARRERO2, J. HADLEY2, B. TAN3, N. DASTURI2, C.P. SHEARMAN1, P.C. CALDER1, E. RAINGER3, G. NASH3, T. LUU4 and R. GRIMBLE1, 1School of Medicine, University of Southampton, Southampton, UK, 2SO16 7PX and 3Department 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 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.

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 BBRC 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 J. SLATER-JEFFERIES1, A. ENGLAND2, B. CICHON2, K. GRIMALDI2, R. GILL-GARRISON2, P.C. CALDER1, W.M. HOWELL2, R. ARMSTRONG2 and R.F. GRIMBLE2, 1Institute of Human Nutrition University of Southampton, Southampton, UK, S016 7PX, 2Scion Ltd, Boulder, CO, USA and 3School of Medicine, University of Southampton, Southampton, UK, S016 7PX.

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 isoprostanes, lipid peroxides and C-reactive protein were measured pre- (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 −308, +252, −174, −511 and −1082 in the TNF-α, lysozyme-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<0.03; Wilcoxon signed ranks test); however, this occurred only in overweight patients (P=0.004 v. 0.674 for BMI<25 kg/m2). Overweight patients with the TNF 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 TNF rather than CT or CC genotype (10.7 (±0.5) v. 3.6 (±2.7) mg/l; P=0.002). 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=0.05 v. 0.044 and patients P<0.001 v. 0.01 for the TNF- v. other genotypic variants of the C-28T SOD SNP).

The B-174 SNP significantly influenced isoprostane concentrations and the change 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. NORMANTON1, J.G.M. HOUDIJK1, D.P. KNOX2 and I. KYRIAZAKIS1,2, 1Animal Health Research Institute, SAC, Edinburgh, UK, EH9 3JG, 2Parastology Division, Moredun Research Institute, Pencait, UK, EH26 0PZ and 3Veterinary 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., 2001). This hypothesis is being addressed in a lactating rat model, as lactating rats show a sensitivity to changes in protein scarcity. Both an increased protein supply and a reduced nutrient demand are sensitive to changes in protein scarcity.

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LP9</th>
<th>HP9</th>
<th>LP3</th>
<th>SED</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAS score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-suppl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pre-suppl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Feeding treatments had a significant effect on the number of eggs found in the colon contents (P<0.03; the Figure shows HP9 and LP3 rats had significantly lower egg counts than LP9 rats. HP9 final litter weight reached 154 g while LP9 reached 115 g, and LP3 68 (±11.6) g (P<0.05) over the whole lactation. Feeding treatment did not affect any of the assessed immunological results as shown in the Table.

The results support the view that the periparturient breakdown of immunity to N. brasiliensis is sensitive to changes in protein scarcity. Both an increased protein supply and a reduced nutrient demand reduced the concentration of nematode eggs in the colon. Since protein was first limiting in the LP9 rats, the response to reducing litter size is most probably attributable to overcoming protein scarcity, and not related to increased availability of other nutrients. Although HP9 and LP3 rats tended to have higher levels of local antibodies and RMCP-II, these responses were not significant, which may be the consequence of the single time point chosen. Hence, further analysis at various points in time post-secondary infection may be required to associate nutritionally improved resistance to parasites during the periparturient period with effects on host immune responses.
The acute effect of triacylglycerols rich in stearic and oleic acid on vascular function. By S.E.E. BERRY1, R. BANERJ2, S. TUCKER3, S.M. CHARLES5, B. JIANG2, P.J. CHOWIENZY2 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’ 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>Test Meal</th>
<th>Plasma TAG (mmol/L)</th>
<th>FMD (%)</th>
<th>PWV (m/s)</th>
<th>PWA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shea butter</td>
<td>28.3 (5.7, 46.9)</td>
<td>83.4 (50.0, 109.8)</td>
<td>-1.3 (-2.7, 0.0)</td>
<td>-3.0 (-4.4, -1.6)</td>
</tr>
<tr>
<td>High-oleic sunflower oil</td>
<td>83.4 (50.0, 109.8)</td>
<td>83.4 (50.0, 109.8)</td>
<td>-1.3 (-2.7, 0.0)</td>
<td>-3.0 (-4.4, -1.6)</td>
</tr>
</tbody>
</table>

FMD (%) was significantly lower following shea butter compared to 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=0.002).

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 differences in response observed in the current study following shea butter and high-oleic sunflower oil. As FMD and postprandial lipemia 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 McNeill at the Cardiovascular Division, King’s College London School of Medicine, St Thomas’ Hospital, London, UK.


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, lepton levels increase during infection and inflammation in many models of disease and this increase has been associated with anorexia in infected dairy cows (Matarse et al. 2005). Whether lepton 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) lepton 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) (CAL), 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 lepton levels using an ovine-specific RIA. FI and lepton data were analysed using RIML. FEC were analysed using ANOVA with repeated measurements. All data were log (x+1) transformed.

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, S 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 lepton levels between lambs fed ad libitum and those fed at 80 and 70% of ad libitum, indicating that lepton levels correlate positively with FI in growing lambs. Lepton 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, lepton 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 lepton levels increase during the acquisition phase of immunity.


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

Fig. 1. Average food intake: S-C AL (●), S-INF (●), B-CAL (□), B-INF (□). FI values (kg DM/day) are presented against weeks of infection.

Fig. 2. Average lepton levels: C AL (●), INF (●), C90 (●), C80 (●), C70 (●), INF (●), CAL (□). Lepton levels (ng/ml) are presented against weeks of infection.

With special thanks to Karen McNeill at the Cardiovascular Division, King’s College London School of Medicine, St Thomas’ Hospital, London, UK.
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By D.I. SHAW, W.L. HALL and C.M. WILLIAMS, Hugh Sinclair Unit of Human Nutrition, School of Food
Medway School of Science, University of Greenwich, UK and

Vascular dysfunction is an abnormality associated with the metabolic syndrome, cardiovascular disease. In vitro studies have compared fatty acids (100 μM) on the expression of a broad spectrum of genes associated with endothelial function. Consequently, findings to date do not allow comparison of the effects of different fatty acids.

Ten healthy Caucasian volunteers (one male and nine females) were recruited in a cross-over design. Three separate 2 h 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, 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 ± 0.26 mmol/l respectively. Predicted GI of the test meal was 46.61 units (Foster-Powell, Holt & Brand-Miller, 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 < 0.05) and post-intervention period (–65%; P < 0.001) compared with control. The AUC for HDL-cholesterol was significantly lower after the test meal in both the pre- (–53%; P < 0.05) and post-dietary period (–58%; P < 0.008) compared with control but fasting serum triacylglycerols, cholesterol or LDL-cholesterol were not significantly different between groups.

Eicosapentaenoic acid (EPA) resulted in increased up-regulation of eNOS (P < 0.05) and MCP-1 (P < 0.001) compared with DHA. Arachidonic acid (AA) significantly reduced the expression of genes encoding for pro-inflammatory proteins. In comparison to DHA, EPA significantly increased up-regulation of eNOS (P < 0.05) and MCP-1 (P < 0.01) compared with AA. Oleic acid (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 significantly higher expression (P < 0.05) compared with AA.

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.

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, Falbourn Road, Cambridge, UK, CB1 9NL

To develop an understanding of the aetiology 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; Hovorka, 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; Soonthornpun 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 sensitivity.

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 g [2H2]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.


Do differences exist between gym and non-gym users’ nutritional knowledge and use of nutrition labels?

Numerous studies have been published describing consumers’ use and understanding of nutrition labels and nutrition overall (Higginson et al. 2002a,b). However, 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 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 salicylic acid in healthy human subjects. By V. RUNGAPAMESTRY 1, A.J. DUNCAN 2, Z. FULLER 3 and B. RATCLIFFE 1, 1The Robert Gordon University, Saint Andrew Street, Aberdeen, UK, AB25 1HG; 2The Macaulay Institute, Craigiebuckler, Aberdeen, UK, AB15 8QH; 3Rowett Research Institute, Aberdeen, UK, AB10 6UX.

In broccoli, salicylic acid, 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, salicylic acid 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 brassicae in vitro, was from lightly cooked compared with raw or fully cooked brassicae (Rungapamestry et al. 2005). Alkyl isothiocyanates have been shown to interact with proteins in vitro (Björklund, 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 150 g lightly cooked broccoli (microwaved for 2 min) or fully cooked broccoli (microwaved for 5.5 min) 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 and excretion of isothiocyanates. Each meal was separated by a wash-out period of at least 48 h. Urine was collected for 24 h following each meal and analysed for excretion of allyl mercaptan acid (AMA) and salicylic acid mercapturic acid (SFMA), the biomarkers of AITC and salicylic acid absorption respectively, by HPLC (Mennick et al. 1987).

Although glucoraphanin intake was not markedly different between broccoli treatments, consumption of lightly cooked broccoli produced a higher yield of salicylic acid 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 (P<0.01) (Fig. 1 (c)). 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 salicylic acid 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).

Björklund R (1973) Physiological 12, 185–190.

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

The anti-inflammatory and anti-neoplastic 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, or is excreted as 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±4 years) ingested a yoghurt flavoured with spice (cumin) containing 134 μg SA and urine was collected at intervals over 12 h. 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>Garcum 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.

As approximately 70–80% of SA is converted to SU in vivo (Needs & Baroès, 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 chemopreventive 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 (Tei et al. 1989, Barkeling et al. 1990; Golay et al. 1997). The present 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 readily available snacks were chosen for this study. Two snacks consisting of 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 (69% 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 altered expression of fat metabolism-regulated genes. By A.M. ERHUMA1, S.C. LANGLEY-EVANS2 and A.J. BENNETT3,
1School of Biomedical Sciences, Queen’s Medical Centre and 2Division of Nutritional Sciences, University of Nottingham, Nottingham, UK, NG7 2UH

Evidence from experimental studies in animals strongly supports the hypothesis that components of the metabolic syndrome are programmed in utero (Langley-Evans, 2006). However, the mechanistic basis of this metabolic programming has not yet been established. The aim of the present study was to investigate whether prenatal exposure to a low-protein diet is associated with altered expression of genes involved in fat synthesis such as fatty acid synthase (FAS), fatty acid oxidation, PPAR-α and its downstream target medium-chain acyl-CoA dehydrogenase (MCAD) as well as PPAR-γ. Preganant Wistar rats were randomly allocated to five treatment groups. A control group was fed 180g casein/kg diet throughout pregnancy and four other experimental groups were fed a 90g 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 throughout pregnancy (LP all; day 0–22 gestation). On delivery of litters all dams were transferred to standard laboratory chow and the same diet was used to wean the offspring at 4 weeks of age. The offspring were killed at the age of 18 months. The mRNA expression of target genes in liver was measured using real-time RT-PCR.

Hepatic FAS mRNA expression at 18 months

<table>
<thead>
<tr>
<th>Diet</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.2 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>LP all</td>
<td>0.6 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>LP early</td>
<td>0.7 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>LP mid</td>
<td>0.5 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>LP late</td>
<td>0.4 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
</tbody>
</table>

Hepatic PPAR-γ expression at 18 months

<table>
<thead>
<tr>
<th>Diet</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.8 ± 0.2</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>LP all</td>
<td>1.4 ± 0.2</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>LP early</td>
<td>1.5 ± 0.2</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>LP mid</td>
<td>1.3 ± 0.2</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>LP late</td>
<td>1.2 ± 0.2</td>
<td>0.8 ± 0.2</td>
</tr>
</tbody>
</table>

Hepatic MCAD mRNA expression at 18 months

<table>
<thead>
<tr>
<th>Diet</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.5 ± 0.5</td>
<td>6.4 ± 0.5</td>
</tr>
<tr>
<td>LP all</td>
<td>5.0 ± 0.5</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td>LP early</td>
<td>4.5 ± 0.5</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>LP mid</td>
<td>3.5 ± 0.5</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>LP late</td>
<td>2.5 ± 0.5</td>
<td>1.5 ± 0.5</td>
</tr>
</tbody>
</table>

Hepatic PPAR-α mRNA expression at 18 months

<table>
<thead>
<tr>
<th>Diet</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.5 ± 0.2</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>LP all</td>
<td>2.1 ± 0.2</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>LP early</td>
<td>2.0 ± 0.2</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>LP mid</td>
<td>1.5 ± 0.2</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>LP late</td>
<td>1.0 ± 0.2</td>
<td>0.5 ± 0.2</td>
</tr>
</tbody>
</table>

As there is evidence to suggest that over-exposure of fetal tissues to glucocorticoids of maternal origin plays a key role in metabolic programming (Langley-Evans, 2006), further studies investigated whether the programmed changes in hepatic SREBP-1c expression were glucocorticoid-dependent. Rats were allocated to be fed isenergetic diets (control; 180g casein/kg diet) or (LP; 90g casein/kg diet) throughout pregnancy. The LP group received an inhibitor of steroid synthesis, metyrapone (5 mg/kg body weight), metyrapone plus corticosterone (15 mg/kg body weight), or vehicle (saline) injections over the first 2 weeks of pregnancy. The pregnant rats remained on the same diets until delivery at 22d gestation. On delivery the animals were transferred to a standard laboratory chow diet. The offspring were weaned at 4 weeks of age on to the same chow diet and killed for collection of liver tissue. The SREBP-1c promoter activity in response to glucocorticoid was assessed using the H4IIE cell line transfected with rat SREBP-1c reporter vector and treated with 1 μg dexamethasone. Diet-mediated suppression of hepatic SREBP-1c expression was removed by prenatal administration of metyrapone, and restored by corticosterone replacement. In SREBP-1c reporter-transfected H4IIE cells, dexamethasone increased reporter activity. These data indicated that not only basal but also stimulated SREBP-1c was altered 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, Greenburn Road, Bucksburn, Aberdeen, UK, AB21 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. 2005). 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 (acyl CoA carboxylase; ACC-1) and fatty acid oxidation (carnitine palmitoyl transferase; L-CPT-1) in the pregnant rat.

Female Roytett 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 folic acid (–F) and one deficient in folic acid 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 Express (Applied Biosystems).

ACC-1 mRNA:18S rRNA ratio in maternal liver (arbitrary values)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Mean ± SEM</th>
<th>n</th>
<th>Mean ± SEM</th>
<th>n</th>
<th>Mean ± SEM</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>–F</td>
<td></td>
<td>–F LM LC</td>
<td></td>
</tr>
<tr>
<td>Zinc-restricted</td>
<td>17.4 ± 0.7</td>
<td>6</td>
<td>12.8 ± 1.2</td>
<td>6</td>
<td>13.0 ± 1.6</td>
<td>6</td>
</tr>
<tr>
<td>Zinc-adequate</td>
<td>26.3 ± 1.6</td>
<td>6</td>
<td>25.5 ± 0.8</td>
<td>6</td>
<td>19.5 ± 2.2</td>
<td>6</td>
</tr>
<tr>
<td>Zinc-supplemented</td>
<td>7.7 ± 0.4</td>
<td>6</td>
<td>9.0 ± 1.8</td>
<td>6</td>
<td>12.5 ± 1.4</td>
<td>6</td>
</tr>
<tr>
<td>ACC-1 mRNA:18S rRNA</td>
<td></td>
<td></td>
<td>ratio in fetal liver (arbitrary values)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc-restricted</td>
<td>11.8 ± 2.6</td>
<td>6</td>
<td>32.3 ± 8.7</td>
<td>6</td>
<td>14.3 ± 2.6</td>
<td>6</td>
</tr>
<tr>
<td>Zinc-adequate</td>
<td>6.66 ± 0.7</td>
<td>8</td>
<td>9.52 ± 2.6</td>
<td>6</td>
<td>244.9 ± 400</td>
<td>7</td>
</tr>
<tr>
<td>Zinc-supplemented</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data were analysed by ANOVA followed by Fisher’s post hoc test. Mean values within a row with unlike superscript letters were significantly different (P<0.05).

There was a significant decrease in ACC-1 mRNA levels and a significant increase in the levels of the mRNA for L-CPT-1 in the maternal liver from both –F and –F LM LC groups. These changes suggest that folate deficiency decreases synthesis and increases the oxidation of fatty acids in the maternal liver. Although there were significant changes in the mRNA coding for proteins involved in lipid metabolism in dams fed –F and –F LM LC diets, liver triacylglycerol concentrations were unchanged in the –F groups and only increased in the livers of dams fed the –F LM LC diet.

Levels of ACC-1 mRNA were unchanged in the fetal liver of the –F group but were significantly lower in the –F LM LC group. Levels of L-CPT-1 mRNA in the fetal liver were increased significantly when dams were fed the –F diet but not when they were fed the –F LM LC diet. This suggests that folate deficiency increases fatty acid oxidation in the fetal liver but does not change synthesis. Folate deficiency combined with low methionine and choline reduces fatty acid synthesis but does not change oxidation. These contrasting results may reflect the development of hepatic steatosis in the dam.

These results show that dietary folate deficiency changes 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-2005–007036).


The present study was funded by BBSRC (C3D18271).


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. Burdge, J. Powell and P.C. Cakker.

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 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).

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, Elsie Oldendorf Laboratory, Falsham 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 (Lingren et al.; 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:Δ6-linolenic acid (LA: LNA) 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: LNA) oil.

There was a significant effect of dietary treatment on TAG (Group x 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: LNA (OF–RS, 10.4 % v. OF–SF, 2.8%) although not significant. The table shows TAG (geometric means and standard deviations; mmol/l).

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.

Supported by Unilever Corporate Research.


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Supported by Unilever Corporate Research.

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. HWAALA 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)–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)–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 14798 MBq 3H₂O, and killed by decapitation 1 h later. Blood and liver were taken for analysis as described by Obeid et al. (2006).

<table>
<thead>
<tr>
<th>Diet</th>
<th>n</th>
<th>Mean (so)</th>
<th>Liver weight (g)</th>
<th>Liver fat (%)</th>
<th>Liver glycogen (mg/g Liver)</th>
<th>Liver lipogenesis</th>
<th>Liver lipogenesisa</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF-LB₆</td>
<td>8</td>
<td>1397.5</td>
<td>126</td>
<td>11.64</td>
<td>0.4</td>
<td>29.49</td>
<td>2.6</td>
</tr>
<tr>
<td>LF-NB₆</td>
<td>8</td>
<td>1490.6</td>
<td>103</td>
<td>12.61</td>
<td>0.5</td>
<td>35.88</td>
<td>2.1</td>
</tr>
<tr>
<td>LF-SNB₆</td>
<td>7</td>
<td>1247.1</td>
<td>17</td>
<td>10.86</td>
<td>0.6</td>
<td>30.76</td>
<td>1.3</td>
</tr>
<tr>
<td>LF-NB₆</td>
<td>7</td>
<td>1343.8</td>
<td>48</td>
<td>11.86</td>
<td>0.5</td>
<td>37.96</td>
<td>2.1</td>
</tr>
<tr>
<td>HF-LB₆</td>
<td>8</td>
<td>1377.1</td>
<td>16</td>
<td>12.05</td>
<td>0.4</td>
<td>34.70</td>
<td>1.5</td>
</tr>
<tr>
<td>HF-NB₆</td>
<td>7</td>
<td>1265.7</td>
<td>19</td>
<td>12.27</td>
<td>0.5</td>
<td>34.72</td>
<td>2.0</td>
</tr>
</tbody>
</table>

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

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: 6n-3) is extremely susceptible to oxidation and animal studies have found that high intakes increase lipid 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 F₂ 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-5,6-dihydro-8-isoprostane F₂ 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>
<thead>
<tr>
<th>Diet</th>
<th>Women (n 16)</th>
<th>Men (n 16)</th>
<th>Women (n 17)</th>
<th>Men (n 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean*</td>
<td>% 95 CI</td>
<td>Mean*</td>
<td>% 95 CI</td>
<td>Mean*</td>
</tr>
<tr>
<td>8-Isoprostane F₂ (nmol/mmol creatinine)</td>
<td>2,3 Dinor-isoprostane F₂ (nmol/mmol creatinine)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Geometric mean. Repeated-measures ANOVA adjusted for treatment order showed no statistically significant differences.

The 2.3 dinor metabolite occurred in five-fold higher concentrations than 8-isoprostane F₂. The immunosass for the 2.3 dinor metabolite gives much higher values (approximately 10–30-fold) than the GC-MS assay (I’yasoua et al. 2004). However, the values reported were similar to those reported in other studies in the literature using immunosassays. 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. GIBBS1, A. JETHA1, D. MELLOR1 and S. HAMPTON2, 1Dietetics, Nutrition and Food and 2Neuromedicine Group, University of Surrey, Guildford, Surrey, UK, GU2 7XH

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 (LG) 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 glucose 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 (10h) following a controlled pre-meal, before each leg of the study. Blood samples were taken using a fingertip method, at baseline (0), and at 15, 30, 45, 60, 90, 120 min postprandially. Samples were analysed for glucose using the YSI 2300 STAT plus glucose and lactate analyzer ( ). 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=58). The observed GI of the meals was calculated from the incremental area under the curve of the glycaemic responses as % 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 (SEM 53)%; LGI 163 (SEM 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 mixed 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. HORGAN2, S. WHYBROW2 and J. STUBBS2, 1Biometrics and Statistics Scotland, Rowett Research Institute, Bucksburn, Aberdeen, UK, AB21 9SB and 2Rowett 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.

It might then be thought that variation over time within the same individual will show a negative correlation.

Days with a higher than usual fat intake should have a lower CHO intake. However, this too is false. In a collection of data (Stubbs et al. 2001) on fifty-nine subjects consuming their normal diet for 12d, the fat-CHO correlation for total amount was positive in the majority of individuals.

However, individuals do show preferences for particular diet compositions. When daily intakes are plotted on a composition triangle, it can be seen that most values are concentrated in a region of the plot corresponding to a distribution ( ). The proportion of food items ranked from lowest to highest ED, by grouping them into intervals from the lowest 10% to the highest, is shown in the following graph. The GI of each food item within that group was calculated from the incremental area under the curve of the glucose responses as % 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 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.
Antioxidant imbalance in ulcerative colitis. By P. RANA1, A.S. ANDERSON1 and J.H. CUMMINGS2, 1 Centre for Public Health Nutrition Research, Ninewells Medical School, Dundee, DD1 9SY and 2 Division of Pathology and Psychoendocrinology, 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 patients with moderately active disease had significantly lower plasma iron, selenium and glutathione peroxidase levels (Sturmiolo 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 oligosaccharides, gum Arabic, vitamin E, vitamin C and selenium found clinical improvement in supplemented subjects with a decreased requirement for steroids (Seidner 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 (DRV) (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 (mg)</td>
<td>54</td>
<td>75</td>
<td>43</td>
<td>60</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>13</td>
<td>8.7</td>
<td>11.6</td>
<td>19.8</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 for females (P<0.01) while intakes were lower in RNI in females 19–50 years (P<0.01).

Copper intake was significantly correlated with sigmoidoscopy score (rs = 0.344, P<0.01). In males, iron (rs = 0.457, P<0.01), copper (rs = 0.556, P<0.001) and zinc (rs = 0.348, P<0.05) were positively correlated with sigmoidoscopy score. In females, iron correlated negatively with sigmoidoscopy score (rs = -0.354, P<0.05).

A regression analysis for the whole group revealed that 9% (r^2 = 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% (r^2 = 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 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. SPURR, 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 artifactual 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 480) 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.

Nutrient Intake RNI Intake RNI

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Intake</th>
<th>RNI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper (mg)</td>
<td>1.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Selenium (mg)</td>
<td>54</td>
<td>75</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>13</td>
<td>8.7</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>8.3</td>
<td>&gt;4mg/d (safe intake)</td>
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</tbody>
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<tr>
<td>Copper (mg)</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Selenium (mg)</td>
<td>75</td>
<td>43</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>8.7</td>
<td>19.8</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>7.4</td>
<td>&gt;3mg/d (safe intake)</td>
</tr>
</tbody>
</table>

Sample n Mean total antioxidant capacity (mmol/l) P

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean total antioxidant capacity (mmol/l)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red wine</td>
<td>10</td>
<td>22.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>White wine</td>
<td>4</td>
<td>20</td>
<td>NS</td>
</tr>
<tr>
<td>Capsules (dissolved in water)</td>
<td>8</td>
<td>14.7</td>
<td>NS</td>
</tr>
<tr>
<td>Capsules (dissolved in 10% (v/v) alcohol)</td>
<td>8</td>
<td>14.2</td>
<td>NS</td>
</tr>
<tr>
<td>Orange juice</td>
<td>5</td>
<td>7.5</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Grape juice</td>
<td>7</td>
<td>5.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Differences between means were analysed by one-way ANOVA (Tukey). Significance levels are for the comparison with red wine.

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. Wrieden1, K.L. Barton1, A.J. ADAMSON2 and L. COCHRANE1, 1School of Medicine, University of Dundee, Ninewells Medical School, Dundee, UK, DD1 9SY and 2Human 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.

Smaller 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.

<table>
<thead>
<tr>
<th>Food</th>
<th>Institutionalised</th>
<th>Free-living</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean value</strong></td>
<td><strong>Median (g)</strong></td>
<td><strong>IQR (g)</strong></td>
</tr>
<tr>
<td>White sugar</td>
<td>8</td>
<td>5–10</td>
</tr>
<tr>
<td>Red cabbage</td>
<td>7</td>
<td>5–12</td>
</tr>
<tr>
<td>Bread, white or softgrain, sliced</td>
<td>38</td>
<td>30–64</td>
</tr>
<tr>
<td>Buns</td>
<td>10</td>
<td>6–14</td>
</tr>
<tr>
<td>Bread, white or softgrain, 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>Custard 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>25–49</td>
</tr>
<tr>
<td>Porridge</td>
<td>77</td>
<td>131–226</td>
</tr>
<tr>
<td>Cornflake-type cereal</td>
<td>30</td>
<td>24–34</td>
</tr>
<tr>
<td>Milk powder</td>
<td>73</td>
<td>54–113</td>
</tr>
</tbody>
</table>

*Differences between means were analysed by one-way ANOVA for the bottled group. A paired t test was used to compare the differences between bottled and draught beers. Mean value was significantly different from that of the corresponding UK beer: *P < 0.05, **P < 0.01.

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. N01028).


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>75 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cont-fed</td>
<td>Wheat-fed</td>
</tr>
<tr>
<td></td>
<td>n  Mean ± SD</td>
<td>n  Mean ± SD</td>
</tr>
<tr>
<td>75 mg/kg</td>
<td>32 22.37 ± 2.10</td>
<td>32 23.72 ± 2.60</td>
</tr>
<tr>
<td>250 mg/kg</td>
<td>32 22.35 ± 3.43</td>
<td>32 23.18 ± 3.19</td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>32 21.96 ± 3.37</td>
<td>32 22.60 ± 3.54</td>
</tr>
</tbody>
</table>

Mean values within a row with unlike superscript letters were significantly different (Fisher’s least significant difference test; P<0.05).

Vitamin E did not appear to affect drip 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.

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 postmenopausal 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 sonometry (McCue CUBA Clinical Ultrasonometer; McCue Plc., Southampton, UK).

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, 959 (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.

This project was supported in part by Lancashire teaching hospitals NHS trust research directorate Seedcorn funding.

A proteomic analysis of aortic proteins in zinc and metallothionein deficiency. By J.H. Beattie1,
M.-J. Gordon1, G.J. Rucklidge1, M.D. Reid1 and I.S. Kwon2, 1Rowett Research Institute, Aberdeen,
UK, AB21 9SB and 2Department 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.

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 B. DE ROOS 1, A. GEELEN 2, K. LILLYCROP 1, 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 Wageningen Centre for Food Sciences and Wageningen University, Wageningen, The Netherlands

Induction of a modified metabolic phenotype in the offspring by feeding a protein-restricted (PR) diet during pregnancy in the rat involves DNA hypomethylation and altered covalent histone modifications leading to increased expression of specific genes (Lillycrop et al. 2005a, b). 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 can be explained by differential expression of long-chain acyl-CoA desaturase and other proteins involved in fatty acid metabolism.

We investigated the effect of feeding a PR diet during pregnancy on the expression of histone methyltransferases and the DNA demethylase MBD2. Rats were fed either a control (18% (w/w) casein) or PR (9% (w/w) casein) diet both containing 1 mg/kg folic acid, or the PR diet supplemented with 5 mg/kg folic acid from conception to delivery. Dams were fed standard chow (AIN-76A) during lactation. Litters were reduced to eight at birth and offspring were weaned onto chow at postnatal day 28 and killed 6 d later. Gene expression was measured by semi-quantitative real-time RT-PCR (Lillycrop et al. 2005a).

There was no significant effect of PR diet on expression of DNMT 3a or 3b (Table 1). However, expression of DNMT 1 was significantly lower (28.6%) in the PR offspring (Table 1). MeCP 2 expression was significantly lower (28.6%) in the PR offspring. The PRF diet prevented reduction in DNMT 1 expression and did not alter the expression of DNMT 3a and 3b.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control (%)</th>
<th>PR (%)</th>
<th>PRF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNMT 1</td>
<td>100.0</td>
<td>82.6*</td>
<td>109.0</td>
</tr>
<tr>
<td>DNMT 3a</td>
<td>100.0</td>
<td>96.7</td>
<td>103.0</td>
</tr>
<tr>
<td>DNMT 3b</td>
<td>100.0</td>
<td>96.3</td>
<td>96.8</td>
</tr>
<tr>
<td>MBD 2</td>
<td>100.0</td>
<td>93.5</td>
<td>ND</td>
</tr>
<tr>
<td>MeCP 2</td>
<td>100.0</td>
<td>71.4</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values significantly different from the control group are indicated by * (P < 0.05). PR, protein-restricted diet; PRF, protein-restricted diet supplemented with 5 mg/kg folic acid from conception to delivery.

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. n-3 fatty acids may protect against CVD in man.
The effects of altering the dietary \( \omega-3 \) fatty acid ratio on insulin sensitivity, lipoprotein size and postmenopausal lipids in older UK men and women. The OPTILIP study. By M.D. BRIGGS, T.A. HADDEN, G. T. ADAMS, F. LEWIS, S. SLOANE, S. BURTON and M. LEWIS, Department of Nutrition and Food Science, University of Surrey, Guildford, Surrey, UK, GU2 7XH and B.A. GRIFFIN, Institute of Human Nutrition, University of Southampton, Southampton, UK.

Fish oil has shown beneficial effects in reducing mortality from CVD. Atherosclerosis, which underlies CVD, has an inflammatory component involving the production of pro-inflammatory cytokines and the formation of atherosclerotic plaques. Postmenopausal women, in whom CVD risk is increased, are often insulin resistant and have raised postprandial lipaemia.

While the CD36 SNP 33137 GG genotype experienced a 1.5-fold increase in postmenopausal lipoprotein particle size, the effect of fish oil intervention on HDL concentration was unchanged. This knowledge of the influence of CD36 SNP may improve the efficacy of fish oil as a hypolipidaemic agent in middle-aged men.

Supported by the Food Standards Agency (project no. N2014/5).

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) were 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 individual 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<sup>-/-</sup> mice are resistant to obesity-induced insulin resistance following a high-fat diet. By S. Toomey, J. Browne and H.M. Roche, Natigenomics 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<sup>-/-</sup> mice, which have a compromised macrophage response. Eight C57BL/6 controls and eight IL-1R1<sup>-/-</sup> 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 adipo 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<sup>-/-</sup> 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<sup>-/-</sup> group (P<0.05). Serum TAG concentrations were also reduced in the IL-1R1<sup>-/-</sup> mice; however, this did not reach significance. Microarray gene expression analysis showed that 462 genes were up regulated in the IL-1R1<sup>-/-</sup> mice, the majority of which were involved in metabolism, signalling, transcription and translation, and transport. In contrast, 318 genes mostly 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 regulation of GLUT4 and IRS-1 and down regulation of TNFα, MCP-1, MIP-1a, and IL-6 in the IL-1R1<sup>-/-</sup> group. The Table shows gene expression analysis.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control Mean SEM</th>
<th>IL-1R1&lt;sup&gt;-/-&lt;/sup&gt; Mean SEM</th>
<th>IL-1R1&lt;sup&gt;-/-&lt;/sup&gt; Mean SEM</th>
<th>P value</th>
<th>IL-1R1&lt;sup&gt;-/-&lt;/sup&gt; Mean SEM</th>
<th>IL-1R1&lt;sup&gt;-/-&lt;/sup&gt; Mean SEM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT4</td>
<td>1.00 ± 0.34</td>
<td>1.00 ± 0.192</td>
<td>1.00 ± 0.222</td>
<td>0.0307</td>
<td>1.00 ± 0.196</td>
<td>1.00 ± 0.279</td>
<td>NS</td>
</tr>
<tr>
<td>IRS-1</td>
<td>1.472 ± 0.514</td>
<td>1.241 ± 0.474</td>
<td>0.366 ± 0.202</td>
<td>0.0440</td>
<td>0.0897</td>
<td>0.0289</td>
<td>NS</td>
</tr>
<tr>
<td>TNFα</td>
<td>0.00</td>
<td>0.002</td>
<td>1.00 ± 0.02</td>
<td>0.0006</td>
<td>0.0006</td>
<td>0.0006</td>
<td>0.0002</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>2.866 ± 0.303</td>
<td>1.685 ± 0.166</td>
<td>0.610 ± 0.096</td>
<td>0.043</td>
<td>0.043</td>
<td>0.043</td>
<td>NS</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. Bremmer1, G. Horgan2 and G.E. Lobley1, 1Rowett Research Institute, Bucksburn, Aberdeen, UK, AB21 9SB and 2Biomathematics 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 (Haltin & 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 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 days 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/kg). 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 vs. 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. 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.


The SH2-B gene is associated with serum leptin and body fat in normal females. By S.D. O’DELL1, Y. JAMSHIDI2, H. SNIEDER1,4, D. GE3 and T.D. SPECTOR1, 1Nutrition Food and Health Research Centre, King’s College London, London, UK, SE1 9NH, 2Division of Clinical Developmental Sciences, St George’s, University of London, London, UK, SW17 ORE, 3Georgia Prevention Institute, Department of Pediatrics, Medical College of Georgia, Augusta, GA, USA and 4Twin Research and Genetic Epidemiology Unit, St Thomas’s Hospital, London, UK, SE1 7EH

Leptin signals nutritional status to centres in the hypothalamus via the JAK-STAT pathway, acting as an important regulator of adiposity. The SH2 domain-containing putative adapter SH2-B binds via its SH2 domain to the leptin receptor, LepRb, which acts as an important regulator of adiposity. The SH2 domain-containing putative adapter SH2-B binds via its SH2 domain to the leptin receptor, LepRb, which acts as a substrate for the Janus kinase (JAK) family of protein tyrosine kinases. LepRb phosphorylation then allows recruitment of the SH2-B interaction partner, SHP-2, to the receptor. SHP-2 is a Grb2-associated SH2-domain containing protein tyrosine phosphatase, which has a role in the regulation of insulin receptor tyrosine kinase activity (Ren et al., 2005). The SH2-B gene is associated with serum leptin and body fat in normal females (Duan et al., 2004), and also with the metabolic syndrome, insulin resistance and type 2 diabetes (Duan et al., 2004b) as well as severe leptin resistance, hyperphagia and obesity (Ren et al., 2005). Thus, SH2-B is a key positive regulator of leptin action.

The aim of the present study was to determine whether single-nucleotide polymorphisms (SNP) in the SH2-B gene are associated with measures of body fat and insulin sensitivity in 2455 normal women from the St Thomas’s UK Adult Twin Registry (TwinUK). We utilised Caucasian data from the twinmap project (www.twinmap.org) to identify tagging SNP. Five SNP (minor allele frequency MAF > 0.06), rs4788102, rs8055982, rs7498665, rs7359397 and rs3888190 were selected and pairwise in perfect linkage disequilibrium (pairwise r² = 1.00), so one tSNP rs7498665 (Ala484Thr) was selected to tag the entire gene. Genotyping was by pyrosequencing (Biotage, Sweden).

<table>
<thead>
<tr>
<th>Variables</th>
<th>No.</th>
<th>11/12/22</th>
<th>Mean</th>
<th>sd</th>
<th>Mean</th>
<th>sd</th>
<th>Mean</th>
<th>sd</th>
<th>Genomic model</th>
<th>Variance (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>931/164/144</td>
<td>24.96</td>
<td>4.40</td>
<td>24.76</td>
<td>4.38</td>
<td>24.85</td>
<td>4.24</td>
<td>Co-dominant</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>931/164/144</td>
<td>64.61</td>
<td>11.76</td>
<td>65.39</td>
<td>11.87</td>
<td>66.06</td>
<td>11.48</td>
<td>Additive</td>
<td>0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total fat (%)</td>
<td>903/115/337</td>
<td>22.90</td>
<td>8.77</td>
<td>23.58</td>
<td>8.90</td>
<td>23.86</td>
<td>8.52</td>
<td>Dominant</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>944/175/347</td>
<td>13.96</td>
<td>11.58</td>
<td>16.67</td>
<td>12.11</td>
<td>17.02</td>
<td>11.64</td>
<td>Additive</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>939/142/333</td>
<td>77.70</td>
<td>10.08</td>
<td>79.51</td>
<td>10.23</td>
<td>78.90</td>
<td>9.85</td>
<td>Dominant</td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central fat (%)</td>
<td>902/143/335</td>
<td>1.29</td>
<td>0.71</td>
<td>1.35</td>
<td>0.74</td>
<td>1.32</td>
<td>0.67</td>
<td>Co-dominant</td>
<td>0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA</td>
<td>352/41/126</td>
<td>1.18</td>
<td>0.68</td>
<td>1.28</td>
<td>1.11</td>
<td>1.32</td>
<td>0.75</td>
<td>Co-dominant</td>
<td>0.39</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HOMA, homeostasis model assessment of insulin resistance.

Carriers of the minor (Thr) allele had significantly higher serum leptin, total fat mass, waist circumference and weight. There was no association with the HOMA insulin resistance score. The Ala484Thr polymorphism appears not to influence protein structure and function and is likely to be in LD with an unidentified functional variant in the 10 kb SHP-2 gene or regulatory regions. The present results support a role for SH2-B in modulating the regulation of body weight and fat by leptin in this female population.

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


Abstact of Original Communications

2006
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>-Cryptoxanthin (%)</th>
<th>-Carotene (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinach</td>
<td>18.0</td>
<td>3.7</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Broccoli</td>
<td>38.4</td>
<td>0.8</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Red pepper</td>
<td>97.7</td>
<td>11.9</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>

Three independent experiments.

The carotenoids analysed included lutein, zeaxanthin, -cryptoxanthin, lycopene and -carotene. Digested spinach contained the highest amount of lutein (2531 µg/100 g); however, there was a low transfer of lutein into lipid micelles (18.0%). Digested red pepper contained a much lower amount of lutein (78.6 µg/100 g) but had similar levels of lutein in both the digestate and micellarised fractions (97.7% micellarised). There was a high percentage micellarisation of zeaxanthin from red pepper and sweet potato. Though zeaxanthin was detected in spinach and -cryptoxanthin in both spinach and broccoli, there was no detectable transfer of these carotenoids into micelles. Similarly, lycopene was detected in red pepper only but none of this carotenoid was micellarised. There were similar levels of -carotene transferred from the digestate to the micellar fractions of spinach, red pepper and sweet potato.

Previous studies have demonstrated the potential health benefits associated with carotenoids; however, information regarding the digestion and absorption of these compounds remains limited. The present study simulated the human digestion process in order to estimate the amount of carotenoids from the various vegetables that becomes accessible for absorption by the human mucosa.


Replacing breakfast and snacks or desserts 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, 1 Department of Human Sciences, Loughborough University, Leicester, UK, LE11 3TU and 2 Nutritional Epidemiology Group, Centre for Epidemiology and Biostatistics, University of Leeds, Leeds, UK, LS2 9EN

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 11.7) 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 cereals 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 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 day dietary records, completed on one weekend day and two week days. The first day 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 < 0.001) (control = 740 kJ/d, cereal = 1418 kJ/d, cereal + exercise = 1092 kJ/d). Significant reductions in weight, body fat and body shape were observed in the two intervention groups (about 0.82 kg, cereal = 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.
Preliminary study to investigate what characteristics underlie successful weight loss? By C.J. BYE1,2, J.H. LAVIN2, S. WHYBROW3 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 (Diogenes; 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 emotional 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 diet preoccupations, e.g. awareness of the energy and macronutrients 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. 2004) 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.

Table: Activity data

<table>
<thead>
<tr>
<th>Activity</th>
<th>Successful (n=5)</th>
<th>Less successful (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Number of steps taken per day</td>
<td>9312</td>
<td>8475</td>
</tr>
<tr>
<td>Percentage of time spent active</td>
<td>21.4%</td>
<td>8.5</td>
</tr>
<tr>
<td>Physical activity level</td>
<td>0.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* Time spent active consisted of walking, running, standing and climbing stairs.
† Actual time 5h 13 min.
‡ Actual time 3h 54 min.

The higher level of diet preoccupation found in successful respondents suggests that successful dieters are more aware of the nutritional composition of foods, leading them to consciously choose foods lower in fat and energy. There appeared to be differences in dietary intake and activity levels; however, further research with a larger sample is required to explore this further.

Results showed that G. lucidum has a synergistic or additive effect with pencillin against both MRSA and MSSA, as evidenced by an up to 32-fold lowering of the MIC of pencillin. 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).

ALL CHANGE: design and implementation of a primary school-based lifestyle intervention. By A.M. CRAIGIE1, J.J.P. BELCHET2, A. GREENE1, S.A. GREENE1, G. KENNEDY2, F. KHAN1, E.M. ROBERTS3 and A.S. ANDERSON1, 1Centre for Public Health Nutrition Research and 2Vascular Diseases Research Unit, University of Dundee, Ninewells Hospital, Dundee, UK, DDI 9ST

The intervention approach was developed using ‘action research’ methods and targeted dietary intake (increasing fruit, vegetable and n-3 fatty acid intake and reducing saturated fat, soft drink and non-milk extrinsic sugar intake), physical activity and inactivity.

The formative research comprised a review of the relevant literature, two focus groups with 8–10-year-old children, and four focus groups with teachers in order to identify barriers and opportunities to encourage healthful diet and physical activity practices. The children’s focus groups explored current understanding and views on: ‘healthy eating’, sport, exercise, activity and inactivity, perceived control over lifestyle choices, and methods of promoting behavioural changes in diet and physical activity. Focus groups with teachers examined existing school practices, and sought feedback on intervention concepts.

The following themes emerged from the formative research:

1. Children reported some degree of control over their food choices and perceived the presentation of food to be important. Tasting new foods and trying recipes was highlighted;
2. To encourage the achievement of ‘5-a-day’, children suggested ‘reference guides’ to define what would be considered a fruit or vegetable (i.e. not potatoes), as well as ‘record cards’ to record their fruit and vegetable portions;
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;
4. Teachers raised the issue of trying to fit extra health lessons into an already busy curriculum and did not have confidence in the uptake of after-school activities;
5. Literature regarding the encouragement of n-3-rich fish consumption in children was sparse, and suggested ideas from the focus groups were limited;
6. Teachers considered the involvement of the parents important to the success of the intervention, and this was supported by the literature.

These findings informed the development of the ALL CHANGE intervention which was implemented in two schools over 8 months. Teachers were provided with eight classroom lessons incorporating take-home activities and two physical education 5-min mini-lessons. These comprised both new and tried-and-tested materials from interventions including ‘5 a day the Isaiah Street Way’ (Anderson et al. 2005) and ‘Eat Well and Keep Moving’ (Gortmaker et al. 1999). The lessons incorporated take-home challenges including supermarket visits to identify and try new fruit and vegetables, ‘5-a-day’ record sheets, and classroom lessons incorporating tasting sessions and food preparation. Children also received a minimal-contact summer intervention incorporating quizzes and tasks that encouraged parental involvement. ‘Supajus’, an orange juice fortified with n-3 fatty acids was also provided as a healthier alternative to soft drinks.

While the published literature forms an important source on which to base intervention designs, the findings of the present study highlight the importance of carrying out further formative research with target groups. Defining contemporary challenges, child preferences, teacher views and the practicalities of implementation are vital steps in informing the design of a school-based intervention.

Funded by the Chief Scientist Office (CZB/0496).


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

Very little data are available on the nutritional status and food intakes of the African-Caribbean population living in the UK. In the most recent National Diet and Nutrition Survey for adults the results have not been analysed according to ethnic group and anthropometric data are not collected in the Expenditure and Food Survey. However, there is evidence that African-Caribbeans are more likely to suffer from certain diet-related conditions such as stroke (Department of Health, 2001). It was therefore decided to conduct a study on food intakes and the nutritional status of African-Caribbeans living in Staffordshire, in collaboration with Staffordshire County Council. Findings on the health status of the subjects and some of the food intake results are presented here.

Volunteers were recruited by placing posters in public places in Staffordshire and contacting local community groups. Data were collected using face-to-face interviews. A questionnaire was developed to collect demographic, health and lifestyle data. A food-frequency questionnaire, previously developed by Sharma et al. (1996) for a similar population, was modified to assess the dietary intakes 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 44% of subjects were obese (BMI ≥30 kg/m²), with only 18% falling in the normal range. Although the risk of complications for central obesity may vary according to ethnicity (Garrow, 2000) over two-thirds of subjects were in the very high-risk category (WC>102 cm in men and >88 cm in women). One-third of the sample (n 14) suffered from at least one health condition and seven of those suffered from two or more conditions. The most commonly reported health conditions were diabetes (n 9) and hypertension (n 8). Only two subjects reported that they smoked whereas twenty-five subjects (64%) consumed alcohol. However, intakes were low, with none of the subjects exceeding government recommendations.

Preliminary analysis of the dietary intake data showed that although intakes of fruit and vegetables exceeded average intakes of UK adults (Henderson et al., 2002), only 31% of the sample were meeting the daily recommendation of five portions of fruit and vegetables. The majority of subjects (82%) used fresh, rather than convenience foods, when preparing meals. Traditional West Indian dishes, such as rice and peas, patties and soups, formed an important part of the diet. Aspects of the diet that need to be addressed include high energy intakes and the widespread use of salt or All-Purpose seasoning.

Although this was a small study and volunteers may have had a particular interest in their diet and health status, the high prevalence of obesity is a matter of concern. It is important to take the positive aspects of the diet into account when developing health education strategies for this group.

This study was supported by funding from Staffordshire County Council.


Development of a computer-based tool for measuring schoolchildren’s diets. By M.S. TAYLOR, C. SUMMERBELL, A. ADAMSON, B. LANG and S. CROOKS, Food And Nutrition Group, University of Teesside, Middlesbrough, UK, TSI 3BA and Human Nutrition Research Centre, School of Clinical Medical Sciences, William Leech Building, University of Newcastle, Newcastle upon Tyne, UK, NE2 4HH.

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.

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 (Rickett & 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).

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. It does, however, agree with 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 (Petty & Cacioppo, 1986).

The public can best be persuaded by different types of wording in nutrition education messages.
n-3 Long-chain polyunsaturated fatty acid intake from fish and depressed mood: non-linear or confounded association? By H. AL-SHAMARI 1, P. AMUNA 1, I. TEWFIK 1, A. BUMEJjad 2 and F. ZOTOR 3.

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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 sub-sample of 994 (ninety-nine male; ninety-five female) were measured over a 12-week period between 2003 and 2004 and also using a 3-d diet 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 32.99% classified as overweight (85th percentile, i.e. BMI >25 kg/m2) and 31.96% as clinically obese (90th percentile, i.e. BMI >30 kg/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’ 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 activity 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-communable diseases unless appropriate interventions are implemented to reverse the trend.
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. BERRY1, U. MULLA1, P.J. CHOWIENZCYK2 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’ 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. 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 subjects were used to estimate the RNAE of young British individuals as shown in the Table below.

<table>
<thead>
<tr>
<th>NEAP (g/mEq per d)</th>
<th>n</th>
<th>Mean</th>
<th>Median</th>
<th>Range</th>
<th>Spearman’s ρ correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1704</td>
<td>48</td>
<td>45.7</td>
<td>45.1</td>
<td>6.4–103.10</td>
<td>R 0.584; P&lt;0.001</td>
</tr>
<tr>
<td>NAEindirect (mEq/d)</td>
<td>1684</td>
<td>44.9</td>
<td>14.3</td>
<td>4.3</td>
<td>2.14–102.84</td>
</tr>
</tbody>
</table>

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 (N02010) was funded by the Food Standards Agency.

References

- Vokes L, R.H.T. Gannon, D.J. Millward, D.P. Lovell, H.M. Macdonald, L. A. Frassetto, T. Remer and S.A. Lanham-New. Department of Nutrition and Diabetics, Queen Alexandra Hospital, Cosham, Portsmouth, UK. P06 SLY. 2Centre for Nutrition and Food Safety. 3Post Graduate Medical School, University of Surrey, Guildford, UK. GU2 7XH. 4Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen, UK. AB25 ZD. 5Department of Medicine and General Clinical Research Center, University of California, San Francisco, CA, USA. 6Department of Nutrition and Health, Research Institute of Child Nutrition, Dortmund, Germany.

Indirect estimates of net acid excretion and net rate of endogenous non-carboxylic 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. MILLWARD2, D.P. LOVELL3, H.M. MACDONALD2, L.A. Frassetto3, T. Remer3 and S.A. Lanham-New2. 1Department of Nutrition and Diabetics, Queen Alexandra Hospital, Cosham, Portsmouth, UK. P06 SLY. 2Centre for Nutrition and Food Safety. 3Post Graduate Medical School, University of Surrey, Guildford, UK. GU2 7XH. 4Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen, UK. AB25 ZD. 5Department of Medicine and General Clinical Research Center, University of California, San Francisco, CA, USA. 6Department of Nutrition and Health, Research Institute of Child Nutrition, Dortmund, Germany.

Adults ingest approximately 50–100 mEq H+ ions from sulfur-containing amino acids and other dietary components in the Western diet, resulting in mild but chronic metabolic acidosis. Increased dietary H+ ingestion consequentially increases renal net indirect 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-carboxylic 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 increased 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.

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 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 alkali contributors. The difference in the major whole food alkali contributors identified by the two models reflects important but subtle differences in dietary nutrient sensitivity. These differences and previous work (Pynnö 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.

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

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 USA that portion sizes for specific foods, for example, soft drinks, hamburgers, and French fries have increased over the last three decades (Niedens & 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 compared using the Mann–Whitney U test. To test the use of the calculated portion weights, 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 weights 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, 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 also 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).


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 funding the European Food Information 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., 1995; Greenfield & Southgate, 2003). The present research aims to identify how the key user group of nutrition researchers use FCD, 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 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 between national based 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 (PPE/519.441). 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. BORGES2 and A. CROZIER3, 1Centre for Public Health Nutrition Research, Ninewells Hospital, University of Dundee, Dundee, UK, 2Dundee Institute of Biological and Life Sciences, University of Dundee, Dundee, UK, and 3Institute 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) 2000–2001 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. The mean AOC intake of the UK population from fruit and vegetables was 670 μmol/d. 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 2000–2001, it was found that of the 123 subjects in Scotland, 113 subjects consumed less than 400 g fruit and vegetables per d. 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 top five fruit and vegetables in the Scottish NDNS sample.

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.

Table: Effects of Standard Mixed Meal and High-Fat Meal on Plasma Adiponectin Concentrations (ng/ml).

<table>
<thead>
<tr>
<th>Time</th>
<th>Baseline</th>
<th>1h</th>
<th>3h</th>
<th>4h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Mean (ng/ml)</td>
<td>Mean (ng/ml)</td>
<td>Mean (ng/ml)</td>
<td>Mean (ng/ml)</td>
</tr>
<tr>
<td>High-fat meal</td>
<td>Young lean</td>
<td>9.9 0.9</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Young obese</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Older lean</td>
<td>10.7 0.7</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Older obese</td>
<td>7.4 0.6</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Standard mixed meal</td>
<td>Young lean</td>
<td>10.3 1.2</td>
<td>9.8**</td>
<td>9.0 0.9</td>
</tr>
<tr>
<td></td>
<td>Young obese</td>
<td>7.3 0.6</td>
<td>7.3 0.6</td>
<td>7.4 0.6</td>
</tr>
<tr>
<td></td>
<td>Older lean</td>
<td>10.5 0.8</td>
<td>10.2 0.9</td>
<td>10.4 0.8</td>
</tr>
<tr>
<td></td>
<td>Older obese</td>
<td>7.7 0.6</td>
<td>7.7 0.6</td>
<td>7.9 0.6</td>
</tr>
</tbody>
</table>

** 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.

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 influence 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 fasting 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 (SE 0.4) kg/m²; n = 14) and obese (BMI 32.5 (SE 0.5) kg/m²; n = 13), young men (age 31 (SE 0.8) years; and lean (BMI 23.6 (SE 0.3) kg/m²; n = 13) and obese (BMI 32.5 (SE 0.5) kg/m²; n = 16) older men (age 57 (SE 0.8) years) were fasted overnight and received either a high-fat meal or a standard mixed meal separated by a 2 d interval. To replicate times of peak adiponectin secretion previously published in the literature, plasma adiponectin was measured at baseline and at 4 h after ingestion of the high-fat meal and at 1 and 3 h after ingestion of the standard meal. Fasting plasma adiponectin concentrations were significantly lower in obese than in lean men (7.9 (SE 0.5) v. 10.4 (SE 0.5) ng/ml, P<0.001, respectively). There was no significant difference in fasting plasma adiponectin concentrations between young and older men (9.2 (SE 0.6) v. 9.8 (SE 0.5) ng/ml, P=0.5, respectively). In young lean men only, plasma adiponectin concentrations decreased significantly compared with baseline 1 h after consumption of the standard mixed meal but returned to baseline levels 3 h postprandially. Plasma adiponectin concentrations did not change significantly after consumption of the high-fat meal in any group.

The Table shows the effects of standard mixed meal and high-fat meal on plasma adiponectin concentrations (ng/ml).

The present study is funded by the European Commission, Quality of Life and Management of Living Resources, Action line 6.1, Age-related illnesses and health problems (QLK6-2002-02288). L.M.W.’s laboratory is funded by the Scottish Executive Environment and Rural Affairs Department (GEERAD).
Red grape juice protects against exercise-induced DNA damage.

By A.M.C.E. JENKINSON1, S.J. DUTHIE1, J.A.M. KYLE2, and G.G. DUTHIE1,2

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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 (SCG) version 5.4). Their children have been followed up for 5 years. A FFQ was completed by 1751 mothers during pregnancy, a respiratory questionnaire was 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 carried out 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.

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. McNEILL1, S. MARTINDALE1, P.J. HELMS1 and A. SEATON1

Department of Environmental and Occupational Medicine, University of Aberdeen, Aberdeen, UK; AB25 2ZP and Department of Child Health, University of Aberdeen, Aberdeen, UK; AB25 2ZG

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 flexors 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 (CK) (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.

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 intake were 3.5 (95% CI 1.4, 8.7) (P=0.008) times more likely to be of the persistent wheeze 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 carotenoids). No associations were found with the children’s own diet for these nutrients. Previous results from the study show 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.

This research was funded by Asthma UK.


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Plasma CK activity (U/l)  
<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>Red grape juice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>49.5</td>
<td>8.4</td>
<td>–</td>
<td>72.0</td>
<td>13.2</td>
<td>87.1</td>
</tr>
<tr>
<td>Placebo</td>
<td>51.0</td>
<td>9.0</td>
<td>–</td>
<td>111.2*</td>
<td>18.8</td>
<td>135.4*</td>
</tr>
<tr>
<td>Placebo</td>
<td>66.2</td>
<td>10.4</td>
<td>–</td>
<td>111.2*</td>
<td>18.8</td>
<td>135.4*</td>
</tr>
<tr>
<td>Placebo</td>
<td>66.2</td>
<td>10.4</td>
<td>–</td>
<td>111.2*</td>
<td>18.8</td>
<td>135.4*</td>
</tr>
</tbody>
</table>

Mean value was significantly different from baseline: *P<0.05, **P<0.01.

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 (SEERRAD).
Low-carbohydrate diets have proved popular with the public as a weight-loss strategy. Limited evidence suggests that when followed over the long-term, low-carbohydrate diets may have beneficial effects on diet-related diseases such as type 2 diabetes and the metabolic syndrome, though risks are also associated with such diets. 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 prostate cancer, even after adjustment for age and geographical location. We did not find any support for this hypothesis. Our analyses did not confirm any association between exposure to low Se environments and risk of prostate cancer (P = 0.38). There was 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 polymorphism and prostate cancer risk. 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 areas entering the large bowel.
Intracellular trafficking and functional activity of splice variants of the zinc transporter SLC30A5. By R.A. VALENTINE1, K.A. JACKSON2, J.C. MATHERS3 and D. FORD1, 1School of Applied Sciences, Northumbria University, Newcastle upon Tyne, UK, NE1 8ST, 2Institute for Cell and Molecular Biosciences and 3School of Clinical Medical Sciences, University of Newcastle upon Tyne, UK, NE2 4HH

Zn is an essential micronutrient and is involved in a wide range of cellular processes through functions that include playing a critical structural role in many proteins and also acting as a catalytic component in enzymes. It is therefore important to elucidate the molecular mechanisms of Zn homeostasis. Mammalian Zn transporters are classified in two major families: the SLC30 (ZnT) family and the SLC39 (ZIP) family. The function of Zn transporters in the SLC30 family is to reduce cytosolic Zn concentration, either by intracellular sequestration in subcellular compartments or by efflux across the plasma membrane, and members of the SLC39 family work in the opposite direction, to increase cytoplasmic Zn concentration, but detailed functional characterisation, particularly of the SLC39 family, is lacking.

The aim of the study was to examine the functional properties and the role in Zn homeostasis of two splice variants of the SLC30A5 gene product by examining the effect of their over-expression in Caco-2 cells on the activity of a Zn-responsive reporter gene and by determining their patterns of intracellular localisation in response to changes in the extracellular Zn concentration.

To examine the effect of the SLC30A5 splice variants on intracellular Zn concentration and/or distribution, Caco-2 cells (human small intestinal cells) were transfected with a plasmid construct (pEGFP-Clontech) from which one or other of the SLC30A5 splice variants, including a C-terminal green fluorescent protein (GFP) tag, was expressed, plus a β-galactosidase reporter construct (pBLUE-TOP11; Invitrogen) driven by the Zn-responsive metallothionein Zn (MT2a) promoter. Control cells were co-transfected with the reporter construct plus vector (pEGFP) only. β-Galactosidase activity was measured using the substrate chloro-methyl β-D-galactosidase in cell lysates prepared 48 h post-transfection.

Expression of either splice variant of SLC30A5 increased β-galactosidase activity, indicating increased transfection from the Zn-responsive MT2a promoter (Fig. 1).

<figure>
  <image>
  <caption>Fig. 1. Mean β-galactosidase activity (in 6) in Caco-2 cells transiently co-transfected with a β-galactosidase reporter construct driven by the Zn responsive MT2a promoter alone or MT2a promoter plus SLC30A5 splice variant A or B with either (i) 5 μM or (ii) 100 μM ZnCl₂ added to the extracellular medium. Error bars represent SEM. *** P≤0.001 one-way ANOVA and Bonferroni's post-hoe test</caption>
</figure>

To examine the intracellular distribution of both SLC30A5 splice variants at different extracellular Zn concentrations, Caco-2 cells transfected with only the plasmid constructs driving expression of the corresponding GFP-tagged proteins, as described earlier, were fixed using methanol and viewed by confocal laser scanning microscopy 48 h post-transfection. Cells expressing the SLC30A5 splice variant A showed staining consistent with plasma membrane localisation under depleted Zn conditions (nominal concentration 0 μM), but at 100 μM extracellular Zn there was no apparent staining of the plasma membrane and most staining appeared to co-localise with rhodamine-labelled wheat germ agglutinin (Rh-WGA), a marker of the Golgi apparatus. Conversely, the data indicated that increasing the Zn concentration of the extracellular medium had an opposite effect on the subcellular localisation of SLC30A5 splice variant B, which appeared to localise partially to the plasma membrane at 100 μM Zn but co-localised with Rh-WGA at the lower Zn concentration.

The increased activity of the Zn-responsive MT2a promoter when either splice variant of SLC30A5 was co-expressed in Caco-2 cells indicates increased total intracellular Zn concentration or altered intracellular Zn partitioning. Neither splice variant appeared to localise to the nuclear membrane at the extremes of extracellular Zn concentration tested, so the findings would not support the premise that either of the splice variants acts to sequester Zn in intracellular compartments or to efflux Zn across the plasma membrane. The accepted dogma that Zn transporters of the SLC30 family act in such a way to reduce cytosolic Zn concentration must, therefore, be challenged. The opposing patterns of redistribution in response to changes in extracellular Zn concentration suggest distinct functions of SLC30A5 splice variants in the control of either systemic and/or intracellular Zn homeostasis.

The apolipoprotein B signal peptide insertion/deletion polymorphism and within-pair differences in lipid levels in twins. By L.F. MASSON1, A. CUMMING2, C. TUYA3, S. WOOD4, K.A. RANCE4, 1Department of Public Health, 2Molecular and Cell Biology, 3Medicine and Therapeutics, 4Rowett Research Institute, Aberdeen, UK, AB21 9SB and 5Environmental & Occupational Medicine, University of Aberdeen, Aberdeen, UK, AB25 2ZD

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 a 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>Within-pair differences in MZ pairs</th>
<th>Within-pair differences in MZ and DZ pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total cholesterol (mmol/l)</td>
<td>LDL-cholesterol (mmol/l)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>95% CI</td>
</tr>
<tr>
<td>I/I</td>
<td>38</td>
<td>0.37</td>
</tr>
<tr>
<td>I/D and D/D</td>
<td>40</td>
<td>0.30, 0.51</td>
</tr>
</tbody>
</table>

* P value for difference 0.051 0.063 0.083 0.133

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, 30/32 Hyde Terrace, Leeds, UK, LS2 9LN

Genetic sensitivity to the bitter taste of phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP) has been linked with 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., 2000; 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 super-tasters 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.

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 cruciferous 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 super-tasters 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. FULLER1, V. RUNGAPAMESTY2, B. RATCLIFFE3, P. LOUIS4 and A.J. DUNCAN1, 1The Macaulay Institute, Craigiebuckler, Aberdeen, UK, AB15 8QH, 2The Robert Gordon University, Aberdeen, UK, AB25 STB 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 (Krod 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 day for 21 days (period 1) and a further six volunteers received no supplementation (control). Treatment allocations were reversed for period 2 which also lasted 21 days. Faecal samples were obtained before, and 16 days after, the start of the supplementation during each period. At the end of each period each volunteer consumed two meals, separated by 2–4h, 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.

Facial 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).

Prolonged cooking of brassica vegetables reduced the conversion of glucosinolates to isothiocyanates, and may thus diminish the health benefits of brassica. Enhancing colonic populations of Bifidobacteria did not result in increased uptake of isothiocyanates following the consumption of cooked cabbage.

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


Does ascorbic acid protect type 2 diabetes subjects from hyperglycaemia-induced endothelial dysfunction? By S.W. CHOI1, I.F.F. BENZIE1, C.S.Y. LAM2, S.W.S. CHAT3, D.C. YU2, J.J. Kwan2, Y.H. TAN2, G.S.P. YEUNG2, V.T.F. YEUNG2, G.C. WOO1, J.J. STRAIN2 and B.M. HANNIGAN3, 1Faculty 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 atherosclerosis. Increased ICAM-1 is believed to pre-date lesion formation. Plasma levels of cleaved 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 (s.d. 10.6) years. HbA1c, plasma glucose (FPG), AA, soluble ICAM-1 and VCAM-1 were measured (see Table). Pearson's correlational analysis was performed.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c (%)</td>
<td>7.8</td>
<td>1.5</td>
<td>5.0–14.0</td>
</tr>
<tr>
<td>FPG (mM)</td>
<td>8.0</td>
<td>2.7</td>
<td>3.3–17.1</td>
</tr>
<tr>
<td>AA (µM)</td>
<td>47.5</td>
<td>20.1</td>
<td>6.0–136.0</td>
</tr>
<tr>
<td>Soluble ICAM (ng/ml)</td>
<td>639</td>
<td>207</td>
<td>237–1371</td>
</tr>
<tr>
<td>Soluble VCAM (ng/ml)</td>
<td>1195</td>
<td>438</td>
<td>580–4432</td>
</tr>
</tbody>
</table>

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 mM (‘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 mM/1 (‘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=0.857; P<0.0001) and HbA1c (r=0.482; P<0.001). No such relationship was seen in men with acceptable glycemic control, or with higher AA even if glycemic control was poor. No correlation of FPG or AA with VCAM-1 was seen.

The observed association between poor glycemic 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 glycemic control and AA status is better. Good glycemic 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 glycemic control may help identify type 2 DM men at high risk of vascular complications. Long-term follow up is underway.


Cu is an important cofactor in many different biological processes. Despite this, suitable biomarkers for predicting the Cu status of an individual have yet to be identified. This makes dietary recommendations difficult to set. To compound this, there is currently little information available on the genetic control of the homeostatic mechanisms regulating Cu status.

Our ultimate aim is to define the genetic regulation of Cu homeostasis using in vitro cell systems representative of key tissues (liver and intestine) involved in Cu homeostasis, and to identify Cu status biomarkers. In addition by comparing results obtained from a lymphocyte cell line with those from the liver and intestine cell, we are seeking to identify a robust biomarker that can be measured in an accessible tissue (such as blood) that can be functionally related to Cu-induced effects in remote tissues.

The outcomes of this project should provide a mechanistic understanding of Cu homeostasis and the means of determining and/or predicting deficiency or overload in individuals.

By optimising our in vitro cell-culture system using low O2 tensions, the experiments are performed in an environment that more accurately mimics the physiological conditions of the cells in vivo. We have combined this design with transcriptomic and proteomic analyses, to study time- and dose-dependent affects of Cu on the gene expression in these systems.

Transcription analysis of several genes known to be critical to Cu homeostasis revealed no clear-cut response to varying physiological Cu exposures, with the notable exception of the MT2a gene which displayed both time- and dose-dependent changes (Fig. 1 and Fig. 2). Comparison between Caco-2 and HepG2 cell models revealed cell-line-specific differences in the scale and time course of the response.
铁吸收在有炎症性肠病的患者中是正常的，但在没有贫血的情况下，铁的缺乏是无法检测到的。这可能是由于铁的需要，或者患者有铁缺乏时没有补充铁。在这种情况下，铁的吸收可能与其他（未知）的饮食成分有关。如果一组持续的膳食补充可以被识别出来，那么就可以推断出这种饮食补充是铁缺乏的标志。

1Nutrition and Epigenetics Group, Division of Vascular Health, Rowett Research Institute, Aberdeen, UK. 2Aberdeen University, 11200 SW 8th Street, Miami, FL 33199, 2Cancer Research UK Carcinogenesis Group, Paterson Institute for Cancer Research, University of Manchester, Manchester, UK. M20 4BX

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 under 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 Oδ-alkylguanine-DNA alkyltransferase (MGMT), which repairs mutagenic and toxic Oδ-guanine damage were determined by [3H]-labelled oligonucleotide 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).

<table>
<thead>
<tr>
<th>Folate status</th>
<th>Mean (SEM)</th>
<th>Depletion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma (ng/ml)</td>
<td>96.5 (2.2)</td>
<td>96</td>
</tr>
<tr>
<td>Whole blood (ng/ml protein)</td>
<td>1.95 (0.08)</td>
<td>40</td>
</tr>
<tr>
<td>Dorsal colon (ng/mg tissue)</td>
<td>24.6 (1.0)</td>
<td>60</td>
</tr>
<tr>
<td>Liver (ng/mg tissue)</td>
<td>136.2 (6.9)</td>
<td>31</td>
</tr>
</tbody>
</table>

Results are for eight to ten rats per control (F) or folate-deficient (F−) group (* P<0.005 Student's t-test).

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.

Macular pigment response to a xanthophyll supplement of lutein, zeaxanthin and meso-zeaxanthin By R.A. BONE, J.T. LANDRUM, Y. CAO, A.N. HOWARD and D.I. THURNHAM. Florida Int'l University, 11200 SW 8th Street, Miami, FL 33199, University of Ulster, Coleraine, BT52 USA

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 soybean oil, with a meal for 120 days.

Macular pigment optical density (MPOD) was measured in each eye by ficker photometry at least four times prior to supplementation, then twice weekly during the 120 day supplementation period and for the week 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=0.42, P=0.044; Z, R=0.56, P=0.013).

Two additional subjects were recruited for a detailed carotenoids analysis at 42 days. Baseline concentrations of L, Z and MZ for the two subjects respectively were (1) 0.229, 0.044, 0 and (2) 0.148, 0.021 0 and the changes at day 42 were (1) 0.01, 0.043, 0.044 and (2) 0.306, 0.131, 0.145 respectively. 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 6-alkylguanine-DNA alkyltransferase (MGMT), which repairs greater amount of MZ than Z in the supplement, the within-subject plasma responses were similar for the two isomers of Z but very different between subjects. Evidence from a number of studies indicates that meso-zeaxanthin in the retina may enhance the eye's protection against AMD, possibly more so the other two carotenoids (Bhosale & Bernstein, 2005) and these data provide evidence of the plasma responses to a mixed MZ:L supplement.

Age related macular degeneration treatment responds to a xanthophyll supplement of lutein, zeaxanthin and meso-zeaxanthin By R.A. Bone, J.T. Landrum, Y. Cao, A.N. Howard and D.I. Thurnham Florida Int'l University, 11200 SW 8th Street, Miami, FL 33199 University of Ulster, Coleraine, BT52 USA

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.

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Assessment of skin colour pigmentation in patients undergoing ultraviolet B treatment. By A. MAVROEID1,2, F. ONEILL1,2, C. THIND3, A. ORMEROD1, D.M. REID1,2 and H.M. MACDONALD1,2. Osteoporosis Research Unit, University of Aberdeen, Wolfson Hall Hospital, Aberdeen, UK, AB25 2ZD. School of Medicine, University of Aberdeen, Aberdeen, UK, AB25 2ZD and 3 Dermatology Department, Aberdeen Royal Infirmary, Aberdeen, UK, AB25 2ZU.

Vitamin D has long been considered an anomaly in the nutrition world since cutaneous synthesis through sunlight exposure (wavelength 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 sunlight 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 cheekbones 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 how 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. McKEINLEY, S.E.C.M. GILCHRIST, J.V. WOODSIDE, U. CHAKRAVARTHY 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. Carotenoid and vitamin C status may be possible biomarkers. Few studies have been carried out to investigate the relationship between dietary intakes of fruit, 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, ß-cryptoxanthin, ß-carotene, ß-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 number of portions per d and summed to provide a total fruit, total 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, ß-cryptoxanthin, ß-carotene, ß-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 Vuillemier & Keck (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 ß-cryptoxanthin, no other carotenoids were significantly associated with fruit, vegetable and juice intake. Serum ß-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 ß-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 ß-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.

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. 1. Predominant cinnamic acids present in fruits, vegetables and cereals.

Fig. 2. Availability and metabolism of cinnamic acids from strawberries.

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.

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 those without this condition (Barton-Wright & Ellis, 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.

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Dietary protein restriction in the pregnant rat induces altered antioxidant modifications to histones


Diabetes mellitus (DM) is associated with increased oxidative stress (King & Loeken, 2004). However, 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 gene expression control sequences of the GR 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 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 in the liver of the offspring after weaning.

Feeding a protein-restricted 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 gene expression control sequences of the GR 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 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 in the liver of the offspring after weaning.

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 seen in healthy and type 2 DM subjects as expected; fasting, peak and 2 h glucose levels were 5.4 (SD 0.2), 8.8 (SD 1.6) and 5.8 (SD 0.9) mM, respectively, for healthy subjects and 8.3 (SD 2.9), 17.4 (SD 5.5) and 14.6 (SD 2.7) mM, respectively, for DM subjects. The procedure was repeated within 2 weeks with the other treatment. Type 2 DM patients also underwent a standard oral glucose tolerance test followed by oral glucose load, and plasma samples were obtained and assayed for markers of oxidative stress and antioxidant status.

**Materials and Methods**

Healthy and type 2 DM subjects were studied. Healthy subjects and type 2 DM patients were recruited with their informed consent. Healthy subjects took 75 g glucose (25 g/m) in 30 ml water or 30 ml water at 8.30 am, and venous blood was taken at fasting, 30, 60 and 120 min post-ingestion. Type 2 DM patients also underwent a standard oral glucose tolerance test and plasma samples were obtained and assayed for markers of oxidative stress and antioxidant status.

**Results**

For healthy subjects, significant post-ingestion increases (P<0.05) in insulin, C-peptide, FFA and triglycerides were seen in healthy and type 2 DM subjects. Significant post-ingestion increases (P<0.05) in ANOVA for repeated measures were seen in healthy and type 2 DM subjects as expected, fasting, peak and 2 h glucose levels were 5.4 (SD 0.2), 8.8 (SD 0.5) and 14.6 (SD 2.7) mM, respectively, for healthy subjects and 8.3 (SD 2.9), 17.4 (SD 5.5) and 14.6 (SD 2.7) mM, respectively, for DM subjects. Type 2 DM patients also underwent a standard oral glucose tolerance test and plasma samples were obtained and assayed for markers of oxidative stress and antioxidant status.

**Discussion**

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 and H4 is decreased. These new findings suggest that induction of persistent phenotypes in the offspring involves altered DNA modification and specific modifications to the histone structure.

**References**

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. WAHLI¹, 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 t) 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 isoforms 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. GEISSLER², 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, Fallowfield 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 (± 54.7) g/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 7 d. Serum Fe was determined in blood collected before and at 30 min intervals up to 4 h 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.

First, the quantity of blood removed in order to maintain serum ferritin at about 50 microg/l per annum was significantly less (P < 0.001) during treatment with PPI compared with before (see Fig. 1); this equated to removal of 0.95 (± 0.08) v. 0.18 (± 0.06) g Fe before and during PPI. The duration of each recording period was similar (6.3 ± 0.6 (SE) years before PPI v. 4.2 ± 0.3 (SE) years during PPI; P = 0.2). Second, gastric acid suppression, induced by taking a PPI for 7 d, was associated with a significant reduction in serum Fe increase (P < 0.01) following ingestion of the test meal (see Fig. 2). The present results suggest that if the speciation of Fe in the intestinal lumen is disrupted through inhibition of gastric acid then there is the potential to limit the high Fe absorption in HH. Administration of a PPI could be a useful adjunct to phlebotomy in the management of HH.

Fig. 1. Average amount of blood removed per annum.

Fig. 2. Serum Fe increase.
Oral ferrous sulfate is associated with an increase in serum non-transferrin-bound iron and a decrease in plasma ascorbate. By C. HUTCHINSON 1, J. POWELL 2, I. MUDWAY 3 and C. GEISSLER 1. 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. 3Gastrointestinal Laboratory, The Rayne Institute, St Thomas’s Hospital, London, UK, SE1 7EH and 4Pharmaceutical Science Research Division, School of Biomedical and Health Sciences, King’s College, London, UK, SE1 9NN.

We previously found serum non-transferrin-bound Fe (NTBI) after the ingestion of 200 mg FeSO 4 (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 defense system. Ascorbate is a major contributor to plasma antioxidant capacity. In the present study we aimed to (i) confirm the occurrence of serum NTBI after oral Fe, (ii) investigate the change in plasma ascorbate following 200 mg FeSO 4 and (iii) determine whether there is diurnal variation in plasma ascorbate.

Twelve women with IDA completed a study of NTBI and plasma ascorbate after FeSO 4 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 FeSO 4 (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 FeSO 4 was determined using a simple linear fit.

Ascorbate (AA) is a vital nutrient in human physiology. However, due to the potential of Fe and Cu to catalyse AA auto-oxidation (Satoh et al. 1997) its determination presents significant challenges. It is therefore important to eliminate interference from transitional metals during the preparation, storage and the assay of AA. This is particularly important in plasma samples obtained from Fe-supplemented subjects, where significant concentrations of non-transferrin bound-Fe (NTBI) may exist (Hutchinson et al. 2004). The aim of the present study was to investigate whether the metal chelator diethylenetriamine penta-acetic acid (DTPA) would prevent oxidative losses of AA from plasma collected from FeSO 4-supplemented volunteers.

We thank Dr D.Y. Liu for analysis of serum NTBI, Mr H.J.J. Mohamed for assistance in the preparation of plasma ascorbate samples and Mrs C. Dunster for analysis of plasma ascorbate.


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Gene expression in rat liver exposed to acute alcohol and daidzein. By J.Ci. LIN, M.J. ARNO, M.C.Y. WONG, V.R. FREDDY 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 15923 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 transportation 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. MULGREW1, 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 25 μM. However, there was little change in VCAM-1 or ICAM-1 expression with 50 μM-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.

The effect of maternal nutrient restriction between 30 and 80 d 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 Reproductive and Early Life, Schools of Human Development University Hospital and Veterinary Medicine and Science, Sutton Bonnington, University of Nottingham, UK, NG7 2UH

Maternal nutrient restriction between 30 and 80 d 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; n 7) or an NR diet (50% of C; n 11) until 80 d gestation. Thereafter all sheep were fed to 100% calculated metabolisable energy requirements to term (about 12–13 MJ/d near term). Offspring were delivered spontaneously and were reared by their mothers to weaning. From 4 to 12 months of age they were group-housed in a barn (to restrict activity) with increased energy-dense food available to promote fat deposition. At 1 year of age a proportion of these offspring (C, n 7; NR, n 3) had jugular catheters inserted and glomerular filtration rate (GFR) measured by injection of 100 MBq Tc-99m DTPA and 3, 4 and 5 h later heparinised plasma was collected and γ counted. GFR was determined by extrapolation from the decline in radioactivity over time (Gleadhill et al. 1999). All sheep were then humanely euthanased with electrocortical stunning and exsanguination. Renal GR mRNA abundance was quantified by real-time PCR. Results are expressed as mean values and standard errors and were analysed using independent samples t test using SPSS version 11 (SPSS Inc., Chicago, IL, USA).

At 1 year of age mean body weight (C 91 (SE 2) kg (n 7); NR 88 (SE 1) kg (n 11)) and composition were similar between groups. GR mRNA abundance was, however, significantly reduced in the kidneys of NR offspring (C 20.8 (SE 3.2) ng/μl; NR 5.8 (SE 1.3) ng/μl (P<0.0001)). Plasma cortisol was similar between groups but GFR was lower in the NR group (C 134 (SE 17) ml/min (n 7); NR 114 (SE 17) ml/min (n 3)).

Maternal nutrient restriction, targeted over the period of maximal placental growth, results in offspring that as young adults show reduced renal glucocorticoid sensitivity which may act to compromise kidney function during obesity. The present findings emphasise the importance of the juvenile environment in determining the magnitude of adverse outcome following in utero exposure to a reduction in nutrient supply.

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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 more 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 9585 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, age, 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, and 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 (Silvers & 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 nutrients, 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 greater affluence; potatoes, eggs and offal by comparison are cheaper items, typically consumed by those more deprived (Gregory et al., 1990).

In conclusion, for the first time we have shown 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); cytosolic SOD from a mean of 35.8 U/mg protein in group A to 45.4 U/mg protein in group B; (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 spectroscopy was used to measure the activities of hepatic superoxide dismutase (SOD) (total and CuZn), 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 significantly increased (total SOD from a mean of 37.5 U/mg protein in group A to 45.7 U/mg protein in group B; P < 0.01; cytosolic SOD from a mean of 35.8 U/mg protein in group A to 45.4 U/mg protein in group B; P < 0.01). Increases in SOD activity may represent a compensatory adaptation to the increased reactive oxygen species load.


The cytotoxic 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 polyunsaturated fatty acid (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 more 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.

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Pre-treatment with the dietary phyto-oestrogen daidzein significantly ameliorated levels of MDA–protein adducts (mean MDA–protein adduct score of 1.25 in group F compared with 2.17 for group B; P < 0.01), and the activities of cytosolic SOD were decreased by daidzein (mean activity of 39.2 U/mg protein in group F compared with 45.4 U/mg protein for the control in group B; P < 0.01). Other enzyme activities were unaffected by daidzein (P > 0.05). Furthermore, ATC pre-treatment had no significant effect on the level of either MDA–protein adducts, nor on the activities of any of the antioxidant enzymes (P > 0.05).
Reduced maternal folate supply decreases genomic DNA methylation in the offspring of C57BL/6J mice. By K.J. WALTHAM1, E.A. WILLIAMS2 and J.C. MATHERS1, 1Human 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 DNA)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>High (n = 15)</td>
<td>13.305</td>
<td>8061, 19855</td>
</tr>
<tr>
<td>High</td>
<td>Low (n = 14)</td>
<td>12.390</td>
<td>7368, 19203</td>
</tr>
<tr>
<td>Low</td>
<td>High (n = 16)</td>
<td>18.307</td>
<td>12.170, 25692</td>
</tr>
<tr>
<td>Low</td>
<td>Low (n = 12)</td>
<td>30.091</td>
<td>21.306, 40391</td>
</tr>
</tbody>
</table>

*Probability of effect: maternal diet P = 0.003; weaning diet P = 0.014; maternal diet × weaning diet P = 0.012

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 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).

Nucleotide excision repair (NER) is responsible for the repair of bulky DNA lesions caused by UV light, food-derived heterocyclic amines, polycyclic aromatic hydrocarbons and many other genotoxins. Sufferers of the genetic syndrome xeroderma pigmentosa are deficient in NER and have up to a 1000-fold increased NER capacity (% NER) 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 13/05721. T. 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, School of Health 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 carotenes, for example, lycopene and β-carotene, which contain only carbon and hydrogen, and the xanthophylls, for example, zeaxanthin and lutein, which are their oxygenated derivatives. The absorption of carotenoids by the intestine is dependent on these fat-soluble substances 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). Before the simulated gastric and intestinal digestion procedure, digesta were homogenised to simulate the suprachloro micellar fraction. The carotenoids from whole fruit, homogenate, digestate and micelles were extracted twice using a solvent mixture of hexane, acetone and ethanol.

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>Carotenoid</th>
<th>Fruit</th>
<th>Mean %</th>
<th>SD</th>
<th>Mean %</th>
<th>SD</th>
<th>Mean %</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lutein</td>
<td>20.1</td>
<td>16.4</td>
<td>8.0</td>
<td>18.7</td>
<td>10.0</td>
<td>14.9</td>
<td>7.0</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>20.0</td>
<td>16.4</td>
<td>8.0</td>
<td>18.7</td>
<td>10.0</td>
<td>14.9</td>
<td>7.0</td>
</tr>
<tr>
<td>Lycopene</td>
<td>20.0</td>
<td>16.4</td>
<td>8.0</td>
<td>18.7</td>
<td>10.0</td>
<td>14.9</td>
<td>7.0</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>20.0</td>
<td>16.4</td>
<td>8.0</td>
<td>18.7</td>
<td>10.0</td>
<td>14.9</td>
<td>7.0</td>
</tr>
</tbody>
</table>

The present research was funded by Science Foundation Ireland.
Elevated urinary F$_2$-isoprostane concentrations are associated with congestive heart failure. By G.C. McKeeMANN$^1$, C.M. HUGHES$^2$, P.P. McKEDOWN$^2$, J.V. WOODSIDE$^1$ and I.S. YOUNG$^1$, $^1$Nutrition and Metabolism Research Group, CCPS, Queen’s University Belfast, Belfast, UK, BT12 6BJ and $^2$Epidemiology 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 (Nonaka-Sarukawa et al. 2003). F$_2$-isoprostanes, isomers of prostaglandin 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). Urinary 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 failure. A new method for extracting F$_2$-isoprostanes from urine using ammonium-exchange solid-phase extraction (Lee et al. 2004) was used before a double derivatisation procedure involving pentafluorobenzyl bromide followed by bis-(trimethylsilyl)methylacetaimide. F$_2$-isoprostanes were analysed by GC-MS-NCl (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. F$_2$-isoprostane concentrations were expressed per mg of urinary creatinine.

Median urinary F$_2$-isoprostane concentrations were 61.9 (interquartile range (IQR) 40.1, 85.5) pg/mg creatinine in the control group and 20.7 (IQR 12.0, 28.5) 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 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 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 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 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.


M.M. Raats, M. Lumbers 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 = 240, 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.

There is of course a potential for cultural bias at all stages of the research process and a potential for over-simplification of cultural contrasts. However, the fact that data were collected and the first stage of analysis was country specific, carried out in the original language by culturally appropriate and methodologically competent researchers strengthens the integrity of the results. Linguistic and cultural equivalence are difficult, if not impossible, to maintain at all stages of qualitative research, from data collection through to analysis and interpretation. It is, however, important to avoid taking a top-down approach to cross-cultural qualitative research. Analysis must be grounded in the data, otherwise the true strength of qualitative data will be lost.

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’, QLK1-2002-02447, ‘Choosing foods, eating meals: sustaining independence and quality of life in old age’. It does not necessarily reflect its views and in no way anticipates the Commission’s future policy in this area.
Association of serum "antioxidant power" with myocardial infarction: a case–control study. By K.D.R.R. SILVA, C.B. LIYANAGE and D.L.L. MUNASINGHE, Department of Applied Nutrition, Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka, Makandura, Gampaha 60470, Sri Lanka

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: Comparison of MI patients and Controls

<table>
<thead>
<tr>
<th></th>
<th>MI patients (n = 30)</th>
<th>Controls (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55.5 ± 10.5</td>
<td>54.9 ± 10.2</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>46.7</td>
<td>30.0</td>
</tr>
<tr>
<td>Ex-smoker (%)</td>
<td>26.7</td>
<td>16.7</td>
</tr>
<tr>
<td>Non-smoker (%)</td>
<td>26.7</td>
<td>53.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.5 ± 4.1</td>
<td>22.4 ± 4.1</td>
</tr>
<tr>
<td>FRAP value (μmol/l)</td>
<td>1296.6*</td>
<td>1372.1*</td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td>4.5 ± 1.2</td>
<td>4.6 ± 0.9</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.9 ± 1.1</td>
<td>2.8 ± 1.0</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>0.9* ± 0.2</td>
<td>1.2 ± 0.5</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.7* ± 0.6</td>
<td>6.4 ± 0.4</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td></td>
<td></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 first and second 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, 14.2)) 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 defense, 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 ≥35 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.


Reported weight loss from a 1-year randomised controlled trial examining three dietary interventions for obesity management: initial findings. By C. ROLLAND1, M. HESSION1, C. TUYA1, S. MURRAY2, K. JARRETT2, A. WISE1 and J. BROOM1, 1Robert Gordon University, Aberdeen, UK, AB25 1HG and 2LighterLife, Harlow, UK, CM18 7BL

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Comparison of body composition, body fat distribution and cardiovascular risk factors in healthy pre- and postmenopausal women. By K.D.R.R. SILVA, S. THANGARAJA and R.J. KAMAL, Department of Applied Nutrition, Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka, Makandura, Gonawila 60170, Sri Lanka

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-one postmenopausal women were the subjects. Weight, height, waist circumference (WC) and hip circumference were measured. Biocoelectrical impedance analysis was used to assess body composition. Blood pressure, fasting levels of glucose and lipid risk markers of CHD were determined.

Postmenopausal women had significantly higher waist:hip ratio (WHR) and waist:height ratio (WHTR) ($P<0.05$) than premenopausal women, although BMI was similar in both groups (see Table). Postmenopausal women had significantly higher body fat percentage, fat mass and significantly lower lean body mass compared with premenopausal women ($P<0.03$). Also, postmenopausal women had significantly higher triacylglycerols (TAG) ($P=0.0001$), glucose ($P<0.04$) and diastolic blood pressure ($P<0.001$). Total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), TC:LDL-C and LDL-C:HDL-C of the two groups showed no significant difference. High WHR and WHTR of the postmenopausal women signified high abdominal adiposity and showed a positive correlation with biochemical risk markers and diastolic blood pressure when adjusted for age. Weight, WC and BMI showed positive correlations with lipid risk markers and systolic and diastolic blood pressure (data not shown).

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.


A novel indirect calorimeter. By A.P. BRADLEY. MeditrEn Limited Daresbury Innovation Centre Daresbury WA4 4FS, UK

The aim of this study was to evaluate the performance of a new compact indirect calorimeter from MeditrEn Limited.

The GEM2 is a compact, fully integrated and portable indirect calorimeter, measuring flow, oxygen concentration and CO$_2$ content of expired air. The breath collection method is either a face mask or a disposable breathing tube.

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).

The resting metabolic rate (RMR) of the same person was measured once a day over a 23 day period. The testing protocol required the subject to be measured firstly on the GEM2 and secondly with the original GEM. The subject was rested for 30 minutes before the trial. A 10 minute measurement was made with the GEM2 system and then immediately followed by a 10 minute measurement using the original GEM system.

Recuperation trials using a known gas (80.16% N$_2$, 19.84% CO$_2$) yielded >98% for the original GEM and >95% for the GEM2 of theoretical recovery.

Using original GEM the mean RMR was 8.948 MJ/day, ranging from 7.991 to 10.498 MJ/day. Using GEM2 the mean RMR was 8.994 MJ/day, ranging from 6.941 to 10.724 MJ/day. Expressing these differences as percentage yielded an average error of 0.25%, with a standard deviation of 1.50%.

Using GEM2 it was found that the daily RMR for a single subject has a variation of 23.9% across the duration of the study. This reflects the oscillations in the daily RMR that the daily RMR for a single subject has a variation of 23.9% across the duration of the study. This reflects the oscillations in the daily RMR that the daily RMR for a single subject has a variation of 23.9% across the duration of the study. This reflects the oscillations in the daily RMR that the daily RMR for a single subject has a variation of 23.9% across the duration of the study.

**Table:**

<table>
<thead>
<tr>
<th></th>
<th>Postmenopausal women (n=30)</th>
<th>Premenopausal women (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td><strong>sd</strong></td>
<td><strong>Mean</strong></td>
</tr>
<tr>
<td>Age (years)</td>
<td>54*</td>
<td>3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57.4</td>
<td>8.9</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>24.8</td>
<td>3.4</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>85.1</td>
<td>10.5</td>
</tr>
<tr>
<td>WHR</td>
<td>0.90*</td>
<td>0.08</td>
</tr>
<tr>
<td>WHTR</td>
<td>0.96*</td>
<td>0.07</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>22.8</td>
<td>4.3</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>30.5*</td>
<td>2.5</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>35.4</td>
<td>5.3</td>
</tr>
<tr>
<td>Lean body mass (%)</td>
<td>60.5*</td>
<td>2.5</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>3.02</td>
<td>1.07</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>3.98</td>
<td>1.10</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>0.94</td>
<td>0.31</td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td>1.00*</td>
<td>0.33</td>
</tr>
<tr>
<td>TC:LDL-C</td>
<td>3.91</td>
<td>2.04</td>
</tr>
<tr>
<td>LDL-C:HDL-C</td>
<td>4.63</td>
<td>1.91</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.12*</td>
<td>1.11</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>134</td>
<td>14</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79*</td>
<td>8</td>
</tr>
</tbody>
</table>

*Significantly different from premenopausal women ($P<0.05$).
Reduction of growth rate of infants after the age of 6 months due to parents’ smoking.

By A. WALKER and H.J. FLINT, Microbial Ecology Group, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, UK, AB21 9SB

School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, Tehran, Iran

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 colonic microflora colonise starch, or how inter-individual variation influences the formation of SCFA, mainly acetic, propionic and butyric acids. In the colonic colonising bacteria, butyrate is important in the maintenance of a healthy colon and is the major fuel for the colonocytes.

In the present study we developed a single-stage fermentor system to examine the colonisation of resistant starch (Hylon VII). Following incubation of starch in fermentors that were inoculated separately with faecal samples from four different donors, the indole was recovered and analysed. By FISH analysis, using a panel of fluorescently labelled probes, it was possible to examine the colonising bacteria.

The 226 randomly selected infants included in the study had grown normally until the age of 6 months. The case children (mean age 5.8 years) and controls (mean age 5.3 years) were those whose growth rate had decreased afterwards. The body weight of the case group was lower than that of the controls (mean SD = 3280.9 ± 623.9 g; P < 0.001). At the age of 9 months, however, the body weight of the case group was lower than that of the controls (mean SD = 3211.2 ± 541.0 g; P = 0.013). The statistical analysis was largely consistent with these findings with the exception of the estimate of the abundance of the major colonising bacteria (by the inoculum attached bacteria varied with faecal donor. Overall, Eubacterium rectale (mainly B. adolescentis and Ruminococcus bromii) 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 major colonising bacteria (by the inoculum inoculated from one donor). Although resistant starch is widely reported to be butyrogenic, among the primary colonising species, contributes to butyrate formation in the mixed gut ecosystem.

In conclusion, the present study suggests that the colonisers of insoluble starch found in the gut are restricted to relatively few specialist species of bacteria, most of which have been culturable and are restricted to relatively few specialist species of bacteria, most of which have been culturable. These species, Bacteroides thetaiotaomicron, Bacteroides uniformis, Bifidobacterium breve, Bifidobacterium adolescentis and Ruminococcus bromii, contribute to butyrate formation in the mixed gut ecosystem.

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.

Variable

| Variable | n | Mean (SD) | 95% CI | t | df | P
|----------|---|-----------|-------|---|----|---
| Mothers’ age group | 0.8017 | 0.5072 | 2.4987 | 1 | 0.1139 | 2.2290, 6.0238
| Fathers’ smoking | 0.3697 | 0.3606 | 1.0507 | 1 | 0.3053 | 1.4472, 2.9343
| Procedure of supplementary food preparation | 0.7619 | 0.4791 | 2.5293 | 1 | 0.1117 | 2.1424, 5.4790

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

By S.W. MA 1, B. DUTHIE 2 and F. THIES 1, 1College of Life Science and Medicine, University of Aberdeen, Aberdeen, UK, AB25 2ZD, 2BioSS, Rowett Research Institute, Aberdeen, UK, AB10 6UX and 3Rowett Research Institute, Aberdeen, UK, AB10 6UX

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 day 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 (Pulse 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 = 0.18; 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).

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-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.

Funded by the Food Standards Agency. G. D. is grateful to SEERAD for support.

Influence of breast-feeding and birth weight on body mass index and waist:hip ratio in Greek women. By E. ECONOMOU1, G. BEGUM2 and S. MESTANA1, 2Department of Human and Health Sciences, School of Biosciences, University of Westminster, 115 New Cavendish Street, London, UK, W1W 6UW and 2Private dietician clinical, 23 Kanari street, 10673, Athens, Greece

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.

Dep. variable

<table>
<thead>
<tr>
<th>Boys (%)</th>
<th>Girls (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time 1</td>
</tr>
<tr>
<td>Snacks weekdays (≥ twice daily)</td>
<td>0.008*</td>
</tr>
<tr>
<td>Fruit (≥2 m per day)</td>
<td>0.00</td>
</tr>
<tr>
<td>Soft drinks (≥2 m per day)</td>
<td>0.00</td>
</tr>
<tr>
<td>Whole milk (≥2 m per day)</td>
<td>0.00</td>
</tr>
<tr>
<td>Chips (≥2 m per day)</td>
<td>0.00</td>
</tr>
<tr>
<td>Food poverty (not enough food)</td>
<td>0.00</td>
</tr>
<tr>
<td>Dieting (on a diet)</td>
<td>0.00*</td>
</tr>
<tr>
<td>Vegetarian (vegetarian)</td>
<td>0.00*</td>
</tr>
</tbody>
</table>

Although different on some items, the effect sizes were very low, indicating that, although statistically significant, seasonality did not have a strong influence on these variables, and thus many not be of practical relevance.

These findings may be partly explained by the availability of most foods throughout the year, and the relatively minor changes in daylight and other weather factors related to Spring and Autumn in Ireland. Moreover this study did not take into account the effect, if any, of the summer holiday period on these variables. These findings should also be considered in the light of previous work identifying differences in nutrient intake/status by season, with an emphasis on measurement and methodological differences between studies.

HBSC Ireland was funded by the Health Research Board. We thank all the children and schools for taking part.


