# Proceedings of the Nutrition Society

## Abstracts of Communications

*A Scientific Meeting was held at University College, Cork, on 22–24 July 1998, when the following papers were presented.*

*All abstracts are prepared as camera-ready material by the authors.*

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**Nutrient intakes of female adolescents attending a single-sex compared with a mixed secondary school.** By SUSANNE T. LEECH, YVONNE M. RYAN, and MARY A.T. FLYNN, *Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Ireland*

Adolescent girls require diets of high nutritional quality to support the increased rates of growth that characterize puberty and the teenage years, and therefore, represent a nutritionally vulnerable group. The purpose of the present study was to investigate if there were any differences in the dietary intakes between girls attending a mixed secondary school and girls attending a single-sex secondary school.

Subjects (*n* 48) were randomly selected from first and second years in the mixed school and first year in the single-sex school. Girls attending the mixed school were significantly older than girls attending the single-sex school (13.4 *v.* 13.1 years, $P<0.05$) and more of them were in socioeconomic classes (SEC) 5 and 6 (30 *v.* 13%). However, both age and SEC were comparable in the random sample selected for this study. Body weight concerns were assessed by a questionnaire and were found to be comparable for the two groups. The nutrient intakes of the subjects were determined using the 7 d diet history method of the Irish National Nutrition Survey (Lee & Cunningham, 1990) and body weight was measured. BMR was estimated and reported energy intakes (EI) were expressed as a multiple of BMR (EI:BMR). A study-specific cut-off value of 1.48 was used to determine underreporting (Goldberg *et al*, 1991). In the Table, the nutrient intakes of the two groups are compared, including those who underreported their energy intakes.

| | Mixed school (*n* 26) | | Single-sex school (*n* 22) | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| Energy (MJ) | 12.0*** | 3.2 | 9.0 | 1.6 |
| EI:BMR (MJ/ 24 hours) | 2.17*** | 0.6 | 1.59 | 0.3 |
| Carbohydrate (% energy) | 49.6 | 3.3 | 50.4 | 3.5 |
| Protein (% energy) | 11.4 | 1.4 | 11.7 | 3.3 |
| Fat (% energy) | 38.7 | 3.0 | 37.0 | 3.8 |
| Fibre (Southgate) (g) | 25.1*** | 6.4 | 18.3 | 3.3 |
| Iron (mg) | 13.0*** | 3.3 | 9.7 | 1.9 |
| Calcium (mg) | 981 | 376 | 833 | 298 |
| Folate (ug) | 251** | 77.6 | 200.6 | 46.2 |

Mean values were significantly different from girls attending single-sex school: ** $P < 0.01$, *** $P < 0.001$

Eight per cent (*n* 2) of girls attending the mixed school underreported their EI compared with 32% (*n* 7) of girls attending the single-sex school. Compared with Irish recommended daily allowances (RDA), mean intakes were low in both schools for Fe (RDA 14 mg), Ca (RDA 1200 mg) and folate (RDA 300 ug) (IUNA, 1997). The main food sources of Fe for girls attending the mixed school compared with girls attending the single-sex school were bread (19 *v.* 20%), meat, fish, and poultry (14% for both schools), fortified breakfast cereals (10 *v.* 16%, $P=0.03$), potatoes (9 *v.* 6%, $P=0.02$), chocolate (8 *v.* 6%) and crisps (6 *v.* 4%). The main food sources of calcium were dairy products (41 *v.* 50%), bread (18 *v.* 16%), and chocolate (10 *v.* 7%). The main food sources of folate were fortified breakfast cereals (15 *v.* 21%), potatoes (19 *v.* 16%), fruit (17 *v.* 14%), bread (13% for both schools), milk (8 *v.* 11%), vegetables and pulses (4 *v.* 7%, $P=0.03$) and crisps (7 *v.* 5%). This study demonstrates the wide variety of foods which can contribute to the high nutritional requirements of the adolescent period; however, the more bioavailable sources of micronutrients should be emphasized.

Lee P & Cunningham K (1990) *Irish National Nutrition Survey*. Dublin: INDI.
Goldberg GR, Black AE, Jebb S A, Cole T J, Murgatroyd P R, Coward WA & Prentice AM (1991) *European Journal of Clinical Nutrition* **45**, 569-581.
IUNA (1997). *Irish Universities Nutrition Alliance Report: Nutrition and Women- A Guide for Health Professionals*. Nutriscan Limited.

**A comparison of the dietary and lifestyle habits and nutritional knowledge of cardiology and non-cardiology hospital staff.** By (1)HELEN R. CASEY, (1)MARY A.T. FLYNN, (2)SHIELA KING and (2)JOHN H. HORGAN, (1)*Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Ireland,* (2)*Department of Cardiology, Beaumont Hospital, Dublin 9, Ireland.*

Diet is recognized as an important preventative factor in cardiovascular disease (Ulbricht & Southgate, 1991) and interest in nutrition is increasing among health professionals (Glanz, 1997). The present cross-sectional study examined nutritional factors among cardiology and non-cardiology hospital-based health professionals. Sixty subjects (doctors 35%, nurses 40%, electrocardiogram technicians 8.5%, occupational therapists 11.5%, psychologists 3% and pharmacists 2%) working in a Dublin teaching hospital (Beaumont) completed the study. Thirty were recruited from the cardiology department (cardiology group, mean age 30 years) where staff receive regular lectures from a dietitian. Thirty subjects of similar professional backgrounds were recruited from non-cardiology areas of the hospital (non-cardiology group, mean age 29 years) where nutrition does not play a role in therapy. Dietary intakes were assessed using the fat intake questionnaire (Cantwell *et al.* 1997). Nutritional knowledge on heart disease prevention was assessed using a standardized questionnaire designed for the study. Height, weight, waist and hip measurements were recorded and used to estimate B.M.I. ($kg/m^2$) and waist:hip ratio (WHR). Smoking, alcohol and exercise habits were also assessed.

The groups were comparable in terms of demography, anthropometry and lifestyle habits; however, significantly more of the cardiology group took regular exercise during leisure time (43 *v.* 26%, $P=0.02$). Nutritional knowledge was better in the cardiology group where significantly more subjects could: (a) identify dietary sources of monounsaturated fatty acids (87 *v.* 67%, $P=0.04$) and (b) were aware of the benefits of antioxidant vitamins on cardiovascular health (67 *v.* 37%, $P=0.02$).

| | Cardiology group (*n* 30) | | Non-cardiology group (*n* 30) | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| Energy intake (MJ) | 11.0 | 3.2 | 11.7 | 3.1 |
| Energy intake/BMR (MJ/24 h) | 1.68 | 0.6 | 1.65 | 0.5 |
| Total fat (% energy) | 37.4 | 5.8 | 37.2 | 8.9 |
| Saturated fatty acids (% energy) | 12.9 | 3.0 | 15.2** | 4.1 |
| Monounsaturated fatty acids (% energy) | 11.8 | 2.5 | 11.8 | 2.2 |
| Polyunsaturated fatty acids (% energy) | 7.1 | 3.0 | 5.6* | 1.7 |

Mean values were significantly different from those for the cardiology group: * $P < 0.03$, ** $P < 0.02$.

Significantly higher intakes of polyunsaturated fatty acids and significantly lower intakes of saturated fatty acids were found in the cardiology group compared with the non-cardiology group. The groups were comparable for intakes of all other nutrients. Comparison of food intakes of both groups showed that significantly more of the cardiology group chose to eat vegetables (97 *v.* 57%, $P=0.001$), oily fish (70 *v.* 43%, $P=0.04$) and full fat polyunsaturated spread (40 *v.* 7%, $P=0.002$). Significantly more of the non-cardiology group chose full fat milk (67 *v.* 40%, $P=0.04$), red meat (90 *v.* 67% $P=0.02$) and butter (50 *v.* 23%, $P=0.03$). Median intakes of wholemeal bread ($P=0.03$), processed meats ($P=0.02$), low fat mayonnaise ($P=0.02$) and cream ($P=0.02$) were significantly higher in the non-cardiology group compared with the cardiology group. Knowledge appears to be an important factor in behaviour. The cardiology group had greater nutritional knowledge and this had a positive impact on their personal dietary and lifestyle habits.

Cantwell M, Gibney MJ, Cronan D, Younger K, Hogan L & Flynn MAT (1997) *16th International Congress of Nutrition-Book of abstracts* Pp183 (PT2).
Glanz K (1997) *American Journal of Clinical Nutrition* **65**, Suppl., 2016S-2019S.
Ulbricht T & Southgate D (1991) *Lancet* **338**, 985-992.

**Taurine modulates immune response, and reduces bacterial translocation and gut atrophy in a murine model of sepsis.** By LEÁN O'FLAHERTY, PHILIP P. STAPLETON, HENRY P. REDMOND and DAVID J. BOUCHIER-HAYES, *Royal College of Surgeons in Ireland, Department of Surgery, Beaumont Hospital, Dublin 9, Ireland*

Trauma and critical illness have been shown to impair host immune defences and gut barrier function. This compromised state provides favourable conditions for bacterial translocation, which has been implicated in the development of sepsis. Research has suggested that optimal nutritional support should provide nutrients to promote gut function. Taurine is one of the most abundant amino acids in the gut; however, levels are depleted after surgery and during catabolic stress (Ahlman *et al.* 1995A, b). The consequences of this on host defences have not been investigated.

We examined the effect of enteral taurine supplementation on immune response in two murine models of sepsis (using CD-1 mice), caecal ligation and puncture (CLP) and intraperitoneal lipopolysaccharide (LPS) injection (25 mg/kg). Immune response was assessed by isolating peritoneal macrophages and measuring their release of tumour necrosis factor-α (TNFα) and superoxide anion. In a separate set of experiments, we determined the influence of taurine supplementation on gut morphology and bacterial translocation in thirty CD-1 mice who had received an endotoxin challenge (500 μg LPS by intraperitoneal injection).

| | | | Sepsis | | | | Sepsis + taurine | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | LPS | | CLP | | LPS | | CLP | |
| | Mean | se | Mean | se | Mean | se | Mean | se | Mean | se |
| TNFα (pg/ml) | 4.1 | 0.8 | 26.6* | 10.3 | 54.8* | 11.9 | 16.2 | 10.7 | 17.5† | 6.7 |
| Superoxide (pmol/l) | 13.7 | 3.9 | 22.9 | 5.4 | 17.4 | 2.6 | 29.2* | 4.6 | 30.8*† | 3.5 |

Mean values were significantly different from control, $*P < 0.05$ (ANOVA).

Mean values were significantly different from CLP sepsis group, $\dagger P < 0.05$ (ANOVA).

From the Table it can be seen that induction of sepsis significantly increased TNFα production by macrophages, and this was attenuated by taurine supplementation. Furthermore, mice who received taurine had a significantly higher antimicrobial capacity represented by superoxide anion release. In the second study, taurine supplementation was associated with lower bacterial translocation rates to the liver (5.05 (se 0.3) *v.* 6.02 (se 0.2) log CFU /g, $P = 0.03$, t-Test) and spleen (2.31 (se 0.12) *v.* 2.92 (se 0.22) log CFU /g, $P = 0.04$, t-Test), and with smaller numbers of aerobic organisms in the caecum (12.3 (se 0.25) *v.* 13.0 (se 0.14) log CFU /g, $P = 0.025$, t-Test). On histological examination, measurements of villus height in the proximal jejunum were significantly higher in the supplemented group ($P = 0.04$, t-Test).

These findings suggest that taurine supplementation in catabolic states may improve outcome by down regulating the inflammatory response whilst up regulating antimicrobial function. These immunomodulatory benefits, together with the observed gut trophic effects and associated reduction in bacterial translocation may have clininal implications for the development of sepsis.

Ahlman B, Ljungvist O, Andersson K & Wernerman J (1995*a*) *Clinical Nutrition* **14**, 54-55

Ahlman B, Ljungqvist O, Persson B, Bindslev L & Wernerman J (1995*b*) *Journal of Parenteral and Enteral*

**Resting metabolic profile of patients with quiescent ulcerative colitis and comparison with controls.** By ELIZABETH J. SIMPSON[1], MARK A.S. CHAPMAN[2], DAVID. BERRY[2], ANDREW T. COLE[2] and IAN A. MACDONALD[1], *University of Nottingham Schools of [1]Biomedical Sciences and [2]Medical and Surgical Sciences, Queen's Medical Centre, Nottingham NG7 2UH*

During periods of active inflammation, patients with ulcerative colitis (UC) frequently modify their diet, by reducing consumption of complex-carbohydrate-containing foods, to avoid diarrhoea. As part of a study looking at *in vivo* metabolism of colonically administered butyrate in quiescent UC, we compared the resting metabolic rate and substrate utilisation of patients with UC and controls.

Ten control patients (eight male, median age 61 (range 34-78) years, BMI 27.0 (SEM 0.87) kg/m$^2$) and eleven with quiescent disease (five male, 53 (range 28-66) years, BMI 25.4 (SEM 1.07) kg/m$^2$) were studied 3 h after a phosphate enema bowel preparation and 1 h after a standard light lunch (approximately 1674 kJ, % energy: protein 20, fat 25, carbohydrate 55). Measurements were made of resting energy expenditure (REE) and respiratory exchange ratio (RER), by ventilated hood indirect calorimetry, for 4 h. At the start of the monitoring period, all subjects received an enema containing 5 mmol butyrate, of which 1.25 mmol was labelled with $^{13}C$. The results of the labelled butyrate metabolism have been presented elsewhere (Simpson *et al.* 1998).

Mean REE over the 4 h period was 0.070 (SEM 0.001) kJ/min per kg in UC patients and 0.060 (SEM 0.001) kJ/min per kg in controls (*Fig.1*), although these differences were not significant when age was taken into account. By contrast, RER was lower in UC patients than in the controls ($P<0.001$) (*Fig.2*) and there was a larger RER response to the previous meal over the first hour of monitoring in the control group ($P<0.001$).

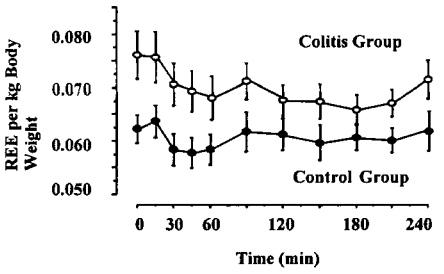


*Fig.1*

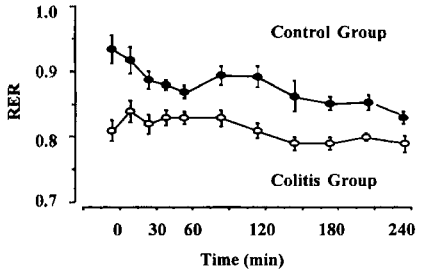


*Fig.2*

Patients with quiescent UC do not appear to have increased overall REE. The low RER in these patients is consistent with a higher fat content in their antecedent diet and requires further investigation.

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Simpson EJ, Chapman MAS, Dawson J, Berry D & Cole AT (1998) *Gut* **42**, A42.

**The evaluation of an undernutrition risk score to identify surgical patients at risk of malnutrition on admission.** By MARGARET DOYLE[1,3], ELIZABETH BARNES[2] and MARY MOLONEY[3], [1]*University of Dublin, Trinity College, Dublin, Ireland;* [2]*St Vincent's Hospital, Dublin, Ireland and* [3]*Dublin Institute of Technology, Kevin Street, Dublin, Ireland*

Hill *et al.* (1977) described the incidence of undernutrition among surgical patients who have been hospitalized for more than 1 week after surgery as being 50%. The objective of the present study was to evaluate an undernutrition risk score (URS) developed by the Dietetic Department of an acute tertiary referral teaching hospital in Dublin, with the aim that the URS could be used by nursing staff to identify surgical patients at risk for malnutrition on admission. Forty surgical patients (sixteen males and twenty-four females) were recruited. The standard objective nutritional assessment which was carried out on each patient consisted of a 3 d diet history. Anthropometric indices included weight, armspan, mid upper arm circumference, mid arm muscle circumference and hand grip dynamometry. A nutrition risk index (NRI) screening tool was used by a single observer to categorize patients as having low, moderate or severe risk of malnutrition. The indices used for the NRI were serum albumin and percentage weight loss (Veterans Affairs Total Parenteral Nutrition Cooperative Study Group, 1991). The URS assessed patients with respect to changes in weight and appetite, gut function and disease status and was completed by nursing staff by interview for each of the patients recruited.

| | Undernutrition risk score | | | |
|---|---|---|---|---|
| NRI Category | Low risk % (*n*) | Moderate risk % (*n*) | Severe risk % (*n*) | Total % (*n*) |
| Low risk | 52.9 (18) | 2.9 (1) | 2.9 (1) | 58.8 (20) |
| Moderate risk | 11.8 (4) | 14.7 (5) | 0.0 (0) | 26.5 (9) |
| Severe risk | 0.0 (0) | 0.0 (0) | 14.7 (5) | 14.7 (5) |
| Total | 64.7 (22) | 17.6 (6) | 17.6 (6) | 100 (34) |

The URS was found to be successful in detecting 71.4% (*n* 10) of surgical patients who were classified as being at some risk (moderate/severe) for undernutrition by the NRI. However, 11.8% (*n* 4) of the patients who were categorised by the NRI as being at moderate risk for undernutrition were classified as being at low risk by the nursing staff using the URS. Thus, the URS was found to be most sensitive in the detection of those at low or severe risk for undernutrition and least sensitive for those at moderate risk.

*The URS in this study provided an accurate and ,hence, useful screening tool that could be used for surgical patients who are capable of feeding themselves independently.*

Hill G L , Pickford I , Young G A , Schorah C J , Blackett R L , Burkinshaw L , Warren JV & Morgan D B (1977) *Lancet* **2,** 689-692.

Veterans Affairs Total Parenteral Nutrition Cooperative Study Group (1991) *New England Journal of Medicine* **325,** 525-532.

**Assessment of energy content of menus, energy intakes and wastage of food in two Irish teaching hospitals.** By CIARA M. BROWNE[1,2] and MARY MOLONEY[2], [1]*University of Dublin, Trinity College, Dublin, Ireland and* [2]*Dublin Institute of Technology, Kevin Street, Dublin, Ireland*

Malnutrition refers to all deviations from adequate nutrition including energy undernutrition (Shetty, 1994). Undernutrition has serious implications for health and recovery from illness or surgery (Lennard-Jones, 1992). The present study analysed the provision of energy in the menus of two Hospitals, A and B. Analysis of 3 d weighed food intakes on a total of fifty-one subjects was undertaken. Food wastage from all subjects was also recorded. Only hospital food consumed by subjects was included. Snacks from other sources were excluded.

| | Hospital A | | | | Hospital B | | | |
|---|---|---|---|---|---|---|---|---|
| | Males (*n* 16) | | Females (*n* 10) | | Males (*n* 13) | | Females (*n* 12) | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Energy provision (MJ/d) | 7.12 | 0.54 | 7.12 | 0.54 | 9.37* | 1.6 | 9.37* | 1.6 |
| Energy intake (MJ/d) | 5.5 | 1.2 | 5.4 | 0.8 | 6.7* | 1.9 | 6.8* | 1.9 |
| Patients not achieving BMR (%) | 87 | | 70 | | 62* | | 8* | |

Mean values were significantly different from those from hospital A, *$P<0.05$.

Energy intakes were below the calculated BMR (Schofield, 1985) requirements in 87% of males and 70% of females in hospital A. In addition, the difference between energy provided by the menu and actual energy intake for the same males and females was highly significant.

A low provision of staples, e.g. potatoes, in hospital A was noted. Hospital B had a high percentage wastage for fresh fruit (27 *v.* 0%), vegetables (29 *v.* 19%), potatoes (19 *v.* 11%), bread (24 *v.* 15%) and breakfast cereals (17 *v.* 4%) than hospital A. A statistically significant difference ($P = 0.046$) between the two hospitals was found in relation to wastage of potatoes, with hospital A at 11% and hospital B at 19%. However, the small serving of potatoes (75g) in hospital A was noteworthy. Interestingly, fruit did not feature on the menu in Hospital A. This study supports a previous finding on low energy intakes among hospitalized patients (Fenton *et al.* 1995).

Fenton J, Eves A, Kipps M & O'Donnell CI (1995) *Journal of Human Nutrition and Dietetics* **8,** 239 – 248.

Lennard-Jones JE (1992) The Kings Fund Report: London. *The Kings Fund Centre*.

Schofield WN, Schofield C & James WPT (1985) *Human Nutrition: Clinical Nutrition* **39** (suppl), 1-96.

Shetty P (1994) *Medicine International* **22,** 392-395.

**Nutritional characteristics of Irish patients undergoing resection of major carcinoma.** By C. CORISH[1], P. FLOOD[2], J.V. REYNOLDS[3] and N. P. KENNEDY[1], *[1]Department of Clinical Medicine, Trinity College Dublin, [2]Department of Clinical Nutrition, St James's Hospital, Dublin and [3]Department of Surgery, St James's Hospital, Dublin, Ireland*

This study was undertaken to examine the nutritional status of Irish patients admitted to hospital for major resection of carcinoma and to determine how nutrition status and current nutritional management affect both nutritional and clinical outcomes.

A total of 244 nutritional assessments were carried out on fifty-nine patients (thirty-seven male, twenty-two female), aged 31-90 (mean 66, SD 12) years. Cases in hospital were assessed weekly, from admission until day of operation and weekly, thereafter, until discharge. Assessments were carried out at 6 and 12 weeks post-discharge when possible.

Nutrition risk index (NRI=1.519 albumin (g/l) + 0.417%usual body weight) was calculated where possible to identify patients requiring nutritional support. Thirty-three (63%) of fifty-two patients were found to be at nutritional risk and seven (13%) were severely malnourished. BMI estimates identified three (6%) of these using the standard threshold value of <20 kg/m$^2$.

Involuntary weight loss in the 6 months before admission to hospital occurred in forty-three of the fifty-seven patients who had data on weight before admission, with twenty-one (37%) losing more than 10% of body weight. In those patients who lost weight, the mean weight loss was 11.2%. Patients with a BMI <20 kg/m$^2$ on admission had a mean weight loss of 13.4%.

Variables found to have no effect on survival included sex, age ≥65years and weight loss of >5% over the month before admission. Weight loss >10% in the 6 months before admission significantly decreased postoperative survival (P = 0.024).

Reduced appetite on admission to hospital was reported by twenty-one (36%) patients, with eleven (19%) reporting that their appetite was virtually non-existent. Vomiting and/or diarrhoea was reported by fifteen (25%) patients, difficulties in swallowing requiring food consistency modification by eleven (19%) while three (5%) were unable to take food orally.

| Type of cancer . . . | Lung | Head & neck | Bowel | Oesophageal | Ovarian | Gastric | Pancreatico-duodenectomy |
|---|---|---|---|---|---|---|---|
| | (*n* 16) | (*n* 13) | (*n* 12) | (*n* 10) | (*n* 4) | (*n* 2) | (*n* 2) |
| No. with poor appetite | 9 | 6 | 5 | 7 | 2 | 2 | 1 |
| No. with swallowing problems | 0 | 6 | 0 | 8 | 0 | 0 | 0 |
| No. with vomiting/diarrhoea | 1 | 1 | 10 | 0 | 2 | 0 | 1 |
| Mean BMI (kg/m$^2$) | 25.2 | 24.9 | 25.3 | 21.6 | 24.8 | 27.6 | 28.4 |

Of the patients, 76% lost weight in hospital, mean 4.3 (SD 3.3)kg, representing a mean 5.6% of their weight on admission. Two patients with a normal BMI on admission lost sufficient weight in hospital to reach a BMI <20 kg/m$^2$. Of six patients who had two preoperative assessments, four lost weight (mean 1.9 (SD 2.0)kg, mean 2.2% body weight before surgery). Following discharge, twenty-six (70%) patients lost weight within 3 months (mean 3.8 (SD 2.7)kg, mean 5.9% body weight). Three patients with a normal BMI on discharge reached a BMI <20 kg/m$^2$ subsequently.

Forty-four (75%) patients were referred for nutritional support and/or advice during the study period while two additional patients received nutritional supplements from the nursing staff. Referral occurred if there was an obvious inability to eat or when clinical complications occurred. A history of weight loss >10% did not automatically result in early referral on admission (only in sixteen cases of twenty-one eligible). Neither the use of BMI (<20 kg/m$^2$) nor NRI (≤100) identified all patients in this study who required nutritional intervention. It seems that a careful history including details of weight, appetite and ability to eat remains an important indicator of the need for nutrition support.

C. Corish is the recipient of a studentship sponsored by Abbott Laboratories (Irl.) Ltd.

**Home enteral tube feeding: a retrospective review of discharges from a Dublin hospital over 6 years (1992-8).** By EDEL Mc NAMARA[1], PHILOMENA FLOOD[2] and NICHOLAS P. KENNEDY[1], *[1]Department of Clinical Medicine, Trinity Centre for Health Sciences, St James's Hospital, Dublin 8, Ireland and [2]Department of Clinical Nutrition, St James's Hospital, Dublin 8, Ireland*

Home enteral tube feeding (HETF) is recognized as a valuable therapeutic option for patients requiring nutritional support following discharge from hospital. However, little is known about the numbers or characteristics of patients on HETF in the Republic of Ireland.

The medical and dietetic records of 161 patients discharged on HETF between July 1992 and April 1998 were examined in the present study. There were similar numbers of men (53%) and women (47%) in the sample, with an average age of 70.6(SD 14.3) years. HETF was predominantly used in neurology and oncology patients, with cardio-vascular-accident (CVA) (35.6%) and cancers affecting swallowing (31.1%) being the most common indications. Of patients, 83% were fed through a gastrostomy tube, with only 7.4% fed through a nasogastric or nasoduodenal tube. Most patients were fed using a standard, polymeric feed (4.18 kJ/ml). The usual method of feeding was continuous feeding (i.e. ≥16 h/d). Some patients were able to consume small amounts of food orally, but most of their nutrient requirements were provided by enteral feeding.

The degree of dependence of patients in this sample is illustrated by the number discharged to institutional care (45% to nursing home facilities, 8% to hospice care, and 6% to other hospitals) rather than to their own homes (41%). Patients on HETF in the Dublin area (*n* 116) were followed up. Information on completed HETF episodes (i.e. the length of time spent in the community on HETF before death or resuming full oral nutrition) was obtained for fifty-four patients (see Table). The remaining patients either continued to be fed artificially, or dates for their death or resumption of oral feeding were not available.

| | Diagnosis | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Cancer | | | CVA | | | Other | | |
| Time on HETF (d) | median | IQR | n | median | IQR | n | median | IQR | n |
| When on HETF until death | 30 | 174 | 15 | 193 | 502 | 18 | 199 | 245 | 8 |
| Before resuming oral intake | 13 | 58 | 6 | 477 | 59 to 605* | 3 | 17.5 | 1 to 34 | 4 |

IQR, inter-quartile range.
*Range is given where n was insufficient to yield a meaningful IQR.

Patients with cancer did not survive as long as those with CVA and spent significantly less time on HETF (P=0.001). The long-term outcome of patients on HETF requires further research, and should include consideration of quality of life in addition to cost issues and disease outcomes.

Edel McNamara is funded by Nutricia Ireland.

**Response of erythrocyte glutathione reductase activation coefficient (EGRAC) and plasma pyridoxal-5' phosphate (PLP) to riboflavin supplementation (a pilot study).** By S.M. MADIGAN[1], F. TRACEY[2], H. McNULTY[1], M.J. EATON-EVANS[1] and J.J. STRAIN[1], *[1]Northern Ireland Centre for Diet and Health (NICHE), University of Ulster, Coleraine BT52 1SA and [2] Elderly Rehabilitation Unit, Coleraine Hospital, Causeway HSS Trust BT52 1JA*

EGRAC is now the most widely used test of riboflavin status. Values greater than 1.2 are considered to indicate suboptimal status of the vitamin but there is some uncertainty regarding the validity of high EGRAC values with ageing. One possible way of resolving whether or not high EGRAC values in the elderly are indicative of riboflavin deficiency is by assessing the response of plasma PLP (a functional index of vitamin $B_6$ status) to riboflavin supplementation. The formation of PLP in tissues requires riboflavin in the form of FMN. The objective of the present study was to investigate the effect of riboflavin supplementation on EGRAC and plasma PLP in elderly people.

Forty-five healthy, free-living elderly subjects (m 8, f 37, 65-91y) were randomly assigned to treatment groups to receive either 1.6 or 25 mg riboflavin daily, or to the placebo group, for a 12-week supplementation period. In the total sample of subjects who completed the intervention ($n$ 41), a significant EGRAC response was only observed in those individuals who received 25 mg riboflavin/d ($P$=0.041) (not shown), suggesting a pharmacological effect of riboflavin. Individuals who were classified as suboptimal in riboflavin (EGRAC ≥1.2), vitamin $B_6$ (plasma PLP <20nmol/l), or both at baseline were examined separately (see Table).

| | | EGRAC | | | | | Plasma PLP (nmol/l) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $n$ | Pre- | | Post- | | $P$ value* | Pre- | | Post- | | $P$ value* |
| | | Mean | SD | Mean | SD | | Mean | SD | Mean | SD | |
| Suboptimal $B_2$ | 20 | | | | | | | | | | |
| Placebo | 7 | 1.28 | 0.08 | 1.23 | 0.09 | 0.130 | 30.2 | 24.8 | 36.6 | 30.9 | 0.204 |
| Riboflavin Supplementation (all) | 13 | 1.28 | 0.06 | 1.16 | 0.10 | <0.001 | 35.0 | 22.1 | 33.5 | 17.5 | 0.641 |
| 1.6 mg/d | 10 | 1.29 | 0.07 | 1.19 | 1.10 | 0.003 | 33.1 | 22.9 | 29.7 | 12.3 | 0.243 |
| 25 mg/d | 3 | 1.24 | 0.05 | 1.08 | 0.05 | 0.051 | 41.4 | 22.4 | 46.1 | 28.9 | 0.345 |
| Suboptimal $B_6$ | 12 | | | | | | | | | | |
| Placebo | 4 | 1.23 | 0.15 | 1.23 | 0.13 | 0.455 | 13.7 | 2.2 | 15.5 | 4.4 | 0.241 |
| Riboflavin Supplementation (all) | 8 | 1.17 | 0.14 | 1.13 | 0.11 | 0.118 | 14.1 | 2.2 | 25.6 | 19.4 | 0.020 |
| 1.6 mg/d | 4 | 1.28 | 0.10 | 1.19 | 0.14 | 0.042 | 14.3 | 8.1 | 20.7 | 5.6 | 0.035 |
| 25 mg/d | 4 | 1.05 | 0.01 | 1.07 | 0.03 | 0.829 | 14.1 | 5.9 | 38.5 | 25.4 | 0.054 |
| Suboptimal $B_2$ & $B_6$ | 7 | | | | | | | | | | |
| Placebo | 3 | 1.29 | 0.12 | 1.28 | 0.07 | 0.459 | 12.6 | 0.3 | 15.1 | 5.3 | 0.257 |
| Riboflavin Supplementation (all)+ | 4 | 1.28 | 0.10 | 1.19 | 0.14 | 0.042 | 14.3 | 8.1 | 20.7 | 5.6 | 0.035 |

*Differences were assessed using a one tailed $t$ test for paired data. $P$ values <0.05 were considered significant
+All four subjects received 1.6 mg riboflavin / d

The results show that in subjects deemed to have inadequate biochemical status of either nutrient at baseline, supplementation with riboflavin resulted in a significant response in the status of whichever nutrient was low. In the limited number of subjects classed as having low status of both vitamins ($n$ 7), riboflavin supplementation appeared to improve the status of both vitamins. Although the subject numbers are small, these results show that riboflavin supplementation at physiological and /or dietary levels, can correct abnormality of not only EGRAC but also plasma PLP, confirming the biochemical interdependency of these vitamins and suggesting that riboflavin is the limiting nutrient. Since dietary intakes of both nutrients in this sample were found to be adequate (results not shown), our data suggest that older people may have increased requirements for riboflavin and that current dietary recommendations may be insufficient for their needs.

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**Relationship between riboflavin status, methylenetetrahydrofolate reductase (MTHFR) genotype and plasma homocysteine in healthy elderly subjects.** By M.C. McKINLEY[1], H. McNULTY[1], J. McPARTLIN[2], J.J. STRAIN[1], D.G. WEIR[2] and J.M. SCOTT[3], *[1]Northern Ireland Centre for Diet and Health (NICHE), University of Ulster, Coleraine BT51 1SA and Departments of [2]Clinical Medicine and [3]Biochemistry, Trinity College, Dublin, Ireland.*

Elevated plasma homocysteine levels have recently been identified as an independent risk factor for vascular disease. Homocysteine is metabolized either by remethylation to methionine or by a transsulfuration reaction which forms cysteine. One enzyme of notable interest in the remethylation of homocysteine is MTHFR which catalyses the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate; this, in turn, acts as a methyl donor in the remethylation of homocysteine to methionine. Recently a common point mutation (C677 to T) in MTHFR, which results in thermolability with reduced enzyme activity, has been discovered. Since MTHFR requires riboflavin in the form of FAD as a prosthetic group, it is possible that low riboflavin status, particularly in combination with homozygosity for the C677T mutation, may be associated with elevated plasma homocysteine levels; however, to date this has not been investigated. This possible relationship is especially pertinent in an elderly population given that homocysteine levels are markedly raised and sub-optimal riboflavin status is common in the elderly (Madigan *et al.* 1998).

A total of ninety-six healthy elderly subjects (mean age 74 years, 27M, 69F) who were not taking multi-vitamin preparations were recruited for this study. MTHFR genotype (by polymerase chain reaction techniques), plasma homocysteine (by HPLC), and riboflavin status (by erythrocyte glutathione reductase activation coefficient, EGRAC) were measured. The Table shows plasma homocysteine levels (μmol/l) in those subjects homozygous for thermolabile MTHFR (TT), heterozygous (CT), and those without the variant (CC), both for individuals who were riboflavin replete (EGRAC < 1.20) and individuals who had sub-optimal riboflavin status (EGRAC ≥ 1.20).

| | Plasma homocysteine (μmol/l) | | | | | |
|---|---|---|---|---|---|---|
| MTHFR Genotype | EGRAC < 1.20 (Riboflavin replete) | | | EGRAC ≥ 1.20 (Riboflavin sub-optimal) | | |
| | Mean | SD | $n$ | Mean | SD | $n$ |
| CC | 11.29 | 4.03 | 25 | 11.42 | 2.52 | 19 |
| CT | 12.61 | 5.16 | 17 | 12.12 | 3.68 | 23 |
| TT | 13.20 | 7.27 | 3 | 12.17 | 2.53 | 9 |
| Total | 11.95 | 4.65 | 45 | 11.85 | 3.05 | 51 |

ANOVA indicated that plasma homocysteine levels were not significantly elevated in association with sub-optimal riboflavin status, even in the presence of thermolabile MTHFR. However, the prevalence of sub-optimal riboflavin status was significantly higher ($P$ < 0.05) in homozygotes for thermolabile MTHFR (75.0%) compared with those without the variant (43.0%). This may indicate that in individuals who are homozygous for thermolabile MTHFR, riboflavin status is diminished by a mechanism yet to be established. Alternatively it is possible that elevated plasma homocysteine levels will only be observed in association with profound riboflavin deficiency (which is extremely rare in man) as suggested by Bates & Fuller (1986) who demonstrated in rats that MTHFR activity was only decreased in association with moderate to severe riboflavin deficiency (EGRAC 1.80 - 2.03). Ongoing research at this centre should help to clarify this issue.

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**Impact of methylenetetrahydrofolate reductase (MTHFR) genotype and folate status on plasma homocysteine levels in healthy male and female adults**. By B. WILSON[1], H. McNULTY[1], J. McPARTLIN[2], J.J. STRAIN[1], D.G. WEIR[2] and J.M. SCOTT[3], [1] *The Northern Ireland Centre for Diet and Health (NICHE), University of Ulster, Coleraine BT52 1SA and Departments of* [2] *Clinical Medicine and* [3] *Biochemistry, Trinity College Dublin, Ireland*

Elevated plasma levels of the amino acid homocysteine (hyperhomocysteinaemia) are implicated in a number of pathologies including premature vascular disease and neural tube defects. Homocysteine (hcy) levels are influenced by both B-vitamin status and genetic factors, notably a thermolabile variant of MTHFR with impaired ability to catalyse the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. This common C677T mutation is established as being associated with mild hyperhomocysteinaemia but the relationship between MTHFR genotype and folate status is unclear as recent studies have obtained conflicting results, finding significantly higher (van der Put *et al.* 1995) or lower (Molloy *et al.* 1997) RCF status in individuals with thermolabile MTHFR compared to the other genotype groups. The aim of the present investigation was to establish the nature of the relationship between MTHFR genotype, hcy and RCF in males and females.

For this study, 125 healthy adults (19-59 years) were recruited. MTHFR genotype was identified in whole-blood extracts by polymerase chain reaction amplification followed by *HinF1* restriction digestion, hcy levels were measured by HPLC with fluorometric detection, and RCF status was established by microbiological assay. The Table shows differences (Independent *t* test) in mean hcy and RCF levels in males and females homozygous for thermolabile MTHFR (TT) (males *n* 7; females *n* 10), heterozygotes (CT) (males *n* 21; females *n* 36) and those with no variant (CC) (males *n* 17; females *n* 34).

| | CC | | CT | | TT | | Total Sample | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| hcy (μmol/l) M+F | 8.4 | 2.5 | 9.9 | 5.4 | 15.1 ** | 10.9 | 10.0 | 6.0 |
| hcy(μmol/l) M | 9.4 | 3.0 | 12.7 | 7.0 | 20.9** | 13.4 | 12.4 | 8.1 |
| hcy (μmol/l)F | 7.9 | 2.1 | 8.6 | 3.7 | 11.0* | 6.7 | 8.6 | 3.8 |
| RCF (μg/l) M+F | 321 | 147 | 294 | 16 | 243* | 101 | 298 | 129 |
| RCF (μg/l) M | 265 | 103 | 260 | 96 | 225* | 90 | 258 | 94 |
| RCF (μg/l) F | 348 | 159 | 312 | 126 | 256* | 107 | 320 | 141 |

Mean values were significantly different from CC group: * $P<0.05$, ** $P<0.01$

Hcy levels were significantly higher in males ($P<0.05$) than in females in both CT and CC groups but not in the TT group; RCF levels in males were significantly lower ($P<0.05$) than in females in all genotype groups. These results show that homozygosity for thermolabile MTHFR is associated with lower RCF levels, confirming the findings of Molloy *et al.* (1997), and is most marked in the male TT genotype group. Therefore, individuals with thermolabile MTHFR may have increased folate requirements in order to maintain equivalent folate status, an issue currently under investigation at this centre.

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**Factors affecting the erythrocyte folate status of Irish female adolescents.** By Y.M. RYAN[1], J. MCPARTLIN[2], M.J GIBNEY[3] and M.A.T. FLYNN[1], [1]*Department of Biological Sciences, Dublin Institute of Technology, Kevin St., Dublin 8, Ireland,* [2]*Vitamin Research, Sir Patrick Duns Trinity College Laboratory, St James's Hospital, Dublin 8 and* [3]*Unit of Nutrition and Dietetics, Department of Clinical Medicine, Trinity Medical School, St James's Hospital, Dublin 8*

Erythrocyte folate levels of 906 nmol/l (400 ng/ml) or higher are associated with a reduced risk of having a neural tube defect-affected pregnancy (Daly *et al.* 1995). The purpose of the present study was to identify factors which predict erythrocyte folate levels in Irish female adolescents.

Female students (*n* 305), aged 14-17 years were recruited at random from nine single-sex, Dublin secondary schools (four fee paying and five non-fee paying). Finger-prick blood samples were taken for measurement of the erythrocyte folate concentration using a microbiological assay described by Molloy & Scott (1997). Food and nutrient intakes were assessed using the 7 d diet history method of the Irish National Nutrition Survey (Lee & Cunningham, 1990). Social class (O'Hare, 1986) and the use of folic acid-containing dietary supplements was recorded. Subjects with erythrocyte folate concentrations < 906 nmol/l (group I, *n* 229, 75% of the total group) were compared with subjects with erythrocyte folate concentrations ≥ 906 nmol/l (group II, *n* 76).

Compared with group I, group II subjects had significantly higher mean intakes of total folate (233.1 *v.* 299.1 μg/d respectively, $P < 0.001$) and significantly higher median intakes of folic acid (35.7 *v.* 85.6 μg/d respectively, $P < 0.001$). The groups were comparable with regard to their mean energy (8.8 *v.* 8.5 MJ/d respectively) and food folate intakes (183.8 *v.* 184.8 μg/d respectively).

In relation to their food intakes, a significantly smaller proportion of group I subjects consumed foods fortified with folic acid (i.e. breakfast cereal, milk and bread) compared with group II subjects (75 *v.* 95% respectively, $P < 0.001$). Compared with group I, group II subjects reported significantly higher median intakes (for consumers only) of fortified breakfast cereal (17 *v.* 27 g/d respectively, $P < 0.001$), fortified milk (137 *v.* 290 ml/d respectively, $P = 0.008$), milk (217 *v.* 289 ml/d respectively, $P < 0.05$) and fruit (143 *v.* 177 g/d respectively, $P < 0.05$).

Significantly fewer of group I subjects compared with group II subjects reported taking folic acid-containing dietary supplements (25 *v.* 42% respectively, $P = 0.004$). The groups were comparable with regard to their socio-economic backgrounds; however, more subjects with erythrocyte folate concentrations < 340 nmol/l (150 ng/ml) (5% of the total group) were from social classes 5 and 6 compared with group II subjects (40 *v.* 7% respectively, $P = 0.008$). Multiple regression analysis showed that supplement use ($P = 0.001$), folic acid intake from fortified foods including breakfast cereal ($P < 0.001$), milk ($P = 0.003$) and bread ($P = 0.028$) and fruit intake ($P = 0.011$) significantly positively predicted erythrocyte folate levels (adjusted $R^2$ 29%).

Education about good food sources of folate, particularly the role of fortified foods, is paramount if the erythrocyte folate status of Irish female adolescents is to be improved.

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**The relationship between plasma and dietary phylloquinone (vitamin $K_1$) in Scottish adults.** By C. BOLTON-SMITH[1], R.J.G. PRICE, S.T. FENTON[1], D.J. HARRINGTON[2] and M.J. SHEARER[2]
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Phylloquinone ($K_1$) is required for the gamma-carboxylation of a number of coagulation, bone and other proteins (Shearer, 1997). Dietary sources are primarily green leafy vegetables and vegetable fats in baked products (Fenton *et al.* 1997). The relationship between dietary intake and plasma levels has not been previously reported in healthy individuals on their usual diets and with repeated measurements over a whole year. Thirty-one men (aged 20-55 years) and thirty-four women (aged 24-45 years) each provided a fasted blood sample at the beginning, middle and end of a 7 d weighed food record at each season of a year. Seven subjects were previously excluded due to incomplete data-sets or confirmed incomplete recording of food intake (weight gain plus energy intake:BMR <1.2). The mean plasma $K_1$ values for each week, quantified by HPLC with electrochemical detection, were correlated with the estimated mean $K_1$ intake, using the food database developed for that purpose (Bolton-Smith *et al.* 1998). Plasma $K_1$ values (nmol/l) were also adjusted for triacylglycerols (TAG, mmol/l) as a ratio ($K_1$:TAG). Mean values over the year are reported in the Table.

| | Men (*n* 31) | | Women (*n* 34) | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| Plasma $K_1$ (nmol/l) | 0.75 | 0.28 | 0.68 | 0.23 |
| Plasma $K_1$:TAG | 0.66** | 0.21 | 0.85 | 0.35 |
| Dietary $K_1$ (μg/d) | 72.1 | 64.7 | 63.5 | 33.0 |
| Dietary $K_1$ (μg/5 MJ) | 34.4 | 30.3 | 40.9 | 22.3 |

**Mean value was significantly different from that of women, $P<0.01$ (ANOVA)

Spearman rank correlation coefficients between plasma $K_1$ (nmol/l) and dietary $K_1$ (μg/d) were 0.28 ($P<0.05$) for both men and women. Expressing dietary $K_1$ in relation to total energy intake did not affect the results; however, expressing plasma $K_1$ in relation to TAG levels greatly improved the correlation coefficients: men $r$ 0.45, $P<0.001$; women $r$ 0.41, $P<0.001$. Multiple regression analysis indicated that 44 % of the variance in plasma $K_1$ was explained by dietary $K_1$ + total energy intake + plasma TAG. Other variables entered (sex, age, smoking habit and BMI) were not significant. Apolipoprotein-E phenotype (Kohlmeier *et al.* 1996) and the bioavailability of $K_1$ from different foods (Gijsbers *et al.* 1996) may be further confounding factors in the plasma-dietary $K_1$ relationship.

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**The effect of β-carotene on repair of DNA strand breaks in a human tumour epithelial cell line (HepG2).** By N.M. MILLAR, J.A.WOODS and N.M. O'BRIEN, *Department of Nutrition, University College, Cork, Republic of Ireland*

A consistent association between fruit and vegetable consumption and reduced risk of many types of cancer has been well documented. Many of these studies have focused on β-carotene (BC) as the potentially active micronutrient in fruit and vegetables. BC can act as an antioxidant under physiological conditions. Oxidative processes are believed to play a role in carcinogenesis and hence it was plausible to propose that this compound might be of value in cancer prevention. However, results from recent intervention trials indicate that supplementation with BC is not beneficial in the prevention of cancer in Western populations. Moreover, lung cancer rates in heavy smokers were unexpectedly higher in the supplemented groups (Heinonen & Albanes, 1994; Omenn *et al.* 1996). The objective of the present study was to examine the effect of BC on repair of oxidatively induced DNA damage in human hepatoma (HepG2) cells.

HepG2 cells were preincubated with BC (5 μmol/l dissolved in dimethyl sulphoxide) for 24 h followed by a challenge with 100 μM-$H_2O_2$ for 5 min at 4°. The reaction was quenched by adding 200 ml/l foetal calf serum. The cells were then allowed to repair over a period of 24 h (at 37° and 50 ml/l $CO_2$). The extent of DNA damage and repair was assessed using the single cell gel electrophoresis assay (Comet assay) (Tice *et al.* 1990). Results (Table) represent the mean and standard error for N≥2 independent experiments. Control cells were not challenged with $H_2O_2$.

**Table 1.** Percentage of unrepaired DNA strand breaks

| Repair time (h) | 0 | | 0.16 | | 0.5 | | 9.5 | | 24 | | Control | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| 5 μM-BC present | 100 | 0.3 | 98.5 | 1.7 | 95.6 | 2.2 | 49.1 | 0.9 | 45.1 | 0.0 | 8.3 | 1.2 |
| 5 μM-BC absent | 100 | 0.3 | 85.1 | 1.5 | 75.7 | 0.9 | 9.2 | 2.1 | 6.2 | 2.2 | 7.9 | 0.7 |

After a 24 h recovery period the percentage of unrepaired DNA strand breaks was considerably higher (45%) in cells pre-treated with BC before the $H_2O_2$ challenge. The corresponding control cells preincubated with BC but not challenged with $H_2O_2$ had a much lower percentage of unrepaired strand breaks (8.3%) after the same recovery period. In the absence of BC pre-treatment the cells, after the 24 h recovery period, had similar levels of unrepaired DNA strand breaks compared with their corresponding controls (6.2 V. 7.9% respectively). The initial damage (0 h) following $H_2O_2$ challenge was similar whether the cells were preincubated or not for 24 h with BC.

These data indicate that BC pretreatment of human tumour epithelial cells at a concentration of 5 μmol/l for 24 h delayed repair of oxidatively damaged DNA.

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**Green tea and *in vivo* antioxidant status; post-ingestion changes in plasma ferric reducing/antioxidant power (FRAP) confirm absorption and systemic distribution of polyphenolic tea antioxidants.** By I.F.F. BENZIE[1], Y. T. SZETO[1] and J.J. STRAIN[2], [1]*Department of Nursing and Health Sciences, Hong Kong Polytechnic University, Kowloon, Hong Kong;* [2]*Northern Ireland Centre for Diet and Health (NICHE), University of Ulster, Coleraine BT52 1SA*

Plant-based foods and beverages are rich in antioxidant compounds, including the antioxidant vitamins C and E, and flavonoids. Absorption of these compounds may increase *in vivo* antioxidant status and, thereby, help lower risk of chronic disease associated with oxidative damage (Strain & Benzie, 1998). Tea, particularly green tea, contains very high levels of antioxidant polyphenolic compounds (Graham, 1992). However, results of previous studies of absorption of tea antioxidants show no agreement (Maxwell & Thorpe, 1996; Serafini *et al.* 1996), and the question of whether the antioxidant potential of tea can be realized, in terms of improving *in vivo* antioxidant status, remains unanswered. The aim of the present study was to investigate absorption and systemic distribution of tea antioxidants by monitoring acute changes in the plasma antioxidant potential in response to ingestion of tea. Post-ingestion changes in urinary polyphenolic content and antioxidant potential were also monitored.

The study made use of the high precision and sensitivity of an automated test of 'total antioxidant potential', the ferric reducing/antioxidant power (FRAP) assay (Benzie & Strain, 1996, US patent pending). Blood and urine samples were collected from ten healthy, consenting adults (five men, five women) immediately before and at timed intervals after ingestion of freshly prepared, strong (50 g dry tea leaves/l), green tea. Volunteers arrived fasting and took nothing during the course of the test apart from tea and sips of distilled water. At least 4 weeks later, seven of the volunteers repeated the study, drinking water in place of tea. All blood samples (heparinized) were separated within 3 h of collection, and the FRAP assay performed on plasma immediately thereafter. Urine samples were collected without preservative, and total phenolics by Folin Ciocalteau method and FRAP were measured within 4 h of collection.

Results showed that plasma antioxidant potential increased significantly ($P < 0.01$) after ingestion of green tea, with peak increases seen at 20-40 min post-ingestion. Mean increase in plasma FRAP value was 44 (SE 9) μmol/l, which represents an increase of approximately 4% in the plasma antioxidant potential. Mean 2 h post-ingestion area under the curve for plasma FRAP, calculated by the trapezoid method, was 61.6 (SE 14.4) μmol/l. Post-ingestion increases in urinary FRAP values mirrored those of total phenolics in urine. There was a significant correlation ($r$ 0.845; $P < 0.001$) between FRAP and phenolics in urine, with peak excretion at 60-90 min after ingestion of tea. No increases were seen in plasma or urine when water, rather than tea, was taken.

This study shows that polyphenolic antioxidants in green tea are absorbed quickly after ingestion, and cause a significant increase in the antioxidant potential, as FRAP, of circulating plasma. The response was relatively small, however, and most, though not all, plasma FRAP values had returned to near baseline (fasting) levels by 2 h post-ingestion, i.e. tea-related increases in plasma antioxidant potential are of fairly short duration. Nevertheless, regular consumption of green tea may lead to enhanced *in vivo* antioxidant status and lowered risk of chronic disease.

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**Bioavailability of *n*-3 polyunsaturated fatty acids (*n*-3 PUFA) in microencapsulated fish oil.** By S. HIGGINS, Y. CARROLL and P.A. MORRISSEY, *Department of Nutrition, University College Cork, Ireland.*

The incidence of CHD is particularly low in populations with a high fish intake. This has been attributed to the high levels of *n*-3 PUFA especially eicosapentaenoic acid (20:5*n*-3) (EPA) and docosahexaenoic (22:6*n*-3) (DHA), found in fish (Kromhout *et al.* 1985). At present in the European Union (EU), dietary intake of *n*-3 PUFA contributes on average 0.23% of dietary energy, which is only half the value recommended by the EU Scientific Committee for Food (1993). It appears that most people have difficulty achieving the recommended dietary levels of *n*-3 PUFA by increasing their fish intake. The successful incorporation of fish oil into ordinary foods may increase intakes of *n*-3 PUFA. Microencapsulation of fish oil protects the highly sensitive *n*-3 PUFA from oxidation. However, as the technology involved in microencapsulating fish oil is relatively new, little is known regarding the bioavailability of *n*-3 PUFA supplied to the body in this way. The aim of the present study was to evaluate the bioavailability of *n*-3 PUFA in microencapsulated fish oil compared with a fish oil capsule.

Twenty-eight healthy volunteers (thirteen males and fifteen females), aged 19-30 years, were randomly assigned to two groups of fourteen and supplemented with microencapsulated fish-oil or a fish oil capsule. The fish oil used in both supplements was supplied by the Danish Institute for Fisheries Research. Microencapsulation of fish oil was carried out by the National Dairy Products Research Centre, Co. Cork, Ireland. Volunteers consumed either a milk-shake (150 ml) containing 9 g food ingredient which was enriched with microencapsulated fish-oil or three 1 g fish-oil capsules each day for 4 weeks. Both supplements supplied 0.9g *n*-3 PUFA daily. Compliance was excellent in both groups. Fasting blood samples were obtained at baseline and following the 4-week supplementation period. Lipid was extracted from plasma according to the method of Lepage & Roy (1986) and fatty acids were analysed by GC (Varian). Plasma total cholesterol levels (Chol) were analysed using an enzymatic, colorimetric kit (CHOD-PAP) supplied by Boehringer Mannheim on a Cary Spectrophotometer (Varian).

| | Fish-oil capsule group (*n* 14) | | | | Microencapsulated fish-oil group (*n* 14) | | | |
|---|---|---|---|---|---|---|---|---|
| Fatty acid (g/100g | Week 0 | | Week 4 | | Week 0 | | Week 4 | |
| total fatty acids) | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 16:0 | 16.9 | 4.5 | 15.4 | 4.5 | 13.6 | 4.0 | 14.4 | 4.3 |
| 16:1 | 2.5 | 0.7 | 2.4 | 0.8 | 3.2 | 0.6 | 2.7* | 0.6 |
| 18:0 | 4.8 | 1.7 | 4.3 | 1.7 | 3.4 | 1.4 | 3.8 | 1.4 |
| 18:1*n*-9 | 21.5 | 3.0 | 21.3 | 3.3 | 23.6 | 2.9 | 21.2* | 2.5 |
| 18:2*n*-6 | 38.8 | 5.5 | 39.6 | 5.9 | 40.0 | 4.1 | 40.2 | 5.4 |
| 20:4*n*-6 | 7.5 | 1.8 | 7.2 | 1.4 | 7.2 | 1.7 | 7.3 | 1.9 |
| 20:5*n*-3 | 0.9 | 0.2 | 1.8*** | 0.5 | 1.0 | 0.4 | 1.9*** | 0.4 |
| 22:6*n*-3 | 1.9 | 0.4 | 2.8*** | 0.6 | 2.2 | 0.6 | 3.3*** | 0.7 |
| Chol (mmol/l) | 3.6 | 0.9 | 3.6 | 0.9 | 4.0 | 0.9 | 4.1 | 0.8 |

Mean values were significantly different from baseline: *$P \leq 0.05$, ***$P \leq 0.001$ (paired $t$ test)

The Table shows that both EPA and DHA were significantly increased ($P \leq 0.001$) in plasma following supplementation in both groups. However, there were no significant differences in EPA or DHA concentrations following supplementation between the groups (adjusting for week 0 values) (ANCOVA). Plasma total cholesterol levels were not significantly altered by *n*-3 PUFA supplementation in either group.

The results of this study show that there was no difference in the bioavailability of *n*-3 PUFA given as microencapsulated fish oil compared with *n*-3 PUFA delivered as a fish oil capsule. Fortification of ordinary foodstuffs with microencapsulated fish oil, therefore, offers the potential to increase intakes of *n*-3 PUFA in line with current recommendations.

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**Contribution of foods to *trans* fatty acid intake in a group of Irish adults.** By M.M. CANTWELL[1], M.J. GIBNEY[2], D. CRONIN[3], and M.A.T. FLYNN[1], *Department of [1]Biological Sciences, Dublin Institute of Technology; [2]Clinical Medicine, University of Dublin, Trinity College, [3]University College Dublin, Republic of Ireland*

*Trans* fatty acids (*t*FA) have been shown to raise LDL-cholesterol and lower HDL-cholesterol levels (Mensink & Katan, 1990). The source of *t*FA may be an important factor; whether they result from the industrial hydrogenation of vegetable oils (H-*t*FA) or are produced by biohydrogenation of linoleic or linolenic acid by bacteria in ruminant animals (N-*t*FA). The main *t*FA formed by hydrogenation of vegetable oils is elaidic acid (*t*9C18:1), while vaccenic acid (*t*11C18:1) is the predominant *t*FA formed by hydrogenation in ruminants. However industrial hydrogenation of marine oils used in the food industry in Ireland produces *t*FAs with sixteen to twenty-four C atoms in the form of a variable number of positional and geometric isomers. The effects of these *t*FA remain largely unknown.

Dietary intake data were collected from 105 men and women aged 23-63 years, using a fat intake questionnaire which was validated in this study group. The fat intake questionnaire included eighty-eight food items or food groups and was structured to follow a typical daily meal pattern. The frequency of consumption of a particular food item was recorded on a per day, per week, per fortnight, or per month basis. The types and brands of fats, oils, margarines, biscuits, cakes, bread, crackers and snack foods used were identified by using photographs of these foods. The individual fatty acid and total *trans* fatty acid contents of 220 Irish processed foods were analysed (Cronin & O'Neill, 1995) and the information was incorporated into the UK food/nutrient database (FOODBASE). The Table shows the percentage contributions of foods to total *t*FA intake.

| | Total *t*FA | | | Total *t*FA | |
|---|---|---|---|---|---|
| Food | Mean (%) | SD | Food | Mean (%) | SD |
| Confectionery | 7.4 | 14.0 | Cheese | 5.5 | 5.7 |
| Spreads | 35.7 | 47.1 | Milk | 9.79 | 10.0 |
| Patisserie | 25.9 | 57.6 | Butter | 9.83 | 18.5 |
| Sauces | 3.6 | 16.9 | Meat | 3.18 | 3.5 |
| Bread | 0.35 | 1.7 | | | |

H-*t*FA accounted for 71% of the total *t*FA intake mainly from spreads, patisserie and confectionery. The remaining 29% came from N-*t*FA (in butter, milk, cheese and meat). The mean *t*FA intake of the group assessed was 5.4 (SD 3.3) g/d (1.9% of total energy intake), comparing closely with the UK recommendation of 5 g/d, or 2% of total energy intake. However, total fat intake estimated for this group was only 34% of total energy intake which is unlikely to be representative of the total Irish population. Nonetheless, a comparison of food intakes of those in the lowest quartile of *t*FA intake (0-1.27% energy) and highest quartile of *t*FA intake (>2.3% energy) showed significant differences. Cakes, chips, monounsaturated full-fat spreads, plain biscuits and polyunsaturated full-fat spreads were eaten in significantly higher amounts by those in the highest quartile of *t*FA intake while root and salad vegetables, tomatoes, soup and wine were eaten in significantly higher amounts by those in the lowest quartile of *t*FA intake.

Although these data do not indicate that partially hydrogenated fats and oils represent a serious health risk in Ireland, they do raise the question of their continued use to replace saturated fats in processed foods. Additional and continued analysis of processed foods is necessary to assess the *t*FA intake of Irish adults in more detail.

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**Effect of genetic variation of apolipoprotein B (apoB) on postprandial lipidaemia.** By I.L. BLACK[1], A.M. TULLY, E. NOONE, H.M. ROCHE[2], A.S. WHITEHEAD and M.J. GIBNEY, *[1]Smurfit Institute of Genetics, Lincoln Gate, Trinity College, Dublin 2, Ireland, [2]Trinity Centre for Health Sciences, St James' Hospital, James' Street, Dublin 8, Ireland*

ApoB is the main structural lipoprotein of triacylglyceriol (TAG)-transporting lipoproteins (chylomicrons and VLDL) and their remnants (chylomicron remnant and LDL). Therefore the protein must have domains which bind to TAG, facilitate TAG hydrolysis by lipoprotein lipase and allow the removal of chylomicron remnants and LDL from the circulation. In recent years many frequently occurring polymorphisms of apoB have been identified, with three having the most effect on fasting plasma lipid levels. The insertion/deletion polymorphism occurs in exon 1 of the apoB gene causing the deletion of three amino acids in the signal peptide. The deletion (D) allele causes inefficient translocation, resulting in reduced efficiency of secretion of the mature protein. Individuals carrying the D allele have lower plasma TAG postprandial peaks (Xu *et al.* 1990). The XbaI restriction fragment length polymorphism (RFLP) which is a silent mutation in exon 26, is believed to be linked to a functional mutation. Both alleles of the XbaI polymorphism have been associated with increased risk of CHD. The EcoRI RFLP causing a missense mutation in exon 29, is thought to be close to the LDL receptor binding site and so affect LDL clearance, although no differences in fasting lipid or lipoprotein levels have been associated with this polymorphism. Previous postprandial studies have found the insertion/deletion polymorphism to be the most useful for explaining lipoprotein and lipid variation. Regis-Bailly *et al.* (1995) found that deletion homozygotes (DD) had the lowest concentrations of apoB-containing lipoproteins throughout the postprandial response. Peacock *et al.* (1995) found that individuals carrying the D allele had a smaller postprandial response for chylomicron remnants and VLDL compared with insertion homozygotes (II). Byrne *et al.* (1996) showed that the II individuals had significantly higher TAG-rich lipoprotein TAG for the highest non-esterified fatty acids quartile compared with the other genotypes. The present study set out to investigate how these three polymorphisms affect both the lipoprotein and lipid postprandial responses in a single study group.

The insertion/deletion polymorphism, XbaI RFLP and EcoRI RFLP were defined, in a population of 119 Caucasians, using the polymerase chain reaction and restriction enzyme digestion. Lipid levels were analysed colorimetrically for individuals ($n$ 72-82) who fulfilled the study criteria. The results were analysed by two-sample-t-test and chi-squared tests to explain the genotype:phenotype relationship.

The I allele of the insertion/deletion polymorphism was found to be significantly associated with larger BMI values ($P$=0.027). Analysis of the fasting and postprandial plasma TAG levels showed that the R- allele of the EcoRI polymorphism tended to be distributed in the upper end of the TAG-fasting, -maximum and -area under the curve distributions. Analysis of the TAG concentration of TAG-rich lipoproteins found a larger difference between the two alleles. Again, the R- allele being associated with higher plasma TAG-rich lipoprotein-TAG-fasting, -maximum, and -area under the curve . The chi-squared test showed the R- allele is located at a higher frequency in the top tertile of all of the TAG distributions.

The XbaI RFLP did not have any effect on lipid distributions in this population. In this study the insertion/deletion polymorphism had little or no effect on the postprandial response, however effects on BMI were observed. When the fasting and postprandial apoB protein concentrations have been measured the insertion/deletion polymorphism may be seen to cause variation in the apoB levels; this effect may be indirectly seen in the BMI distribution. In this study population the EcoRI polymorphism had effects on the fasting and postprandial TAG levels, thus it is suggested that individuals carrying the R+ allele have a healthier postprandial lipid profile than R- carriers.

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**Postprandial lipaemia in response to sucrose intake in middle-aged men with CHD risk factors and matched controls.** By AUDREY E. BRYNES, C. MARK EDWARDS and GARY S. FROST, *Department of Nutrition & Dietetics, Hammersmith Hospital, London W12 0HS*

Evidence suggests that excessively high intakes of sucrose (>100 g) increase postprandial lipaemia. It is unknown if this is true of moderate intakes of sucrose (75 g) or if people at risk of CHD have exaggerated lipaemia. There is also still some debate over how much of the postprandial hypertriacylglycerolaemia is due to the fructose content rather than the overall carbohydrate content. The present study was designed to measure postprandial triacylglycerol (TG) and free fatty acid (FFA) levels in response to a glucose test meal (75 g glucose) *v.* a sucrose test meal (75 g sucrose). The meals were otherwise matched for nutrient content (3.68 MJ, 48 g fat of which 30 g saturated, 115 g carbohydrate and 5 g protein).

Twenty male volunteers were recruited and separated into a control and an 'at risk' group depending on the fasting lipid results. They were studied in a randomized order on two separate occasions. To control for fasting lipid levels, identical frozen meals were provided on the evenings before the test, plus written information on exercise and alcohol given. The demographic details are shown in Table 1. The numbers of smokers, ex-smokers and non-smokers were identical in both groups. Alcohol intake was not significantly different between the groups. All men were sedentary (as classified by Department of Health, 1991). There were no significant macronutrient differences (recorded by 3 d diet diary) between the groups and both were similar to the UK national averages (% energy) 15 protein, 43 carbohydrate, 39 fat of which 13 saturated, 10 monounsaturated and 5 polyunsaturated).

| | Age (years) | BMI ($kg/m^2$) | Waist (cm) | Plasma Cholesterol (mmol/l) | Plasma HDL (mmol/l) | Plasma Triglycerides (mmol/l) |
|---|---|---|---|---|---|---|
| Control | 47 | 26.6 | 88 | 4.6 | 1.5 | 1.12 |
| Risk CHD | 51 | 27.4 | 100 | 6.6 | 1.2 | 2.4 |
| *P* value | NS | NS | 0.01 | 0.01 | 0.03 | 0.02 |

After an overnight fast (12-14 h) a cannula was inserted into the antecubital fossa before each meal. Blood samples were taken at -15, 0, 5, 15, 30, 45, 60, 90, 120, 150 min, 3, 4, 5 and 6 h after the start of the meal. The meal was started at time 0 and consumed within 10 min on every occasion. The results were normally distributed and are presented as fasting means and mean incremental areas under the curves with their standard errors (IAUC) 0-360 min. Comparison between glucose and sucrose in men with high or low lipids was by ANOVA. $P<0.05$ was taken as significant.

| | Fasting plasma TG (mmol/l) | | Fasting plasma FFA (μmol/l) | | IAUC TG (0-360) (mmol/l per min) | | IAUC FFA (0-360) (mmol/l per min) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| Sucrose | | | | | | | | |
| Control | 1.10 | 0.1 | 403 | 43 | 89.1 | 23 | -33.5 | 21 |
| Risk CHD | 1.89 | 0.3 | 586 | 55 | 144.9 | 45 | -86.0 | 15 |
| Glucose | | | | | | | | |
| Control | 0.94 | 0.1 | 452 | 85 | 173.6 | 45 | -68.5 | 27 |
| Risk CHD | 2.59 | 0.6 | 563 | 67 | 159.7 | 28 | -82.2 | 23 |

There was no difference in the postprandial TG or FFA response to glucose or sucrose within or between the groups. Our results suggest that 75g of sucrose given as part of a single meal appears to make little difference to the postprandial lipaemic response compared with glucose in men with or without a risk of CHD. This suggests that since the effect of sucrose is small, the risk associated with sucrose ingestion is minimal in comparison with the risk of raised basal levels of TG, NEFA, cholesterol and increased waist circumference observed in the group with CHD risk factors.

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**The postprandial cholesterol response is associated with fasting plasma triacylglycerol concentrations.** By A.M. TULLY, E.J. NOONE, H.M. ROCHE and M.J. GIBNEY, *Unit of Clinical Nutrition, Department of Clinical Medicine, Trinity Centre for Health Sciences, St James' Hospital, Dublin 8, Ireland*

Most postprandial research has been completed in clinical trials with subjects who are either hypertriacylglycerolaemic or have a history CHD. Studies in normolipidaemic people alone have mainly concentrated on fasting triacylglycerol (TAG) levels, due to their well-documented correlation with postprandial TAG response. Less attention has been paid to postprandial cholesterol levels in healthy normolipidaemic subjects, despite the fact that we spend most of our time in the postprandial state. In a recent study Clifton & Nestel (1996) observed a significant, adverse effect of dietary cholesterol on postprandial cholesterol and TAG levels in hyperlipidaemic subjects only. The same effect was not observed in the normolipidaemic group.

In the present study sixty-seven healthy, non-obese, non-smoking volunteers (forty male, twenty-seven female, mean age 33 years, mean BMI 23.8 $kg/m^2$) were recruited according to a list of exclusion criteria. A biochemical screen was completed to ensure that all subjects had blood lipid profiles and glucose within the normal range. Details regarding age, weight and height were also recorded. Postprandial lipaemia was studied after a standardized high-fat meal (40 g fat, 0 g cholesterol) given to volunteers following a 12 h overnight fast. Alcohol was restricted for 24 h before the study day. Venous blood samples were drawn from an indwelling cannula at baseline before the test meal and at 2 hourly intervals thereafter over an 8 h period. TAG-rich lipoproteins (TRL) were separated by ultracentrifugation. Plasma and TRL were analyzed for TAG and cholesterol concentrations. Subjects were divided into four groups according to their fasting TAG concentration.

| | TAG levels, quartiles | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Quartile 1 | | Quartile 2 | | Quartile 3 | | Quartile 4 | |
| Lipid Fraction (mmol/l) | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Fasting TAG | 0.57 | 0.08 | 0.79 | 0.05 | 1.08 | 0.14 | 1.65 | 0.42 |
| TAG AUC | 5.51 | 1.32 | 7.21 | 1.56 | 9.81 | 1.3 | 13.10† | 2.51 |
| Fasting cholesterol | 4.58 | 0.71 | 4.68 | 0.93 | 5.40 | 0.89 | 5.77* | 1.10 |
| cholesterol AUC | 36.42 | 5.84 | 37.44 | 7.18 | 41.32 | 5.68 | 44.56* | 8.87 |
| Fasting TRL - TAG | 0.26 | 0.13 | 0.49 | 0.22 | 0.68 | 0.20 | 0.96† | 0.27 |
| TRL - TAG AUC | 3.07 | 1.94 | 4.86 | 2.62 | 6.84 | 2.64 | 9.88† | 4.38 |
| Fasting TRL - cholesterol | 0.09 | 0.05 | 0.18 | 0.08 | 0.24 | 0.12 | 0.35† | 0.15 |
| TRL - cholesterol AUC | 0.56 | 0.38 | 1.19 | 0.79 | 1.61 | 0.84 | 2.53† | 1.39 |

* Significantly different from Groups 1 & 2 $P < 0.01$.
† Significantly different from Groups 1, 2 & 3 $P < 0.005$.

The plasma and TRL - TAG area under the curve (AUC) values were significantly greater ($P < 0.005$) in the highest quartile. Cholesterol concentrations in the fasted and postprandial states in plasma and the TRL fraction were significantly ($P < 0.01$) greater in the highest TAG quartile in comparison with groups 1 & 2.

These findings demonstrate that healthy, normolipidaemic volunteers, with fasting TAG concentrations at the upper end of the normal range, show significant enrichment of plasma and TRL with cholesterol. This potentially adverse effect could cause cholesterol deposition in the arterial wall. These observations make compelling evidence for strengthening the argument for the inclusion of fasting TAG in the assessment of CHD risk, paying particular attention to those who have TAG levels at the upper end of the normal range. This is supported by the results of an 8 year follow-up to the Copenhagen Male Study where it was revealed that subjects with TAG levels of about 1.6 - 2.5 mmol/l had the highest incidence rate of ischaemic heart disease of the study group (*n* 2372, TAG range 0.44 - 22.4 mmol/l), (Jeppesen *et al.* 1998).

Clifton PM and Nestel PJ (1996) *American Journal of Clinical Nutrition* **64**, 361-367.
Jeppesen J, Ole Hein H, Suadicani P & Gyntelberg F (1998) *Circulation* **97**, 1029-1036.

**The effect of a fat rich test meal on cholesteryl ester transfer protein mass and activity in normolipidaemic individuals during postprandial lipaemia**. By E. NOONE, H.M. ROCHE, A.M. TULLY and M.J. GIBNEY, *Unit of Nutrition, Department of Clinical Medicine, Trinity Centre for Health Sciences, St James's Hospital, Dublin 8, Ireland*

Cholesteryl ester transfer protein (CETP) mediates the transfer of cholesterol ester (CE) from HDL to VLDL and LDL. During alimentary lipaemia there is a significant increase in the rate of CE transfer (Tall, 1986). Previous studies of CETP have measured either CETP mass or activity, in hypercholesterolaemic or diabetic subjects with a small number of normolipidaemic controls. In the present study, thirty seven males and twenty six females (age 32.44 (SD 9.60) years; weight 73.48 (SD 13.65) kg) with a normal lipid profile fasted overnight for 12 h. A fasting blood sample was taken, then a 40 g fat test meal was consumed by each subject. A postprandial blood sample was then taken 6 h after the test meal. Plasma samples were analysed for CETP mass and activity, plasma cholesterol and triacylglycerol (TAG) concentrations and HDL-cholesterol, phospholipid, apolipoprotein A1, and TAG concentrations. Due to the importance of fasting plasma cholesterol on CETP metabolism the study group was divided according to plasma cholesterol concentration (< 5.2 mmol/l; > 5.2 mmol/l).

| | Total cholesterol < 5.2 mmol/l (n 39) | | | | Total cholesterol > 5.2 mmol/l (n 24) | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 h | | 6 h | | 0 h | | 6 h | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| CETP Mass (ug/ml) | 1.96 | 0.61 | 2.09* | 0.71 | 2.22 | 0.81 | 2.67** | 0.75 |
| CETP activity pmol NBD-CE transferred/3h. | 145.51 | 30.66 | 177.50*** | 59.82 | 156.50 | 43.87 | 223.67** | 75.08 |
| Cholesterol (mmol/l) | 4.42 | 0.54 | 4.38 | 0.56 | 6.01 | 0.63 | 5.94*** | 0.75 |
| TAG (mmol/l) | 0.91 | 0.40 | 1.02*** | 0.46 | 1.12 | 0.36 | 1.17 | 0.49 |

Mean values were significantly different from those for 0 h: $*P < 0.05$, $**P = 0.01$, $*** P \leq 0.0001$.

Fasting and postprandial CETP mass, activity, total cholesterol and TAG concentrations of the two groups are presented in the table. Significant increases in CETP mass and activity were observed in both groups during postprandial lipaemia. An increase in plasma TAG concentration was observed in both groups during postprandial lipaemia but was only significant in the low-cholesterol group, while a decrease in total plasma cholesterol was observed in both groups. Both groups showed a significant correlation between ($P \leq 0.038$) mass and activity in fasting and postprandial states. Multiple stepwise regression analysis of the total study group (n 63) showed that fasting CETP mass was the principal determinant of fasting CETP activity ($\beta = 43.002$; $P \leq 0.0001$), postprandial CETP activity was determined by fasting plasma cholesterol concentration ($\beta = 0.055$; $P = 0.0025$) followed by TAG-rich lipoproteins (TRL) TAG concentration ($\beta = 0.013$; $P = 0.005$) and CETP mass at 6 h ($\beta = 0.254$; $P = 0.0070$).

This study shows that there was an increase in CETP mass and activity in response to a test meal. There is a significant correlation between CETP mass and activity in the fasted and postprandial states. Postprandial CETP activity is associated with fasting plasma cholesterol and postprandial TRL TAG concentrations.

Tall AR (1986) *Journal of Lipid Research* **27**, 361-368.

**Confirmation of reduced energy intake after consumption of yoghurt containing Olibra™** . By A.A. Burns[1], M.B.E. Livingstone[1], R.W. Welch [1], L. Lindmark [2], and I. R. Rowland [1], Northern Ireland Centre for Diet and Health (NICHE), *University of Ulster, Coleraine BT52 1SA and Scotia LipidTeknik AB, Box 6686, 11384 Stockholm Sweden*

Investigations of the regulation of food intake and energy balance have shifted from an emphasis on amounts of energy consumed to questions related to how macronutrients differ in their effects on satiety and energy balance. In a previous study (Burns *et al.* 1998; study 1) a novel fat emulsion (Olibra™ , 95% fractionated palm oil, 5% fractionated oat oil, LipidTeknik, Stockholm, Sweden) was shown to increase satiety and significantly reduce short-term energy intake. To determine whether these results could be reproduced, thirty volunteers (sixteen females, fourteen males; 21-36 years; BMI < 30 kg/m 2; non-smokers) participated in the present double-blind, randomized crossover study (study 2). Each volunteer, in a fasted state from the evening before the study day, received a defined breakfast (25% estimated energy expenditure) at 09.00 hours. At 13.00 hours a 200 g portion (800 kJ) of a test (5 g Olibra™, 1 g milk fat) or control (6 g milk fat) yoghurt was randomly given for lunch. Between meals only water was permitted until 17.00 hours when volunteers were given *ad libitum* access to a range of foods. Intake was assessed by weighing the food and remains, and macronutrient intake was determined using Compeat 4.

| | All subjects | | | | Females | | | | Males | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Intake at evening meal | Study 1 (*n* 29) Mean | SEM | Study 2 (*n* 30) Mean | SEM | Study 1 (*n* 15) Mean | SEM | Study 2 (*n* 16) Mean | SEM | Study 1 (*n* 14) Mean | SEM | Study 2 (*n* 14) Mean | SEM |
| Energy (MJ) | | | | | | | | | | | | |
| Test | 6.41** | 0.49 | 6.87*** | 0.36 | 5.26** | 0.43 | 5.62** | 0.36 | 7.66 | 0.78 | 8.30* | 0.38 |
| Control | 7.62 | 0.33 | 7.86 | 0.36 | 6.74 | 0.35 | 6.58 | 0.31 | 8.55 | 0.46 | 9.32 | 0.41 |
| Fat (g) | | | | | | | | | | | | |
| Test | 70.4*** | 6.17 | 72.0*** | 4.95 | 58.1** | 5.90 | 55.2** | 4.39 | 83.5* | 10.2 | 91.1* | 6.24 |
| Control | 91.0 | 4.80 | 84.7 | 5.17 | 80.0 | 5.01 | 68.5 | 4.45 | 103.0 | 7.22 | 103.0 | 7.23 |
| Protein (g) | | | | | | | | | | | | |
| Test | 58.3* | 4.75 | 69.2** | 5.06 | 48.8 | 5.14 | 52.9** | 5.37 | 68.5 | 7.39 | 88.0 | 5.82 |
| Control | 67.0 | 4.49 | 78.9 | 4.72 | 58.0 | 3.99 | 65.5 | 4.19 | 75.9 | 7.64 | 94.3 | 7.02 |
| Carbohydrate (g) | | | | | | | | | | | | |
| Test | 177 | 12.6 | 187** | 7.80 | 142* | 10.7 | 168* | 9.32 | 214 | 19.3 | 209 | 10.3 |
| Control | 196 | 10.0 | 204 | 7.51 | 174 | 13.6 | 184 | 9.13 | 220 | 11.4 | 228 | 8.94 |
| Weight of food (g) | | | | | | | | | | | | |
| Test | 1157* | 78.0 | 1226*** | 54.9 | 955*** | 71.3 | 1071** | 59.7 | 1374 | 119.7 | 1404** | 71.7 |
| Control | 1321 | 50.3 | 1439 | 62.3 | 1214 | 58.3 | 1260 | 68.5 | 1435 | 73.7 | 1645 | 79.3 |

Mean values were significantly different from control: * P < 0.05, ** P < 0.01, *** P < 0.001 (two-tail paired t test)

In the present study energy and macronutrient intakes were significantly reduced in the total group and in the female group by similar magnitudes to the initial study. The male group responded similarly to the male group in study 1 and the energy intake was also significantly lower. An independent t test showed no significant differences between the results of studies 1 and 2. Significant reductions in the total weight of food eaten after the test yoghurt were observed in all groups except the males in study 1. These results confirm that consumption of the Olibra™ yoghurt significantly lowers total food intake and energy intake in the short term but further work is required to assess the medium- and long-term effects of Olibra™ in appetite control and energy balance.

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**Effect of protein and/or carbohydrate ingestion on whole-body creatine retention during creatine supplementation in human subjects.** By GERY R. STEENGE, LIZ J. SIMPSON, IAN A. MACDONALD and PAUL L. GREENHAFF, *School of Biomedical Sciences, University Medical School, QMC, Nottingham NG7 2UH*

Carbohydrate (CHO)-mediated insulin release has been shown to enhance creatine (Cr) accumulation in human skeletal muscle, but the amount of CHO required to modulate such an effect is close to the limit of palatability (Green *et al.* 1996). Several proteins and amino acids are known to stimulate insulin secretion (Spiller *et al.* 1987). Furthermore, simultaneous ingestion of CHO and protein appears to result in greater insulin release than would be expected from the sum of their individual responses (Zawadzki *et al.* 1992). The aim of the present study was to examine whether protein ingested in combination with moderate amounts of CHO would increase serum insulin concentration above that observed with CHO alone and thereby increase the extent of whole-body creatine retention towards that seen with larger quantities of CHO.

Eight healthy men visited the laboratory at noon on four randomized occasions after a 4 h fast. Each then consumed 5 g Cr dissolved in 250 ml water, followed 30 min later by either 500 ml placebo (sugar-free Lucozade); 47 g CHO together with 50 g protein (Protein Forte); 47 g CHO (Lucozade) or 94 g CHO (Lucozade). Blood samples were obtained before and at regular intervals for 220 min following Cr ingestion and were later analysed for serum insulin and plasma Cr.

**Table.** Peak concentrations and total area (TA) under time curves for serum insulin and plasma Cr

| | Placebo | | CHO+Protein (47+50 g) | | CHO (47 g) | | CHO (94 g) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Peak Cr (μmol/l) | 996 [a] | 94 | 1008 [a] | 57 | 986 [a] | 79 | 766 [b] | 91 |
| TA Cr (mmol/l per min) | 114 | 8 | 114 | 8 | 110 | 8 | 98 | 9 |
| Peak insulin (mU/l) | 12 [a] | 2 | 78 [c] | 6 | 60 [b] | 6 | 78 [c] | 7 |
| TA insulin (mU/l per min) | 680 [a] | 223 | 5639 [bc] | 782 | 3805 [b] | 439 | 7088 [c] | 604 |

[a,b,c] *Mean values within a row not sharing a common superscript letter were significantly different, $P<0.05$ (one-way ANOVA and Tukey post-hoc test).*

Peak plasma Cr concentration was significantly lower after 94 g CHO compared with the other three treatments and TA under Cr time curve tended to be lower (about 14%). Peak insulin concentration was not lower when comparing CHO+protein and 94 g CHO, but TA under insulin time curve approached significance ($P<0.10$). These data suggest that ingesting protein in combination with moderate amounts of CHO does not augment whole-body Cr retention to the same extent as seen with higher amounts of CHO.

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**Comparison between the *in situ* and the Cornell methods of estimating protein degradability in a variety of ruminant feedstuffs.** By FRANKLIN K. AVORNYO and ERIC L. MILLER, *Nutrition Laboratory, 307 Huntingdon Road, Cambridge CB3 OJQ*

European systems of evaluation of protein feed for ruminants depend on *in situ* determination of protein degradability (dg) requiring rumen-cannulated animals. An alternative is the use of the Cornell computer simulation model to estimate dg from laboratory analysis of N fractions of the feed. Experiments and data were analysed to evaluate the Cornell model against the *in situ* system for dg.

In Expt 1, the regression of fractional *in situ* effective dg ($y$) on the Cornell estimate ($x$) for ten concentrate feeds was $y=1.22x-0.03$ ($r^2$ 0.76; $P<0.001$). In Expt 2, analyses of six variously protected concentrate feeds gave $y=1.27x-0.18$ ($r^2$ 0.96; $P<0.001$). Combining the two experiments gave $y=1.36x-0.16$ ($r^2$ 0.82; $P<0.001$). Regression of *in situ* effective dg ($y$) on soluble protein ($x$) (Cornell fractions A+B1) was also significant ($y=1.02x+0.35$; $r^2$ 0.65; $P<0.001$), but using the Cornell model accounted for a further 17% of the variation in effective dg.

The Cornell sub-model was also used to calculate effective dg at 0.05/h rumen outflow rate from published analyses of N fractions of a range of feeds. These estimates were compared with reported data sets for *in situ* effective dg at 0.05/h rumen turnover rate. The findings are presented in the following three points.

(1) Data on thirty-three nominally similar samples listed by Alderman & Cottrill (1993) for *in situ* and Mansbridge (1996) for Cornell N analyses. When forages were included in the data set, the relationship was $y=0.58x+0.34$ ($r^2$ 0.36; $P<0.001$). However, the relationship for the nine forages found in the data set was not statistically significant; $y=-0.34x+1.08$ ($r^2$ 0.03; $P=0.672$). Excluding the nine forages, the equation for the remaining cereals, cereal by-products, brewery by-products, pulps and protein concentrates was $y=0.74x+0.25$ ($r^2$ 0.33; $P<0.01$).

(2) From data published by McAllister *et al.* (1993) on nine heat- and chemically treated canola (rapeseed) meals, the relationship was $y=1.06x+0.09$ ($r^2$ 0.97; $P<0.001$).

(3) From data reported by Moshtaghi Nia & Ingalls (1992) on samples of canola meal given six different moist-heat treatments, the relationship was $y=1.07x-0.20$, $r^2$ 0.79 ($P<0.05$).

The lack of agreement with the forages may be due to the narrow range of their dg values and to the fact that nominally similar, rather than the same forages, were compared. In addition, the *in situ* values for forages may be affected by microbial contamination of bag residues, which apparently reduces the effective dg. Current work has shown significant microbial contamination of forages, which substantially affects the calculated dg.

For the concentrates and by-products analysed, estimates of dg obtained using the Cornell model were highly significantly correlated with the *in situ* estimates.

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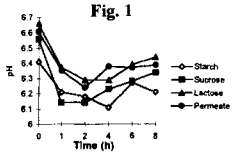
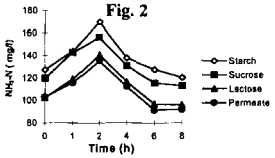
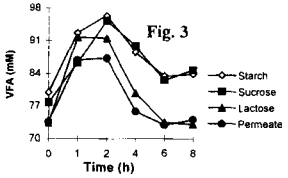
Moshtaghi Nia S A & Ingalls J R (1992) *Canadian Journal of Animal Science* **72**, 83-88.

**Effects of isoenergetic supplements of starch, sucrose, lactose and whey permeate on rumen metabolism and digestion.** By A. HUSSAIN and E. L. MILLER, *University of Cambridge, Nutrition Laboratory, 307 Huntingdon Road, Cambridge CB3 OJQ*

Whey permeate (850 g lactose/kg) is a commercial by-product obtained from the manufacture of cheese. Its rate of fermentation and effect on rumen metabolism are similar to those of pure lactose (Hussain & Miller, 1998). The main objective of the present study was to examine the effect of isoenergetic amounts of starch, sucrose, lactose and whey permeate supplementation (equivalent to 50 g glucose) on digestion and metabolism in ruminants. For this purpose four different diets containing the supplement, 350 g concentrate and 600 g hay were fed to four wethers in five periods using a 5×4 incomplete Latin-square design. Concentrate along with supplement was offered twice daily in two equal meals. Hay was given every 2 h, $^{35}S$ was infused continuously. During each period samples from rumen, duodenum, faeces and urine were taken to measure rumen fermentation, digestion and microbial protein synthesis. Data obtained were analysed using the ANOVA procedure of Genstat 5. The main results are given below.




| Diets | Starch | Sucrose | Lactose | Whey permeate | SED |
|---|---|---|---|---|---|
| Rumen protozoa (x$10^4$/ml) | 129.90 | 119.20 | 79.50 | 82.10 | 5.52 |
| Total tract nutrient digestion % | | | | | |
| Organic matter (OM) | 69.03 | 69.16 | 69.17 | 69.60 | 0.96 |
| Neutral detergent fibre (NDF) | 62.34 | 62.42 | 62.82 | 64.20 | 1.26 |
| Acid detergent fibre (ADF) | 57.14 | 57.72 | 57.89 | 59.38 | 1.51 |
| Nutrient flow at the duodenum (g/d) | | | | | |
| OM flow | 410.40 | 402.50 | 372.10 | 375.30 | 10.21 |
| NDF flow | 221.20 | 217.20 | 191.80 | 188.50 | 8.20 |
| Microbial N (MN) flow from $^{35}S$ | 10.27 | 10.41 | 11.51 | 11.83 | 0.50 |
| MN supply from purine derivatives (PD) | 10.18 | 10.75 | 11.58 | 12.15 | 0.65 |

At different times after feeding, lactose and whey permeate resulted in less depression in rumen pH (Fig. 1), reduced rumen $NH_3$ (Fig. 2) and reduced total volatile fatty acid (VFA) concentration (Fig. 3) compared with starch and sucrose. Lactose and whey permeate significantly reduced the number of rumen protozoa ($P<0.001$) compared with starch and sucrose. Sucrose also tended to reduce total protozoa compared with starch ($P=0.08$). Total tract nutrient digestion was not affected by the different treatments. Compared with starch and sucrose, lactose and whey permeate resulted in less OM and NDF flow (g/d) at the duodenum ($P<0.05$), indicating better rumen fibre digestion, while MN flow ($^{35}S$) was increased ($P<0.05$). MN supply, measured by PD, gave similar results to the $^{35}S$ measurement. When feeding concentrate twice daily, replacing 50 g starch with lactose and whey permeate improves rumen pH and fibre digestion, reduces rumen protozoa with a consequent decrease in rumen $NH_3$ and increases microbial (bacterial) protein supply.

Financial support from Cambridge Commonwealth Trust and the supply of lactose and whey permeate from Volac International are acknowledged.

Hussain A & Miller E L (1998) *Proceedings of the British Society of Animal Science, 1998,* 66.

**Intake of selected nutrients from vitamin and mineral supplements by subjects in the EPIC Study, Norfolk, UK.** By AILSA A. WELCH, ANGELA A. MULLIGAN, ROBERT N. LUBEN AND SHEILA A. BINGHAM, *EPIC Study, Institute of Public Health, University of Cambridge, Strangeways Research Laboratory, Cambridge CB1 4RN*

We have developed a new database (VIMS; vitamin and mineral supplements) using data supplied by manufacturers which, to date, contains 900 individual types and brands. These data have been converted for concentration so that the values are compatible with nutrients as given in the UK food tables.

Preliminary results from the first 1860 participants (coding 98% complete) to enrol in the EPIC (European Prospective Investigations into Cancer) Study in Norfolk indicate that 40.6% of participants had consumed a supplement during the past year, 37.5% of them regularly. (Regular consumption is defined by questionnaire response, from EPIC participants, indicating that supplements were consumed on a regular basis.)

| Nutrient | Intake from food | Intake from VIMS | | Subjects consuming VIMS | |
|---|---|---|---|---|---|
| | Mean * | Mean | % food intake | N | % |
| Calcium (mg) | 1044 | 171 | 16 | 100 | 5 |
| Magnesium (mg) | 330 | 25.2 | 8 | 57 | 3 |
| Manganese (mg) | 5.6 | 1.3 | 23 | 68 | 4 |
| Potassium (mg) | 3758 | 29.9 | 1 | 36 | 2 |
| Selenium (µg) | 54 | 37.7 | 70 | 20 | 1 |
| Zinc (mg) | 10 | 4.9 | 49 | 89 | 5 |
| Total carotene (µg) | 2917 | 1956 | 67 | 16 | 1 |
| Vitamin A retinol (µg) | 866 | 806 | 93 | 596 | 32 |
| Vitamin $B_6$ (mg) | 2.1 | 9.5 | 453 | 222 | 12 |
| Vitamin C (mg) | 114 | 200 | 176 | 291 | 16 |
| Vitamin D (µg) | 3.7 | 6.1 | 165 | 600 | 32 |
| Vitamin E (mg) | 6 | 24.4 | 407 | 565 | 30 |
| Folic acid (µg) | 310 | 258 | 83 | 147 | 8 |

* Values derived from the EPIC food-frequency questionnaire used with 204 male and female subjects aged 45 – 74 years in the UK (Bingham *et al.* 1997).

The Table shows that average daily consumption of nutrients from supplements was close to or greater than that from food for vitamins A (retinol), $B_6$, C, D, E and folic acid.

In the VIMS database the percentages of supplements containing amounts greater than average daily intake were β-carotene 81, retinol 9, vitamin C 45 and vitamin E 55.

Unless accounted for, nutritional supplements can lead to gross misclassification of exposure in epidemiological studies.

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**The effect of suboptimal and deficient dietary magnesium intakes on bone composition and bone metabolism in the rat.** By ANNETTE CREEDON, ALBERT FLYNN and KEVIN CASHMAN, *Department of Nutrition, University College, Cork, Ireland*

Mg status influences osteocyte proliferation, tissue organization and mineralization of bone (Kenney *et al.* 1994) and Mg deficiency is associated with abnormal bone formation in experimental animals (Carpenter *et al.* 1992). However, the effect of Mg deficiency on bone turnover is unclear. The objective of the present study was to examine the effects of dietary Mg intakes on bone mineral composition and on the biochemical markers of bone formation (serum osteocalcin) and bone resorption (urinary excretion of the pyridinium crosslinks) in a rat model.

Forty 3-week-old male rats, Wistar strain, average weight 59 g, were randomized into five groups (*n* 8/group) and housed individually in metabolism cages. Three groups were fed *ad libitum* on AIN-76 (American Institute of Nutrition 1977) diets containing 400 (adequate), 200 (suboptimal) or 20 (deficient) mg Mg/kg for 3 weeks while two groups were pair-fed with the adequate diet in the same quantities as those consumed by the groups fed on the suboptimal and deficient diets respectively. Pyridinium crosslinks (Pyr and Dpyr) were measured by an HPLC method in 24 h urine samples taken for three consecutive days during the final week of the study. Femur mineral content and serum Mg concentration were measured using atomic absorption spectrophotometry after dry ashing and appropriate dilution respectively. Osteocalcin was measured by an ELISA method in serum samples taken at the end of the study.

| Group ... | Deficient | | Pair-fed/D* | | Suboptimal | | Pair-fed/S* | | Adequate | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Weight gain (g/21 d) | 46.7$^{c}$ | 5.1 | 58.9$^{c}$ | 5.2 | 86.6$^{b}$ | 7.1 | 86.2$^{b}$ | 6.0 | 105.8$^{a}$ | 7.6 |
| Serum Mg (mg/dl) | 0.73$^{c}$ | 0.04 | 2.39$^{a}$ | 0.12 | 2.13$^{b}$ | 0.13 | 2.39$^{a}$ | 0.14 | 2.58$^{a}$ | 0.14 |
| Femur Ca (mg/g dry wt) | 213.7$^{a}$ | 5.9 | 218.6$^{a}$ | 2.5 | 218.0$^{a}$ | 2.8 | 219.0$^{a}$ | 4.5 | 223.1$^{a}$ | 3.7 |
| Femur P (mg/g dry wt) | 74.0$^{a}$ | 1.3 | 81.7$^{a}$ | 1.6 | 76.5$^{a}$ | 2.5 | 76.8$^{a}$ | 1.4 | 76.1$^{a}$ | 1.4 |
| Femur Mg (mg/g dry wt) | 1.7$^{c}$ | 0.1 | 3.6$^{a}$ | 0.1 | 3.2$^{b}$ | 0.1 | 3.7$^{a}$ | 0.1 | 3.7$^{a}$ | 0.1 |
| Urinary Pyr (nmol/d)† | 12.7$^{b}$ | 1.7 | 22.8$^{a}$ | 2.5 | 15.2$^{b}$ | 1.8 | 23.0$^{a}$ | 1.9 | 27.8$^{a}$ | 1.8 |
| Urinary Dpyr (nmol/d)† | 17.7$^{b}$ | 3.4 | 29.4$^{a}$ | 3.2 | 19.8$^{b}$ | 3.7 | 29.5$^{a}$ | 1.9 | 37.5$^{a}$ | 3.7 |
| Serum osteocalcin (ng/ml) | 46.9$^{b}$ | 3.7 | 87.4$^{a}$ | 7.4 | 62.9$^{b}$ | 2.2 | 87.4$^{a}$ | 8.2 | 93.6$^{a}$ | 7.4 |

$^{a,b,c}$ Mean values within a row with unlike superscript letters were significantly different, $P<0.05$ (ANOVA and Fisher's LSD for follow-up).
*Pair-fed/D and Pair-fed/S refer to the pair-fed litter mates of the animals in the deficient and suboptimal groups respectively.
† Mean values for 3 d collection during week 3 of the study.

Body-weight gains over the 3-week study period were significantly less in the suboptimal and deficient Mg intake groups and their pair-fed controls compared with the *ad libitum*-fed adequate group. Femur Ca and P contents were similar in all groups. Serum and femur Mg levels, urinary excretion of Pyr and Dpyr, and serum osteocalcin were significantly lower in the suboptimal and deficient groups compared with the pair-fed and *ad libitum*-fed adequate group. In conclusion, reducing Mg intake from the recommended level to either a suboptimal or deficient level significantly reduced Mg status of the animals, had no effect on bone mineral composition, except for Mg content and significantly lowered the rates of both bone formation and bone resorption.

This work was supported by the Department of Agriculture, Food and Forestry, Dublin.

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**Use of glutathione peroxidase-1 (GPX1) activity and mRNA levels and other selenium-dependent parameters to assess selenium requirements in female rats throughout the life-cycle.** By ROGER A. SUNDE, KEVIN M. THOMPSON, JACQUELINE K. EVENSON and SHERRI L. WEISS, *Nutritional Sciences, University of Missouri, Columbia, MO 65211 USA*

We hypothesize that the Se regulation of GPX1 expression is a component of GPX1 function, and thus GPX1 activity and mRNA levels are most useful for determining Se requirements (Sunde, 1997). In young rapidly growing female rats (Weiss *et al.* 1996), the Se requirement for growth is <0.007 μg Se/g diet, liver GPX1 activity and Se fall most dramatically in Se deficiency, and ≤0.1 μg Se/g diet is sufficient to raise levels of seven Se-dependent parameters to plateau levels (Table). We are now focusing on Se requirements in later periods of the life-cycle that are less amenable to traditional approaches.

To evaluate the National Research Council (1995) recommendation of at least 0.4 μg Se/g for pregnant and lactating rats, female weanling rats were fed on a Se-deficient diet supplemented with graded levels of dietary Se from 0 to 0.3 μg Se/g as $Na_2SeO_3$ and mated at >10 weeks. The Table shows summary values for dams killed on day 18 of pregnancy and day 18 of lactation. At all times, the liver Se and liver GPX1, plasma GPX3 and erythrocyte GPX1 activities reached plateau level at ≤0.1 μg Se/g diet.

**Table**: Minimal dietary selenium requirements in female rats

| | Weanling rats | | Pregnant rats | | Lactating rats | | One-year old rats | |
|---|---|---|---|---|---|---|---|---|
| Parameter | Def* (%) | Req† (μg/g) | Def* (%) | Req† (μg/g) | Def* (%) | Req† (μg/g) | Def* (%) | Req† (μg/g) |
| Growth | 100 | <0.007 | 101 | <0.007 | 92 | <0.007 | 126 | <0.007 |
| Erythrocyte GPX1 activity | 40 | 0.1 | 17 | 0.05 | 19 | 0.05 | 26 | ≤0.05 |
| Plasma GPX3 activity | 8 | 0.07 | 14 | 0.05 | 4 | 0.1 | 18 | ≤0.05 |
| Liver Se | 4 | 0.1 | 4 | 0.075 | 4 | 0.075 | 5 | ≤0.05 |
| Liver GPX1 activity | 2 | 0.1 | 7 | 0.05 | 5 | 0.075 | 3 | ≤0.05 |
| Liver GPX1 mRNA | 11 | 0.05 | 19 | 0.05 | 18 | 0.05 | -- | -- |
| Liver GPX4 activity | 29 | 0.05 | 35 | 0.05 | 23 | 0.05 | 39 | ≤0.05 |
| Liver GPX4 mRNA | 58 | ≤0.02 | 77 | ≤0.02 | 87 | ≤0.02 | -- | -- |
| Liver Sel P mRNA | -- | -- | 92 | ≤0.02 | 86 | ≤0.02 | -- | -- |
| Liver 5'DI mRNA | -- | -- | 87 | ≤0.02 | 68 | ≤0.02 | -- | -- |

* Level of parameter in Se-deficient rats relative to rats given 0.2 μg Se/g diet
† Minimal dietary requirement (plateau breakpoint) necessary to achieve plateau level for specified parameter (Weiss *et al.* 1996).

To evaluate Se requirements using GPX1 mRNA levels, we used a ribonuclease protection assay (RPA) to simultaneously quantitate mRNA levels for a number of the Se-dependent enzymes. RPA showed that 0.05 μg Se/g diet was necessary for maximal GPX1 mRNA levels throughout pregnancy and lactation.. RPA also readily showed that Se regulation of GPX1 mRNA is unique and specific as compared with regulation of mRNA level for GPX4, plasma selenoprotein P (Sel P) and thyroxine 5'-deiodinase type-1 (5'DI). In an extended trial, female rats were fed on these diets for 365 d. Measured enzyme activities (Table) indicate that Se requirements in these older, slower growing rats are reduced.

These studies demonstrate that the Se requirement for female rats is highest immediately following weaning, does not increase above 0.1 μg Se/g diet during lactation or pregnancy, and is lowest in old rats.

Supported by MO AES and USDA 95-37200-1799.

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Weiss SL, Evenson JK, Thompson KM and Sunde RA (1996) *Journal of Nutrition* 126, 2260-2267.

**The effect of dietary copper on bone metabolism in healthy adult males.** By A. BAKER[1], A. FLYNN[1], K. CASHMAN[1], L. HARVEY[2], G. MAJASK-NEWMAN[2], and S. FAIRWEATHER-TAIT[2], [1]*Department of Nutrition, University College Cork, Republic of Ireland,* [2]*Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA*

The incidence of osteoporosis is increasing in Europe, in both females and males. A recent study (Eaton-Evans *et al.* 1995) reported that Cu supplementation of the usual diets of peri-menopausal women reduced the rate of bone loss, suggesting that inadequate dietary Cu intake may contribute to age-related bone loss in this population group. The objective of the present study was to examine the effects of dietary Cu intake, ranging from inadequate to above adequate levels, on bone metabolism in healthy adult males.

Nine healthy male volunteers (aged 18-60 years) participated in a longitudinal dietary intervention study in which they were given medium (1.6 mg/d), low (0.7 mg/d) and high (6.0 mg/d) intakes of Cu over consecutive 8-week periods with 4-week washout periods. The volunteers lived in a residential human nutrition unit in the Institute of Food Research, Norwich and were given low-Cu diets (0.7 mg/d) supplemented with the appropriate level of Cu (as $CuSO_4$); all other nutrients met with the dietary reference values (Department of Health, 1991).

Fasting blood and morning urine samples were taken at the end of week 6 of each dietary period and were analysed for serum osteocalcin (a biomarker of bone formation) and urinary pyridinium crosslinks of collagen (biomarkers of bone resorption) respectively. Plasma Cu and total caeruloplasmin (Cp) and urinary creatinine were also determined. Urinary pyridinium crosslinks (pyridinoline (Pyr) and deoxypyridinoline (Dpyr)) were expressed relative to creatinine excretion.

| | Dietary Cu (mg/d) | | | | | |
|---|---|---|---|---|---|---|
| | 0.7 | | 1.6 | | 6.0 | |
| Variable | Mean | SE | Mean | SE | Mean | SE |
| Plasma Cu (μmol/l) | 14.5 | 0.7 | 13.8 | 0.7 | 15.3* | 0.6 |
| Plasma total Cp (g/l) | 0.20 | 0.01 | 0.24 | 0.03 | 0.30 | 0.05 |
| Serum osteocalcin (ng/ml) | 24.9 | 3.5 | 22.8 | 4.0 | 25.4 | 4.0 |
| Urinary Pyr (nmol/mmol creatinine) | 48.8 | 6.4 | 39.3 | 5.6 | 37.8 | 6.7 |
| Urinary Dpyr (nmol/mmol creatinine) | 17.6* | 2.3 | 13.5 | 1.2 | 14.7 | 2.6 |

Significantly different from medium Cu intake (1.6 mg/d), *$P<0.05$ (Student's $t$ test, paired).

Increasing dietary Cu from a medium to high intake significantly increased plasma Cu but had no effect on either total Cp or biomarkers of bone metabolism. Decreasing dietary Cu from a medium to a low intake had no effect on plasma Cu, total Cp or serum osteocalcin but increased urinary Pyr, although not significantly ($P<0.06$), and significantly increased urinary Dpyr ($P<0.05$). This indicates that low-Cu diets increase the rate of bone resorption. Such an increase, if continued, could lead to increased bone turnover and reduced bone mass.

This research was supported by funding from the European Commission (CT95-0813, FOODCUE).

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**No effect of copper supplementation on mononuclear leukocyte DNA damage as assessed by the Comet assay: FOODCUE Project.** By J.M. O'CONNOR, M.P. BONHAM, E. TURLEY, A. MCKEOWN, V.J. MCKELVEY-MARTIN, C.A. KEHOE, M.S. FAUGHNAN, J. COULTER, J. EATON-EVANS, M. CHOPRA, W.S. GILMORE and J.J. STRAIN, *Northern Ireland Centre for Diet and Health (NICHE), University of Ulster, Coleraine BT52 1SA*

Cu is known to act as a pro-oxidant to biological molecules *in vitro* but recent evidence suggests that adequate body Cu status is required for the maintenance of antioxidant defences *in vivo*. The purpose of the present study was to determine the effect of increased dietary Cu on DNA damage as assessed by the Comet assay in twenty four healthy adult non-smokers (twelve males and twelve females) aged 22-45 years. The Comet (single-cell gel electrophoresis) assay gives a measure of DNA strand breaks and alkali labile sites McKelvey-Martin *et al.* 1993). A measure of oxidative base damage, in addition to DNA strand breaks, can be obtained by digesting the mononuclear leukocyte DNA with endonuclease III, before processing for the Comet assay.

The study design was a double-blind repeated crossover trial with treatment and intervening placebo periods each of 6 weeks duration. The following active supplementations were given orally in sequence: $CuSO_4$ at a dose of 3 mg/d and Cu amino acid chelates (AAC) at doses of 3 mg Cu/d and 6 mg Cu/d. Blood sample collection took place at the beginning and end of each 6-week supplementation and placebo period. Mean (SE) food Cu intake by diet history was 1.48 (0.1) mg/d for males and 1.03 (0.1) mg/d for females.

A number of putative indices of body Cu status were measured and serum diamine oxidase (DAO) activity was the only index which was consistently and significantly altered during all three Cu supplemental periods studied. The Comet data (mean %tail DNA values with their standard errors) for the endonuclease assay are shown in the Table. There was no significant alteration in DNA damage following Cu supplementation. Similarly no significant alterations were observed for the Comet data without endonuclease III pre-treatment for the whole group or for men and women analysed separately (results not shown).

| | 3mg $CuSO_4$ | | Placebo period 1 | | 3mg Cu AAC | | Placebo period 2 | | 6mg Cu AAC | | Placebo period 3 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| *n* | 17 | | | | 16 | | | | 15 | | | |
| % tail DNA | 4.40 | 0.76 | 3.25 | 0.33 | 2.85 | 0.25 | 2.93 | 0.26 | 3.98 | 0.47 | 2.79 | 0.49 |
| *n* | 12 | | | | 14 | | | | 12 | | | |
| DAO (U/l) | 1.01** | 0.28 | 0.20 | 0.07 | 1.63** | 0.39 | 0.29 | 0.09 | 1.38** | 0.30 | 0.41 | 0.17 |

Mean values were significantly different from the corresponding placebo, **$P<0.01$ (independent $t$ test).

It is concluded that Cu supplementation, even at these high intakes for 6 weeks (2-6 fold above normal), does not appear to cause any biologically significant DNA damage *in vivo*.

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**Copper supplementation and LDL susceptibility to oxidation: FOODCUE Project.** By M. BONHAM, E. TURLEY, A. McKEOWN, J. M. O'CONNOR, W. S. GILMORE, M. CHOPRA, J. EATON-EVANS, V.J. McKELVEY-MARTIN and J.J. STRAIN, *Northern Ireland Centre for Diet and Health (NICHE), University of Ulster, Coleraine BT52 1SA*

Evidence suggests that oxidative modification of LDL is one of the major processes involved in the pathogenesis of atherosclerosis. The oxidant mediating these effects *in vivo* is unknown although Cu ions have been suggested. However, there is appreciable evidence arising from *in vivo* studies to indicate an antioxidant rather than a pro-oxidant role for Cu through maintenance of antioxidant defences. Furthermore, it seems unlikely that free $Cu^{2+}$ ions would be available *in vivo* to promote oxidation. An alternative and more physiological oxidant may be peroxynitrite ($ONOO^-$), formed by the simultaneous generation of NO and superoxide, and which has emerged as a potential candidate for mediating LDL oxidation *in vivo* (Beckman *et al.* 1994). The present study examined the effects of Cu supplementation on LDL susceptibility to oxidation ($Cu^{2+}$ and $ONOO^-$ induced) in twenty-four free-living healthy men and women (aged 22-45 years).

The study protocol was a double-blind randomized crossover supplementation trial. Subjects took Cu supplements (supplied by Thompson & Joseph, Norwich Ltd.) of $CuSO_4$ (3 mg/d) and Cu glycine chelates (3 mg/d and 6mg/d; Cu GC) for periods of 6 weeks with three intervening placebo periods. Blood samples were taken at the beginning of the trial and at the end of each 6-week period. Mean food Cu intakes, as assessed by diet history, were 1.48 mg/d (SE 0.1) for males and 1.03 mg/d (SE 0.1) for females. The susceptibility of isolated LDL to $Cu^{2+}$-induced oxidizability was assessed using the *in vitro* method (Esterbauer *et al.* 1989) monitored by conjugated diene formation at 234 nm. The rapid oxidation of the LDL fraction is preceded by a lag phase which is used as an indication of the intrinsic resistance of the LDL to oxidation. The $ONOO^-$ induced oxidation was measured using lipoprotein gel electrophoresis kits (Beckman) and the relative electrophoretic mobility (REM) of the oxidised LDL sample assessed relative to its native LDL control.

Lag times (min) and REM at each crossover (mean values with their standard errors) are displayed in the Table. Crossover differences were independently *t* tested by supplementation regimen to examine the effect of treatment. Statistical analysis showed no effect of Cu supplementation on lag times or REM. These results indicate that dietary Cu supplementation (to levels between 2-6 fold greater than average daily intake) do not promote LDL susceptibility to induced oxidation *in vitro*.

| | 3 mg $CuSO_4$ | | Placebo period 1 | | 3 mg Cu GC | | Placebo period 2 | | 6 mg Cu GC | | Placebo period 3 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| *n* | 24 | | 24 | | 19 | | 19 | | 18 | | 18 | |
| Lag time (min) | 39 | 2 | 39 | 3 | 37 | 2 | 38 | 2 | 44 | 4 | 40 | 3 |
| REM | 3.1 | 0.5 | 3.2 | 0.6 | 3.0 | 0.5 | 3.1 | 0.6 | 3.1 | 0.6 | 3.1 | 0.8 |

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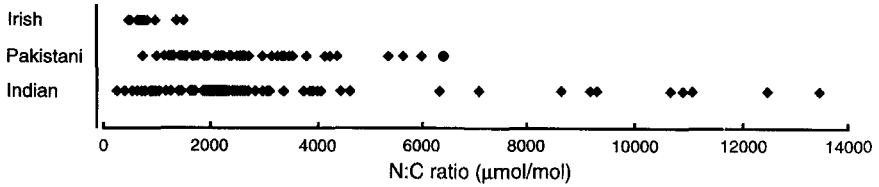
**Urinary neopterin as a marker of infection and the lack of influence of vitamin A supplementation in infants.** By S.F. TSANG[1], L.C. DOWEY[1], F.S.W. MCCULLOUGH[1], C.A. NORTHROP-CLEWES[1], P.I. PARACHA[2], B.S. DAS[3] and D.I. THURNHAM[1], [1]*Northern Ireland Centre for Diet and Health, University of Ulster, Coleraine BT52 1SA,* [2]*Department of Human Nutrition, Agricultural University, Peshawar, North West Frontier Province, Pakistan,* [3]*Ispat General Hospital, Rourkela 769005, Orissa, India*

Urinary neopterin is an early, sensitive and non-specific marker of cellular activation in the immune system (Drenth *et al.* 1995). Vitamin A supplements have been shown to reduce morbidity in infants. The objective of the present study was to compare the urinary neopterin:creatinine (N:C) ratios obtained from forty-five Pakistani, eighty Indian and twelve Irish infants. In addition, N:C ratios were measured in some of the infants following a double-blind, randomized vitamin A supplementation study. Weekly treatment was either 2.5 mg (8,333 IU) or 5.0 mg (16,666 IU) retinyl palmitate plus 2.5 mg α-tocopherol or placebo (2.5 mg α-tocopherol) (Hoffman-La Roche, Switzerland) given in 5 ml maize oil for 4 weeks. Urinary neopterin and creatinine were measured by reverse phase HPLC (Palmisano *et al.* 1995).

Basal neopterin and creatinine ratios (μmol/mol) obtained from Pakistani, Indian and Irish infants.




At baseline, the N:C ratios were significantly higher in the Pakistani (median 2200) and Indian (median 2350) infants in comparison with the Irish (median 723) infants ( $P = 0.001$, ANOVA, logged values). There was no difference between treatment and placebo N:C ratios at baseline or at any other time point in either Indian or Pakistani groups. However, at 4 weeks N:C ratios were reduced in both groups compared with baseline (Pakistani NS, Indian $P = 0.001$, repeated measures ANOVA). These results confirm that the urinary N:C ratio is higher in infants living in environments where exposure to disease is high. The lack of a difference in N:C ratios between the vitamin A and placebo treatments, does not support the idea that vitamin A suppresses infection although the dose may have been too small. It has been noted previously that it took 2 months after conventional treatment with vitamin A for impression cytology in previously-deficient children to return to normal (Amedee-Manesme *et al.* 1988). Seasonal or human factors may have been responsible for the parallel decreases in the N:C ratio in both groups following the vitamin A or placebo treatment. Alternatively α-tocopherol, which was present in both placebo and the vitamin A supplements, should be more closely examined for possible influences on neopterin metabolism.

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**Comparison of breast-milk concentrations and dietary intakes of retinol, carotenoids and α-tocopherol between Irish and Pakistani mothers.** By L.M.A. McDERMOTT[1], F.S.W. McCULLOUGH[1], P.I. PARCHA[2], C.A. NORTHROP-CLEWES[1] and D.I. THURNHAM[1], [1]*Northern Ireland Centre for Diet and Health (NICHE), University of Ulster, Coleraine, BT52 1SA,* [2]*Department of Human Nutrition, Agricultural University, Peshawar, North West Frontier Province, Pakistan*

The WHO recently recommended breast-milk vitamin A as a unique indicator to monitor vitamin A status in lactating women, breast-fed infants and children aged 1-3 years. Breast milk concentrations of retinol, carotenoids and α-tocopherol from mothers living in a developed and developing country were compared. Irish lactating mothers (*n* 20) were recruited and requested to express manually one 10 ml milk sample between 12.00 and 15.00 hours. The milk samples were frozen at –40°C until analysis. A general socio-economic and a 24 h dietary recall questionnaire were completed. Samples were available from twenty Pakistani mothers. Milk samples were analysed in duplicate by reverse phase HPLC, dietary information was analysed using Compeat and statistical analysis performed using SPSS.

| | Irish samples (*n* 20) | | Pakistani samples (*n* 20) | |
|---|---|---|---|---|
| μmol/kg fat | Mean | SD | Mean | SD |
| Retinol | 56.00 | 22.26 | 53.58 | 47.96 |
| Lutein | 4.88 | 2.82 | 5.63 | 3.84 |
| ß-Carotene | 1.00 | 0.60 | 0.0† | 0.0 |
| α-Tocopherol | 318.01 | 219.73 | 102.71** | 67.72 |

† Concentration too low to be measured.
** $P < 0.001$, Mean value was significantly different from that for Irish samples.

The retinol values reported for the Irish and Pakistani milks were sufficient to meet infant requirements. A retinol concentration of 1.0 μmol/l (approximately 20 μmol/kg fat) is considered sufficient for an infant's metabolic requirements, while a concentration of 2.3 μmol/l (approximately 46 μmol/kg fat) will also allow for the accumulation of safe and adequate stores (Stoltzfus & Underwood, 1995). The significantly higher α-tocopherol concentration in the Irish samples suggests a greater intake of vitamin E-rich foodstuffs.

Mean dietary retinol intake for Irish mothers was 768 μg retinol equivalents (RE)/d (range 198-1586 μg RE/d), which was lower than expected. Stoltzfus & Underwood (1995) reported mean values of 660 μg RE/d and 1540 μg RE/d in developing and developed countries respectively. However the dietary analysis method used in this study was not representative of habitual intake and daily fluctuations of retinol-rich foodstuffs would be expected. Unfortunately dietary data from Pakistan were not available.

Interestingly a significant correlation was found between the dietary lutein intake and breast-milk lutein concentrations in the Irish milks ($r$ 0.68, $P = 0.001$). In addition there was also a significant correlation between breast-milk retinol and breast-milk lutein in the Pakistani samples ($r$ 0.81, $P = 0.0001$) but not in the Irish samples ($r$ 0.16, $P = 0.53$). Lutein is an abundant carotenoid in green vegetables and these observations may be further evidence that lutein is a useful biomarker of the sources of vitamin A in the diet (Thurnham *et al.* 1997).

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**Vitamin D receptor gene polymorphisms and circulating osteocalcin in healthy young Irish women.** By D. SHEEHAN, T. BENNETT, A. FLYNN and K. CASHMAN, *Department of Nutrition, University College Cork, Republic of Ireland*

Osteocalcin, a vitamin D-responsive protein, is the most abundant non-collagenous protein in bone and its circulating level is a widely used marker of bone turnover in normal and disease states. In studies of twins, variation in serum osteocalcin levels has been shown to have a major genetic component and to be closely correlated with the known genetic diversity in bone mineral density (BMD) (Pocock *et al.* 1987). Morrison *et al.* (1992, 1994) showed that variability in circulating osteocalcin levels and BMD was reflective of allelic variation in the vitamin D receptor (VDR) gene in Australian subjects of UK-Irish descent. No such study has been carried out in a resident Irish population. The aim of the present study was to examine the relationship between serum osteocalcin and restriction fragment length polymorphisms (RFLP, detected by the *Taq* I endonuclease) that define human VDR alleles in a cohort of healthy young Irish females.

Fifty-three healthy Irish females aged 19-33 years (mean age 24.1 years) were recruited. They had no history of bone disease, and had not taken any medication that could affect bone. Two fasting blood samples (10 ml) were collected from each subject. One blood sample was processed to serum which was subsequently analysed for osteocalcin by enzymeimmunoassay (EIA), while the other blood sample was used for isolation of genomic DNA from leukocytes by a standard method. RFLP for the VDR gene were determined by the method of Riggs *et al.* (1995). The association between the VDR genotype and serum osteocalcin concentration was assessed by ANOVA comparing the categorical classes (RFLP) against the continuous variable (osteocalcin). Fisher's protected least-significant-difference test was used for the pairwise comparison of the continuous variable means of each categorical (RFLP) class.

| | | Serum osteocalcin (μg/l) | | | | |
|---|---|---|---|---|---|---|
| *Taq I* RFLP | *n* | Mean | SE | Sig. 1 | Sig. 2 | *P* value |
| *tt* | 6 | 23.0 | 1.0 | *tt* vs. *TT* | *tt* vs. *Tt* | 0.003 |
| *Tt* | 31 | 14.2 | 1.0 | <0.01 | <0.001 | |
| *TT* | 16 | 15.5 | 1.5 | | | |

Sig., significance (probability that such a difference could occur by chance) referring to the difference between the means of the homozygotes (Sig. 1) and to the difference between the means of the homozygotes (presence of RFLP site (t)) and the heterozygotes (Sig. 2). *P* value is for the *F* test on the overall effect.

The *tt* VDR genotype was the least prevalent genotype in this group, in agreement with frequency data in females from other Caucasian or ethnic groups, and was associated with significantly higher serum osteocalcin levels compared with the other two genotypes. The findings of this preliminary study suggest that healthy young adult Irish females with the *tt* VDR genotype have higher rates of bone turnover than those with Tt or TT genotypes. Higher rates of bone turnover in adults are associated with increased risk of low BMD and osteoporosis in later life.

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**The effect of amount and composition of added oil on the ability to trap the geometric isomers of lycopene from tomato juice after boiling.** By A.E. TAGGART, S. BEATTIE, M. CHOPRA and D.I. THURNHAM, *Northern Ireland Centre for Diet and Health, University of Ulster, Coleraine, BT52 1SA*

Intake of processed tomato products has been linked with a decreased risk of prostate cancer; however, unprocessed tomato products did not show the same effect (Giovannucci *et al.* 1995). Boiling maize oil with tomato juice (10 ml/l) for 1 hr has been shown to enhance absorption of the lycopene (Stahl & Sies, 1992). This could be due to two main reasons; first, the breakdown of the food matrix and second, an increase in the proportion of the *cis*-lycopene isomer found in the boiled juice. On further investigation it was observed that the majority of the *cis*-isomers were to be found in the oil fraction of this system. Without the presence of oil, the isomeric ratio equilibrated to that found in untreated (i.e. unboiled) tomato juice. It is suggested that the oil acts in such a way as to trap the more lipophilic *cis*-isomers and prevent or delay them converting back to the *trans* form.

Therefore, experiments were carried out to examine the effects of boiling tomato juice with various amounts of maize oil and with oils of different fatty acid compositions, on the *cis*- and *trans*-isomer content. Oil (10-100 ml/l) was added to tomato juice and the mixture boiled for 1 hr. The concentration of the *trans*-isomer in the oil fraction was significantly correlated with the amount of oil added ($r$ 0.96, P<0.001, $n$ 8). Although the increase in the *cis*-isomer was not significant ($r$ 0.58, $P$=0.131, $n$ 8), it did follow a similar trend. Overall, there was approximately a 2-3 fold increase in isomers trapped for a 10-fold increase in the amount of oil used.

The influence of fatty acid composition was investigated using a 100 ml/l oil-tomato juice suspension. Five commercially available oils were tested. They spanned the spectrum from highly saturated (coconut oil) through highly monounsaturated (extra virgin olive oil) to highly polyunsaturated oils (safflower oil), and included cod liver oil (high in $n$-3 polyunsaturated fatty acids), and maize oil as used by Stahl and Sies (1992). After boiling, the amount of each lycopene isomer found to be present in the oil was correlated against the percentage of saturated, monounsaturated and polyunsaturated fatty acids contained within each oil. Inverse relationships for both the *cis*- ($r$ –0.75, $P$=0.1, $n$ 5) and the *trans*- ($r$ –0.86, $P$=0.06, $n$ 5) lycopene were found, but were not significant when the amount of each type of isomer was correlated with the percentage of saturates present in the oils. A trend in the opposite direction was observed for the relationship between the amount of lycopene isomers and the percentage monounsaturated (*trans*-, $r$ 0.57, $P$=0.3, $n$ 5; *cis*-, $r$ 0.3, $P$=0.6, $n$ 5) and polyunsaturated (*trans*-, $r$ 0.4, $P$=0.5, $n$ 5; *cis*-, $r$ 0.5, $P$=0.4, $n$ 5) fatty acids, in the oils. The overall ratio of *cis*- to *trans*-isomers remained fairly constant at 1:2.

The information gained from these experiments indicates that the best system for increasing the proportion of *cis*-lycopene contained in tomato juice, was the addition of 10-20 ml of an oil, low in saturated and high in unsaturated fatty acids per litre of juice.

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**The effect of ascorbic acid supplementation on $H_2O_2$-mediated nuclear factor κB (NFκB) activation in human lymphocytes.** by LISA A. BRENNAN, GERARD M. MORRIS, YVONNE A. BARNETT AND BERNADETTE M. HANNIGAN, *Cancer and Ageing Research Group, School of Biomedical Sciences, University of Ulster, Coleraine, N.Ireland, BT52 1SA.*

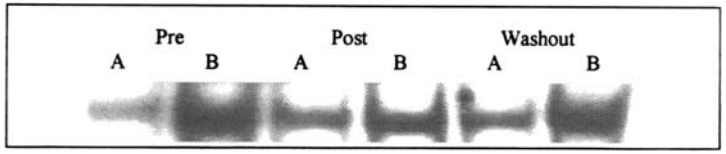
There is increasing evidence that reactive oxygen species (ROS) such as $H_2O_2$ may function in intracellular signalling pathways through the activation of transcription factors such as NFκB. Activation of NFκB initiates the transcription of a number of genes encoding cytokines, cell surface receptors, cell adhesion molecules and acute-phase proteins. NFκB activation by a variety of stimuli has been recently shown to be inhibited in vitro by antioxidants such as ascorbic acid (Bowie & O'Neill, 1997).

As part of a larger study seven healthy non-smokers were asked to supplement their otherwise unchanged diets with ascorbic acid for 42 d followed by a 6-week washout period. Peripheral blood lymphocytes (PBL) were separated from whole-blood samples taken presupplementation, postsupplementation and following the washout peroid. Intracellular ascorbic acid was measured after lysing PBL in ice cold metaphosphoric acid (10g/100ml) using the method of Vuilleumier & Keck (1989). Separated PBL were treated for 4 h with a 50 μM-$H_2O_2$ dose known to activate NFκB. Electrophoretic mobility shift assays (EMSA) were performed to detect NFκB activation. The table shows intracellular ascorbic acid levels in μmol/μg protein.

| | Pre | | Post | | Washout | |
|---|---|---|---|---|---|---|
| Supplementation | Mean | SE | Mean | SE | Mean | SE |
| Vitamin C (4 X 250mg daily) | 165.2 | 72.2 | 482.2** | 65.2 | 481.4* | 177.8 |

Mean values were significantly different from presupplementation: *$P$<0.05, **$P$<0.02.

Intracellular levels were significantly increased supplementation. PBL accumulated μMolar amounts of ascorbic acid, which persisted for at least 6 weeks following withdrawal of the supplement.




Blots show autoradiographs indicating NFκB activation in a particular sample over the supplementation period. Sample A in each blot shows untreated control PBL (displaying basal activation) and sample B in each blot shows $H_2O_2$-treated PBL. PBL show enhanced activation following $H_2O_2$ treatment. This was unchanged following supplementation and washout, despite significant increases in intracellular ascorbic acid. Bowie & O'Neill (1997) previously showed that mM concentrations of ascorbic acid were required to inhibit activation of NFκB in primary and transformed endothelial cells *in vitro*; it may be that such high concentrations would also be needed to have this effect *in vivo*.

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*This work was carried out under a MAFF contract.*

**Effects of oleic acid, eicosapentaenoic acid and docosahexaenoic acid on genotoxin-induced frequencies of sister chromatid exchanges (SCE) in Indian Muntjac fibroblasts.** By S. HIGGINS[1], M.H. VASCONCELOS[2] and N.M. O'BRIEN[1], [1]*Department of Nutrition, University College Cork, Ireland*, [2]*IPATIMUP, Rue Dr. Roberto Frias, 4200 Porto, Portugal*

*n*-3 Polyunsaturated fatty acids (*n*-3 PUFA) may have anti-carcinogenic potential in man (Schloss *et al.* 1997) and animals (Ramesh and Das, 1998). The aim of the present study was to investigate if the *n*-3 PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), or the *n*-9 MUFA oleic acid (OA), have an effect on background and genotoxin-induced frequencies of SCE in Indian Muntjac fibroblasts.

Muntjac cells were cultured in Dulbecco's Modified Eagle's Medium supplemented with foetal calf serum (200 ml/l) and 2 mM L-glutamine and maintained in a humidified atmosphere at 37° with 50 ml/l $CO_2$. Muntjac cells were cultured at 5 x $10^5$ cells/100 mm petri dish for 24 h before addition of fatty acids (50 μM) which were delivered to the cells complexed to 20 g/l bovine serum albumin (fatty-acid free) and incubated for a further 24 h. In order to verify the uptake of the fatty acids by the cells, parallel dishes were processed for lipid extraction and GC analysis. Genotoxins, $H_2O_2$ (75 μM), *tert*-butyl hydroperoxide (TBH) (20 μM) and mitomycin C (MMC) (2.4 μM), were added to the cells for 1 h before the end of the 24 h fatty acid incubation period. Blank (no genotoxin or fatty acid) and control (genotoxin and fatty acid carrier systems only) treatments were included. The SCE assay (Latt and Schreck, 1980) was carried out following the incubation period. The frequency of SCE was scored as the number of exchanges in twenty metaphases per slide and expressed as the average number of SCE per chromosome. Results are expressed as means with their standard errors of three independent experiments.

| Treatment | SCE | | Treatment | SCE | | Treatment | SCE | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | | Mean | SEM | | Mean | SEM |
| Blank | 1.07 | 0.03 | Blank | 1.07 | 0.03 | Blank | 1.07 | 0.03 |
| Control | 1.20 | 0.12 | Control | 1.20 | 0.12 | Control | 1.20 | 0.12 |
| $H_2O_2$ | 3.50* | 0.00 | TBH | 2.40* | 0.12 | MMC | 3.57* | 0.03 |
| $H_2O_2$+OA | 3.83* | 0.09 | TBH+OA | 3.20*† | 0.00 | MMC+OA | 4.38*† | 0.21 |
| $H_2O_2$+EPA | 4.19*† | 0.22 | TBH+EPA | 3.37*† | 0.13 | MMC+EPA | 4.53*† | 0.49 |
| $H_2O_2$+DHA | 3.77* | 0.09 | TBH+DHA | 3.17*† | 0.17 | MMC+DHA | 4.60*† | 0.15 |
| LSD($P$<0.05) | 0.69 | | | 0.77 | | | 0.82 | |

Mean values were significantly different from control, *$P$<0.05 (one-way ANOVA, least significant difference).
Mean values were significantly different from cultures treated with genotoxin alone, †$P$<0.05.

GC analysis showed highly significant ($P$≤0.001) uptake of each fatty acid by the cells (results not shown). No difference was observed in background frequencies of SCE between blanks, controls and fatty acid treatments, indicating that these fatty acids *per se* do not cause DNA damage (results not shown). Cells incubated with genotoxins ($H_2O_2$, TBH or MMC) showed increased ($P$≤0.05) frequencies of SCE when compared with blank/control frequencies (Table). Cells incubated with genotoxins in the presence of fatty acids also showed significantly higher ($P$≤0.05) levels of SCE when compared with blank/control frequencies. When cells supplemented with genotoxins in the presence of fatty acids were compared with cells treated with genotoxins alone, higher levels of SCE were observed in the former suggesting that the fatty acids studied exacerbate DNA damage caused by these genotoxins.

This study was funded by an EU shared-cost project (FAIR-CT-95-0085).

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**The effect of the citrus phytochemicals limonin and nomilin on xenobiotic metabolizing enzyme activity in the rat liver and lung.** By C. JEWELL and N.M. O'BRIEN, *Department of Nutrition, University College Cork, Republic of Ireland.*

Limonin and nomilin are two phytochemicals found primarily in citrus fruit. In the present study, we report the effect of these phytochemicals in the diet on the xenobiotic metabolizing enzymes in the liver and lung of the rat.

Male Wistar rats (3—4 weeks old) in groups of six were fed on an AIN-76 diet (American Institute of Nutrition, 1977) containing 675 μg/day of either limonin or nomilin for 6 days. This concentration is representative of eating approximately six pieces of citrus fruit daily for human subjects. A control group received the AIN-76 diet alone. All animals received 15 g of diet/d and were not fasted prior to sacrifice, which was by cervical dislocation. Tissues were excised and used to prepare subcellular fractions. Cytochrome P450 activity was assessed using the substrates ethoxyresorufin for cytochrome P4501A1, methoxyresorufin for cytochrome P4501A2, pentoxyresorufin for cytochrome P4502B1/2 and benzyloxyresorufin for cytochrome P450 types 1A1/2, 2B1/2 and 3A (Burke *et al*, 1985). Glutathione-S-transferase (GST) was measured using dinitrochlorobenzene as a substrate (Habig *et al*, 1974).

| | Liver | | | | | | Lung | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | Limonin | | Nomilin | | Control | | Limonin | | Nomilin | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| EROD† | 121.3 | 10.2 | 76.0* | 7.1 | 63.0* | 6.4 | ND | | ND | | ND | |
| MROD† | 31.2 | 3.0 | 26.0 | 2.6 | 22.6* | 1.9 | ND | | ND | | ND | |
| PROD† | 15.1 | 1.4 | 12.5 | 0.8 | 11.7 | 0.7 | 11.6 | 2.8 | 3.5* | 0.8 | 1.8* | 1.5 |
| BROD† | 40.1 | 5.9 | 39.4 | 4.3 | 31.2 | 3.3 | 100.4 | 6.1 | 32.6* | 3.3 | 31.0* | 2.3 |
| GST‡ | 616.3 | 31.6 | 645.4 | 46.3 | 741.1 | 51.1 | 76.5 | 4.6 | 71.4 | 2.2 | 70.9 | 2.9 |

ND = not detectable.
Mean values were significantly different from control, *P<0.05 (n=6, ANOVA, Dunnett's test).
†pmoles/min/mg microsomal protein.
‡nmoles/min/mg cytosolic protein.

Results showed that treatment with limonin and nomilin led to a significant decrease in liver ethoxyresorufin-O-deethylation (EROD) activity compared with controls. Nomilin led to a significant decrease in liver methoxyresorufin-O-demethylation (MROD) activity. However, no decrease in activity was seen in pentoxyresorufin-O-dependentylation (PROD) or benzyloxyresorufin-O-dearylation (BROD) in the liver. PROD and BROD activitites were significantly decreased in the lung compared with control, while EROD and MROD were below the level of detection. Liver GST activity increased after treatment with nomilin only but was not significantly different from control (P=0.053). Lung GST activity was unaffected by either phytochemical.

Many antimutagenic or anticarcinogenic compounds are inducers or inhibitors of xenobiotic metabolizing enzymes. To the best of our knowledge this is the first study of the effects of the citrus phytochemicals limonin and nomilin on liver and lung cytochrome P450 subtypes. Further investigation of the effects of these and other citrus phytochemicals on a broad range of xenobiotic metabolizing enzymes is required to elucidate their effects and potential implications on health.

This work was supported by the Department of Agriculture, Food and Forestry, Dublin.

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**Inter-individual variation in soyabean isoflavone metabolism: the role of habitual diet.** By ELIZABETH A. BOWEY[1], I. R. ROWLAND[1], H. ADLERCREUTZ[2], T.A.B. SANDERS[3] and HELEN WISEMAN[3] *[1]BIBRA International, Woodmansterne Road, Carshalton, SM5 4DS, [2]Department of Clinical Chemistry, University of Helsinki, Meilahti Hospital, FIN-00290, Helsinki, Finland, [3]Nutrition, Food and Health Research Centre, King's College London, Campden Hill Road, London W8 7AH*

Soyabeans and soyabean products such as textured vegetable protein (TVP) are a rich source of the conjugated isoflavones, genistin and daidzin. After ingestion these compounds are hydrolysed, and further metabolized by the gastrointestinal flora (Xu *et al.* 1995). The aglycone, daidzein can be converted to the oestrogenic equol, or to the more biologically inert O-desmethylangolensin (O-Dma). The metabolism of these compounds is not well understood in man. Studies have shown a wide inter-individual variation in isoflavone metabolite excretion after soyabean consumption (Setchell *et al.* 1984; Kelly *et al.* 1995). The reasons for the variation are unclear, but in a recent study (Lampe *et al.* 1998), females who excreted equol were found to consume a significantly higher percentage of energy as carbohydrate and greater amounts of plant protein and NSP. In order to investigate the effect of habitual diet on isoflavone metabolism, we carried out a *post hoc* analysis on data from twenty-three subjects who had consumed 30 g TVP/d for 17 d (56 mg/d total isoflavones). A 24 h urine sample was collected at the end of the experiment and analysed for isoflavonoids by isotope dilution gas chromatography mass spectrometry (Adlercreutz *et al.* 1991). Pre-experimental 3 d weighed food intake diaries were completed and analysed by computer program. Good equol producers (35% of total subjects) were categorized as those excreting more than 1000 nmol/24 h.

| | Good equol producers (*n* 8) | | Poor equol producers (*n* 15) | |
|---|---|---|---|---|
| | Mean | SEM | Mean | SEM |
| Equol (nmol/24 h) | 10 905 | 2147 | 47 | 7 |
| Fat (% energy) | 26 ** | 2.3 | 35 | 1.6 |
| Carbohydrate (% energy) | 55 * | 2.9 | 47 | 1.7 |
| Starch (% energy) | 30 | 2.9 | 27 | 1.9 |
| NSP (g/MJ) | 2.4 | 0.3 | 1.9 | 0.2 |
| Protein (% energy) | 16 | 1.2 | 14 | 0.9 |
| Energy (MJ/d) | 8.8 | 1.1 | 8.6 | 0.6 |

Mean values were significantly different from the poor equol producers, *P<0.05, **P<0.01 (unpaired *t*-test).

Good equol producers excreted about 200 times more equol than the poor equol producers. Excretion rates ranged from 25-118 nmol/24 h in the poor-equol-producing group to 1459-16 588 nmol/24 h in good equol producers. Energy and protein intakes were similar in both groups. However, the good equol producers consumed significantly less fat and more carbohydrate than the poor equol producers. Higher amounts of NSP were consumed by the good equol producing group but this difference did not reach significance. These results suggest diet-related differences in gut microflora metabolism of isoflavones as seen for other xenobiotic compounds (Mallett *et al.* 1984). The relationship between high-fat diets and the gut flora metabolism of soyabean isoflavones warrants further attention.

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**Absorption of flavonol conjugates in ileostomy subjects.** By A. CAHILL[1], M.S. MCDONALD[2], A. CROZIER[2], and M.E.J. LEAN[1], *[1]Department of Human Nutrition, Glasgow Royal Infirmary, Glasgow G32 2ER, [2]Department of Botany, University of Glasgow, G12 8QQ,*

Flavonoids are polyphenolic compounds found ubiquitously in a wide variety of plants. Flavonoids possess anti-inflammatory properties and are of potential benefit in reducing atherosclerosis (Hertog *et al.* 1993). Approximately 50% of quercetin glucosides are absorbed from the small intestine in human subjects (Hollman *et al.* 1995).

We set out to estimate the absorption of the flavonol conjugates in onions in eight otherwise healthy ileostomy subjects (mean age 53 yr, range 34-69 yr). Each subject received a 200 g meal of lightly fried white onions after an overnight fast. This followed a 48 h low flavonoid diet. Plasma samples were collected at baseline, 1, 1.5, 2, 3, 5 and 24 h. Urine samples were collected over the periods 0-5, 5-12 and 12-24 h, and ileostomy effluent over the periods 0-2, 2-5 and 5-24 h. Extraction of conjugates was performed with methanol (500 ml/l) and 1.2 M-HCl. The non-hydrolysed samples were analysed by HPLC with UV and fluorescence detection, using a 15-40% gradient of acetonitrile in water adjusted to pH 2.5 with trifluoroacetic acid.

| Flavonol | Intake (mg) | | Peak concentration in plasma (ng/ml) | | 24 h urine excretion (μg) | | 24 h ileostomy excretion (μg) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Isorhamnetin-4`-glucoside | 2.8 | 0.3 | 115.85 | 18.1 | 236.8 | 71.5 | 2111.7 | 252.7 |
| Quercetin-3-glucoside | 3.8 | 0.8 | Not detected | | 189.6 | 46.3 | 550.6 | 202.6 |
| Quercetin-4`-glucoside | 39.8 | 5.4 | 25.25 | 2.64 | 294.1 | 218.9 | 1967 | 4048 |
| Quercetin-3,4`-glucoside | 108.4 | 10.7 | Not detected | | 216.9 | 79.3 | 14068 | 12430 |

Lightly fried onions contain quercetin-3,4`-diglucoside, quercetin-4`-glucoside, isorhamnetin-4`-glucoside, quercetin-3-glucoside and free quercetin (in order of decreasing concentration). The peak accumulation of flavonol conjugates in plasma was greatest for isorhamnetin-4`-glucoside and quercetin-4-glucoside. These peak levels of flavonol absorption occurred within 2 h, with subsequent levels falling within 5 h towards baseline. As a percentage of intake, the flavonol conjugates detected in greatest amounts in urine and ileostomy samples were isorhamnetin-4`-glucoside and quercetin-3-glucoside (in urine 11.7% and 7.3%, and in ileostomy effluent 88% and 21% respectively). Despite not detecting significant quantities in plasma, 7.3% of the intake of quercetin-3-glucoside and 0.33% of quercetin-3,4`-glucoside was found in urine. A significant proportion of the conjugates quercetin-3,4`-glucoside, quercetin-4`-glucoside and to a lesser extent quercetin-3-glucoside were unaccounted for by the amounts detected in plasma or excreted in urine or ileostomy effluent.

Flavonols are absorbed as glucoside conjugates. Of the flavonol conjugates in onions, isorhamnetin-4`-glucoside and quercetin-4-glucoside are detected in plasma in the greatest amounts. Levels appear rapidly in plasma but then appear to be taken up or metabolized within 3 h. When viewed in conjunction with previous work (Aziz *et al.* 1998), it appears that the small intestine is the main site of absorption for flavonol conjugates. Despite the appearance of only small amounts in ileostomy effluent, insignificant amounts of quercetin-3-glucoside and quercetin-3,4`-glucoside were detected in the plasma samples. This suggests that flavonol conjugates may either be taken up by or metabolized by the liver. This metabolism may either be to isorhamnetin-4`-glucoside or to another form which we have not detected in our analysis.

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**The effect of flavonoids on background and hydrogen peroxide-induced DNA damage.** By S.A. AHERNE, J.A. WOODS and N.M. O'BRIEN, *Department of Nutrition, University College, Cork, Republic of Ireland*

Flavonoids are naturally occurring polyphenolic compounds found in many fruits, vegetables and beverages and are an integral part of the human diet. Flavonoids are reported to exhibit a wide variety of biological effects, including antioxidant and free-radical scavenging activities. Reactive oxygen species (ROS) have been implicated in a range of human diseases such as atherosclerosis and certain cancers. When an imbalance between ROS generation and antioxidants occurs, oxidative damage to cell targets (DNA, lipids, proteins) can be aggravated (Halliwell, 1997). $H_2O_2$, a ROS, is a well-documented genotoxin which induces DNA damage by way of single-strand breaks. In the present study, the antioxidant and geno-protective effects of three flavonoids, myricetin, quercetin and rutin were investigated in human hepatoma (HepG2) cells.

HepG2 cells were cultured in Dulbecco's modified Eagles' medium and maintained in a humified atmosphere at 37°. In all experiments, a low (10 μmol/l), medium (50 μmol/l) and high (200 μmol/l) concentration of each flavonoid was used. HepG2 cells were supplemented with myricetin, quercetin or rutin for 24 h. Cells were scored for DNA damage either immediately after the 24 h supplementation period or following exposure to $H_2O_2$ (50 μM) for 30 min at 37°. DNA damage was measured using the alkaline single-cell gel electrophoresis assay (Comet assay) (Tice *et al.* 1990). One hundred nuclei on each slide were scored at random and given a value of 0, 1, 2, 3 or 4 (from undamaged, 0, to maximally damaged, 4). In order to express results as fold difference, values were divided by the negative control value.

| | Flavonoid concentration (μM) | Fold difference (relative to control) Myricetin Mean | SE | Quercetin Mean | SE | Rutin Mean | SE |
|---|---|---|---|---|---|---|---|
| Without $H_2O_2$ | 0 | 1.0 | 0 | 1.0 | 0 | 1.0 | 0 |
| | 10 | 1.1 | 0.1 | 1.2 | 0.1 | 1.1 | 0.04 |
| | 50 | 1.2 | 0.1 | 1.1 | 0.1 | 1.2 | 0.1 |
| | 200 | 1.3 | 0.1 | 1.2 | 0.1 | 1.1 | 0.1 |
| LSD ($P < 0.05$) | | NS | | NS | | NS | |
| With 50 μM $H_2O_2$ | 0 | 8.0 | 0.2 | 8.0 | 0.2 | 8.0 | 0.2 |
| | 10 | 7.3 | 0.1 | 6.7 | 0.1 | 7.2 | 0.1 |
| | 50 | 6.4 | 0.2 | 5.8 | 0.2 | 6.2 | 0.2 |
| | 200 | 4.7 | 0.2 | 4.3 | 0.2 | 4.6 | 0.2 |
| LSD ($P < 0.05$) | | 0.5 | | 0.5 | | 0.5 | |

Statistical analysis was by one-way ANOVA, followed by least significant difference (LSD), *n* 5 for treatments.

Neither the flavonoids nor $H_2O_2$ were cytotoxic to the cells at the concentrations used as assessed by the neutral-red uptake assay. In the absence of $H_2O_2$, the flavonoids themselves were not genotoxic at levels up to 200 μM. In the presence of 50 μM-$H_2O_2$, increasing levels of the flavonoids decreased the genotoxic effects of $H_2O_2$ as assessed by the Comet assay. Myricetin, quercetin and rutin protected the cells against $H_2O_2$-induced DNA damage at all concentrations tested ($P < 0.05$).

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**Dietary intake of selenium in the UK.** By SUSAN M. CHURCH[1], GILLIAN E. SMITHERS[1], HELEN CREWS[2] and SELVARANI ELAHI[3], [1]*Nutrition Unit, Ministry of Agriculture, Fisheries and Food (MAFF), London SW1P 3JR,* [2]*CSL Food Science Laboratory, Norwich NR4 7UQ,* [3]*Laboratory of the Government Chemist, Teddington, TW11 0LY*

Concerns have been raised that intakes of Se in the UK are falling, with the trend towards using European wheat rather than North American wheat, which has a higher Se content, being suggested as a contributory factor (Barclay *et al.* 1995; Rayman, 1997). The Total Diet Study (Peattie *et al.* 1983), which is carried out continuously, is a model of the national average domestic diet in the UK used to monitor intakes of food constituents. Foods representative of the UK diet are purchased, prepared and combined into twenty food groups for analysis. MAFF commissioned the CSL Food Science Laboratory (CSL) and the Laboratory of the Government Chemist (LGC) to determine Se levels in samples from the 1995 Total Diet Study. CSL used hydride generation-inductively coupled plasma-mass spectrometry while LGC used hydride generation-atomic fluorescence spectrometry.

Average intakes of Se were calculated from the concentrations of Se found in each food group and the population average consumption of each food group (estimated mainly from MAFF's National Food Survey). Results of analyses by CSL and LGC agreed well, giving middle level intakes of 34 μg/d and 33 μg/d respectively (range 33-34 μg/d and 29-39 μg/d respectively). The Table shows the concentration of Se in each food group and relative contribution to intake.

| Food group* | CSL Se content (mg/kg) | CSL Se intake† (mg/person per d) | LGC Se content (mg/kg) | LGC Se intake† (mg/person per d) |
|---|---|---|---|---|
| Bread | 0.053 | 0.006 | 0.05 | 0.005 |
| Miscellaneous cereals | 0.022 | 0.002 | 0.02 | 0.002 |
| Carcass meat | 0.08 | 0.002 | 0.08 | 0.002 |
| Meat products | 0.099 | 0.004 | 0.07 | 0.003 |
| Poultry | 0.16 | 0.003 | 0.11 | 0.002 |
| Fish | 0.32 | 0.004 | 0.3 | 0.004 |
| Eggs | 0.16 | 0.002 | 0.21 | 0.003 |
| Sugars and preserves | 0.009 | 0.001 | <0.004 | 0.001 |
| Potatoes | 0.007 | 0.001 | 0.02 | 0.003 |
| Other vegetables | 0.015 | 0.001 | 0.02 | 0.002 |
| Beverages | <0.001 | 0.000 | <0.004 | 0.002 |
| Milk | 0.010 | 0.003 | <0.01 | 0.001 |
| Dairy products | 0.031 | 0.002 | 0.03 | 0.002 |
| Nuts | 0.52 | 0.001 | 0.54 | 0.001 |
| Total | - | 0.034 | - | 0.033 |

* Excludes food groups not contributing to middle level intake.
† Middle level intake, i.e. concentrations below limit of detection taken as 0.5 x limit of detection.

These new data show that population average Se intakes (i.e. including adults and children) in the UK have fallen below the reference nutrient intake for adults (75μg/d for men) (Department of Health, 1991). The Committee on Medical Aspects of Food and Nutrition Policy (COMA) considered these data and agreed that: (i) there is, at present, no evidence of adverse health consequences from current intakes; (ii) monitoring of Se intakes and measurements of Se status sho.0 uld continue; (iii) further research should be encouraged to investigate whether the current levels of intake are adequate, and whether the body adapts to changing intakes (Ministry of Agriculture, Fisheries and Food & Department of Health, 1998).

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**Effects of replacing starch with whey permeate, lactose and sucrose under steady-state conditions on rumen metabolism and digestion.** By A. HUSSAIN and E. L. MILLER, *University of Cambridge, Nutrition Laboratory, 307 Huntingdon Road, Cambridge CB3 OJQ*

Sugars appear to be superior energy supplements for ruminants compared with starch (Chamberlain *et al.*,1993). Feeding of lactose increased DM intake and milk yield in dairy cows (Garnsworthy, 1996). Whey permeate (850 g lactose/kg) is a commercial by-product obtained from the manufacture of cheese. Being a source of lactose it can be used as an energy supplement in the feeds of ruminants. In order to examine the suitability of whey permeate as an energy supplement, 50 g readily fermented maize starch in concentrate (A) was replaced with 6.25, 12.5, 25 or 50 g whey permeate (B-E). Whey permeate (C) was also compared with equivalent amounts (10 g) of lactose (F) and sucrose (G). A 7×4 incomplete Latin-square design was conducted using seven wethers in four periods. Animals were given 600 g hay and 400 g concentrate per day continuously at 2 h intervals. During each period samples of rumen contents, faeces and urine were taken to measure rumen fermentation, nutrient digestion and microbial protein synthesis. Data obtained were analysed using the ANOVA procedure of Genstat 5. The main results are given in the Table.

| Diets | A | B | C | D | E | F | G | SED |
|---|---|---|---|---|---|---|---|---|
| Rumen fermentation | | | | | | | | |
| pH | 6.33 | 6.31 | 6.30 | 6.38 | 6.33 | 6.31 | 6.34 | 0.06 |
| Ammonia-Nitrogen (mg/l) | 198.4 | 196.5 | 195 | 175.1 | 194.4 | 172.3 | 185.6 | 16.20 |
| Total VFA concentration (mM) | 90.6 | 94.72 | 91.49 | 85.36 | 93.59 | 93.14 | 89.05 | 3.54 |
| Total Protozoa (x$10^4$/ml) | 127 | 83 | 101 | 139 | 98 | 96 | 94 | 12.1 |
| Total tract nutrient digestion (%) | | | | | | | | |
| Dry matter (DM) | 65.48 | 67.05 | 66.55 | 65.66 | 66.37 | 66.27 | 63.92 | 0.94 |
| Organic matter (OM) | 68.62 | 70.12 | 69.24 | 68.52 | 69.48 | 69.08 | 66.71 | 0.96 |
| Neutral detergent fibre (NDF) | 60.82 | 62.74 | 61.57 | 61.1 | 61.67 | 61.71 | 57.99 | 1.71 |
| Nitrogen (N) | 73.58 | 75.73 | 75.13 | 73.67 | 74.22 | 74.28 | 72.87 | 0.63 |
| Microbial protein synthesis measured by urinary excretion of purine derivatives (PD) | | | | | | | | |
| Allantoin (mmol/day) | 8.76 | 8.82 | 8.58 | 8.98 | 8.84 | 8.94 | 8.82 | 0.57 |
| Uric acid (mmol/day) | 3.36 | 3.32 | 3.05 | 3.24 | 3.4 | 3.29 | 3.47 | 0.25 |
| Total PD (mmol/day) | 13.15 | 13.47 | 12.7 | 13.66 | 13.73 | 13.44 | 13.65 | 0.64 |
| Microbial N supply (g/d) | 11.41 | 11.62 | 10.89 | 11.76 | 11.83 | 11.59 | 11.77 | 0.57 |

Rumen pH and total volatile fatty acid (VFA) concentration were not affected by the supplements. All the sugar supplements, however, gave lower mean values for rumen $NH_3$ (non significant). Total rumen protozoa were reduced on diets B ($P$=0.004), C ($P$=0.05) and E ($P$=0.03) compared with diet A but there were no significant differences between the sugars. Except for sucrose, total tract nutrient digestion was not affected by the different treatments. Sucrose resulted in significantly lower total tract DM ($P$=0.03), OM ($P$=0.03), NDF ($P$=0.05) and N digestion values ($P$=0.05) compared with lactose. Total PD excretion in urine and microbial N supply calculated from that were not affected by different treatments. From these results it is concluded that whey permeate can be included at up to 125 g/kg concentrate, replacing starch without any adverse effect on rumen metabolism. In most cases replacing starch with sugars (whey permeate, lactose and sucrose) reduces rumen protozoa.

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**A spreadsheet programming model for linear and stochastic feed formulation problems.** By M.Y. BAIG and E.L. MILLER, *University of Cambridge, Nutrition Laboratory, 307 Huntingdon Road, Cambridge CB3 OJQ*

In general, least cost feed formulation softwares are too expensive to be afforded by many nutritionists and also they may not fulfil the exact requirements of the user. Moreover, most available softwares work on the basis of Linear Programming. A major problem in this method is the variability in nutrient content of ingredients coming from different batches and sources, and variation attributed to the laboratory procedure and human error. Conventionally, mean values are used for formulation. As a simple statistical rule, if a normally distributed random element exists in a population and only its mean value is accounted for, while ignoring the variability, then the probability of occurrence of the mean value will only be 50% *i.e.* $P(A) \geq 0.5$. A solution to the problem is Stochastic Programming, which can raise the probability of meeting the specified requirements up to a desired level. In this method non-linear deterministic equations are constructed as a function of the mean and variance of the random element and are solved using iterative quadratic optimization.

All these problems could be solved if nutritionists were able to write their own programme but they do not, generally, have expertise in computer languages to develop their own package. This problem was solved by writing codes for computer spreadsheets, which are widely available and easy to operate. Using these codes nutritionists can develop their own feed formulation software either in Linear or Stochastic form according to their specific requirements.

A standard linear programming model, in matrix form, can be presented as follows.

$$\text{Minimize } \sum_{j=1}^{n} c_j x_j \quad \text{subject to } \sum_{j=1}^{n} a_{ij} x_j \geq b_i \qquad \sum_{j=1}^{n} a_{ij} x_j \leq b_i \qquad \sum_{j=1}^{n} x_j = 1 \qquad x_j \geq 0,$$

where $c_j$ is the cost per unit for $j$th ingredient, $x_j$ is the quantity of $j$th ingredient, $a_{ij}$ is the quantity of $i$th nutrient per unit of the $j$th ingredient, $b_i$ is the requirement for the $i$th nutrient in the diet. If we wish to increase the success rate of meeting the $i$th nutrient in the diet up to or to fall below the level $b_i$, to a probability of $P \geq \alpha_i$, then the constraint will be modified as follows

$$P\left(\sum_{j=1}^{n} a_{ij} x_j \geq b_i\right) \geq \alpha_i \qquad \text{or} \qquad P\left(\sum_{j=1}^{n} a_{ij} x_j \leq b_i\right) \geq \alpha_i .$$

If the $a_{ij}$ are independent and normally distributed random variables with respective population mean $\mu_{ij}$ and the standard deviation $\sigma_{ij}$, the above constraints can be derived as follows

$$\sum_{j=1}^{n} \mu_{ij} x_j \geq b_i - Z_i \sqrt{\sum_{j=1}^{n} \sigma_{ij}^2 x_j^2} \qquad \text{or} \qquad \sum_{j=1}^{n} \mu_{ij} x_j \leq b_i - Z_i \sqrt{\sum_{j=1}^{n} \sigma_{ij}^2 x_j^2} ,$$

where $Z_i$ is the standard normal deviate corresponding to the required probability level. The following example worksheet, developed in Microsoft Excel will solve such problems.

b4&b24=CP, b5&b25=CP_SD, b6&b26=ME, b7&b27=Ca, b8&b28=Weight, b9=Cost, b13=Quantity, b21=Total Cost, c3=Fish Meal, d3=Soyabean Meal, e3=Barley, c4=69.4, c5=2.31, c6=14.2, c7=5.62, d4=50.7, d5=1.62, d6=13.4, d7=0.45, e4=12.8, e5=0.53, e6=12.8, e7=0.11, c8:e8=1, c9=335, d9=160, e9=105, c10=(c5^2)*(c13^2), e20=Probability, f20=90, d10=(d5^2)*(d13^2), e10=(e5^2)*(e13^2), c12=c9*c13, d12=d9*d13, e12=e9*e13, c21=sum(c12:e12), c24=sumproduct($c$13:$e$13,c4:e4)+(-normsinv($f$20/100))*sqrt(sum(c10:e10)), c25=sumproduct($c$13:$e$13,c5:e5), c26=sumproduct($c$13:$e$13,c6:e6), c27=sumproduct($c$13:$e$13,c7:e7), c28=sumproduct ($c$13:$e$13,c8:e8), c23=Final value, d23=Requirements, d24=18, d26=12, d27=0.5, d28=1

Using Solver in Tools menu $c$21 was set as target cell and minimum with $c$13:$e$13 as changing cells. The other constraints were adjusted as c24≥d24, c26≥d26, c27≥d27, c28=d28. Additional constraints for minimum inclusion of each feed were arbitrarily set as c13≥0.02, d13≥0.03, e13≥0. When this model was executed for probability of 90%, it resulted in a least cost formula consisting of fish meal 6.75%, soyabean meal 5.33% and barley 87.92% with a cost of £123.45 per ton. In this particular example the optimisation of crude protein followed stochastic programming while the other nutrients followed linear programming. Thus this worksheet model provides a fast and accurate way to solve both linear and nonlinear stochastic feed formulation problems. Many nutritionists, who are already familiar with the use of electronic spreadsheets, will find it a simple and easy to set model for their assessment of the economic ramifications of choices in animal nutrition and feeding.

**Macronutrient intake in normal-weight children at high and low risk of obesity.** By AILEEN F. McGLOIN[1], M. BARBARA E. LIVINGSTONE[1], LUCY C. GREENE[1], SANDRA E. WEBB[1], JOANNA M.A. GIBSON[1], SUSAN A. JEBB[2] and ANDREW M. PRENTICE[2], [1]*Northern Ireland Centre for Diet and Health, Biomedical Sciences, University of Ulster at Coleraine, BT52 1SA,* [2]*MRC Dunn Nutrition Unit, Cambridge CB2 2DH*

Numerous dietary studies have shown an association between adiposity and the consumption of a high-fat diet (Lissner et al., 1992). In children, there has been a limited number of studies investigating the role of macronutrient composition of the diet in the risk of obesity. It has been shown that compared with children of lean parents, children of obese parents consume a significantly greater proportion of energy as fat (Eck et al., 1992). Elevated protein intake in childhood may also predispose to obesity later in life (Rolland-Cachera et al., 1995). This present study examined differences in macronutrient intake in children at high risk (HR) of obesity (parental BMI > 30 kg/m$^2$) and at low risk (LR) of obesity (parental BMI < 25 kg/m$^2$).

Subjects were ninety-six healthy children (67M; 29F) matched for age (mean 7.1 SD 0.9 years). Dietary intake was measured using 7 d weighed records. Anthropometric measurements, energy intake (EI) and macronutrient intake (g/d and % energy) results are shown in the Table.

| | Low Risk | | | | High risk | | | |
|---|---|---|---|---|---|---|---|---|
| | Males (*n* 31) | | Females (*n* 18) | | Males (*n* 31) | SD | Females (*n* 11) | |
| | Mean | SD | Mean | SD | Mean | | Mean | SD |
| Age (years) | 7.1 | 0.6 | 7.2 | 0.6 | 7.0 | 1.0 | 7.1 | 0.6 |
| Weight (kg) | **24.0*** | 3.2 | 21.8 | 2.8 | **26.1*** | 4.7 | 24.0 | 3.5 |
| Height (m) | 1.24 | 0.05 | 1.22 | 0.05 | 1.25 | 0.08 | 1.24 | 0.05 |
| Energy intake (MJ/d | 6.9 | 1.7 | 6.8 | 1.0 | 6.9 | 1.4 | 7.7 | 1.9 |
| Protein (g/d) | 52.6 | 13.7 | **49.6*** | 9.4 | 52.7 | 12.2 | **59.7*** | 14.3 |
| Carbohydrate (g/d) | 222 | 58 | 219 | 36 | 218 | 48 | 243 | 71 |
| Fat (g/d) | 66.1 | 17.7 | 67.0 | 12.2 | 69.9 | 15.6 | 75.8 | 19.3 |
| Protein (% energy) | 12.9 | 1.7 | 12.8 | 1.8 | 12.7 | 1.7 | 13.2 | 2.9 |
| Carbohydrate (% energy) | 50.7 | 4.0 | 50.4 | 4.9 | 49.1 | 5.1 | 49.4 | 6.6 |
| Fat (% energy) | 36.2 | 3.4 | 36.9 | 3.6 | 37.9 | 3.7 | 37.2 | 4.6 |

Mean values were significantly different for corresponding LR and HR groups * $P< 0.05$ (one-way ANOVA)

Although all the children were of normal weight, boys in the HR group were significantly heavier than their LR counterparts ($P < 0.05$). In girls, there was no significant difference. EI was not significantly different between groups. There was a trend for higher fat intakes (% energy) in the HR children that fell short of significance in boys ($P = 0.058$). Although protein (% energy) did not differ between groups, protein intake (g/day) was significantly higher in HR girls compared with LR girls ($P<0.05$).

This study failed to confirm the findings of Eck et al. (1992). However, the trend for a higher body weight in the HR groups in association with higher % fat energy intake boys, and higher protein intake (g/day) in girls may suggest a role for macronutrient composition of the diet in the development of obesity in this group. However, whether these trends are risk markers for obesity later in life remains to be established.

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***Trans* fatty acid levels in adipose tissue of Irish adults.** By L. HOGAN and K. M. YOUNGER, *Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Ireland*

*Trans* fatty acids (TFA) are produced by the partial hydrogenation of vegetable and marine oils. TFA are not synthesized in the human body. The fatty acid composition of adipose tissue reflects the habitual intake of TFA over the previous 1-2 years (Beynen *et al.* 1980). Recent studies associate TFA with an increased plasma level of LDL and therefore an increased risk of CHD (Willett *et al.* 1993; Ascherio *et al.* 1994).

The objective of the present study was to assess the level of TFA in the subcutaneous fat and erythrocyte membranes of subjects from an Irish population. Healthy subjects (fifty-nine female, sixty-three male, aged 23-63 years) were recruited. One fat aspirate and one blood sample were taken from each subject. Adipose tissue fatty acid composition was analysed by capillary GC and by infra red spectroscopy. Individual erythrocyte membrane fatty acids were analysed by gas chromatography-mass spectrometry. Serum LDL, HDL and total cholesterol levels were analysed using enzymic assays.

Results for this population were: mean TFA 4.22 (sd 0.8) g/100 g adipose tissue lipid, mean serum HDL 1.07 (sd 0.4) mmol/l, LDL 3.54 (sd 1.5) mmol/l and total cholesterol 5.53(1.49) mmol/l. These levels of adipose tissue TFA are similar to those levels found in other studies (Enig *et al.* 1984; London *et al.* 1991; Hudgins *et al.* 1991). The 18:1*t* TFA content of erythrocyte membranes was: mean 0.85 (sd 0.39) g/100 g erythrocyte lipid. A positive ($r$ 0.13) non-significant ($P$=0.24) relationship was observed between adipose tissue 18:1*t* and serum LDL levels. A significantly stronger relationship ($r$ 0.33, $P$=0.002) was observed, however between adipose tissue 16:1*t* and serum LDL levels. These results suggest that 16:1*t* may, in fact, be the more offending isomer with respect to CHD risk. There was a significant correlation ($r$ 0.32, P=0.01) between 18:1*t* (adipose tissue) and 18:1*t* (erythrocyte membranes) suggesting that, although levels of TFA in adipose tissue and erythrocyte membranes are different in magnitude, there is a strong relationship between them.

These, the first such data for an Irish population, in agreement with published data, show some evidence that increased levels of TFA in adipose tissue (and thus the diet) are associated with raised serum LDL.

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**Can patients' knowledge of their own weight and height be used to replace measured weight and height in the calculation of BMI?** By M. DOYLE[1], C. CORISH[1], P. FLOOD[2] and N. P. KENNEDY[1], [1]*Department of Clinical Medicine, Trinity Centre for Health Sciences, St James's Hospital, Dublin 8, Ireland,* [2]*Department of Clinical Nutrition, St James's Hospital, Dublin 8, Ireland*

Routine assessment of BMI has been recommended as a useful way of assessing nutritional status in patients attending a general practitioner's (GP) surgery (Edington *et al.*1996). Data suggest that changes in body weight may be associated with increased mortality (Kushner, 1993).

The objectives of the present pilot study were to estimate the nutritional status of GP attendees using BMI and to determine if the patient's self-reported weight and height could be used as surrogates for measured weight and height in the calculation of BMI by the GP.

This study was carried out in three GP practices yielding a total of 235 cases (fifty-eight males, 175 females) with a mean age of 48 (sd 18) years. Patients were asked to complete a nutrition screening questionnaire which included questions on weight and height. Calculation of BMI was possible in 184 cases (78% of the sample) from the self-reported information and in 120 (51%) from actual measures of weight and height. BMI derived from self-reported and from actual weights and heights were compared in ninety-two subjects, of whom eighteen mis-reported their weight by 2.2 kg or more. Patients appeared to have reasonable knowledge of their height, the mean difference between that reported and measured being 23 (sd 19)mm. Garrow's (1981) classification of obesity was used to divide the sample for cross-classification purposes (see Table).

The prevalences of undernutrition, overweight and obesity found in general practice were similar when BMI was calculated using either the reported or measured weights and heights. In almost one-quarter of cases (22% of 235), BMI could not be calculated using self-reported weight and height values. These patients were unable to give information on their own weight, height or both. However, when BMI based on measured values was calculated for 55% (*n* 28) of this group the overall distribution of nutritional status did not alter.

| Nutrition status | Self-reported group (*n* 184) | Measured group (*n* 120) | Under reporters (*n* 10) | Over reporters (*n* 8) | Total mis-reporters (*n* 18) |
|---|---|---|---|---|---|
| Undernourished | 16 (9%) | 9 (8%) | 0 | 2 (22%) | 2 (22%) |
| Normally nourished | 84 (46%) | 46 (38%) | 3 (7%) | 1 (2%) | 4 (9%) |
| Overweight | 60 (32%) | 44 (37%) | 4 (9%) | 1 (2%) | 5 (11%) |
| Obese | 24 (13%) | 21 (17%) | 3 (14%) | 4 (19%) | 7 (33%) |

Predictably, undernourished (BMI <20 kg/m$^2$) or obese (BMI ≥30 kg/ m$^2$) patients were more likely to report their weight incorrectly. A non-significant trend towards more frequent under-reporting of weight in males than in females and towards more over-reporting of weight in females compared with males was observed. Age did not influence accuracy of reporting weight.

In conclusion, self-reported weights and heights were found to be useful surrogates for measured weights and heights in calculating BMI for GP attendees who are of normal weight (BMI 20-24.9 kg/m$^2$) or are overweight (BMI 25.0-29.9 kg/m$^2$). However, it is recommended that GP should measure the weight, rather than rely on self-reported weight, of those who appear to be either underweight or obese.

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**Obesity management in general practice: a pilot qualitative study of attitudes of general practitioners and practice nurses in Glasgow.** By SOPHIE TESSIER and STEWART W. MERCER, *Department of Human Nutrition, University of Glasgow, Royal Infirmary, Glasgow G31 2ER*

The increasing incidence of obesity in the UK, raises a growing concern among health professionals as to where and how obesity prevention and treatment should be managed. Recent reports and guidelines have suggested that the obvious place for this to occur, given the importance of social context and the scale of the problem, is within the primary healthcare setting (Scottish Intercollegiate Guidelines Network, 1996; Scottish Office, 1993). The present study aimed to explore, in depth, views of general practitioners (GP) and practice nurses (PN) on the issues surrounding weight management in the primary health care context.

Audiotaped semi-structured interviews, with defined domains, were conducted among GP (*n* 8) and PN (*n* 10) in their practice. Interviewees were selected from a representative sample (stratified for social class of the practice and year of qualification of the GP) of general practices within the Greater Glasgow area. Transcribed interviews were coded and emerging themes identified (Bryman & Burgess, 1994).

When the perception of the problem was investigated, most of the participants recognized the importance of the problem of obesity, its increasing prevalence and related conditions.

All of the interviewees aimed for lifestyle changes and realistic goals. Most of the GP sent obese patients to the PN for ongoing management, although many were unsure of what the nurse actually did in practice. Underlying attitudes for such management included the view that it is not a GP's role to be dealing with obesity directly, although other factors such as time limitation in consultations were also cited frequently. In general, both GP and PN appeared to have little motivation or enthusiasm for weight management, mainly due to perceived lack of success in the past. Exceptions to this attitude were identified when related conditions (e.g. diabetes) were present or when patients were enthusiastic themselves. The personal experiences of GP and PN of their own weight management also seemed to influence attitude in some cases. Most PN were dealing with weight management on an individual basis, although some were running or had run groups in the past. The need to negotiate with patients as individuals was seen as a very important factor. They repeatedly made the point that the treatment of the overweight/obese patient varies from one to another and, as a result, requires an ability to adapt and be flexible. PN expressed some views about a lack of communication from doctors and felt at times that GP "off-loaded" the weight management problem onto them.

A complex range of barriers to obesity management was identified. These included factors characteristic of the primary healthcare team, patients, environment and dietary changes. GP strongly expressed the need to educate the population, including mothers and children, requiring a greater involvement of government, the media, the medical profession in general and those in health education in particular. PN felt they could benefit from further courses, meetings, and distance learning material. In particular, PN voiced a desire to learn techniques to help them to motivate, or understand the motivation of obese patients.

The study suggests that the primary health care team could benefit from better communication and organization between GP and PN, and better knowledge of behavioural/psychological models of motivational status of patients. The wider question of how realistic it is to target primary care as the focus of effective obesity prevention and treatment in the UK remains to be answered.

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**Obesity and weight management training needs of general practitioners and practice nurses.** By JOSEPH F. MURRAY[1], SUSAN ELEY[2], PATRICIA HAUGHNEY[3] and MICHAEL E.J. LEAN[2], [1]*Department of Human Nutrition, University of Glasgow, Yorkhill Hospitals, Glasgow G3 8SJ,* [2]*Department of Human Nutrition, University of Glasgow, Glasgow Royal Infirmary, Glasgow G31 2ER,* [3]*Biomedical Sciences, University of Glasgow, G12 8QQ*

The report *Obesity in Scotland Integrating Prevention with Weight Management* (Scottish Intercollegiate Guidelines Network, 1996) stated that the continuing education of relevant staff was needed for the implementation of the guideline. This is especially pertinent for the primary health care team. The present study examined the views of general practitioners (GP) and nurses surrounding the extent of the problem of obesity, their clinical role and their obesity and weight management education needs.

The postal survey was conducted in nine Health Boards of Scotland that were, in broad terms, urban (the 'Central Belt' of Forth Valley, Lanarkshire, Greater Glasgow and Lothian) and rural (Argyll, Dumfries and Galloway, Western Isles, Shetland and Orkney). Stratified by a measure of deprivation (Carstairs & Morris, 1991), a systematic sample of 100 GP and 100 practice nurses was drawn from the total population across the health board areas. A response rate of 74.5% (149/200) was achieved, of which 141 questionnaires had complete data. There were no significant differences between responders and non-responders in their practice (deprivation score (Carstairs & Morris, 1991), number of GP, health board, urban/rural) and individual characteristics (sex, duration in present post).

The majority of the sample of GP and practice nurses (96%) reported that obesity was an 'extensive problem' in their practice. When asked about their clinical role, 84.8% reported 'both prevention and treatment', 8.6% reported 'prevention of obesity' and 6.6% reported 'treatment of obesity'.

| | Proportion of GP and practice nurses reporting nutrition training needs | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GP (*n* 66) | | | | | | Nurses (*n* 85) | | | | | | |
| | Essential | | Of interest | | Not very relevant | | Essential | | Of interest | | Not very relevant | | *P* value |
| | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % | |
| The role of nutrition in disease | 24 | 36.9 | 39 | 60.0 | 2 | 3.1 | 55 | 65.5 | 29 | 34.5 | 0 | 0.0 | 0.001 |
| Natural history and causes of obesity | 27 | 40.9 | 37 | 56.1 | 2 | 3.0 | 32 | 38.6 | 50 | 60.2 | 1 | 1.2 | 0.68 |
| Complications of weight gain benefits of weight loss | 44 | 67.7 | 19 | 29.2 | 2 | 3.1 | 73 | 85.9 | 12 | 14.1 | 0 | 0.0 | 0.016 |
| Current health assessment methods | 28 | 42.4 | 35 | 53.0 | 3 | 4.5 | 66 | 78.6 | 18 | 21.4 | 0 | 0.0 | 0.001 |
| Weight management | 49 | 74.2 | 16 | 24.2 | 1 | 1.5 | 75 | 88.2 | 10 | 11.8 | 0 | 0.0 | 0.63 |
| Incorporating prevention routinely into clinical management | 32 | 48.5 | 32 | 48.5 | 2 | 3.0 | 65 | 76.5 | 20 | 23.5 | 0 | 0.0 | 0.001 |

Comparing responses from each profession using chi-square, the Table indicates that GP and practice nurses identified significantly different training needs. The data suggest that a large proportion of the primary health care team could be better equipped in the prevention and treatment of obesity.

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**Patient satisfaction, nutrient content and actual intake of food served in two Dublin teaching hospitals.** By FIONA RUSH[1,2] and MARY MOLONEY[2], [1]*University of Dublin, Trinity College Dublin, Ireland;* [2]*Dublin Institute of Technology, Kevin Street, Dublin, Ireland*

Previous studies have reported low energy and micronutrient intakes among the hospitalized population (Thomas *et al.* 1988; Fenton *et al.* 1995). The present study assessed the provision and intake of hospital food and selected nutrients in fifty one subjects (hospital A, *n* 26; hospital B, *n* 25) aged 19-65 years, who were independently consuming the regular hospital diet. Nutritional analysis using WISP (weighed intake software package, version 1.25 for Windows '95) was carried out on the hospital menus and on the 3 d weighed intake recorded for each subject. Subjects also completed an interview-assisted questionnaire on satisfaction with food services.

| | | Hospital A (*n* 26) | | | Hospital B (*n* 25) | | |
|---|---|---|---|---|---|---|---|
| | | Mean | SD | % RNI* | Mean | SD | % RNI* |
| Vitamin C: (mg) | Provision/d | 32 | 9.7 | 80 | 36 | 15 | 90 |
| | Intake/d | 27 | 10 | 68 | 35 | 22 | 88 |
| Folate : (ug) | Provision/d | 208 | 90 | 104 | 219 | 86 | 110 |
| | Intake/d | 166 | 38 | 83 | 159 | 58 | 79 |
| Calcium : (mg) | Provision/d | 685 | 134 | 98 | 659 | 117 | 94 |
| | Intake/d | 507 | 90 | 72 | 483 | 137 | 69 |
| | | | | %recommendation† | | | %recommendation† |
| Fibre : (g Englyst) | Provision/d | 11.8 | 4.4 | 66 | 12.7 | 1.8 | 71 |
| | Intake/d | 9.2 | 2.4 | 51 | 10.4 | 4.8 | 58 |

RNI values : vitamin C = 40 mg/d, folate = 200 ug/d, calcium = 700 mg/d. (Department of Health, 1991).

†Recommendation refers to 18 g fibre (Englyst)/d as a median of the recommended range (12-24 g/d). (Department of Health, 1991).

Mean energy intakes were found to be low (especially among males) in both hospitals; hospital A: males 5.5 MJ, females 5.4 MJ *v.* hospital B: males 6.7 MJ, females 6.8 MJ. Of subjects in both hospitals, 76% had a mean vitamin C intake below the reference nutrient intake (RNI). A significant difference between hospitals ($P$=0.001, two-tail) was reported for the percentage contribution to vitamin C from fruit; 11% in hospital B *v.* 1% in hospital A, where fresh fruit did not feature on the menu. In both hospitals 100% of females aged 19-50 years had iron intakes below the RNI for this group (14.8 mg/d). Fibre intakes were also low with 85% of subjects in hospital A and 72% of subjects in hospital B consuming amounts below the recommended 12-24 g/d. In both hospitals portion size, temperature of hot food and poor availability of hospital food between 18.00 and 08.00 hours were identified as the main areas of dissatisfaction. In hospital A 50% of subjects considered the standard portion size to be inadequate, 24% claimed that food served was not hot enough and 85% wished to see the introduction of an evening snack after 18.00 hours.

These findings suggest the need for further research into the quality of hospital catering in terms of nutrient adequacy and consumer acceptance.

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**Nutrient intake, income and dietary variety in adults.** By SUSAN ELEY, ANNIE S. ANDERSON*, LINDA MAHER and MICHAEL E.J. LEAN, *Department of Human Nutrition, University of Glasgow, Glasgow Royal Infirmary, Glasgow G31 2ER.*

The diet of the UK population has been documented as one which is high in fat and low in carbohydrate, dietary fibre, fruit and vegetables (Gregory *et al.* 1990). It is accepted that disposable household income influences food choice. A dietary survey (using the 7 d weighed inventory approach) of 160 adults aged 18-65 years was conducted in Glasgow, Scotland, a city with a high rate of diet-related disease and diverse household income range. Macronutrient intakes reported by this population suggest that overall nutrient intakes have improved (Anderson *et al.* 1997). Dietary variety (DV) was defined as total food items reported over the 7 d study period and average fruit and vegetables intake (FV) was measured in g/day. Data analysis using a novel barcode system allowed an examination of nutrient intakes and variety of food items by income.

| | Income group 1 (high) † (*n* 35) | | Income Group 2 (*n* 34) | | Income Group 3 (*n* 56) | | Income group 4 (low) (*n* 30) | | *P* value | Spear. corr with DV | Spear. corr with FV |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | | | |
| Diet variety (DV)* (items/week) | 178 | 7 | 162 | 7 | 151 | 6 | 132 | 7 | 0.0002 | - | 0.3*** |
| Fruits and vegetables (FV) (g/d) | 260 | 24.9 | 254 | 31.4 | 205 | 23.6 | 151 | 24.3 | 0.03 | 0.3*** | - |
| Daily values | | | | | | | | | | | |
| Energy (MJ) | 8.7 | 0.3 | 8.8 | 0.5 | 8.2 | 0.3 | 7.2 | 0.4 | 0.01 | 0.5*** | 0.2** |
| Energy from fat (%) | 35 | 0.7 | 37 | 0.9 | 37 | 0.7 | 39 | 0.9 | 0.004 | -0.2* | -0.34*** |
| Energy from SFA (%) | 12 | 0.5 | 13 | 0.5 | 14 | 0.4 | 15 | 0.4 | 0.001 | -0.2* | -0.47*** |
| Energy from MUFA (%) | 11 | 0.3 | 12 | 0.4 | 13 | 0.3 | 14 | 0.4 | 0.0002 | -0.3*** | -0.3*** |
| Daily intake per 4184J | | | | | | | | | | | |
| Riboflavin (mg) | 0.8 | 0.1 | 0.8 | 0.04 | 0.8 | 0.03 | 0.8 | 0.1 | 0.4 | 0.4*** | 0.3*** |
| Vitamin $B_{12}$ (mg) | 2.3 | 0.3 | 2.8 | 0.5 | 2.4 | 0.3 | 3.1 | 0.8 | 0.6 | 0.3*** | 0.2** |
| Vitamin C (mg) | 42 | 4.6 | 32 | 3.7 | 29 | 3.0 | 27 | 4.7 | 0.05 | 0.3*** | 0.7*** |
| Calcium (mg) | 418 | 17.4 | 442 | 20.4 | 413 | 17.3 | 406 | 15.3 | 0.6 | 0.3*** | 0.2** |

Compared with the higher income groups, those living on a lower income reported significantly greater percentages of total energy from fat, SFA and MUFA in the diet. Decreasing disposable income was also significantly associated with diets less dense in vitamin C. Dietary variety was itself related to fruit and vegetable consumption. Increasing disposable household income was associated with greater dietary variety and higher fruit and vegetable consumption (g/d). Increasing diet variety and fruit and vegetable intake were related to decreasing percentage energy from total fat, SFA and MUFA and increasing intakes of riboflavin, vitamin $B_{12}$, vitamin C and Ca. The data suggest that there were income differences in fruit and vegetable intake and dietary variety in adults. This may have implications for dietary fat intakes as increasing dietary variety was significantly related to decreasing percentage energy from SFA and MUFA in the adult diet.

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MUFA, monounsaturated fatty acids; SFA saturated fatty acids.

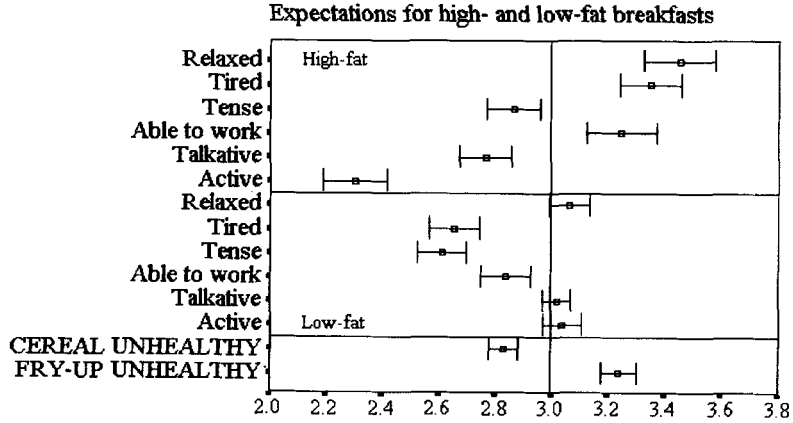
* $P<0.05$, ** $P<0.01$, *** $P<0.001$.

† Annual household incomes were: group 1 >£30 000, group 2 £20 000 - £29 999, group 3 £10 000 - £19 999, group 4 <£9 999.

**A comparison of expectations about high- and low-fat breakfasts.** By MARIE REID[1] and RICHARD HAMMERSLEY, *Department of Psychiatry, University of Sheffield, Northern General Hospital, Sheffield S5 7AU, and Department of Sociological Studies, University of Sheffield, Sheffield S10 2FL*

Expectations about the outcome of drinking alcohol predict drinking behaviour (McMahon & Jones, 1994), so perhaps expectations about the outcome of eating can predict unhealthy food choices, such as consumption of *overtly* high-fat food. University staff (*n* 296, 123 males, 173 females) completed a food-frequency questionnaire (FFQ) (Yarnell *et al.* 1983) and answered questions about what they expected to happen after eating four types of breakfast. Here, expectations about a high-fat full-fried (English/Irish/Scottish) breakfast are compared with expectations about a low-fat breakfast of toast and cereal. Expectations about breakfasts were assessed with twenty-two questions phrased for example "After eating (toast and cereal/ a full fried breakfast) I would expect to feel relaxed."

By paired *t* tests with the criteria set at $P<0.001$ to correct for multiple comparisons, all items but enjoyment and happiness differed between breakfasts; there were few sex differences. Nine items relating to health were summed to produce one score of expected unhealthiness of the breakfast. These data are shown in the Fig., along with expected mood effects. High-fat, fried breakfasts were expected to lead to effects including relaxation, tiredness, reduced activity (but ability to work) compared with low-fat breakfasts, but the former were expected to be less healthy.




Two variables from the FFQ were predicted in regression analysis. Frequency of frying was significantly related to expecting a fried breakfast to result in exercising less (beta=0.18), being more relaxed (beta=0.15) and being less talkative (beta=0.21) (total 8% of variance). Frequency of eating cereal was significantly related to expecting toast and cereal to result in being less able to work (beta=0.22), less full (beta=0.18), more relaxed (beta=0.14), more likely to exercise (beta=0.14) and less thirsty (beta=0.14) (total 12% of variance). It is concluded that expectations about different breakfasts differ substantially and that these expectations are modestly related to dietary intake. At times people may choose food that they perceive to be unhealthy but expect to improve mood.

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**Evaluation of the dental health intervention programme, DEN TV.** By SHARON FRIEL, SIMON COMER, GERALDINE NOLAN, ANNE HOPE and CECILY KELLEHER, *Department of Health Promotion, National University of Ireland, Galway, Ireland*

The present paper reports the results of the evaluation of the DEN TV oral health intervention programme. Under the auspices of the Irish Dental Health Foundation a pilot intervention programme, directed at children in 1st/2nd class and 5th/6th class, was implemented in the Eastern and Western Health Boards. A pre-post repeated measures quasi-experimental research design was used with experimental and control schools chosen before the intervention which reflected the spectrum of schools under the jurisdiction of the Department of Education. The main aims of the intervention programme were to increase the knowledge and change the behaviour of children towards better dental health. The intervention involved use of the DEN TV programme shown on television plus specific visits by a dental nurse during school hours. Both experimental and control children were surveyed using a questionnaire before the intervention and again after the six week intervening period using the same survey instrument. Due to the nature of the intervention, contamination effects may have occurred during the intervening period. For this reason the evaluation consisted of comparisons between those who reported seeing the DEN TV programme and those who did not, plus those who received a visit by a dental health nurse (DHN) and those who did not.

There were no significant differences in responses to most questions between those who said they watched DEN TV and those who did not. The one exception was the question dealing with the reported amount of toothpaste used: children who said they had watched DEN TV were more likely to report that they used the correct, pea-sized amount of toothpaste. The difference was significant for Class 1/2 ($P < 0.05$) and for Class 5/6 ($P < 0.01$). Children who said they had been visited by a DHN registered significant changes in responses to some questions compared with those children who said they had not received such a visit. Class 1/2 reported a change in how often they brushed their teeth, how much toothpaste they used, and the time of day at which they brushed their teeth. The older children who had been visited by a DHN registered a significant change in the amount of toothpaste they used. The proportion agreeing that "fluoride is a better type of toothpaste" was 66% of those who had been visited by a DHN and 47% of those who had not ($P < 0.01$). The Table below summarizes the effect of having watched DEN TV and having a visit by the DHN.

| | Class 1 / 2 | | Class 5 / 6 | |
|---|---|---|---|---|
| **Question topic** | DEN TV (%) | DHN (%) | DEN TV (%) | DHN (%) |
| Use pea-sized amount of toothpaste | 38* | 38** | 50** | 46* |
| Fluoride is a better type of toothpaste | - | - | 67 | 66** |
| Brush for 3 minutes or more | 56 | 59* | 57 | 56 |
| Brush at least twice daily | 74 | 75* | 72 | 73 |

* $P < 0.05$, ** $P < 0.01$

The findings indicate a positive effect of the dental health intervention programme. There appeared to be a greater impact on the children's awareness and behaviour by the DHN compared with that of the television programme. This may be due to a dilution of the oral health messages within the overall television programme.

**Body weight concerns of 13-year-old Irish boys and girls.** By S. SINNOTT, Y.M. RYAN and M.A.T FLYNN, *Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Republic of Ireland*

More adolescent girls compared with boys have been shown to be concerned about their weight (Watt and Sheiham, 1996). The purpose of this present study was to compare the body weight perceptions, body weight satisfaction and weight loss practices of boys and girls attending a mixed school. These variables were also compared between girls attending a single-sex school and those attending a mixed school. Subjects (mean age 13 years, *n* 167, 56% participation rate) were recruited from the first and second years of two south Dublin suburban secondary schools of mixed socio-economic background, one single-sex school (girls) and one mixed school. A self-report questionnaire was administered in a classroom setting to assess perceived relative weight i.e. underweight (uwgt), normal weight (nwgt) or overweight (owgt), and slimming practices of the group. Weight and height measurements were recorded in all subjects and used to calculate percentage relative weight category i.e. underweight, normal weight or overweight as described by Ryan *et al.* (1998). Social class was assessed according to O'Hare (1982). Girls in the single-sex school were found to be significantly younger (13.1 *v.* 13.4 years, $P<0.05$) and significantly fewer of them were in social class 5 and 6 (13 *v.* 30%, $P<0.05$). There was no disparity between actual relative weight and perceived relative weight for either the boys or the girls attending the mixed school. However, girls in the single-sex school perceived themselves to be significantly heavier than they actually were (22% were actually overweight *v.* 34% who perceived themselves to be overweight, $P< 0.05$). The satisfaction of girls and boys with self-perceived weight status is given in the Table.

| | Girls (*n* 94) (single -sex school) | | | Girls (*n* 37) (mixed school) | | | Boys (*n* 36) (mixed school) | | |
|---|---|---|---|---|---|---|---|---|---|
| | uwgt (*n* 3) % | nwgt (*n* 59) % | owgt (*n* 32) % | uwgt (*n* 1) % | nwgt (*n* 23) % | owgt (*n* 13) % | uwgt (*n* 0) % | nwgt (*n* 32) % | owgt (*n* 4) % |
| Satisfied with perceived weight | 0 | 81 | 3 | 0 | 78 | 0 | 0 | 91 | 25 |
| Dissatisfied with perceived weight | | | | | | | | | |
| and (a) want to be lighter | 0 | 17 | 94 | 0 | 22 | 100 | 0 | 3 | 75 |
| (b) want to be heavier | 67 | 0 | 0 | 100 | 0 | 0 | 0 | 6 | 0 |
| Don't know | 33 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |

Significantly more boys than girls attending the mixed school were satisfied with their weight (83 v. 49%, $P< 0.001$) and significantly less boys had attempted to lose weight in the past than girls (22 v. 54%, $P< 0.05$). Girls in the two schools were over three times more likely to perceive themselves as being overweight than boys (35 v. 11%). In fact, although 22% of the boys were actually overweight, only 11% perceived themselves to be overweight. There was no significant difference regarding the level of body weight dissatisfaction and prevalence of weight loss practices among the female students in both schools. Out of the total number of students (167), 49% (*n* 82) reported trying to lose weight in the past. Slimming methods reported by the students included 'exercising' (44% of girls and 9% of boys respectively) 'avoiding eating sugary foods' (35% and 8%) 'dieting' (28% and 6%) 'skipping meals' (19% and 3%) 'avoiding eating red meat' (4% and 0%) 'inducing vomiting' (3% and 3%) 'using diet pills' (2% and 0%) and 'smoking' (2% and 0%). In conclusion, this study suggests that more girls than boys are concerned about their body weight and that the emergence of unhealthy weight loss practices are evident in Irish girls and boys as young as 13 years of age.

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Ryan YM, Gibney MJ, & Flynn MAT (1998) *International Journal of Obesity* **19**, 376-381.

Watt R.G, Sheiham A (1996) *Journal of Human Nutrition and Dietetics* **9**, 451 – 461.

**The effect of in-store music on consumer choice of wine.** By C.M. RYAN, C. A. NORTHROP-CLEWES, B. KNOX AND D.I. THURNHAM, *Northern Ireland Centre for Diet and Health, University of Ulster, Coleraine BT52 1SA*

Supermarket music in this century has gained increased importance in marketing research, as its effects can influence mood states and behaviour within the retailing environment. One study in England reported that music could influence customers in their selection of wines (North *et al.* 1997). The study reported here investigated whether music had a similar influence on the selection of wine by supermarket customers in Northern Ireland.

The effect of French and Italian music, or no music, on sales of French and Italian wines was examined in a large supermarket for six consecutive days. Each treatment was randomly assigned to a day of the week, and carried out on two occasions. The music selected was French accordion music and Italian string music. One in three shoppers buying wines were asked to complete a questionnaire about their views of the in-store music and on their purchases of wine. A total of twenty-five customers per day took part in the study between 10.00 and 18.00 hours. Chi-squared tests within the Statistical Package for Social Sciences were used for the analysis of the data.

| | Wine sales – Mean value of treatment days (day 1, day 2) | | | |
|---|---|---|---|---|
| Music | Total sales (bottles/d) | French wine (%) | Italian wine (%) | Other wine (%) |
| No Music Days | 84 (41,43) | 36 (46, 26) | 13 (7, 19) | 51 (46, 56) |
| French Music Days | 75 (39, 36) | 52 (51,53) | 1 (0, 3) | 46 (49, 44) |
| Italian Music Days | 84 (34, 50) | 36 (44, 30) | 23 (15, 30) | 41 (41, 40) |

The Table shows the total number of bottles sold on the three pairs of treatment days were similar, but the distribution of French, Italian and other wines differed significantly ($P < 0.001$). The Table shows that when French music was played sales increased from 36 to 52% ($P < 0.02$), Italian sales dropped from 13 to 1% and sales of other wines did not change. When Italian music was played sales also increased from 13 to 23% ($P < 0.05$), but sales of French or other wines did not change substantially. From the questionnaire, it was ascertained that only 6% of customers believed that music could influence their purchase of wine and their opinions were similar for fruit, vegetables, meat and cheese. The majority of customers, 83% associated the music with holidays, 11% with a film and 6% with nothing. The data support the observations of North *et al.* (1997) that peoples' purchasing habits are influenced subliminally by in-store music.

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North AC, Hargreaves DJ & McKendrick J (1997) *Nature* **390**, 132.

**Investigating consumer perceptions of the sensory characteristics of ten diverse cheese varieties**

By J. B. P. LAWLOR, C. M. DELAHUNTY and P. A. MORRISSEY, *Department of Nutrition, University College, Cork, Ireland*

People eat regularly the foods that "taste" good to them, although other less-liked foods may have a higher nutritional value. Therefore, the perceived sensory character of a food is an important factor influencing its consumption and consequently the nutritional status of the consumer. Consumer preference mapping is a technique that can be used to optimize the sensory appeal of a food to help increase its consumption.

In the present study the appearance, odour, flavour and texture of ten cheese varieties (Queso Mahon, Cambozola, Gruyère, Wensleydale, Blue Shropshire, Tetilla, Ambassadeur, Fontina, Appenzeller and Chaumes) were objectively evaluated by a trained sensory panel of fifteen assessors using a vocabulary of forty-seven descriptive terms. The cheeses were also evaluated hedonically by 162 "naive" consumers and hierarchical cluster analysis used to group the consumers into seven clusters containing those with similar preferences. These data were mapped onto the descriptive terms using partial least squares regression in order to illustrate how the fundamental sensory characteristics of the cheeses influenced the consumers' preferences. For instance, Gruyère had a "balanced" and "sweet" flavour and "fruity" and "sweet" odour, was most liked by three clusters of consumers and most liked overall (Table). On the other hand, Blue Shropshire scored lowest for "balanced" flavour,

| Cluster | *n* | Queso Mahon | Cambozola | Gruyère | Wensleydale | Blue Shropshire | Tetilla | Ambassadeur | Fontina | Appenzeller | Chaumes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 54 | 6.15 | 6.26 | 5.83 | 5.30 | **6.93** | 4.48 | 4.28 | 3.96 | 5.39 | 4.31 |
| 2 | 32 | 5.94 | 7.44 | 6.78 | 3.50 | **7.47** | 6.19 | 6.03 | 5.94 | 6.56 | 7.09 |
| 3 | 13 | 7.00 | 3.46 | **7.54** | 6.38 | 1.46 | 4.69 | 5.62 | 2.38 | 3.31 | 5.46 |
| 4 | 26 | 4.42 | 5.23 | 5.73 | 4.08 | 1.85 | **7.04** | 5.85 | 5.54 | 5.42 | 6.19 |
| 5 | 21 | 7.67 | 5.67 | **7.71** | 7.00 | 7.62 | 5.86 | 5.62 | 6.00 | **7.71** | 5.33 |
| 6 | 10 | 7.00 | 2.60 | **8.20** | 5.00 | 1.80 | 6.70 | 6.70 | 7.10 | 7.60 | 5.50 |
| 7 | 6 | 4.67 | 3.33 | 6.50 | 2.83 | 1.00 | **7.50** | 2.17 | 1.00 | 2.00 | 3.00 |
| Mean | 162 | *6.12* | *4.85* | ***6.89*** | *4.87* | *4.01* | *6.06* | *5.18* | *4.56* | *5.42* | *5.26* |

Values are mean preference scores of consumers within each cluster; *n*, number of consumers in cluster.
Mean preference scores for each cheese for all clusters are in italics.
The most preferred cheese in each cluster is in bold.
The least liked cheese in each cluster is underlined.

had a "mouldy" appearance, odour and flavour and was least liked overall, but most-liked by two clusters of consumers.

The present study demonstrated the application of sensory evaluation in identifying the key sensory characteristics of cheese that influenced its acceptability. These techniques may be readily applied to any food as a means of increasing its consumption and consequently the nutritional status of the consumer.

**The effect of α- and β-carotene on the survival of human HepG2 and HEL cells challenged with hydrogen peroxide and *tert*-butyl hydroperoxide.** By J.A. WOODS and N.M. O'BRIEN, *Department of Nutrition, University College, Cork, Republic of Ireland*

Many epidemiological studies have demonstrated an inverse association between intake of carotene-rich fruit and vegetables and the incidence of certain cancers. The aim of the present study was to investigate the effects of a 24 h pre-treatment with two pro-vitamin A carotenoids, β-carotene and α-carotene, on the survival of human cells subsequently challenged with either $H_2O_2$ or *tert*-butyl hydroperoxide (tBHP).

Before use, the carotenoids were purified on an alumina column, and the concentration of the compounds determined spectrophotometrically using the published extinction coefficients. The purified carotenoids were divided into portions, dried under nitrogen and stored at -80°. Immediately before use, the dried portions were re-suspended in dimethyl sulfoxide and added to the culture media. Human hepatoma (HepG2) and embryonic lung (HEL) cells were seeded into ninety-six well plates and following adherence, were treated with 1 μM carotenoid for 24 h in reduced serum (25 ml/l v:v fetal calf serum) media. Following removal of the media, the cells were challenged with peroxides (10-500 μM) in serum-free medium for 30 min at 37°/ $CO_2$-air (5:95, v:v). After removal of the culture medium containing the toxin, the cells were allowed to recover in complete media for approximately two cell cycles. Cell survival was measured by the 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay.

| | HepG2 $IC_{50}$ value (μmol toxin/l) | | HEL $IC_{50}$ value (μmol toxin/l) | |
|---|---|---|---|---|
| Treatment | Mean | SEM | Mean | SEM |
| $H_2O_2$ | 300.8 | 45.8 | 283.2 | 79.2 |
| 1 μM α-Carotene + $H_2O_2$ | 305.6 | 15.8 | 215.1 | 16.8 |
| 1 μM β-Carotene + $H_2O_2$ | > 500 | 0.0 | 203.9 | 66.4 |
| tBHP | 126.1 | 24.5 | 307.3 | 67.1 |
| 1 μM α-Carotene + tBHP | 152.6 | 50.0 | 263.8 | 34.9 |
| 1 μM β-Carotene + tBHP | 236.4* | 41.0 | 200.4 | 82.2 |

Mean values were significantly different from control, *P<0.05 (n=3, ANOVA, Dunnett's test)

The $IC_{50}$ value is defined as the concentration of toxin required to decrease cell viability to half its original value. After a recovery period of two cell cycles, HEL cells were found to be significantly more susceptible to the cytotoxic effects of $H_2O_2$ than HepG2 cells. In contrast, the HEL cells were more resistant to a challenge with tBHP than the liver cells. However, whereas both peroxides induced significantly different levels of death in the HepG2 cells, both $H_2O_2$ and tBHP proved to be equally as toxic to the HEL cells. These data reflect not only the distinct modes of action of the two peroxides, but may also imply cell-type differences in the levels of membrane antioxidants.

A 24 h pre-treatment with α-carotene resulted in no significant protection against $H_2O_2$-induced cell death in either cell line. Although a protective trend was observed for β-carotene in the liver cells, this was not significant. In contrast, β-carotene did significantly increase the survival of liver cells challenged with tBHP (P<0.05), with α-carotene showing a small, but non-significant protective effect. These results are consistent with an antioxidant effect of the carotenoids (Palozza *et. al.*, 1996). However no protection by β-carotene against tBHP-induced cytotoxicity was observed in the lung cells.

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Palozza P, Luberto C, Ricci P, Sgarlata E, Calviello G, & Bartoli GM (1996) Archives of Biochemistry and Biophysics **325**, 145-151

**Comparison of Three Different Drying Temperatures for the Preservation of Single Cell Gel Electrophoresis (Comet) Assay Slides.** By K.A. O'Leary, R.P. McCarthy, J.A. Woods, N.M. O'Brien., Department of Nutrition, University College, Cork, Republic of Ireland.

The single cell gel electrophoresis assay (comet assay) is an inexpensive, rapid and highly sensitive method for the determination of DNA single and double strand breaks, crosslinks, and alkaline-labile lesions in individual cells. Any cell from any tissue or organ can be used in the assay, provided a single cell suspension can be obtained. Moreover the technique can be used to assess genotoxic damage in both cycling and non-cycling cells. The assay involves embedding cells in an agarose gel cast on a microscope slide. Following a lysis step, the nuclei are subjected to electrophoresis. In undamaged nuclei, the DNA is too large to migrate in the electric field, however any fragmented DNA in damaged nuclei streams away from the main body of the nucleus, resulting in a "comet"-like appearance and hence the popular name of the assay.

A limitation of the procedure is that the microelectrphoretic gels must be graded rapidly as the comet configuration deteriorates on storage due to dehydration of the agarose and diffusion of DNA (McKelvey-Martin *et al.*, 1993). The objective of this study was to evaluate drying regimes as rapid and simple methods of preservation of the microgels as close to their original fresh state as possible. Preservation of microgels would facilitate their retention as records of an experiment, interlaboratory exchange of gels and the ability to evaluate the microgels over a period of time.

Human hepatoma (HepG2) cells challenged for 30 min with either $H_2O_2$ or *tert*-butyl hydroperoxide (tBHP, data not shown) were used in the study. Microgel slides were prepared and evaluated fresh or after drying at ambient temperature, 37° or at 50°.

For the analysis, 50 ethidium bromide-stained cells were randomly selected from each slide, and given a grade from 0 to 4, depending on the degree of DNA strand breakage observed. A score for each slide was calculated by multiplying the number of comets from each grade by the grade number. Thus a slide of negative control cells would give a score of 50 x 0 (0), whereas a slide of maximally damaged nuclei would have a score of 50 x 4 (200). Fresh slides were analysed within 24 hours following completion of the assay, before being subjected to one of the three drying regimes. Data are expressed as the mean and SEM for n≥3 independent experiments.

| $H_2O_2$ (μM) | Fresh | SEM | Air Dried | SEM | Fresh | SEM | 37° | SEM | Fresh | SEM | 50° | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 48.3 | 11.1 | 51.2 | 10.9 | 37.6 | 11.4 | 47.0 | 11.1 | 57.2 | 11.8 | 61.8 | 12.4 |
| 10 | 94.3 | 9.2 | 98.2 | 8.1 | 53.5 | 10.1 | 70.0 | 16.2 | 92.0 | 7.4 | 93.8 | 17.6 |
| 25 | 149.1 | 10.2 | 155.4 | 6.9 | 137.0 | 18.7 | 140.0 | 17.6 | 122.5 | 19.2 | 131.5 | 18.6 |

Rapid drying at 50° was most effective at preserving the original structure of the gels. Initially, it was hoped to score the dried slides directly. However it proved necessary to re-hydrate the slides with low melting point agarose at 37° prior to scoring. Following re-hydration and scoring, the slides were re-dried and stored over a 2 month experimental period. It was possible to re-hydrate, re-score and re-dry the microgels very successfully during this period. The only problem encountered was fading of the fluorescent dye which was easily rectified by adding more dye with the re-hydrating agarose.

In conclusion, drying of microgels represents a simple, inexpensive and feasible method of preserving analytical data and enhancing the utility of the comet assay.

McKelvey-Martin VJ, Green MHL, Schmezer P, Pool-Zobel BL, De Meo MP, Collins A (1993) *Mutation Research* **288**, 47-63.

**Comparison of gut integrity of hospitalised Indian infants admitted with gastro-intestinal and respiratory infections.** By F.S.W. McCULLOUGH[1], C.A. NORTHROP-CLEWES[1], B.S. DAS[2] and D.I. THURNHAM[1], [1]*NICHE, University of Ulster, Coleraine, BT52 1SA,* [2]*Ispat General Hospital, Rourkela 769005, Orissa, India*

The WHO estimates that approximately 700 million episodes of diarrhoea occur annually amongst children under 5 years old in developing countries, result in 4.6 million deaths. Acute diarrhoeal disease accounts (ADD) for 30% hospital admissions in developing countries and acute respiratory infection (ARI) is also common (Brussow *et al.* 1990).

Baseline gut integrity results are reported from ninety infants aged 4-18 months admittted with ADD and ARI from Ispat General Hospital. Gut integrity was measured using the dual sugar intestinal permeability test. The test is a non-invasive measure of intestinal integrity, dependent on the collection of lactulose (L) and mannitol (M). Lactulose (400 mg) and M (100 mg) are administered in 2 ml water per kg body weight and urine collected for the following 5 h. L and M and expressed as a ratio to creatinine (C) to allow for incomplete urine collections. C was measured colorimetrically.

| | ADD (n = 62) | | ARI (n = 28) | |
|---|---|---|---|---|
| Urinary Marker | Median | Range | Median | Range |
| Lactose (mg) | 21.72 | 4.32-49.33 | 21.13 | 6.03-46.30 |
| Lactose:lactulose | 1.13 | 0.62-5.34 | 1.44 | 0.57-3.51 |
| Lactulose :creatinine | 1.32 | 0.86-5.99 | 0.94 | 0.34-4.56 |
| Mannitol:creatinine | 0.77 | 0.57-4.42 | 1.03 | 0.54-5.82 |
| Lactulose:mannitol | 1.71 | 0.53-23.58 | 0.91 | 0.13-30.99 |

L:M and lactose:L ratios less than 0.12 and 0.4 respectively is considered normal (Lunn *et al.* 1991). The Table shows grossly abnormal L:M values for ADD and ARI patients. These results compare with a study carried out in Tanzania, where the mean L:M value was 0.86 for infants hospitalized with ADD (Willumsen *et al.* 1991). Results have not previously been reported for infants with ARI, however hospitalized adults with pneumonia showed no change in their permeability values (Elia *et al.* 1991). The median L:C ratio of infants with ADD and ARI was much higher than that found in healthy UK infants (0.27), while the M:C ratio was much lower (2.4). The Indian results suggest that mucosal integrity was compromised with an increased loss of L, also a reduction in absorptive surface area and hypolactasia are indicated by reduced M:C and elevated lactose:L ratios.

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