Proceedings of the Nutrition Society

Abstracts of Original Communications

A joint Scientific Meeting of the Nutrition Society and the Association Française de Nutrition and Société de Nutrition et de Diététique de Langue Française was held at the Institute of Child Health, London, UK on 13–14 February 2002, when the following papers were presented.

All abstracts are prepared as camera-ready material.

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

Degree of oxidation of low-density lipoprotein affects gene expression in human monocyte-derived macrophages. By I.C. KAVANAGH[1], A. CUMMING[2], C.E. SYMES[1], D.S. LEAKE[2] and P. YAQOOB[1], [1]*School of Food Biosciences and [2]School of Animal and Microbial Sciences, The University of Reading, Whiteknights, Reading RG6 6AP*

Components of oxidized low-density lipoprotein (oxLDL) have been reported to activate PPARγ in monocytes, which is proposed to induce the expression of the scavenger receptor CD36 through an autoregulatory loop, promoting foam cell formation (Nagy et al. 1998). However, oxLDL is not a single, well-defined entity, but has structural and physical properties that vary depending on the type and degree of oxidation. In experimental situations, LDL can be oxidized to defined degrees, where mildly-oxLDL contains 10–20% of the peak levels of conjugated dienes, moderately-oxLDL contains the peak levels of conjugated dienes and lipid hydroperoxides, and extensive copper oxidation results in decomposition of lipid hydroperoxides and the formation of large quantities of oxysterols. The aim of this study was to investigate the influence of the degree of oxidation of LDL on the expression of PPARγ and the scavenger receptor, CD36, by human monocytes.

LDL from twenty healthy human male volunteers was oxidized using 5 μM cupric sulphate to a mild, moderate or high degree (assessed by levels of conjugated dienes at 234 nm). Monocytes from the same volunteers were cultured with native LDL or oxidized LDL (50 μg protein/ml) for 3 d in medium containing lipoprotein-depleted human serum. Expression of PPARγ and CD36 was assessed by RT-PCR. Since data were not normally distributed, statistically significant differences were assessed using the Wilcoxon rank tests.

Table. Lipid hydroperoxide levels in native and oxidized LDL

| | Lipid hydroperoxides (nmol/mg LDL protein) | | | | | | | |
| | Native | | Mildly oxLDL | | Moderately oxLDL | | Highly oxLDL | |
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
	68.2	11.5	71.7	12.3	526.5	25.7	101.4	13.2

Figure. Effect of mildly, moderately and highly oxLDL on expression of CD36 and PPARγ mRNA by human monocyte-derived macrophages. Results are expressed as arbitrary units relative to native LDL.
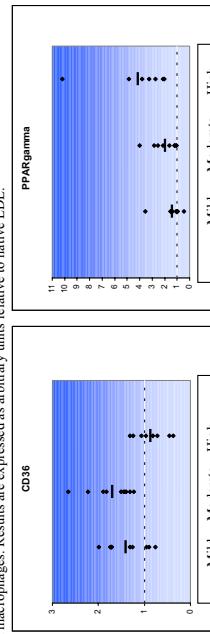
As expected, moderately and highly oxLDL contained peak levels of lipid hydroperoxides, which had begun to decompose in highly oxidized LDL (see Table). Mildly- and moderately oxLDL significantly induced expression of CD36 relative to native LDL ($P = 0.028$ for mild v. native and 0.005 for moderate v. native), but highly oxLDL did not induce expression of CD36 (see Figure. In contrast, moderately and highly oxLDL induced expression of PPARγ relative to native LDL ($P = 0.005$ for moderate v. native and 0.012 for high v. native). These results illustrate differential expression of CD36 and PPARγ by LDL oxidized to different degrees and suggest that the proposed autoregulatory loop for CD36 may be oversimplified.

Nagy L, Tontonoz P, Alvarez JGA, Chen H & Evans RM (1998) *Cell* **93**, 229–240.

The influence of the apolipoprotein B XbaI polymorphism on the association between habitual fat intake and total and LDL cholesterol levels. By L.F. MASSON[1], A. CUMMING[2], C. TUYA[3] and G. MCNEILL[3]. [1]*Dept of Public Health, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, [2]Dept of Molecular and Cell Biology, University of Aberdeen, Zoology Building, Tillydrone Avenue, Aberdeen AB24 2TZ and [3]Dept of Medicine and Therapeutics, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD*

Apolipoprotein (apo) B is the major protein of low-density lipoprotein (LDL) cholesterol, and the presence (X+) of the XbaI cutting site at this gene locus has been associated with significantly higher plasma triacylglycerol, total and LDL cholesterol concentrations (Turner et al. 1995). There is some evidence to suggest that this polymorphism may also influence the plasma lipid response to changes in dietary fat and cholesterol intake. This study aimed to investigate whether the relationship between plasma lipid concentrations and habitual fat intake is influenced by the apoB XbaI polymorphism.

The subjects studied were 169 healthy men and women (72 M, 97 F) aged 18–50 years, who were recruited for a study of coronary heart disease risk factors in twins. Habitual diet was assessed by the Scottish Collaborative Group Food Frequency Questionnaire. A comparison of the questionnaire with 4-d weighed records found Spearman correlation coefficients for energy-adjusted total fat, saturated fat and cholesterol intake ranging from 0.42 to 0.59 in men and from 0.39 to 0.71 in women (Masson et al. 2001). Smoking status and physical activity level were also assessed by questionnaire, and a fasting blood sample was taken for analysis of lipids and DNA extraction. XbaI genotype was determined by polymerase chain reaction followed by XbaI digestion, and digested products were visualized under UV light following ethidium bromide staining of an agarose gel.

Following adjustment for age, sex and body mass index (BMI), total and LDL cholesterol levels were significantly higher ($P < 0.05$) in X+X+ individuals than in X−X+ individuals. High-density lipoprotein (HDL) cholesterol levels were higher in X−X− individuals than in X−X+ or X+X+ genotype groups ($P < 0.05$), but triacylglycerol levels were not significantly different between groups. Total fat, saturated fat and cholesterol intake did not differ significantly between genotype groups. Regression coefficients for the relationship between each dietary variable and total, LDL, HDL or triacylglycerol levels were estimated for each genotype group by multiple regression with adjustment for age, sex, BMI, smoking status, physical activity level and energy intake. There was no significant association between total fat or cholesterol intake and lipid levels in any of the genotype groups, but there was a significant positive association between saturated fat intake and total cholesterol and LDL cholesterol in individuals with the X−X− genotype (see Table).

| | Total cholesterol | | | log$_e$ LDL cholesterol | | |
	X−X− (n 31)	X−X+ (n 86)	X+X+ (n 52)	X−X− (n 31)	X−X+ (n 86)	X+X+ (n 52)
B	0.063	−0.002	−0.002	0.017	−0.001	0.004
[95% CI]	[0.014, 0.112]	[−0.025, 0.022]	[−0.045, 0.040]	[0.002, 0.032]	[−0.010, 0.008]	[−0.007, 0.014]
P	0.013	NS	NS	0.026	NS	NS

The data support the possibility that in this population, individuals with the X−X− genotype are more sensitive to the influence of dietary saturated fat on total and LDL cholesterol levels than those with the X−X+ or X+X+ genotypes.

Masson LF, McNeill G, Tomany JO, Peace HS, Bolton-Smith C & Grubb D (2001) *Proceedings of the Nutrition Society* **60**, 137A.

Turner PR, Talmud PJ, Viswkis S, Ehnholm C & Tiret L (1995) *Atherosclerosis* **116**, 221–234.

Dietary fish oil increases the expression of murine peroxisome proliferator activated receptor genes. By C.E. DONNELLAN[1], M. TADAYYON[2] C. BRISCOE[2], J. ARCH[2] and P.C. CALDER[1], [1]*Institute of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton, SO16 7PX and* [2]*Department of Vascular Biology, SmithKline Beecham Pharmaceuticals, Coldharbour Road, Harlow, Essex CM19 5AD*

Dietary fish oil (FO), which is rich in long-chain *n-3* polyunsaturated fatty acids, partly prevents the elevation in blood lipid concentrations and the development of obesity normally associated with high-fat feeding in rodents (Yaqoob *et al.* 1995; Donnellan *et al.* 1999). Peroxisome proliferator activated receptors (PPAR) appear to have an important role in regulating whole body metabolic responses, particularly in response to dietary fat (Schoonjans *et al.* 1996). Therefore, in this study we investigated whether the effects of dietary FO might be mediated by increased expression of hepatic PPARα and adipose tissue PPARγ in mice.

Male C57BL/6 mice (*n* 6 per diet) were fed *ad libitum* for 6 weeks on a low-fat (LF) diet (25 g corn oil/kg diet) or on one of two high-fat (210 g fat/kg diet) diets which used safflower oil (SO; rich in *n-6* polyunsaturated fatty acids) or FO as the principal fat source. All diets contained identical levels of starch, sucrose, protein, vitamins and minerals. After sacrifice in the fed state, livers and epididymal fat pads were removed. mRNA was extracted from the tissues and Northern blot analysis used to determine the expression of PPARα and acyl CoA oxidase (AOx) in liver, and PPARγ in white adipose tissue. In all cases the amount of mRNA is expressed as density relative to that of β-actin mRNA.
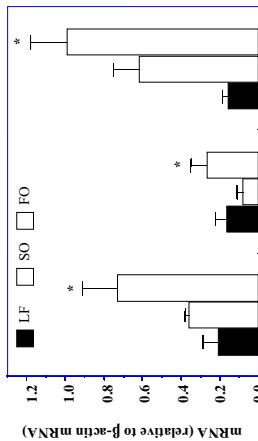
* indicates significantly different from LF and SO (one-way ANOVA; *P*<0.05)

FO-fed mice expressed more PPARα mRNA in the liver and more PPARγ mRNA in white adipose tissue than mice fed on either of the other diets. Furthermore, the livers of FO-fed mice contained a greater amount of mRNA for the PPAR-responsive gene AOx. The activity of hepatic AOx was also higher in these animals (data not shown), indicating that the higher level of PPARα mRNA has a functional consequence. These results suggest that the type of fatty acid in a high-fat diet affects the expression of genes involved in the whole body metabolic response to dietary fat. The effects of FO suggest possible mechanisms by which this diet results in decreased blood lipid concentrations, adipose deposition and weight gain.

Donnellan CE, Wallace FA & Calder PC (1999) *Proceedings of the Nutrition Society* **58**, 25A.
Schoonjans K, Staels B & Auwerx J (1996) *Biochimica et Biophysica Acta* **1302**, 93–109.
Yaqoob P, Sherrington EJ, Jeffery NM, Sanderson P, Harvey DJ, Newsholme EA & Calder PC (1995) *International Journal of Biochemistry and Cell Biology* **27**, 297–310.

The effects of conjugated linoleic acid on the expression of genes involved in cholesterol homeostasis in THP-1 macrophages. By S.M. WELDON, M.J. GIBNEY and H.M. ROCHE, *Unit of Nutrition, Department of Clinical Medicine, Trinity Centre for Health Sciences, St. James's Hospital, James Street, Dublin 8, Ireland*

Arteriosclerosis is hallmarked by cholesterol accumulation in macrophages, which results in their transformation into foam cells and inflammatory activation of cells of the vascular wall. Recent studies suggest that peroxisome proliferator activated receptors (PPARs) regulate cholesterol homeostasis in macrophages, by modulating the expression of key proteins including CD36 (Moore *et al.* 2001), LXRα, and ABCA1 (Chinetti *et al.* 2001)

Fatty acids, including conjugated linoleic acid (CLA), are natural PPAR ligands. Therefore this study investigated the effects of a range of fatty acids and pharmacological PPAR ligands on genes involved in cholesterol homeostasis in the macrophage. The human monocytic leukaemia cell line THP-1 was cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum. For experiments, cells were treated with 100 ng/ml phorbol 12-myristate 13-acetate (PMA) to differentiate monocytes into macrophages. The adherent macrophages were exposed to 50 μM linoleic acid (LA), *cis-9, trans-*11 CLA (*c9,t*11 CLA), *trans-*10, *cis-*12 CLA (*t*10,*c*12 CLA), stearic acid (SA), 1 μM Rosiglitazone (Rosig), 50 μM Wy14643 (Wy) or DMSO only for 48 h. Control cells were left untreated. mRNA levels of ABCA1, CD36, LXRα, PPARα, and PPARγ were quantified using TaqMan real time PCR analysis. The mRNA level of each gene is expressed normalized to GAPDH and is relative to control THP-1 macrophage. Statistical analysis was completed using one-way ANOVA.

Gene	ABCA1 mRNA ABCA1: GAPDH	SE	CD36 mRNA CD36: GAPDH	SE	LXRα mRNA LXRα: GAPDH	SE	PPARα mRNA PPARα: GAPDH	SE	PPARγ mRNA PPARγ: GAPDH	SE
Ctrl	1	0	1	0	1	0	1	0	1	0
DMSO	1.09	0.07	0.92	0.06	1.36	0.06	1.49	0.08	1.19	0.06
LA	1.22	0.08	1.07	0.06	0.98	0.05	1.17	0.1	1.15	0.05
*c9,t*11	1.03	0.07	1.39	0.11	1.39	0.06	1.08	0.09	0.82	0.08
*t*10,*c*12	1.23	0.09	1.23	0.07	1.42	0.13	1.09	0.1	1.05	0.03
SA	1.36	0.07	1.11	0.08	1.64[a]	0.09	1.14	0.13	0.87	0.03
Wy	2.29[a]	0.19	2.68[a]	0.32	2.13[a]	0.16	1.33	0.13	1.13	0.07
Rosig	1.91[a]	0.17	3.7[a]	0.43	1.99[a]	0.08	1.23	0.08	0.98	0.04

[a] indicates significantly different from control (*P*<0.05). Values represent group means of six independent experiments.

This concentration of fatty acids had negligible effects on CD36, LXRα and ABCA1 mRNA expression, with the exception of stearic acid, which was associated with a significant induction of LXRα mRNA. In comparison, the pharmacological PPAR ligands (Wy14643 and Rosiglitazone) caused a significant induction of ABCA1, CD36, and LXRα. No treatment had a significant effect on PPARα and PPARγ expression.

Chinetti G, Lestavel S, Bocher V, Remaley AT, Neve B, Pineda Torra I, Teissier E, Minnich A, Jaye M, Duverger N, Brewer HB, Fruchart J-C, Clavey V & Staels B (2001) *Nature Medicine* **7**, 53–58.
Moore KJ, Rosen ED, Fitzgerald ML, Randow F, Andersson LP, Altshuler D, Milstone DS, Mortensen RM, Spiegelman BM & Freeman MW (2001) *Nature Medicine* **7**, 41–47.

High carbohydrate diet and non-digestible carbohydrates have beneficial effects on endogenous lipid synthesis and plasma lipid levels in humans but do not modify the expression of lipogenic genes in adipose tissue. By M. BEYLOT[1], D. LETEXIER[1], C. VIDON[1], F. DIRAISON[1] and J. VAN LOO[2], [1]INSERM U499, Faculté Laennec, Rue G Paradin, 69008, Lyon, France and [2]Tiense Suikerraffinarij, Tienen, Belgium

Reduction of the contribution of dietary fat to total energy intake is recommended in order to prevent the development of obesity and atherosclerosis. However high-carbohydrate (CHO) diets could promote the development of liver and adipose tissue lipogenesis and induce raised triacylglycerol (TAG) levels; unfavourable side-effects with respect to obesity and atherosclerosis. Therefore we measured plasma cholesterol and TAG levels, cholesterol synthesis and hepatic lipogenesis (deuterated water method) and adipose tissue mRNA levels (RT–competitive PCR) of fatty acid synthase (FAS) and acetyl-CoA carboxylase 1 (ACC1) in five normal subjects studied twice after 3 weeks of a controlled high-fat (HF, fat 45%, CHO 40%, protein 15% of energy intake) or high-CHO (fat 30 %, CHO 55%, proteins, 15%) diets. Cholesterol, fructose and fibre intakes were identical as well as the proportion of saturated, monosaturated and polyunsaturated fatty acids (one-third of total fatty acid intake for each). The study was randomized, with at least 3 months between the two periods of controlled diets. In a second study, we studied eight subjects consuming the high-CHO diet associated with either placebo or inulin (10 g) in a double-blinded, cross-over study. Results are shown as mean and SEM. Comparisons were performed using Student's t-test for paired values. Compared with the high-fat diet, the high-CHO diet lowered plasma cholesterol level (from 5.33 (SE 0.23) to 4.76 (SE 0.24) mM, $P<0.05$) despite a moderate stimulation of cholesterol FSR (from 4.3 (SE 0.3) to 5.3 (SE 0.2)%, $P<0.05$). Hepatic lipogenesis was increased from 6.9 (SE 1.5) to 9.7 (SE 1.6)%, $P<0.05$) without any modification of plasma glucose or insulin levels. Plasma TAG levels were unchanged (0.67 (SE 0.10) v. 0.68 (SE 0.21) mM). Adipose tissue mRNA levels were not modified (FAS: 178 (SE 74) v. 209 (SE 43) atomol/μg total RNA; ACC1: 34.0 (SE 6.5) v. 28.8 (SE 6.6) atomol/μg total RNA). During the second study, the addition of inulin to the high-CHO diet decreased plasma TAG levels (from 0.92 (SE 0.10) to 0.77 (SE 0.08) mM, $P<0.05$) and hepatic lipogenesis (from 9.1 (SE 1.7) to 6.6 (SE 1.2)%, $P<0.05$) but had no effects on either plasma cholesterol level or cholesterol synthesis. Adipose tissue mRNA levels of FAS and ACC1 were again unchanged.

In conclusion, a moderately high CHO diet, compared with a high fat-diet, has beneficial effects on cholesterol metabolism without adverse effects on plasma TAG levels. The addition of inulin has no further beneficial effect on cholesterol but prevents the moderate rise in hepatic lipogenesis and decreases plasma TAG levels, thus reinforcing the beneficial effect of the high-CHO diet. Contrary to what was observed on hepatic lipogenesis, adipose tissue lipogenesis, as reflected by the measurement of FAS and ACC1 mRNA levels, was unaffected.

The effects of fatty acids on PHA-induced cytokine gene expression and production in human peripheral blood mononuclear cells. By A.P. NUGENT, M.J. GIBNEY and H.M. ROCHE, *Unit of Nutrition, Department of Clinical Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8, Ireland*

Cytokines are key regulators of inflammation and immunity, playing an important role in host defence and the elimination of infectious agents (Aggarawal & Puri, 1995). Dietary lipids modulate cytokine production, in particular long-chain unsaturated fatty acids. In this study we investigated the effects of various fatty acids on mitogen-stimulated cytokine production in human peripheral blood mononuclear cells (PBMCs) *in vitro*.

Blood was drawn from a pool of healthy volunteers. PBMCs were isolated and cultured at 5×10^6 cells/5 ml in the presence of 10% heat-inactivated fetal calf serum, 10 μg/ml of the mitogen PHA and fatty acid/ligand dissolved in DMSO. Control cells contained PHA. Final concentrations of DMSO were 0.001% in all conditions and did not have a significant effect on any of the experiments. Ligands used included the CLA isomers *cis-9, trans-11* CLA (*c9,t11*), *trans-10, cis-12* CLA (*t10,c12*), linoleic acid (LA), oleic acid (OA), stearic acid (SA) and palmitic acid (PA). Fatty acids were added at 100 μM. Two identical culture systems were created whereby cells were cultured in the presence of ligand for 48 h, PHA then added and RNA extracted in one set of cells 4 h later and supernatants collected for ELISA in the other cell system 24 h later. mRNA was quantified using RT-PCR and results are expressed relative to GAPDH and normalized to control and PHA cells. Cytokine levels were measured using commercial ELISA kits (R & D Systems, UK). Study results are presented in the Table, values represent means ±SE for six independent experiments.

| | IL-4 | | IL-2 | | TNFα | |
| | mRNA IL-4:GAPDH | ELISA pg/ml | mRNA IL-2:GAPDH | ELISA pg/ml | mRNA TNFα:GAPDH | ELISA pg/ml |
	Mean SE	Mean SE	Mean SE	Mean SE	Mean SE	Mean SE
Cells, PHA	1.00[a] 0	109[a] 29	1.00[a] 0	2417[a] 436	1.00[a] 0	2519[ab] 278
c9t11 CLA	0.41[b] 0.09	108[a] 28	0.50[a] 0.14	1619[a] 431	1.43[a] 0.21	2354[ab] 353
t10c12 CLA	0.63[a] 0.09	105[a] 25	0.64[a] 0.14	1607[b] 334	1.10[a] 0.13	2443[ab] 532
LA	1.04[a] 0.4	99[a] 29	0.58[a] 0.13	1662[b] 458	1.01[a] 0.23	2623[ab] 545
OA	0.43[a] 0.14	88[a] 26	0.52[a] 0.16	1247[b] 395	0.94[a] 0.06	1969[ac] 412
SA	0.38[b] 0.07	90[a] 32	0.51[b] 0.13	1699[a] 524	0.88[a] 0.19	2371[ab] 801
PA	1.06[a] 0.27	150[b] 41	1.29[c] 0.31	2307[a] 462	2.13[b] 0.34	3166[b] 549

[abc] Mean values not sharing a common superscript letter were significantly different: $P<0.05$ (one-way ANOVA).

Most fatty acids affected the production of IL-4 and IL-2 rather than TNFα. The effects of fatty acids were more apparent for mRNA expression. The two isomers of CLA reduced IL-4 and IL-2 expression, the *c9t11* CLA being the most potent. Stearic acid attenuated IL-4 and IL-2 mRNA expression while oleic acid decreased IL-4 mRNA expression but IL-2 cytokine production. The saturated fatty acid, palmitic acid, tended to increase IL-2, IL-4 and TNFα mRNA expression and cytokine production. These results confirm the immuno-immodulatory nature of lipids. Further experiments are needed to elucidate the exact mechanism of this lipid–gene interaction.

Aggarawal B & Puri R [editors] (1995) *Human Cytokines: Their Role in Disease and Therapy.* Boston, MA: Blackwell Science.

A study of CpG island methylation and crypt cytokinetics in human subjects with colorectal cancer. By N.J. BELSHAW[1], J.M. GEE[1], A. POLLEY[1], M. WATSON[2], M. RHODES[2] and I.T. JOHNSON[1], [1]Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA and [2]Norfolk and Norwich University Hospital, Norwich

Crypt cell hyperproliferation is an abnormality observed in the morphologically normal colorectal mucosa of individuals with colorectal cancer. A relatively high rate of crypt cell turnover appears to be an early biomarker of disease in populations at risk of colorectal neoplasia, and the phenomenon appears to be linked to environmental factors, including nutrition (Lipkin et al. 1985; Jubb et al. 2001). Such 'field abnormalities' probably reflect changes in the transcription of genes associated with control of the cell cycle, and may be caused by aberrant CpG island methylation, an epigenetic mechanism that can modulate gene expression in the absence of any alteration to the DNA sequence (Feinberg, 2001). It has been suggested that age-related silencing of the oestrogen receptor gene (ESR1) by CpG island methylation may cause progressive crypt cell hyperproliferation in the human colon (Issa et al. 1994). In the present study we used a novel PCR-based technique to measure CpG island methylation of the ESR1 gene in the flat mucosa of patients with colorectal cancer, and we searched for any correlation between the degree of methylation and the cytokinetics of epithelial cells in adjacent crypts.

One biopsy was obtained from the sigmoid colon of patients diagnosed with colorectal cancer, and a second sample was taken from the same location at surgery. DNA was extracted from the first biopsy, bisulphite-modified, and used as template in PCRs with primers specific for the CpG islands of ESR1 and hMLH1. The degree of methylation at the CpG located +164 bp from the start of translation of the ESR1 gene, or the CpG located −251 bp from the start of translation of the hMLH1 gene was determined by the proportion of PCR product restricted by TaqI or AflIII endonuclease, respectively. The second mucosal sample was fixed (75:25, ethanol:acetic acid) and stained in bulk with Feulgen's reagent. Mean numbers of mitotic and apoptotic figures per crypt were determined by morphological assessment of twenty microdissected crypts from each biopsy and a direct comparison of both variables was made in forty-seven subjects.

For the group as a whole, the hMLH1 gene was uniformly unmethylated, whereas some degree of methylation of the ESR1 gene was detectable in all subjects (mean 5.7%, n 49, SD 3.6, range 1.5–15.7). There was, however, no statistically significant correlation between the level of methylation and the frequency of either mitosis (r 0.127, P 0.394) or apoptosis (r 0.218; P 0.141) in adjacent crypts, nor was there any correlation with the age of the patient.

In this preliminary study, we confirmed the occurrence of varying degrees of CpG island methylation in the promoter region of the ER gene in apparently normal colonic mucosa of patients with colorectal cancer. However, the average level was somewhat lower than previously reported for healthy subjects in this age group, and we observed no statistically significant correlation between ESR1 methylation and the level of mitosis in crypts in the nearby mucosa. We cannot therefore confirm the hypothesis of Issa et al. (1994) that methylation-related silencing of ESR1 expression is a cause of the hyperproliferation of the normal mucosa that predisposes individuals to colorectal neoplasia. In future studies we will extend this approach to genes known to be directly involved in colorectal carcinogenesis, and we will explore the effects of nutrient exposure on age-related CpG island methylation in model systems, in vitro and in vivo.

Feinberg AP (2001) Proceedings of the National Academy of Sciences USA **98**, 392–394.
Issa JP, Ottaviano Y, Celano P, Hamilton SR, Davidson NE & Baylin SB (1994) Nature Genetics **7**, 536–540.
Jubb AM, Bell SM & Quirke P (2001) Journal of Pathology **195**, 111–134.
Lipkin M, Uehara K, Winawer S, Sanchez A, Bauer C, Phillips R, Lynch HT, Blattner WA & Freumeni JF (1985) Cancer Letters **26**, 139–44.

Effect of various diets on gene expression in the liver of mice. By N. DRAGIN[1], P. COSTET[2], V. LE MORVAN[1], L. DUBOURG[1], J.M. GEE[1], M.J. THOMAS[1], A. PALOS-PINTO[1], Y.M. DARMON[1] and E. PEUCHANT[1], [1]Laboratoire de Biochimie 4A, Université de Bordeaux 2, Bordeaux, France and [2]Animalerie Transgénèse, Université de Bordeaux 2, Bordeaux, France

There is some evidence that diet can modulate the ageing process. A caloric restriction diet is known to extend life span in animal models (Sohal et al. 1990) whereas a high-fat diet decreases longevity in mammals by inducing cardiovascular diseases (McGovern et al. 1996). Several mechanisms of action have been postulated, including modifications of DNA repair capacity (Youngman, 1993), gene expression (Lee et al. 1999), metabolic rate and oxidative stress status (Bourdel-Marchasson et al. 2001; Deschamps et al. 2001). To examine the molecular events associated with hypo- and hypercaloric diets on ageing, we used oligonucleotide arrays to define the transcriptional response to diets in mouse liver which play a central role in coordinating mammalian metabolism. For the study, twenty C57BL6/J mice, 8 weeks of age, were divided into three groups according to the type of diet fed for 6 months. Group 1 consisted of mice maintained on a standard diet (ST), group 2 included mice maintained on a cafeteria diet (CD) enriched in lipids and carbohydrates, and group 3 included mice maintained on diet restriction (DR), composed of 70% of standard diet. Hypo- and hypercaloric diets were supplemented with vitamins and microelements to match the percentage found in the standard diet. Each pool of four cDNA samples obtained from livers of the three groups were hybridized to identical membranes (Atlas mouse; Clontech) in order to study known mouse genes. Only genes which displayed a twofold up- or down-regulation or more were considered.
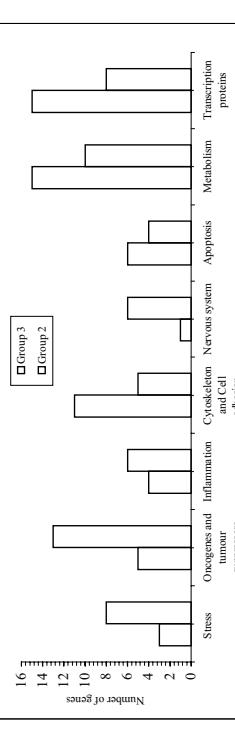
Among the 1176 genes detected in group 2 (CD) as compared to group 1 (ST), 24 were predominantly up-regulated while 36 were down-regulated. Genes that increased in expression are particularly implicated in proinflammatory response, in apoptosis, in metabolism and in oncogenesis, while the down-regulated genes are implicated in tumour suppressor, transcription pathways, oxidative stress and homeostasis systems.

In group 3 (DR), 31 genes were predominantly up-regulated and 30 were down-regulated. Genes that increased in expression were implicated in metabolism and cell homeostasis. The transcription, proinflammation, proapoptosis and stress systems were down-regulated.

When we compared results of group 2 with those of group 3, we observed that a high-fat diet seemed to preferentially up-regulate oncogenes, pro-apoptosis, lipid metabolism and cellular transduction genes and down-regulate genes implicated in antioxidant defence and inflammation.

These results suggest that hypo- and hypercaloric diets can act on the ageing process by modifying gene expression related to cellular cycle, metabolic, inflammation processes and antioxidant defence systems. However, the transcriptional pattern of high-fat diet supports a role of hypercaloric diet in oncogene expression in liver that may have special relevance in hepato-carcinogenesis.

Sohal RS, Svensson I & Brunk UT (1990) Mechanisms of Ageing and Development **53**, 209–215.
McGovern PG, Pankow JS, Shahar E, Doliszny KM, Folsom AR, Blackburn H & Luepker RV (1996) New England Journal of Medicine **334**, 884–890.
Youngman LD (1993) Mutation Research **295**, 165–179.
Lee CK, Klopp RG, Weindruch R & Prolla TA (1999) Science **285**, 1390–1393.
Bourdel-Marchasson I, Delmas-Beauvieux MC, Peuchant E, Richard-Harston S, Decamps A, Reignier B, Emeriau JP & Rainfray M (2001) Age and Ageing **30**, 235–241.
Deschamps V, Barberger-Gateau P, Peuchant E & Orgogozo JM (2001) Neuroepidemiology **20**, 7–15.

The isomer-specific effects of conjugated linoleic acid on gene expression and lipid metabolism in fasting male Ob/Ob mice. By E.J. NOONE[1], S. McBENNETT[2], M.J. GIBNEY[1] and H.M ROCHE[1], [1]*Unit of Nutrition, Department of Clinical Medicine, Trinity Centre for Health Sciences, St James's Hospital, Dublin 8, Ireland and* [2]*Department of Physiology, Trinity College Dublin, Dublin 2, Ireland*

Conjugated linoleic acid (CLA) is the term given to the positional and geometric isomers of linoleic acid. A number of investigations with animal models have revealed that CLA improves lipid metabolism (Nicolosi *et al.* 1997), insulin resistance, improves glucose tolerance (Houseknecht *et al.* 1998), and is anti-atherogenic and promotes the regression of established atherosclerosis (Kritchevsky *et al.* 2000). To date most studies have used heterogeneous blends of CLA isomers; however, there is little information regarding the isomer-specific effects of CLA on lipid metabolism and gene expression.

Sixteen male Ob/Ob mice were randomly allocated to receive a diet high in either the *cis*-9, *trans*-11 CLA (*c9,t11* CLA) isomer, the *trans*-10, *cis*-12 CLA (*t10,c12* CLA) isomer or linoleic acid (control group) for 5 weeks. Animals were sacrificed following a 12-h overnight fast. Animals fed the *c9,t11* CLA isomer had significantly (*P*≤0.002) lower plasma triacylglycerol (TAG) and insulin concentrations compared with those receiving the *t10,c12* isomer of CLA. A number of molecular markers were measured on the basis of these metabolic effects. The *c9,t11* CLA group had significantly (*P*=0.01) lower hepatic microsomal transfer protein (MTP) and (*P*=0.03) diacylglycerol acyltransferase (DGAT) gene expression, when compared with the linoleic acid group. The *t10,c12* CLA significantly reduced (*P*=0.02) lipoprotein lipase (LPL) gene expression in white adipose tissue (WAT) and (*P*=0.004) hepatic DGAT gene expression when compared with the linoleic acid group. There was no difference in hepatic peroxisome proliferator activated receptor α (PPARα) gene expression between study groups.

	Linoleic acid (n 6)		c9,t11 CLA (n 5)		t10,c12 CLA (n 5)	
	Mean	SD	Mean	SD	Mean	SD
Plasma TAG (mmol/l)	1.12	0.17	0.95[a]	0.08	1.25	0.12
Plasma insulin (ng/ml)	1.15	0.79	0.65[a]	0.48	1.82	0.94
Hepatic MTP (MTP/GAPDH)	2.77	0.45	2.13[b]	0.32	2.66	0.73
Hepatic DGAT (DGAT/GAPDH)	2.52	0.96	1.39[b]	0.38	1.00[b]	0.45
Hepatic SREBP 1c (SREBP1c/GAPDH)	0.59	0.56	0.84	0.56	0.76	0.28
Hepatic PPARα (PPARα/GAPDH)	0.47	0.11	0.57	0.22	0.41	0.26
WAT LPL (LPL/GAPDH)	2.04	0.60	1.56	0.23	1.15[b]	0.76

Values represent group means and standard deviations (SD). ANOVA shows significant differences between groups.
[a] Significantly different from *t10,c12* CLA, *P*≤0.002.
[b] Significantly different from linoleic acid group, *P*≤0.05.

This investigation highlights the divergent effects of the isomers of CLA on triacylglycerol and insulin metabolism and gene expression. The plasma TAG-lowering effect of *c9,t11* CLA appears to be mediated through the down-regulation of DGAT and MTP gene expression, both of which play an important role in TAG synthesis and very-low-density lipoprotein (VLDL) secretion. Despite significant decrease in DGAT gene expression, the *t10,c12* CLA isomer decreased WAT LPL gene expression, which may contribute to increased plasma TAG concentrations. The molecular mechanism whereby *c9,t11* CLA improves insulin sensitivity has yet to be elucidated; however, it may be linked to the improvement in TAG metabolism.

Houseknecht KL, Vanden Heuvel JP, Moya-Camarena SY, Portocarrero CP, Peck LW, Nickel KP & Belury MA (1998) *Biochemical and Biophysical Research Communications* **244**, 678–682.
Kritchevsky D, Tepper SA, Wright S, Tso P & Czarnecki SK (2000) *Journal of the American College of Nutrition* **19**, 472S–477S.
Nicolosi RJ, Rodgers EJ, Kritchevsky D, Scimeca JA & Huth PJ (1997) *Artery* **22**, 266–277.

---

c-Fos and HSP70 gene expression in different brain regions of male and female rats after chronic alcohol feeding. By T. NAKAHARA[1], M. HIRANO[2], H. UCHIMURA[2], S. SHIRALI[3], C.R. MARTIN[4], A.B. BONNER[5] and V.R. PREEDY[6], [1]*Faculty of Science, Kyushu University Roppommatsu, Fukuoka, Japan,* [2]*Hizen National Mental Hospital, Kanzaki, Saga, Japan,* [3]*King's College School of Medicine and Dentistry, London,* [4]*Alcuin College, University of York, York,* [5]*University of Kent, Canterbury and* [6]*Department of Nutrition and Dietetics; King's College London, London*

Excessive ingestion of the macronutrient, alcohol, causes devastating complications in the brain, leading to atrophy and impaired cognitive function, with corresponding increases in morbidity and mortality and, as a consequence, reduced quality of life. The pathogenic mechanisms are unknown but various studies have shown that the immediate early genes and heat shock (i.e. stress or chaperone) proteins are increased in alcohol-exposed tissue. However, many of these studies have been carried out *in vitro* or have failed to consider either the nutritional elements in the experimental design by the appropriate use of pair-feeding, or whether there are regional and/or gender differences. We hypothesized that (1) increased expression of heat shock proteins and/or oncogenes occurs as a consequence of alcohol feeding *in vivo*, and sensitivities are related to (2) gender and (3) different brain regions. To test this, we fed male and female rats nutritionally complete diets containing ethanol as 35% of total energy (treated) or energetic amounts of the same diet in which ethanol was replaced by energetic glucose (controls). At the end of 6 weeks, rats were killed and c-Fos and HSP70 mRNA analysed in midbrain, cortex, brainstem and cerebellum, using reverse transcription-polymerase chain reaction (RT-PCR) with an endogenous internal standard, β-actin.

The results showed that there were distinct regional differences (*P*<0.05) in both c-Fos (cerebellum > cortex > midbrain and brainstem) and HSP70 (brainstem and cerebellum > cortex and midbrain). However, the only significant effect of alcohol feeding occurred in the HSP70 mRNA in the midbrain of male rats, which was reduced by *c.* 50% (*P*<0.01). In contrast, no corresponding effect of alcohol feeding was observed in c-Fos mRNA levels in either the midbrain or other regions of female rats.

Midbrain HSP70 mRNA

Midbrain HSP70 of control and ethanol-treated male and female rats. Data are mean ±SEM density unit (*n* 8 female, *n* 10 male) and expressed relative to β-actin mRNA. ** indicates *P*<0.01. Data analysis, 2 (group) × 2 (gender) ANOVA, *a posteriori* analysis using the method of simple effects.
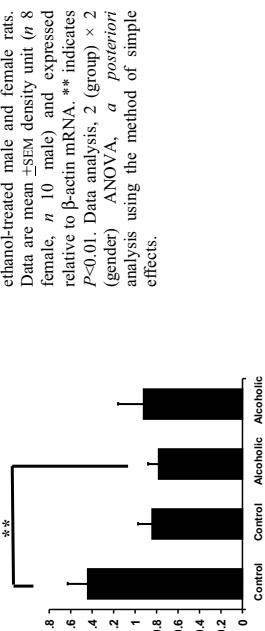
These data show that chronic ethanol feeding has no demonstrable effect on c-Fos mRNA expression in the brain when using nutritionally complete liquid diet regimes with concomitant pair-feeding. HSP70 mRNA, in contrast, is reduced by alcohol feeding and appears to be regional- and gender-dependent. Due to the functional role of HSP70 in protein synthesis, trafficking and cellular protection, a decrease in its encoding mRNA may confer a biological disadvantage to additional metabolic insults or to ethanol itself.

Dietary fat intake in people with familial hypercholesterolaemia is significantly lower than BMI and matched household family members By A.E. BRYNES, J. SHRIMPTON, F. O'NEILL[1], G.S. FROST and G.R. THOMPSON[1], *Departments of Nutrition & Dietetics and Metabolic Medicine[1], ICSM, Hammersmith Hospital, London W12 0HS*

Familial hypercholesterolaemia (FH) is an inherited disorder characterised by raised blood cholesterol, tendon xanthomas and premature ischaemic heart disease. Reduction of plasma LDL cholesterol is the main aim of treatment of this disorder in order to reduce the risk of coronary heart disease. Treatment is based on a low fat healthy eating diet in addition to a lipid lowering drug therapy. There is some concern that with the advent of advanced drug therapies such as statins the FH patient now has little incentive to follow a low fat diet, despite evidence suggesting an additive effect between diet and statin therapy.

This study measured 3-day food intake in 24 households where one volunteer was attending the Hammersmith Hospital Lipid clinic and receiving drug treatment for FH and the other volunteer was an unaffected family member of the same household.

All subjects returned detailed food diaries reporting intake over one-weekend and two-weekdays continuously. Portion sizes were described using handy household measures along with some food labels returned. Analysis was by Dietplan 5. No one was excluded due to reported energy intake being ± 500kcals of calculated energy expenditure (Schofield with activity factor of 1.3). The data were normally distributed and an unpaired t-test was used for statistical analysis. Fasting blood samples were taken for analysis of total cholesterol (TC), LDL, HDL, Total/HDL ratio and triglycerides (TG). The demographic details are shown below. The number of male v females was not different within or between the groups. All subjects reported a stable weight for the previous three months and no recent history of dieting.

	Age (yrs)		BMI (kg/m²)		TC (mmol/l)		LDL (mmol/l)		HDL (mmol/l)		Ratio		TG (mmol/l)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	46	3	28.1	0.9	6.1	0.2	4.0	0.2	1.4	0.1	4.5	0.2	1.6	0.1
FH group	53	2	28.8	1.1	6.0	0.2	4.1	0.2	1.3	0.1	5.0	0.2	1.5	0.1
P value	0.05		NS		NS		NS		0.12		0.09		NS	

The control group was younger than the FH group. The groups were similar for total and LDL cholesterol although there was a trend for the control group to have a lower Total/HDL ratio.

The FH group had a significantly lower total fat intake and saturated fat intake than their partners or family members. Both groups compare favourably to the UK national averages (British Adult survey 1990:15% protein, 43% carbohydrate, 39% fat of which 13% saturated, 10% monounsaturated and 5% polyunsaturated) (Gregory, 1998). All patients had received dietary advice at diagnosis and subsequent continual reinforcement by members of the lipid team.

	Energy (MJ)		% Protein		% CHO		% Fat		Of which % MONO		% POLY		% SAT	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	6.47	0.3	16.2	0.6	45.1	1.7	32.4	0.7	9.4	0.4	4.6	0.3	9.5	0.5
FH group	6.23	0.3	17.3	0.8	48.5	1.9	29.2	1.3	8.5	0.4	4.3	0.3	7.5	0.5
P value	NS		0.3		0.2		0.04		0.1		0.4		0.01	

In conclusion the reported dietary consumption of total and saturated fat is significantly lower in patients with FH than in their unaffected relatives living in the same household or in the population at large.

Gregory J, Foster K, Tyler H and Wiseman M (1998) *The dietary and nutritional survey of British adults*. Office of Population Censuses and Surveys, London, HMSO.

Dietary docosahexaenoic acid (DHA) increases plasma LDL cholesterol concentration and reduces the expression of hepatic sterol regulatory element binding (SREBP) 2 in the hamster. By A.M. SALTER[1], J. BENNETT[2] and A.J. BENNETT[2], *[1]School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD and [2]School of Biomedical Science, University of Nottingham Medical School, Queens Medical Centre, Nottingham NG7 2UH*

Dietary long-chain *n*-3 polyunsaturated fatty acids (*n*-3 PUFAs) can have profound effects on plasma lipid concentrations. One such affect is a lowering of plasma triacylglycerol concentrations. While the mechanism of such effects is not completely understood, it may partly be due to an inhibition of fatty acid synthesis in, and very-low-density lipoprotein (VLDL) secretion from, the liver. The effect of *n*-3 PUFAs on LDL cholesterol is less consistent and, in at least some individuals, they may actually increase LDL. Sterol regulatory element binding proteins (SREBPs) represent a class of nuclear transcription factor that regulate lipid and lipoprotein metabolism. Of the three isoforms identified, SREBP1c is thought to play a major role in regulating fatty acid metabolism, while SREBP2 may play a more important role in regulating cholesterol metabolism. In the present study we have investigated the effects of feeding *n*-3 on plasma lipoprotein concentrations and hepatic SREBP mRNA concentrations in the male golden Syrian hamster.

Three groups of eight hamsters were fed a chow-based diet supplemented with: 2% sunflower oil + 3% olive oil (control), 5% fish oil or 4% algal oil + 1% olive oil. Fish oil contained approximately 18% EPA and 13% DHA, while algal oil contain approximately 40% DHA, thus the total amount of *n*-3 PUFA in the two diets was similar. Cholesterol content of all three diets was adjusted to 0.12%. Diets were fed *ad libitum* for 4 weeks and at the end of the trial whole blood was collected by cardiac puncture and hepatic RNA was isolated. Plasma lipoproteins were isolated by sequential ultra-centrifugation and SREBP mRNA concentrations were measured by nuclease protection assay.

Diet	Chylomicron			VLDL	
	Triacylglycerol	Cholesterol	Triacylglycerol	Triacylglycerol	Cholesterol
Control	0.90±0.23	0.95±0.09	0.73±0.15		0.22±0.06
Fish oil	0.24±0.05***	0.91±0.20	0.21±0.09**		0.05±0.03**
Algal oil	0.29±0.08***	0.85±0.10	0.18±0.09**		0.04±0.03***

All values mean±standard deviation (mM). Data analysed by one-way analysis of variance.
**P<0.01, ***P<0.001.

Both fish oil and algal oil significantly reduced chylomicron triacylglycerol concentration but had no impact on chylomicron cholesterol. By contrast, both oils decreased both VLDL triacylglycerol and cholesterol.

Diet	LDL cholesterol	HDL cholesterol	SREBP1c mRNA	SREBP2 mRNA
Control	0.46±0.08	2.95±0.30	249±43	782±185
Fish oil	1.15±0.25***	1.65±0.16***	76±19***	495±70****
Algal oil	1.03±0.21***	1.52±0.11***	122±36**	406±51***

LDL & HDL cholesterol values are in mM while SREBP mRNA values are in arbitrary units.

LDL cholesterol was increased to a similar extent by both fish oil and algal oil, while both oils significantly decreased HDL cholesterol. Both SREBP1c and SREBP2 mRNA concentrations were reduced by fish oil and algal oil.

The results indicate that feeding a diet containing DHA alone has a similar effect on plasma lipoproteins to one containing an equivalent amount of a mixture of DHA and EPA. Both diets appear to reduce expression of SREBP1c and SREBP2, and reduction in the activity of these transcription factors on their target genes may, at least in part, be responsible for the changes in plasma lipoprotein concentrations.

Copper influences the expression and function of the iron transporter DMT1 in human intestinal epithelial cells. By J.P. TENNANT[1], S. YAMAJI[2], S.K.S. SRAI[2] and P.A. SHARP[1], [1]Centre for Nutrition and Food Safety, School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH and [2]Department of Biochemistry and Molecular Biology, Royal Free and University College Medical School, London NW3 2PF

Dietary metals are absorbed by intestinal enterocytes via a number of transport mechanisms. It has been suggested that the divalent metal transporter (DMT1) represents a common pathway for the uptake of several of these important micronutrients (Gunshin et al. 1997). However, work from our laboratory using human intestinal Caco-2 cells suggests that DMT1 is primarily an iron transporter and has a much lower affinity for other metals (Tandy et al. 2000). Interestingly, we have recently observed that the function and expression of both DMT1 and the proposed enterocyte iron efflux transporter, IREG1, can be regulated not only by iron but also by dietary zinc levels (Yamaji et al. 2001) suggesting that, while other metals may not be major substrates for DMT1, they might contribute to the overall control of intestinal iron absorption. It is also known that high levels of copper in the diet can affect the absorption of iron by the intestine and therefore the aim of our present work was to investigate whether this copper/iron interaction might occur via changes in the expression and transport function of DMT1.

To investigate this problem, we cultured human intestinal Caco-2 cells on Transwell inserts until the cells were fully differentiated (21 d post-seeding). At this time, cells were confluent and fully polarized, forming a distinct brush border (Sharp et al. 2001). Cells were incubated for a further 72 h in the presence or absence of 100 μM FeCl$_3$ or 100 μM CuCl$_2$ and iron transport was measured using ^{55}Fe followed by scintillation counting. In parallel experiments, we measured DMT1 protein levels by Western blotting and mRNA levels by RT-PCR. Data from blotting experiments were semi-quantified using Scion image software. All data are presented as the mean ± SEM of 5–6 separate experiments. Statistical analysis was carried out using one-way ANOVA followed by Scheffé's post hoc test or Student's unpaired t-test where appropriate.

Iron uptake (control cells, 1.21 (SE 0.05) nmol/cm^2.h^{-1}) was inhibited following treatment with 100 μM iron (0.91 (SE 0.04) nmol/cm^2.h^{-1}, P<0.01, ANOVA and Scheffé's post hoc test) and 100 μM copper (0.49 (SE 0.05) nmol/cm^2.h^{-1}, P<0.01). These changes in transport function were accompanied by parallel changes in DMT1 protein and mRNA levels. Regulation of DMT1 mRNA by cellular iron status is thought to occur via differential interaction between an iron-responsive element (IRE) in the 3' untranslated region and cytoplasmic iron regulatory proteins (IRP). There is no evidence that copper can modify this IRE/IRP interaction suggesting that its mode of action is via an alternative pathway, perhaps involving the DMT1 5' promoter region. This possibility is currently under investigation.

Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL & Hediger MA (1997) Nature **388**, 482–487.
Sharp P, Tandy S, Yamaji S, Tennant J, Williams M & Srai SKS (2001) FEBS Letters **510**, 71–76.
Tandy S, Williams M, Leggett A, Lopez-Jimenez M, Dedes M, Ramesh B, Srai SK & Sharp P (2000) Journal of Biological Chemistry **275**, 1023–1029.
Yamaji S, Tennant J, Tandy S, Williams M, Srai SKS & Sharp P (2001) FEBS Letters **507**, 137–141.

The effect of Zn on expression in human intestinal and placental cells of Zn transporter mRNAs. By S.R. PHILLIPS, R.M. RUSSI, R.A. CRAGG, J.M. PIPER, J.C. MATHERS and D. FORD, Human Nutrition Research Centre, Department of Biological and Nutritional Sciences, University of Newcastle, Kings Road, Newcastle upon Tyne NE1 7RU

The involvement of Zn in a wide range of cellular processes highlights the importance of Zn homeostasis. Regulation of intestinal Zn absorption is an important homeostatic mechanism and we hypothesize that regulation of placental Zn transport is important in maintaining fetal Zn status.

We investigated the regulation of intestinal and placental Zn transport by comparing levels of expression of mRNA species coding for the Zn transporters ZnT1 (Palmiter & Findley, 1995), ZnT4 (Huang & Gitschier, 1997), hZIP1 (Gaither & Eide, 2001) and hZTL1 (recently cloned by this group) in the human intestinal epithelial cell line Caco-2 and the placental trophoblast-like cell line JAR.

Caco-2 cells were cultured in Dulbecco modified Eagle's medium supplemented with 10% (v/v) fetal calf serum, 60 μg/ml gentamycin, 2 mM L-glutamine and 1% (v/v) non-essential amino acids over four passages. The Zn^{2+} concentration of the nutrient medium was progressively increased from 3 μM to 100 μM by adding additional ZnCl$_2$ to confluent monolayers, thus avoiding toxicity. Total RNA was harvested after maintenance for 7 d at 100 μM ZnCl$_2$. JAR cells were cultured in RPMI-1640 supplemented with 10% (v/v) fetal calf serum, penicillin/streptomycin (100 units/ml and 100 μg/ml, respectively) and 2 mM L-glutamine. The Zn^{2+} concentration of the nutrient medium was increased from 3 μM to 100 μM by the addition of ZnCl$_2$ to cells 1 d post-seeding. Total RNA was harvested after maintenance for 6 d at 100 μM ZnCl$_2$. Levels of hZTL1 mRNA in samples of total RNA prepared from Zn-supplemented cells and co-passaged controls were compared by semi-quantitative RT-PCR. Signals were normalized against 18s rRNA by including in the PCR reaction a proportion of 18s rRNA-specific primers modified at the 3' end to render them non-extendable, thus reducing the intensity of the 18s rRNA signal to within the range of the transcript measured. Data in the table below are shown as a ratio of the transporter mRNA level to that of 18s rRNA.

		3 μM Zn^{2+}		100 μM Zn^{2+}	
		Mean	SE	Mean	SE
Caco-2	MT (n 5)	0.64	0.03	1.00§	0.04
	ZnT1 (n 5)	0.27	0.06	0.69*	0.07
	ZnT4 (n 5)	0.07	0.02	0.37§	0.04
	hZIP1 (n 5)	0.25	0.04	0.43*	0.03
	hZTL1 (n 5)	0.17	0.06	0.46§	0.03
JAR	MT (n 6)	0.77	0.05	2.58§	0.12
	ZnT1 (n 6)	0.39	0.04	0.38	0.06
	hZIP1 (n 5)	0.52	0.02	0.49	0.02
	hZTL1 (n 6)	0.25	0.03	0.25	0.05

Significantly different from ratio at 3 μM Zn^{2+} by Student's t test: * P<0.01; § P<0.001.

Expression of metallothionein (MT) mRNA, a well-characterized Zn-regulated transcript, was significantly increased in both Caco-2 and JAR cells at 100 μM compared with 3 μM Zn^{2+}. Increasing the Zn^{2+} concentration of the nutrient medium from 3 μM to 100 μM resulted in higher levels of expression in Caco-2 cells of all other transcripts quantified whereas expression in JAR cells of ZnT1, hZIP1 and hZTL1 was not affected by this treatment. ZnT4 mRNA was not detectable in JAR cells.

We conclude that the enterocyte responds to changes in Zn availability by regulating the expression of Zn transporters and suggest that this mechanism is important in Zn homeostasis. In contrast, placental expression of Zn transporters is unresponsive to Zn availability, indicating that regulation of maternal Zn status is the primary mechanism for ensuring an adequate fetal supply of Zn.

Gaither LA & Eide DI (2001) Journal of Biological Chemistry **276**, 22258–22264.
Huang L & Gitschier J (1997) Nature Genetics **17**, 292–297.
Palmiter RD & Findley SD (1995) EMBO Journal **14**, 639–679.

Zinc chelation amplifies thyroid hormone action, but has variable effects on zinc efflux in cultured cells. By H.C. FREAKE, M. SCHALLER, A. TRZCIENSKI and S.A. ZINN, *Departments of Nutritional Sciences and Animal Science, University of Connecticut, Storrs, CT 02269-4017, USA*

Thyroid hormone (T3) functions through a nuclear receptor protein, thought to be one of the many transcription factors requiring zinc for binding to DNA response elements in target genes. In GH3 rat pituitary tumour cells, T3 induces growth hormone gene transcription through a well-defined transcriptional mechanism. Addition of diethylenetriaminepentaacetic acid (DTPA), a high affinity, membrane impermeable chelator of zinc, increases the response to T3, an effect opposite to that predicted (Chattopadhyay & Freake, 1998). This action of DTPA is blocked or reversed by equimolar addition of zinc, but not other divalent cations. We have continued these studies in attempts to understand the mechanisms involved. 65-Zn and 14C-DTPA were used to test the effects of the chelator on cellular zinc homeostasis. When cells were incubated with 14C-DTPA, greater than 99% of the radioactivity was recovered from the media, confirming that this chelator is membrane impermeable. Labelling of media with 65-Zn showed that DTPA (50 µM) reduced entry of zinc into the cells to about 4% of that seen in the absence of the chelator. However, when the cells were preloaded with 65-Zn prior to treatments, DTPA resulted in 59% greater retention of that zinc by the cells ($P<0.05$). When expressed as a percentage of total cellular zinc, nuclear zinc was not altered by these manipulations. This suggests a transport protein responsible for zinc efflux, which is down regulated by the zinc stress induced by the chelator.

Primary cultures of rat hepatocytes were also used, to determine whether effects of DTPA were also found with a different T3 target gene in non-transformed cells. Treatment with DTPA enhanced the induction of S14 mRNA by T3, as measured by Northern analysis, although the effect (about twofold) was less than that observed in GH3 cells. The chelator also resulted in a similar reduction of 65-Zn entry into hepatocytes. However, in contrast to GH3 cells, when hepatocytes were pre-labelled with 65-Zn, DTPA also induced greater efflux of zinc from the cells ($P<0.05$). Thus, while the effects of DTPA on T3-induced gene expression were concordant between the two cell types, they differed in their response in terms of zinc homeostasis. This difference may reflect the situation *in vivo*, where tissues vary in retention of zinc in response to dietary deficiency.

| | GH3 cells | | Primary hepatocytes | |
| | Media labelled | Cells labelled | Media labelled | Cells labelled |
		cpm in cells/total cpm		
Control	0.206 ± 0.019	0.293 ± 0.027	0.304 ± 0.028	0.312 ± 0.022
DTPA (50µM)	0.009 ± 0.002	0.415 ± 0.047	0.033 ± 0.006	0.115 ± 0.008

Chattopadhyay S & Freake HC (1998) *Molecular and Cellular Endocrinology* **136**, 151–157.

Genotype and iron intake in British women. By B. BRATLEY[1], D.GREENWOOD[1], V. BURLEY[1], J. RANDERSON-MOOR[2], K. KUKALIZCH[2], J. MORETON[2] and J. CADE[1], [1]*Nutrition Epidemiology Group, Nuffield Institute for Health, 71–75 Clarendon Road, Leeds LS2 9PL and [2]CRF Genetic Epidemiology Laboratory; ICRF Cancer Medicine Research Unit, St James's Hospital, Beckett Street, Leeds LS9 7TF*

Regulation of iron levels in the body occurs at the point of absorption and at present five genes have been identified which are thought to be implicated in iron metabolism (Lieu *et al.* 2001). Coordinated study of nutritional and genetic factors must be undertaken in order to gain further understanding of iron-related disorders and develop possible therapies (Burke *et al.* 2001). The aims of this study are to genotype a subgroup of the UK Women's Cohort Study (UKWCS) (Greenwood *et al.* 2000) and evaluate the effect of gene mutations C282Y and H63D on iron absorption from the diet in a free-living population.

The C282Y mutation, which is common in Caucasian populations, has been implicated in the aetiology of hereditary haemochromatosis (HH). H63D is also associated with this condition. We are mailing buccal brushes for collection of DNA to ~20,000 UKWCS participants. To date (*n* 1000), allele frequencies are C282Y: G 0.92, A 0.08; H63D: C 0.87, G 0.13. These results are consistent with published UK values (Jackson *et al.* 2001) and are in Hardy-Weinberg equilibrium (P=0.2 and 0.32).

UKWCS subjects were selected to represent diverse dietary patterns, with around one-third being vegetarian, one-third fish eaters and one-third meat eaters. This produces a wide range of intakes. The range between the 2.5 and 97.5 percentiles of iron intake in the UKWCS is 25.9, compared with 16.5 in the National Dietary and Nutritional Survey (Gregory *et al.* 1990). This will allow us to explore the effects of genotype and diet on iron status in subjects with very high and low intakes of dietary iron. Iron stores will be determined in ~2000 women who have provided a blood sample.

Analysis of preliminary data has identified the principal food sources of iron in the cohort. All of these foods contain only non-haem iron. Consequently, it will be important to measure potential absorption modifiers. We are developing protocols for coding and analysis of food diaries that take account of absorption-modifying factors. For example, we have developed a program that includes timing of meal events. This is necessary, as it is the co-consumption of absorption modifiers with meals, not total consumption, which is an important determinant of iron status (Fleming *et al.* 1998).

Total iron	%	Cumulative %
All Bran, Branflakes	10.8	10.8
Wholemeal bread	6.5	17.3
Ready Brek, porridge	4.1	21.4
Wine	3.0	24.4
Potatoes	3.0	27.4
Muesli	3.0	30.4
Brown bread	2.6	33
Weetabix, Shredded Wheat	2.6	35.6
White bread	2.5	38.1
Non-sugar-coated cereals	2.4	40.5

	Hb (g/dl)	Serum ferritin (µg/l)	UIBC (µg/l)	TIBC (µg/l)
n	1772	1216	1768	1709
Mean	13.57	53.15	35.09	55.82
Median	13.60	42.00	34.00	55.00
SD	1.02	48.10	9.99	8.66
Q1	13.00	26.00	29.00	50.00
Q3	14.20	65.00	40.75	60.00
Minimum	6.20	3.00	2.00	22.00
Maximum	17.20	582.00	108	163.00

By linking information on genotype and diet to body iron stores we hope to provide a basis for population dietary recommendations that reduce the risk of iron overload in susceptible individuals.

Burke W, Imperatore G & Reyes M (2001) *Proceedings of the Nutrition Society* **60**, 73–80.
Fleming D, Jacques PJ, Dallal GE, Tucker KL, Wilson PWF & Wood RJ (1998) *American Journal of Clinical Nutrition* **67**, 722–733.
Greenwood DC, Cade JE, Draper A, Barrett JH, Calvert C & Greenhalgh A (2000) *European Journal of Clinical Nutrition* **54**, 314–320.
Gregory J, Foster K, Tyler H & Wiseman M (1990) *The Dietary and Nutritional Survey of British Adults.* London: HMSO.
Jackson HA, Carter K, Darke C, Guttridge MG, Ravine D, Hutton RD, Napier JA & Worwood M (2001) *British Journal of Haematology* **114**, 474–484.
Lieu P, Heiskala M, Peterson P & Yang Y (2001) *Molecular Aspects of Medicine* **22**, 1–87.

Assay and comparison of Δ9 desaturase kinetic parameters in adipose tissue microsomes (rat, human and 3T3-F442A cells). By Y. AMET, E. PLEE-GAUTIER, Y. DREANO, F. BERTHOU and J. DELARUE, *Laboratoire de Biochimie-Nutrition, EA 948, Faculté de Médecine, 22 Avenue Camille Desmoulins, F-29285 Brest Cedex, France*

The rate-limiting step in the biosynthesis of monounsaturated fatty acids is the insertion of a *cis*-double bond in the Δ9 position of fatty acyl-CoA substrates. This oxidative reaction is catalysed by the stearoyl-CoA desaturase (SCD or Δ9 desaturase) and involves cytochrome b5, cytochrome b5 reductase and molecular oxygen. The preferred substrates are palmitoyl- and stearoyl-CoA, which are converted to palmitoleoyl- and stearoyl-CoA, respectively. The balance and/or the ratio between saturated and monounsaturated fatty acids directly influences the membrane fluidity and its physical properties, and alterations in the ratio of these fatty acids have been implicated in a range of disease states including diabetes, obesity, hypertension, alcoholism, heart and vascular diseases. The regulation of the SCD is therefore of considerable physiological importance.

Here we report our experience in separating and assaying the Δ9 desaturase in microsomal preparations from rat and human adipose tissue, or from 3T3-F442A murine cell line. The kinetic parameters (K_m and V_{max}) of the enzyme were also determined in microsomes.

Microsomal fraction was obtained by ultracentrifugation of adipose tissue homogenate, and resuspended in phosphate buffer (pH 7.4) containing 20% glycerol (v/v). Radioactive substrate ^{14}C-stearate was incubated with microsomal protein in presence of phosphate buffer containing MgCl2, and cofactors (ATP, CoA, NAD). Substrate concentration was assayed between 7.5 and 60 μM. The enzymatic reaction was performed at 37° for 10–20 min. After saponification, lipids are extracted and separated by liquid chromatography coupled to a radiometric detection device.

Results reported that the SCD enzyme was detected in all the preparations at levels comparable with those of liver preparations, and the K_m values (25–30 μM) showed high affinity of the enzyme. Insulin increased the SCD enzyme activity in murine cells, whereas 24h glucose deprivation inhibited this enzymatic activity.

In conclusion, our method applied to the study of Δ9 desaturase can be readily assayed in various microsomal preparations, and can be extended to the other desaturases. The high level of the enzyme in murine cell line and in human adipocytes indicate that they could be useful models for further studies on Δ9-desaturase regulatory mechanisms.

Regulation of growth hormone (GH) binding to cultured pig hepatocytes by certain amino acids. By J.M. BRAMELD and P.J. BUTTERY, *Division of Nutritional Biochemistry, University of Nottingham, School of Biosciences, Sutton Bonington Campus, Loughborough LE12 5RD*

The mechanisms of how amino acids may influence gene expression are still unclear. We have previously demonstrated direct effects of individual amino acids on insulin-like growth factor-I (IGF-I) mRNA abundance in cultured pig hepatocytes (Brameld *et al.* 1999). Decreasing concentrations of arginine, proline, threonine, tryptophan and valine were found to inhibit GH-stimulated IGF-I mRNA in a dose-dependent manner, with little or no effect on GH-receptor (GHR) mRNA (Brameld *et al.* 1999). Three mechanisms are possible for these amino acid effects: (1) lack of certain amino acids may inhibit translation of GHR mRNA into protein; (2) amino acids may regulate transcription of the IGF-I gene via interactions with unidentified transcription factors; or (3) amino acids may regulate the stability of IGF-I mRNA, via regulation of IGF-I mRNA-binding proteins. The studies described were carried out to investigate the first of these mechanisms, regulation of translation of GHR mRNA.

Pig hepatocytes were isolated and cultured as described previously (Brameld *et al.* 1999). Hepatocytes were cultured overnight in Hepes buffered Williams' medium E supplemented with 2g/l BSA and 100 nmol/l insulin (basal medium), with or without the various nutrients (glucose, arginine, proline, threonine, tryptophan, valine or leucine). The cells were then maintained on the same basal medium (with or without glucose and individual amino acids), in the presence or absence (control and lacking glucose only) of 3,3′,5-tri-iodothyronine (T3, 10 nmol/l) and dexamethasone (Dex, 100 nmol/l). GH-binding or intracellular concentrations of DNA, protein, glycogen and free glucose and amino acids were measured 24 h later. Bovine GH (bGH) was radiolabelled with ^{125}I via the lactoperoxidase method and purified via gel filtration (Sephadex G50 Fine). GH binding to cultured hepatocytes in the presence or absence of excess unlabelled bGH (as a means of quantifying specific binding), was as previously described (Niimi *et al.* 1990, 1991). The effects of removing individual nutrients on intracellular concentrations of DNA, protein, glycogen, free glucose and amino acids were also investigated, in order to check cell viability and the specificity of any effects. Means of four experiments (four pigs) are shown along with the SED, number of degrees of freedom and ANOVA *P* values.

Treatment	NSB (cpm)	Total binding (cpm)	DNA (μg/dish)	Specific binding (cpm/μg DNA)[a]	Protein (μg/μg DNA)	Glycogen (μg/μg DNA)	Glucose (μg/μg DNA)
Control	7543	14051	115.72	233.9	55.46	126.5	0.972
− T3/Dex	7596	12447	116.96	164.9	56.34	173.3	1.077
− Gluc	5872	10347	109.71	174	55.78	130.9	0.315
− Gluc & T3/Dex	5684	8510	110.5	110.5	54.6	107.5	0.25
− Arg	6417	917	111.78	95.1	52.78	119.4	1.08
− Pro	6708	8923	111.39	83.3	51.86	118.4	1.08
− Thre	7058	10114	110.54	118	54.71	130.5	1.081
− Tryp	7140	10312	106.04	121	53.39	105.4	1.116
− Val	7444	11156	106.47	148.6	55.47	109.3	1.026
− Leu	7278	11704	105.89	182	54.61	116.7	1.022
SED	692.1	1101.3	4.17	26.7	1.85	19.11	0.099
DF	25	25	25	25	25	25	25
P value	0.089	<0.001	0.155	<0.001	0.353	0.068	<0.001

[a] Specific binding is mean of 4×(Total − NSB)/DNA, due to cpm being measured in 0.5 ml from a total of 2 ml.

The presence of glucose and the combination of T3 and Dex had previously been shown to increase GHR mRNA (Brameld *et al.* 1999) and, as expected, removal decreased GH-binding. Leucine, unlike the other amino acids being studied, had no effect on GH-stimulated IGF-I mRNA (Brameld *et al.* 1999) and, as expected, removal had no effect on GH-binding. A lack of certain individual amino acids (arg, pro, thre, tryp and val) reduced specific binding of bGH to cultured pig hepatocytes, with no effect on intracellular DNA, protein, glycogen or free glucose. There were fluctuations in the intracellular free amino acid pools (data not shown), such that removal of an amino acid tended to reduce its intracellular free concentration. Removal of arg, pro, thre and tryp also resulted in imbalances of the intracellular free amino acid pool, but the significance of this is not known. Certain amino acids may therefore interact with the GH-IGF axis by regulating translation of GHR mRNA.

Brameld JM, Gilmour RS & Buttery PJ (1999) *Journal of Nutrition* **129**, 1298–1306.
Niimi S, Hayakawa T & Tanaka A (1990) *Endocrinology* **127**, 688–694.
Niimi S, Hayakawa T, Tanaka A & Ichihara A (1991) *Endocrinology* **129**, 2734–2739.

Regulation of retinol binding protein gene expression in adipocytes differentiated from fibroblastic preadipocytes in primary culture. By C.R.M. KEELEY[1], D.V. RAYNER[1], A. MERRYMAN-SIMPSON[2] and P. TRAYHURN[3], [1]Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, [2]BASF-Pharma Research and Development, Nottingham NG1 1GF and [3]Department of Medicine, University of Liverpool, Liverpool L69 3GA

Retinol binding protein (RBP) is involved in the transport of retinol from the liver, the main storage site of vitamin A derivatives, to target cells where it is utilized. Adipose tissue can also store retinol, and adipocytes have been shown to contain high levels of the mRNA encoding RBP (Makover *et al.* 1989; Montague *et al.* 1998). It is recognized that the sympathetic nervous system plays a central role in the regulation of lipolysis and of the production of important secretory factors from adipose tissue, such as leptin and plasminogen activator inhibitor-1 (Gottschling-Zeller *et al.* 2000; Trayhum & Beattie, 2001). The present study has examined whether expression of the RBP gene in adipose tissue is also under sympathetic control.

Fibroblastic preadipocytes were harvested from the subcutaneous fat of 2-week-old Hooded Lister rats and induced to differentiate. Nine days after the induction of differentiation the adipocytes were treated for 24 h with either 1 μmol BRL37344, 1 μmol noradrenaline, 1 μmol isoprenaline, 100 nmol dexamethasone or 100 nmol leptin. Total RNA was isolated from the cells, and RBP mRNA was detected by Northern blotting using an antisense oligonucleotide with chemiluminescence detection. Densitometry was used to quantify the relative mRNA levels.

In initial studies, tissues obtained from adult male Aston strain mice were also analysed for RBP mRNA. The highest level of RBP mRNA was from the liver, followed by epididymal adipose tissue. A faint signal was seen from the kidney and lung, but no RBP mRNA was detected from the spleen and heart. RBP mRNA was detected in all the fat depots analysed (subcutaneous, epididymal, perirenal, omental and interscapular brown fat), but the levels varied between sites, the highest being the perirenal and epididymal fat. RBP mRNA was only found in mature adipocytes; there was no signal in the stromal vascular fraction. In the primary cell culture system, the highest level of RBP mRNA was found 10 d after the induction of differentiation. Addition of noradrenaline or the β-adrenoceptor agonists isoprenaline or BRL37344 (β3–selective) to the culture medium had no significant effect on the level of RBP mRNA in the adipocytes (*P*>0.05). Leptin also had no effect on RBP mRNA levels; however, dexamethasone significantly (*P*<0.05) reduced adipocyte RBP mRNA.

These studies show that stimulation of β-adrenoceptors within white adipocytes has no effect on RBP mRNA level. Thus, in contrast to lipolysis and the production of leptin and plasminogen activator inhibitor-1, the sympathetic system does not play an important role in regulating the expression of the RBP gene in adipocytes. Other factors, such as glucocorticoids, are likely to be involved in determining adipocyte RBP synthesis.

Gottschling-Zeller H, Aprath I, Skurk T & Hauner H (2000) *Hormone and Metabolic Research* **32**, 509–514.
Makover A, Soprano DR, Wyatt ML & Goodman DS (1989) *Journal of Lipid Research* **30**, 171–180.
Montague CT, Prins JB, Sanders L, Zhang JL, Sewter CP, Digby J, Byrne CD & O'Rahilly S (1998) *Diabetes* **47**, 1384–1391.
Trayhurn P & Beattie JH (2001) *Proceedings of the Nutrition Society* **60**, 329–339.

Effects of dietary vitamin E depletion on colon gene transcription profiles. By J.E. DREW[1], G.G. DUTHIE[1] J.R. ARTHUR[2], K. PICKARD[1], P.C. MORRICE[1], S.J. DUTHIE[1] and C. MAYER[3], [1]Phytochemicals and Genomic Stability, [2]Lipid and Redox Regulation, [3]BIOSS, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB

Numerous epidemiological studies indicate an inverse relationship between fruit and vegetable consumption and the pathogenesis of colorectal cancer. Many of the health benefits of fruit and vegetables are attributed to the antioxidant properties of a number of plant phytochemicals. However, the mechanisms whereby fruit and vegetables influence the pathogenesis of colorectal cancer are still poorly understood. Gene expression profiling strategies were therefore investigated to determine modulation of gene expression in rat gut mucosa and colon tissue in response to dietary deficiency of vitamin E, the major plant-derived, lipid-soluble antioxidant in the diet.

Plasma and liver concentrations of vitamin E in rats consuming the vitamin E (+E) diet were 5.6 (SE 0.4) μg/ml and 51 (SE 4) ng/mg protein, respectively. Comparable values in the rats eating the vitamin E deficient (–E) diet were 0.4 (SE 0.03) μg/ml and 2.2 (SE 0.2) ng/mg protein. Gene expression profiles were obtained by Atlas cDNA array of gut mucosa taken from three rats maintained on the +E and –E diets. Image and data analysis were performed using Atlas image (Clontech) and S-plus statistical software (Insightful, UK).

Gene changes greater than twofold from at least two rats fed on the dietary treatments.	Fold changes
Up-regulated genes –E > +E	
Liver fatty acid binding protein (LFABP)	5.4/3.4
Calponin	10.6/2.2
Down-regulated genes –E < +E	
Sertoli cell cytochrome c oxidase polypeptide I	2.1/2.2
Peptide YY precursor	2.4/2.6
Nuclear tyrosine phosphatase (PRL1)	2.3/2.5
Aminopeptidase B	2.7/3.2

RNA amplification techniques and Northern blot analyses were used to verify differences in RNA levels of LFABP, calponin and PRL1, as measured using image and data analysis, in the RNA template used on a cDNA array filter and in whole colon tissue. Rat gut mucosal total RNA (2 μg) from –E and +E rats was reverse transcribed using a T7 oligodT primer and mRNA amplified via T7 RNA polymerase. The amplified mRNA (1 μg) was Northern blotted and hybridized with ^{32}P labelled LFABP, calponin and PRL1 cDNA fragments. Linear amplification of the samples was verified using a ^{32}P-GAPDH (glyceraldehyde-3-dehydrogenase phosphate) fragment, shown to be expressed at similar levels by the Atlas macroarray image analysis. Densitometric analysis normalized against GAPDH verified up-regulation of LFABP and CAL transcripts in the –E gut mucosa. In contrast, levels of PRL1 were similar in both the –E and +E gut mucosa amplified RNA samples. Northern blot hybridization of entire colon total RNA (20 μg) from –E and +E rats revealed slightly elevated FABP levels in the whole colon tissue in rats on the +E diet with no significant difference in calponin or PRL-1 transcript levels in whole colon.

The image analysis methods correctly identified two genes of the three predicted to be up/down-regulated greater than twofold in gut mucosa samples from vitamin E supplemented and depleted rats. The differential regulation of these genes was not observed by Northern blotting of whole colon tissue, perhaps reflecting regional patterns of variable expression. This is a potentially confounding factor when using array methodology in nutritional models of micronutrient deficiency.

Folic acid supplementation increases genomic DNA methylation in patients with colorectal adenoma. By M. PUFULETE[1], R. AL-GHNANIEM[2], A. KUSHAL[2], A.J.M LEATHER[2], J.A. RENNIE[2], P.W. EMERY[1] and T.A.B. SANDERS[1], [1]Nutrition Food and Health Research Centre, King's College London, Stamford Street, Waterloo, London SE1 9NN and [2]Academic Department of Surgery, King's College Hospital, Denmark Hill, London SE5 9RS

Low dietary folate intake is inversely associated with increased risk of colorectal adenoma and cancer. Folate deficiency results in global DNA hypomethylation, which may increase the risk of carcinogenesis. This study tested the hypothesis that folic acid supplementation would increase global DNA methylation in leucocytes and colonic mucosal cells of patients with colorectal adenoma, using a randomized double-blind placebo controlled parallel design.

Patients with histologically confirmed colorectal adenoma were randomized to receive either a daily 400 µg folic acid supplement (n 15) or placebo (n 16) for 10 weeks. Genomic DNA methylation in leucocytes and colonic mucosa, serum and red cell folate, serum vitamin B_{12} and plasma homocysteine concentrations were determined at baseline and on completion of the study. DNA methylation status was measured by the *in vitro* methyl acceptance assay, based on the ability of DNA to incorporate [³H] methyl groups from *S*-adenosylmethionine.

Folate supplementation increased serum and red cell folate ($P=0.01$) and decreased plasma homocysteine ($P=0.01$) but had no effect on serum vitamin B_{12}. Genomic DNA methylation status increased following folate supplementation; in leucocytes the increase was significantly greater than that in the placebo group ($P=0.04$), whereas in the colonic mucosal cells the increase was not significantly different between the folic acid and placebo groups ($P=0.16$).

The finding that supplementation with a physiological dose of folic acid increases DNA methylation status supports the hypothesis that folate status is suboptimal in patients at risk of colorectal cancer. Further evaluation of the role of dietary folate in the causation of colorectal cancer is warranted.

	Folic acid (n 15)		Placebo (n 16)	
	Baseline	Week 10	Baseline	Week 10
Serum folate (µg/l)				
Mean	7.7	14.0[a]	8.0	6.7
95% CI	(5.6–9.8)	(11.8–16.2)	(6.2–9.8)	(5.2–8.2)
Red cell folate (µg/l)				
Mean	280	444[b]	318	305
95% CI	(216–344)	(336–552)	(255–381)	(247–362)
Serum vitamin B_{12} (ng/l)				
Median	453	431	350	354
Range	(184–1069)	(216–991)	(211–981)	(149–802)
Plasma homocysteine (µmol/l)				
Median	12.7	11.1[c]	11.2	11.4
Range	(6.8–36.2)	(7.5–19.5)	(5.8–17.5)	(5.1–18.9)
[³H] methyl group incorporation in colonic mucosal cells (Bq/µg DNA)				
Mean	574	457	499	452
95% CI	(480–669)	(365–549)	(421–577)	(368–535)
[³H] methyl group incorporation in leucocytes (Bq/µg DNA)				
Mean	748	530[d]	679	618
95% CI	(681–814)	(467–594)	(588–770)	(557–679)

[a,b] Change from baseline significantly different between folic acid and placebo groups ($P=0.001$, independent samples t test).
[c] Change from baseline significantly different between folic acid and placebo groups ($P=0.01$, Mann–Whitney U test).
[d] Change from baseline significantly different between folic acid and placebo groups ($P=0.04$, independent samples t test).

Differential gene expression in DNA mismatch repair proficient and deficient colorectal cancer cell lines exposed to butyrate. By J.M. COXHEAD[1], E.A.WILLIAMS[1], C. GASPAR[2], W. BAL[1], R. FODDE[2] and J.C. MATHERS[1], [1]Human Nutrition Research Centre, Department of Biological and Nutritional Sciences, University of Newcastle upon Tyne NE1 7RU and [2]Department of Human and Clinical Genetics, Leiden University Medical Centre, 2300 RA, Leiden, The Netherlands

Hereditary non-polyposis colorectal cancer (HNPCC) results from an inherited mutation in one of the DNA mismatch repair (MMR) genes responsible for the correction of mispaired nucleotides. Approximately 70% of germline mutations in HNPCC are found in the genes *hMLH1* and *hMSH2*. Microsatellite instability (MSI) is the mutator phenotype of HNPCC and is caused by the incorrect replication of repeated DNA sequences. MSI is present in >90% of HNPCC cases and approximately 13% of sporadic colorectal cancer (CRC) cases.

Treatment of MMR-deficient CRC cell lines with aspirin selects for cells that are microsatellite stable (MSS) (Rüschoff *et al.* 1998). This effect was reversible, and time and dose dependent. The short-chain fatty acid (SCFA) butyrate produced from carbohydrate fermentation in the large bowel has anti-neoplastic effects through inhibiting proliferation and inducing apoptosis in CRC cells. This study was designed to test the hypothesis that MMR-proficient and deficient cell lines are differentially sensitive to butyrate and to investigate changes in gene expression in response to butyrate.

The CRC cell lines HCT116 (*hMLH1⁻/⁻*) and SW480 (MMR-proficient) were grown for 20 d in 1 mM butyrate-enriched media or normal media followed by a recovery period of 20 d in normal media. Media were changed every 2 d and cells were harvested, counted and re-seeded at the original density every 4 d. At days 2, 4, 20, 22 and 40, cells were harvested for gene expression analysis using the Human Genome U95Av2 GeneChip (Affymetrix) with a threefold change in expression considered significant.
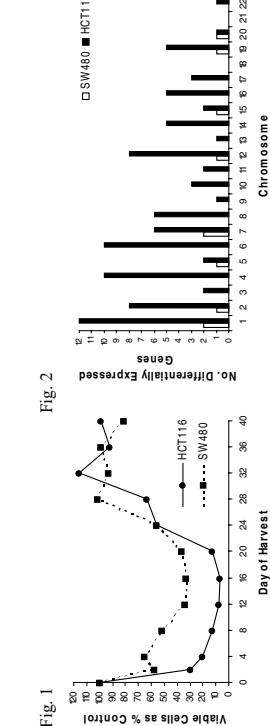
Fig. 1
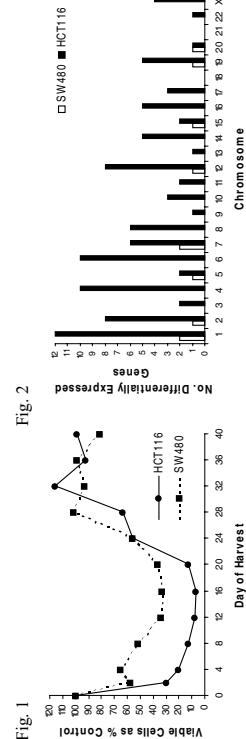
Fig. 2

Butyrate treatment reduced proliferation of both cell lines (Fig. 1) with HCT116 cells (MMR-deficient) showing greater sensitivity. This effect was sustained for 20 d. On day 2, butyrate treatment resulted in differential expression of ten genes by SW480 cells (MMR-proficient) and ninety-seven genes by HCT116 cells (Fig. 2). Approximately equal numbers of genes were up- and down-regulated and these genes were located on almost all chromosomes for both cell lines. This work is now focusing on interpretation of this differential gene expression analysis in terms of function, and MSI analysis will be carried out to determine whether there has been a genetic selection for MSS cells.

J.M.C. holds a studentship from the BBSRC.

Rüschoff J, Wallinger S, Dietmaier W, Bocker T, Brockhoff G, Hofstädter F & Fishel R (1998) *Proceedings of the National Academy of Sciences USA* **95**, 11301–11306.

Micronutrients and cancer prevention: effect of sulphoraphane, an isothiocyanate from broccoli, in the regulation of human colon cancerous cell growth and death. By P. ROUMI, C. ASSOUMAYA, G. PARNAUD, A. CARPENTER, N. GASC, J. TULLIEZ and L. GAMET-PAYRASTRE, *UMR 1089 Xénobiotiques, INRA, 180 Chemin de Tournefeuille, BP3, 31931 Toulouse Cedex 9, France*

Over the past 10–15 years, convincing scientific evidence has accumulated on the relationship between nutrition and cancer. In addition, several epidemiological studies correlate plant-based diets with low risk of cancer, with special attention paid to examining the protective properties of fruits and vegetables. Effects of the latter on colorectal carcinogenesis have been associated with their micronutrient contents (i.e. polyphenolic compounds, vitamins, carotenoids, glucosinolates). Indeed, glucosinolates from dietary cruciferous vegetables provide substantial amounts of isothiocyanates (ITC), deriving from their hydrolysis by vegetal or bowel microfloral myrosinases. ITCs are effective inhibitors of chemical carcinogenesis in a wide variety of animal models. This property is at least in part due to their effects on metabolizing enzyme expression. Thus, they inhibit carcinogen-activating phase I cytochrome P450 (CYP450) and induce detoxifying phase II glutathione S-transferases (GST) enzymes. In addition, ITCs have been identified as GST substrates *in vitro*.

We have recently demonstrated the induction of apoptosis in cultured human colon cancerous cells by sulphoraphane (SR, 1-isothiocyanato-4-(methyl-sulphinyl)butane), the major ITC from broccoli (Gamet-Payrastre *et al.* 2000). A concomitant cell cycle arrest at the G2/M phase occurred in these cells. In order to understand the way in which SR acts at the molecular level, we investigated its effects on proteins involved in early signalling events of apoptosis (mitogen-activated protein kinases), in cell cycle regulation (cyclins) and in isothiocyanate metabolism (GST) in the human colonic carcinoma cell line Caco-2. Using Western blot analysis, we clearly observed a sustained induction of the MAP kinase ERK-1 and ERK-2 by SR in exponentially growing Caco-2 cells at very early stages of culture. Moreover, exposure of the cells to specific inhibitors of these kinases (i.e. PD 098059 or SB 203580) resulted in a decreased rate of SR-induced apoptosis ($P<0.001$ and $P<0.01$, respectively; n 5), thus showing interference with the ERK but also the p38 pathways. Additional examination of expression and phosphorylation of proteins involved in cell cycle events, showed that cyclin B1 and p21 are induced and the cyclin-dependent kinase cdc2 is maintained in an activate state under SR exposure. These results are in accordance with the cell cycle arrest at the G2/M phase previously observed. Examining the expression of enzymes involved in cellular detoxification and stress, we observed a strong induction of GST α and, to a lesser extent, of GST π on exponentially growing cells exposed to sulphoraphane in the same conditions. It is noteworthy that GST α, which has previously been shown to be associated with intestinal cell differentiation (Vecchini *et al.* 1997), is overexpressed in untreated post-confluent differentiated (intestinal-like) Caco-2 cells. In addition, it should be noted that a GST has recently been demonstrated to participate in the regulation of the stress-activated c-Jun N-terminal kinase (Adler *et al.* 1999).

Our results show that the anticarcinogenic properties of sulphoraphane *in vitro* are related to both changes in the early signals of the apoptotic cascade and cell cycle regulation, but also in the induction of GST α, a class of GST whose expression may be associated with the endogenous mechanism of intestinal differentiation.

Adler V, Yin Z, Fuchs SY, Benezra M, Rosario L, Tew K, Pincus MR, Sardana M, Henderson CJ, Wolf CR, Davis RJ and Ronai Z (1999) *EMBO Journal* **18**, 1321–1334.

Gamet-Payrastre L, Li P, Lumeau S, Cassar G, Dupont M-A, Chevolleau S, Gasc N, Tulliez J & Tercé F (2000) *Cancer Research* **60**, 1426–1433.

Vecchini F, Pringault E, Billar TR, Geller DA, Hausel P & Felley-Bosco E (1997) *Cell Growth and Differentiation* **8**, 261–268.

Selenoprotein expression in the rat colon during Se deficiency. By V. PAGMANTIDIS[1], G. BERMANO[2], I. BROOM[2], J.R. ARTHUR[3] and J.E. HESKETH[1], [1]*Department of Biological and Nutritional Sciences, University of Newcastle Upon Tyne NE1 7RU,* [2]*University of Aberdeen and* [3]*Rowett Research Institute, Buckshurn, Aberdeen AB21 9SB*

Selenium is an essential trace element, which is present in several proteins, called selenoproteins, that have various biological roles. In particular cytosolic glutathione peroxidase (GPX1), phospholipid hydroperoxide glutathione peroxidase (GPX4), and gastrointestinal glutathione peroxidase (GPX2) are involved in the cell's antioxidant system. Selenium is incorporated into these proteins as the amino acid selenocysteine (Se-cys), via recognition of the stop codon UGA as a codon for Se-cys. The incorporation of Se-cys at specific UGA codons requires a specific structure (SECIS) in the 3' untranslated region (3' UTR) of the mRNAs coding for selenoproteins. When selenium supply is limiting, the expression of the selenoproteins is decreased, which is also reflected in termination of translation due to the recognition of the UGA codon as a normal stop codon, increased mRNA instability and alteration of the mRNA levels. Colon cancer is a major cause of death in the UK. There is strong evidence linking diet to the development of colon cancer. Selenium has been suggested to protect against colon cancer, but relatively little is known of how Se deficiency affects the colon. The aim of this work was to investigate how Se deficiency affects expression of the glutathione peroxidases in the rat colon.

Previous studies have shown that in rats Se deficiency affects different selenoproteins to various extents and also that their regulation varies between tissues (Bermano *et al.* 1995). Particularly in the liver, GPX1 mRNA levels were greatly reduced under severe Se deficiency, whereas GPX4 mRNA levels showed little or no change. In the present study, rats were fed diets with different amounts of selenium from 402.8 ng/g (superoptimum), 110.8 ng/g (normal), 62.5 ng/g, 33.3 ng/g to 7.6 ng/g (severely deficient). Total RNA was extracted from the colon by a phenol–guanidinium–chloroform procedure and GPX1, GPX2 and GPX4 mRNA abundances were analysed by Northern hybridization. The results show that under severe Se deficiency the mRNA abundance for GPX1 decreased by about 80% ($P<0.05$), GPX4 mRNA levels decreased by 25% ($P<0.05$), whereas for GPX2 there was no significant change in the mRNA levels. Preliminary data from a cell culture experiment suggest that under Se depletion the GPX1 and GPX4 mRNA levels are reduced, whereas the mRNA abundance for GPX2 is not. The results imply that, in the colon as is also found in the liver, GPX4 is less sensitive than GPX1 to Se deficiency. However in the liver GPX4 mRNA abundance is unaffected by severe Se deficiency, whereas it is reduced in the colon. In the colon GPX2 expression appears highly conserved when Se supply is low; this has been found previously in studies with Caco-2 cells (Wingler *et al.* 1999), but has not been observed before *in vivo*. Overall the data show that the pattern of change in glutathione peroxidase gene expression in response to Se deficiency is different in the rat colon compared with the liver.

Bermano G, Nicol F, Dyer JA, Sunde RA, Beckett GJ, Arthur JR & Hesketh JE (1995) *Biochemical Journal* **311**, 425–430.
Wingler K, Böcher M, Flohe L, Kollmus H & Brigelius-Flohe R (1999) *European Journal of Biochemistry* **259**, 149–157.

The effects of high-fibre breakfast cereals on subjective reports of energy and mood. By A. SMITH, *Centre for Occupational and Health Psychology, Cardiff University, 63 Park Place, Cardiff CF10 3AS*

Fibre consumption has declined substantially over the last 150 years and there has been a reciprocal rise in the prevalence of constipation. Constipation affects quality of life and patients with constipation report increased psychopathology (Preston *et al.* 1984) and fatigue (Benton, 2002). Studies have shown that wheat fibre has especially significant gastrointestinal effects and is the most effective treatment for constipation (Graham *et al.* 1982). These pieces of evidence suggest that dietary fibre may also reduce the feeling of fatigue, and the present investigation was conducted to examine this. For the study, 142 members of the general public (mean age 52 years, range 30–80; males *n* 50, females *n* 94) were recruited. An exclusion criterion was regular consumption of a high-fibre breakfast cereal (>6%). An intervention study was then carried out with volunteers being randomly assigned to one of three cereal conditions. Two of the cereals were commercially available high-fibre products (Kellogg's All Bran: 29% fibre and Kellogg's Bran Flakes: 15% fibre) and the third was a specially produced low-fibre cereal (3% fibre). A cross-over design was used with half of the volunteers having the 40 g of test cereal for 14 d followed by their normal breakfast for 14 d and the other half having their normal breakfast for 14 d followed by the test cereal. Compliance with the cereal consumption instructions was high and 139 volunteers completed the study. During each day of the study the volunteers rated how energetic they had felt that day and how refreshed they felt.

Analyses of covariance were conducted with the baseline measures as covariates. Initial analyses revealed no differences between the two high-fibre cereal groups. However, significant differences were found between the high and low fibre conditions. The results, shown below, revealed that those who consumed the high-fibre cereals felt more energetic and refreshed than those in the low fibre condition (both *P*s<0.05). This effect was apparent on 13 out of 14 days.

	High-fibre cereal	High-fibre group, normal breakfast	Low-fibre cereal	Low fibre group, normal breakfast
Ratings of how energetic they felt[1]	2.25	2.05	2.11	2.08
Ratings of how refreshed they felt[1]	2.05	1.87	1.96	1.95

[1] Mean ratings made on a 5-point scale, 0=not at all to 4=extremely.

The present study shows that consumption of high-fibre breakfast cereals is associated with an increase in reported energy. This effect was apparent for most of the study and was not restricted to individuals with low regular fibre intake. Similarly, it did not merely reflect changes in the clinical symptoms of constipation but was also observed in those with no constipation. Further research is now required to elucidate the underlying mechanisms responsible for the observed effects. However, from a practical point of view, the findings suggest that consumption of high-fibre cereals may be one way of reducing fatigue.

Benton D (2002) *Proceedings of the Nutrition Society* **60**, 190A.
Graham DY, Moser SE & Estes MK (1982) *American Journal of Gasteroenterology* **77**, 599–603.
Preston DM, Pfeffer JM & Lennard-Jones JE (1984) *Gut* **25**, A582–A583.

Rats fed a high-sucrose diet have altered heart antioxidant enzyme activity and gene expression. By I. BUSSEROLLES, E. ROCK, E. MAZUR A. GROLIER P. & RAYSSIGUIER Y., W. ZIMOWSKA, E. ROCK, Y. RAYSSIGUIER and A. MAZUR, *Centre de Recherche en Nutrition Humaine d'Auvergne, Unité des Maladies Métaboliques et Micronutriments, INRA, Theix, 63122 Saint-Genès-Champanelle, France*

A high-sucrose diet was used in animal models to induce the metabolic changes observed in Syndrome X, a disorder in which insulin resistance, hypertension, dyslipidaemia and high incidence of cardiovascular diseases are described (Reaven, 1988). The underlying mechanisms for the detrimental consequences of a high-sucrose diet are not clear. However, the possibility exists that fructose feeding facilitates oxidative damage (McDonald, 1995). This hypothesis is supported by previous findings showing that sucrose has a deleterious effect both when antioxidant defences are decreased (Rayssiguier *et al.* 1993) or when free radical production is increased (Rayssiguier *et al.* 1981). This was corroborated by recent data obtained in our laboratory on lipid peroxidation measurements (Busserolles *et al.* 2002). The evaluation at the molecular level of the changes consequent to high sucrose consumption should help to produce a better understanding of the causes and the mechanisms involved.

Besides oxidative stress parameters, we assessed differential gene expression in the cardiac tissue of rats fed a sucrose-rich diet (65% sucrose) for 2 weeks compared with those fed a starch diet (65% starch) by using an array designed to analyse 207 transcripts of genes involved in the cell stress. 8 rats per group were used for biochemical analysis. Microarray analysis was obtained by pooling total RNA of 8 animals per group.

Results indicate that, after exposure of tissue homogenates to iron-induced lipid peroxidation, TBARS (thiobarbituric acid-reacting substances) were significantly higher in heart (*P*<0.01) from the sucrose group compared with the starch group. Whereas no difference in heart zinc content was observed, a significantly lower heart copper level (*P*<0.01) was found in the sucrose group compared with the starch group. Plasma nitrite and nitrate levels were significantly higher in the sucrose group compared with the starch group (*P*<0.001). Heart Cu-Zn-superoxide dismutase (SOD) activity was significantly decreased (*P*<0.05) in the sucrose group compared with the starch group whereas heart Mn-SOD, GPX and catalase activities were no different between the groups. The up-regulated genes in sucrose-fed rats included genes associated with the stress response regulation: the sucrose:starch ratio being 2.2 for vimentin and 2.3 for HSP70. The down-regulated genes included a gene associated with drug and xenobiotic metabolism (sucrose:starch ratio of 0.5 for catechol-*O*-methyltransferase (COMT). Among the antioxidant enzyme genes; whereas Cu-Zn-SOD was up-regulated twofold, neither Mn-SOD nor GPX and catalase gene expression were altered by a high sucrose consumption.

In conclusion, the utilization of cDNA array in the present study confirms dietary sucrose-induced oxidative stress, monitored by increased TBARS and Cu-Zn-SOD activity. It also underlines its hypertensive effect, as suggested by altered HSP70, vimentin and COMT gene expression, and suggests a possible causal relation between these phenomena. The utilization of a cDNA array to determine the time course of events occurring in this experimental model of Syndrome X may thus be the tool of choice for understanding the mechanisms involved.

Busserolles J, Rock E, Gueux E, Mazur A, Grolier P & Rayssiguier Y (2002) *British Journal of Nutrition* **87**, 337–342.
Reaven GM (1988) *Diabetes* **37**, 1595–1607.
McDonald RB (1995) *American Journal of Clinical Nutrition* **62**, 284S–293S.
Rayssiguier Y, Gueux E & Weiser D (1981) *Journal of Nutrition* **111**, 1876–1883.
Rayssiguier Y, Gueux E, Bussière L & Mazur A (1993) *Journal of Nutrition* **123**, 1343–1348.

Does intensive feeding support improve nutritional status and outcome in acutely ill older in-patients? By M. HICKSON[1], C. BULPITT[2], M. NUNES[2], R. PETERS[2], J. COOKE[2], C. NICHOLL[3] and G. FROST[1], [1]Department of Nutrition and Dietetics, Hammersmith Hospitals NHS Trust, Du Cane Road, London W12 0HS, [2]Care of the Elderly Section, Imperial College, Faculty of Medicine, Hammersmith Campus, Du Cane Road, London W12 0HS and [3]Department of Medicine for the Elderly, Addenbrooke's Hospital NHS Trust, Hills Road, Cambridge CB2 7QQ

Malnutrition is particularly common in elderly people and has been shown to increase morbidity and mortality (Corish & Kennedy, 2000). Most attempts to reverse malnutrition in this group have used liquid supplements but the findings are inconsistent (Potter et al. 1998). There are many factors that influence the development of malnutrition, so the provision of an adequate supply of nutrients can only be part of the solution. Another approach is needed. We examined, using a randomized controlled design, whether health care assistants, trained to provide support with all aspects of feeding, could make a significant impact on nutritional status of acutely ill elderly in-patients and whether this improved outcome. For the study, 592 patients were recruited from three Acute Medicine for the Elderly wards at Hammersmith Hospitals NHS Trust, London. Patients were all over 65 years old and had been admitted between 1998 and 2000. Those with a poor prognosis (not expected to survive admission), unable to take food orally, with discharge planned in 4 d, re-admitted and already participated in the study were excluded. Protocol published (see Hickson et al. 1999).

The intervention of intensive feeding support was provided by three health care assistants trained in nutrition, one placed on each ward. Their role included identifying feeding problems, encouraging and helping patients to eat and drink, and monitoring intake.

The main outcome measures were body mass index (BMI), mid-arm muscle circumference, grip strength, Barthel score, antibiotic use, artificial fluid use, laxative use and length of stay.

The median time that patients received the feeding support was 16 d; however, at the end of the trial the groups did not differ in markers of nutritional status, Barthel score, laxative use, grip strength or length of stay. The assisted group was given less IV fluids ($P=0.03$) and less IV antibiotics ($P=0.02$).

Variable	Treatment		Control		P
	Median	(Range)	Median	(Range)	
BMI (kg/m²)	21.5	(11.8–41.2)	21.6	(13.0–36.5)	0.37
MAC (cm)	24.9	(16.5–38.9)	24.8	(16.2–39.3)	0.91
TSF (mm)	11.3	(3.1–33.9)	10.1	(2.7–28.4)	0.07
MAMC (cm)	21.1	(14.6–29.8)	21.7	(14.6–32.3)	0.11
Barthel score	16.0	(0–20)	16.0	(0–20)	0.36
No. of IV antibiotics given	1.0	(1–4)	2.0	(1–5)	0.02
No. of oral antibiotics given	2.0	(1–9)	2.0	(1–6)	0.67
Volume of IV fluids given (l)	3.5	(1–37)	7.0	(1–87)	0.03
No. of days on laxatives	9.0	(1–232)	9.0	(1–63)	0.59
Days from admission to discharge	21.0	(1–286)	23.0	(3–169)	0.41
Days from admission to medically fit for discharge	17.0	(3–136)	17.0	(1–127)	0.45

The use of health care assistants in this specialized role in an acute setting, without change to the food provision or without targeting higher risk patients, reduced the need for intravenous fluids and antibiotics. However the intervention did not improve nutritional status or have an effect on other clinical outcomes in the time span studied. The evidence provided by this trial does not support the use of health care assistants in this role, on Acute Medicine for the Elderly wards. The results highlight the difficulties of improving the intake of sick elderly patients.

Corish CA & Kennedy NP (2000) British Journal of Nutrition **83**, 575–591.
Hickson M, Nicholl C, Bulpitt C, Fry M, Frost G & Davies L (1999) Journal of Human Nutrition and Dietetics **12**, 53–59.
Potter J, Langhorne P & Roberts M (1998) British Medical Journal **317**, 495–501.

Duplicate diet comparison of total fat and fatty acid intake between British Sikhs and Caucasians. By L.M. BRADY, S. LESAUVAGE, C.M. WILLIAMS and J.A. LOVEGROVE, Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, The University of Reading, Reading RG6 6AP

The increased risk for cardiovascular disease (CVD) among Asian Indians living in the UK compared with their Caucasian counterparts may be influenced by dietary factors (McKeigue et al. 1985). Because many foods eaten by the UK Sikh population are not well represented in currently available dietary databases, the aim of the present study was to use the duplicate diet approach to compare the fatty acid intakes of typical Caucasian (n 9, mean age 59.5 years) and Sikh (n 10, mean age 61.8 years) volunteers. Participants were requested to collect a duplicate portion of all foods consumed over a 3 d period, including foods from main meals and all snacks. Total fat was analysed using soxhlet extraction and subsequently the fatty acid content of the diet, including all polyunsaturated fatty acids (PUFA), was determined by gas chromatography.

Although the mean fat intake was higher in Caucasian (67.4 g/d) compared with Sikh (55.1 g/d) volunteers, the difference did not reach statistical significance ($P=0.23$). When the main fatty acid classes were determined, Caucasians were shown to have a significantly higher intake of saturated fatty acids (SFA) than Sikhs (see Figure). No significant differences were reported for intake from the other main fatty acid classes. Intakes of long-chain n-3 PUFA tended to be higher and n-6 PUFA to be lower in Caucasians than Sikhs (see Table and Figure) but differences in the n-6:n-3 PUFA ratio between the two groups Sikhs 8.0 (SE 3.9) v. Caucasians 5.3 (SE 4.0); $P=0.15$, did not reach statistical significance.

Fatty acid	Caucasians (n 9)		Sikhs (n 10)		
	Mean (SEM)		Mean (SEM)		P
EPA (mg/d)	259.3 (77)		115.7 (51)		0.12
DHA (mg/d)	524.1 (115)		257.0 (58)		0.10
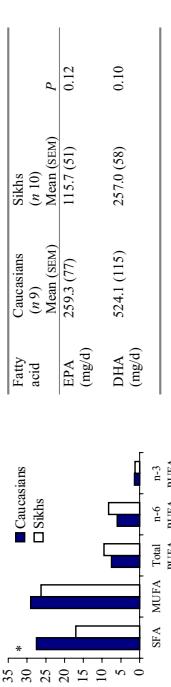
*$P<0.05$

The lower intake of SFA found in the Sikh volunteers is consistent with a previous study in Asian Indians (Miller et al. 1988). Although the present study has shown the mean n-6:n-3 PUFA ratio to be higher in Sikh than Caucasian subjects, unlike our previous study in which PUFA intakes were estimated using diet records, the differences between Sikhs and Caucasians were not found to be statistically significant (Lesauvage et al. 2001). In view of the marked increased risk of CVD in the UK Asian Indian population, further work is needed to verify differences in fatty acid intakes in this ethnic sub-population.

Lesauvage SVM, Lovegrove JA, Minihane AM & Williams CM (2001) Proceedings of the 17th International Congress on Nutrition, Vienna, Austria, 27–31 August 2001.
McKeigue PM, Marmot MG, Adelstein AM, Hunt SP, Shipley MJ, Butler SM, Riemersma RA & Turner PR (1985) Lancet **16**, 1086–1090.
Miller GJ, Kotecha S, Wilkinson WH, Wilkes H, Stirling Y, Sanders TAB, Broadhurst A, Allison J & Meade TW (1988) Atherosclerosis **70**, 63–72.

Web-based quiz program to teach food composition. By A. WISE, *The Robert Gordon University, Queen's Road, Aberdeen AB15 4PH*

Nutrition students need to learn the main features of food composition in order to give suitable dietary advice. 'WinDiets' (formerly 'Diet5') was developed to include a range of educational tools in addition to dietary analysis (Wise, 1998). One of the screens presents a quiz about the composition of foods in which nutritional analyses are shown in bar charts compared to dietary reference values (DRV). Most students now frequently use the Internet to obtain information and this provides many potential advantages for use by nutrition educators (Wise, 1999). Students are unlikely to be able to buy specialist dietary analysis programs, but many of them have computers at home and use them for study. The quiz screen has been adapted for the Internet. Students can easily take it home on disk and others can access it at http://www.rgu.ac.uk/windiets.

A sample screen of the program is shown in the figure. A browser with advanced JavaScript is required, at least version 5 of Internet Explorer or Netscape 6. Foods were classified into eight groups and ten representative foods were chosen for each group. The program contains data on % DRV for fourteen dietary components supplied by 100 g of each food based on estimated average requirements for energy and on reference nutrient intakes for other nutrients by adult males. Analysis of a randomly selected food is presented on screen and students choose from a list. The figure shows how feedback is given when the wrong food is selected. Scores are presented to enhance a competitive spirit. Second-stage students were shown the program in a tutorial and improvements were made after observing how they used it. The altered program was used in a tutorial by a second group of students. Sixteen of the students completed an anonymous questionnaire about their attitudes to the program. Most (*n* 15) found it user-friendly and agreed that they were more motivated to learn by having the score for their attempts than having no score (*n* 14). Fewer agreed that a quiz enhanced their motivation (*n* 11) and some (*n* 6) would prefer to do a quiz in class so that they compete with other students. There was little agreement with the proposition that they would prefer to learn about nutritional composition from a book rather than a computer (*n* 1), but most (*n* 9) were neutral to this attitude. There was overwhelming disagreement (*n* 15) with a statement that 'food composition is such a simple subject that it does not deserve time in an Honours nutrition course'.

Research is required to further develop programs that can be applied to their education. Collaborative research in this area could better establish how students learn. For example, in a simple study during the second tutorial, the number of puzzles students attempted per minute was counted and students differed widely. Some students appeared to improve their score during the tutorial and others did not. More research is needed to help improve the strategies for learning.

Wise A (1998) *British Journal of Nutrition* **79**, 547–550.
Wise A (1999) *Association for Learning Technology Journal* **7**, 46–56.

Body composition and resting energy expenditure in a small group of young professional footballers. By A.L COLLINS and H.D McCARTHY, *School of Health and Sports Science, University of North London, 166–220 Holloway Road, London N7 8DB*

In the football industry, over recent years, the development and nurturing of young talent has become a major priority for lower league clubs keen to survive and progress. Body composition is one area that has a bearing on general fitness, and changes within and between seasons may affect player's performance. Resting energy expenditure can also provide useful information about the energy needs of individuals and help to ascertain dietary strategies. In this small study, twenty-two young professional footballers from 1st, 2nd and 3rd division youth and first teams in England were assessed for body composition using the BodPod measurement system, as well as via anthropometry at seven sites. Resting metabolic rate was measured using open-circuit indirect calorimetry after an overnight fast and 24 h since exercise.

	Mean	Range
Age	18.1	16–23
Height (cm)	180.5	169.1–189.4
Weight (kg)	74.2	59.9–84.5
BMI (kg/m)	22.6	19.6–25.0
% Body fat (Siri)	9.7	4.4–20.0
FM (kg)	7.2	3.7–14.9
FFM (kg)	67.0	53.1–80.9
REE (kJ/day)	8442	7330–10420

FM, fat mass; FFM, fat-free mass; REE, resting energy expenditure.

The mean percentage body fat reported was 9.7%, which compares favourably with other studies in youth players (Franks *et al.* 1999). However, using multi-site skinfolds, mean body fat was 14.7%, which was significantly higher than that derived from the BodPod ($P<0.001$). One reason for this could be the inappropriateness of the predictive equations of density from skinfolds in athletes, perhaps due to the assumed fixed proportion of internal to external fat. One suggestion is that skinfolds may be used predominantly as an index of subcutaneous fat in athletes rather than a predictor of total body fat (Telford *et al.* 1988). Densitometric determination of total body fat (using the BodPod) may not be entirely appropriate without taking into account the possibility of increased bone mineral density due to high amounts of weight-bearing exercise (Wittich *et al.* 1998) This has been demonstrated in soccer players (Calbet *et al.* 2001) and may explain the extremely low body fat values obtained. Percentage body fat may be underestimated if a two-compartment view is assumed, i.e. using the BodPod. Nevertheless, the BodPod provides a useful way of assessing total body fat repeatedly over time; for example, within and between seasons, due to its practical advantages over underwater weighing.

Resting energy expenditure (REE) in this group was found to be significantly greater than predicted REE ($P=0.012$), derived from height, weight and age (Mifflin *et al.* 1990). This may be due to a large proportion of muscle mass in these players, which will contribute substantially to REE (Martin, 1993). In addition, skeletal muscle in athletes such as footballers may be metabolically more active (per kg), elevating REE further (Sparti *et al.* 1997). If REE is predicted from FFM using the equation of Wang *et al.* (2000), underestimation still occurs, but this difference is not significant ($P=0.08$). This highlights the importance of measuring resting energy expenditure in athletes rather than predicting it, unless you have information on muscle mass or at least FFM (Wang *et al.* 2000). The observed high REE will be of significant importance as it will correspond to an elevated energy requirement, probably in the region of 12.5–16.7 MJ/d which should be recognized when considering dietary adequacy of individual players.

Calbet JAL, Dorado C, Diaz-Herrera P & Rodriguez-Rodriguez LP (2001) *Medicine and Science in Sports and Exercise* **33**, 1682–1687.
Franks AM, Williams AM, Reilly T & Nevill A (1999) *Journal of Sports Science* **11**, 3–8.
Martin AD (1993) *Journal of Sports Science* **17**, 812.
Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA & Koh YO (1990) *American Journal Clinical Nutrition* **51**, 241–247.
Sparti A, DeLany JP, de la Bretonne JA, Sander GE & Bray GA (1997) *Metabolism* **46**, 1225–1230.
Telford RD, Egerton WJ, Hahn AG & Pang PM (1988) *Excel* **5**, 21–26.
Wang Z, Heshka S, Gallagher D, Boozer CN, Kotler DP & Heymsfield SB (2000) *American Journal of Physiology* **279**, E539–E545.
Wittich A, Mautalen CA, Oliveri MB, Bagur A, Somoza F & Rotemberg E (1998) *Calcified Tissue International* **63**, 112–117.

Capillary electrophoresis as a tool in urinary profiling: application to a nutritional disorder. By C. GULLO[1], D. PERRETT[2], V. PREEDY[3] and M. HANNA-BROWN[1], [1]*Department of Pharmacy, King's College London, Franklin-Wilkins Building, 150 Stamford Street, Waterloo, London SE1 9NN,* [2]*Dept Medicine, St. Bartholomew's and The Royal London School of Medicine and Dentistry, West Smithfield, London EC1A 7BE and* [3]*Department of Nutrition and Dietetics, King's College London, Franklin-Wilkins Building, 150 Stamford Street, Waterloo, London SE1 9NN*

Capillary electrophoresis (CE) is a widely accepted and useful tool in the separation science arena. The technique encompasses a range of modes allowing for the separation and identification of a wide variety of molecules. One mode of CE which has found numerous applications is that of micellar electrokinetic capillary chromatography (MECC) which utilizes micellar electrolytes and allows for the separation of positive, negative and neutral moieties in one analysis. This paper describes how MECC methodology combined with UV diode array detection at low wavelength has been developed in order to separate a wide range of small unknown urinary components and to assess the potential for a non-invasive diagnostic tool which will provide a rapid indication of metabolic disorders, or abnormalities.

The methodology has been applied to urine samples collected from glucose-fed (control) and ethanol-fed rats in order to ascertain the effect of chronic alcohol administration on the urinary profile. In this study, six ethanol-fed rats were paired with six control rats (same initial body weight, identical diet composition/amount). Urine samples collected were analysed by MECC on a Beckman P/ACE 5510 instrument with UV diode array detection, using a 47 cm capillary (50 μm internal diameter, 40 cm to the detector window), an electrolyte consisting of 25 mM sodium tetraborate containing 75 mM SDS and 6.25 mM sulphated-β-cyclodextrin, pH 9.50, 20°, 18 kV applied voltage and an 8-s hydrodynamic injection of filtered urine. Profiling and identification of the small endogenous urinary components was achieved by virtue of spectral data matching using a 'normal' urinary component UV spectrum database.

Over forty analytes could be detected in rat urine at 195 nm and the results obtained showed the profiles to be significantly different in the ethanol-fed rats. Seven known urinary metabolites were quantified and comparisons between the mean urinary levels (total excretion (mg)/24 h) of urea, creatinine, uracil, phenylalanine, allantoin, hippuric acid and uric acid in control and ethanol-fed rat urine samples are shown in the Table.

	Glucose-fed rats (n 6)	Ethanol-fed rats (n 6)	P
Urea	283.31	236.30	0.525
Creatinine	4.60	3.58	0.064
Uracil	0.47	0.48	0.311
Phenylalanine	3.22	3.41	0.033
Allantoin	28.62	20.30	0.200
Hippuric acid	0.44	0.46	0.334
Uric acid	1.77	0.66	0.005

Statistical analysis of the data (Student's *t-test* for paired samples, two-tailed distribution) was performed in order to assess the differences between means. The results obtained showed the level of uric acid to be significantly decreased (*P*<0.01) with urinary excretion of phenylalanine significantly increased (*P*<0.04) in the ethanol-fed rats compared with the controls. Previously, it has been shown (Preedy *et al.* 1991) (via NMR analyses) that there is a significant increase in uric acid and urea levels and a slight decrease in creatinine levels in ethanol-fed rats compared with controls. It will be interesting therefore to investigate the levels of these components in the samples used in this study by NMR in order to investigate the correlation between the results from each technique. However, it can be concluded at this stage that the CE technique offers many advantages over NMR, such as the required sample volume (<10 nl as compared with 1 ml for NMR), the minimal sample preparation (filtering only as compared with freeze-drying for NMR) and the minimal cost of electrolytes (aqueous buffers as opposed to expensive deuterated solvents for NMR). Such methodology should prove invaluable in the rapid comparison of urinary profiles and indication of metabolic disorders or 'abnormalities'.

Preedy VR, Hammond B, Iles RA, Davies SEC, Gandy JD, Chalmers RA & Peters TJ (1991) *Clinical Science* **80**, 393–400.

Association between nutritional anthropometric parameters and vitamin A status of neonates at birth in Varamin, Iran. By A. DIAZAYERY[1], A.A. SOHEILI[2] and M. DIALALI[1], [1]*Department of Nutrition and Biochemistry, School of Public Health and Institute of Public Health Research,Tehran University of Medical Sciences. O. Box 14155-6446, and* [2]*Abu-Reihan College,Ghods Street, Tehran, Iran*

Vitamin A plays important roles in vision, growth, and development, as well as in immunity and protection of the body against infection and the general health of man. Vitamin A deficiency is associated with increased morbidity and morbidity (Ross & Hammerling, 1994). Even a mild deficiency with no apparent or detectable undesirable effects on vision can increase risk of diseases such as measles, pneumonia, and diarrhoea and of death in infants and children. A knowledge of vitamin A status of infants at birth can help in designing strategies aimed at reducing morbidity and mortality during subsequent growth. Dietary vitamin A supplementation can reduce risk of death in children by one-third. The available information on the vitamin A status of infants at birth and its association with their nutritional status is meager. The objective of this study was to determine the nutritional (anthropometric) status of neonates at birth and its association with their vitamin A (plasma retinol) status in Varamin, a small town in the south of Tehran, Iran.

A total of 226 randomly selected neonates born during April and May 1999 in the only hospital of Varamin, were included in the study. Their nutritional status was determined anthropometrically (weight and length) using standard techniques. In addition, placental blood samples were taken for haemotological and fluorometric retinol (Thompson et al. 1971) determinations. The anthropometric reference standards used were the US National Centre for Health Statistics (NCHS) standards, and vitamin A deficiency was defined as a plasma retinol concentration <20 mcg/dl, a marginal and severe deficiency being 10–20 and <10 mcg/dl, respectively. The SPSS software was used for data analysis.

The results showed that:

1. 2.4% and 24.1% of the neonates were severely and marginally deficient in vitamin A, respectively. The proportion of deficient boys (37.3% of which 3.2% were severely deficient) was nearly twice that of girls.

2. The average body weights and lengths were 98.4% and 102.0% of the respective standards. However, distributions show that 5.0% of the girls and 10.4% of the boys were malnourished on the basis of body weight. The corresponding values on the basis of length were 6.0% and 11.3%. The proportion of low birthweight (LBW) was 5.0% in girls and 10.4% in boys.

3. The LBW infants' serum retinol level (19.5±8.1mcg/dl) was significantly lower than that of normal weight babies (26.7%±9.4mcg/dl, *P*<0.01).

4. The proportions of the lymphocytes and eosinophils, which increase in infection, and in the formation of which vitamin A plays a role, are significantly higher in the LBW infants (*P*<0.04), while the polymorphonuclear count was lower in the former group (*P*<0.01).

5. Regression analysis of the data showed that there exists a statistically significantly positive association only between the placental plasma vitamin A level and birthweight, the regression equation being birthweight=3.02+0.096retinol; no other variable showed a relation with the vitamin. Considering that LBW% is about 8 in the country and that PEM has been shown to be associated with vitamin A deficiency (Torun & Chew, 1999), strategies should be planned and programmes designed to improve the vitamin A status of neonates at birth, namely, increase the vitamin A intake of pregnant women.

Ross AC & Hammerling UG (1994) Retinoids and the immune system. In *The Retinoid Biology, Chemistry and Medicine*, 2nd ed., pp.521–524 [MB Sporn, AB Roberts and DS Goodman, editors]. New York: Raven Press.
Thompson JN, Erddoy P & Brien R (1971) *Biochemistry and Medicine* **5**, 67–89.
Torun B & Chew F (1999) Protein-energy malnutrition. In *Modern Nutrition in Health and Disease*, 9th ed., p. 980 [ME Shils, JA Olson, M Shike and AC Ross, editors]. Philadelphia: Lippincott Williams and Wilkins.

Iron status, diet and cognitive ability in schoolboys aged 11–14 years. By C.S. WILLIAMSON, H. AL-HAMAD and M. NELSON, *Department of Nutrition and Dietetics, King's College London, 150 Stamford Street, London SE1 9NN*

Previous studies in infants and children have shown that iron deficiency anaemia (IDA) is associated with impaired performance in tests of mental development and learning ability (Pollitt, 1993). Recent research in British adolescent girls has shown that poor iron status, even in the absence of anaemia, is associated with lower scores in tests of cognitive function (Nelson *et al.* 2001). The present study set out to investigate whether poor iron status in 11–14-year-old boys has a significant detrimental effect on cognitive ability.

A group of 320 boys aged 11–14 attending two South London schools were asked to complete a questionnaire to assess diet and eating habits (such as vegetarianism, use of vitamin and mineral supplements and dieting to lose weight), ethnic origin and social class background. They provided a single finger prick blood sample for analysis of haemoglobin (Hb) and packed cell volume (PCV) to determine their iron status. Then 137 boys from the top and bottom thirds of the distribution of Hb and PCV values were asked to provide venous blood samples to confirm their iron status. Of 121 subjects who gave venous blood samples, twenty-eight were classified as having good iron status (Hb <12.5 g/dl *and* serum ferritin (SF) <20 µg/l), thirty-three were classified as borderline (Hb <12.5 g/dl *or* SF <12.5 g/dl *and* SF >20 µg/l) and sixty were classified as borderline (Hb <12.5 g/dl *or* SF <20 µg/l). The prevalence of IDA in the total sample was 8% (Hb <12 g/dl) while 20% were found to have iron deficiency (based on SF <20 µg/l). Diet and eating habits (vegetarianism, use of vitamin and mineral supplements and dieting to lose weight) were not found to have a significant effect on Hb and PCV values.

Cognitive ability was assessed using the six school age core scales of the British Ability Scales Second Edition (BAS II). Three separate composite scores were calculated for each subject, reflecting their verbal ability, non-verbal reasoning and spatial ability and a general conceptual ability (GCA) score reflects their overall cognitive functioning. In total sixty-eight subjects completed BAS tests; it was found that boys classified as having poor iron status achieved overall GCA scores that were, on average, 7 points lower than boys with good iron status. Analysis of composite scores showed that pupils with poor iron status scored significantly lower than boys with either good or borderline iron status on the tests of spatial ability.

	n	Verbal ability		Non-verbal reasoning		Spatial ability		Overall GCA score	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Iron status									
Poor	24	107.5	11.3	111.9	9.1	102.0	11.3	108.7	9.7
Borderline	13	108.8	12.6	116.5	10.6	108.9	10.4	113.5	11.7
Good	31	109.4	9.8	118.3	12.9	111.4	15.3	115.5	12.7
P*		0.812		0.119		0.036		0.101	

* Statistically significant differences assessed by one-way analysis of variance.

Hb and PCV values were found to be significantly associated with non-verbal reasoning scores (P 0.045 and P 0.038), spatial ability (P <0.001 and P 0.001) and overall GCA scores (P 0.008 and P 0.015). Finally, when age, social class and ethnic background were taken into account in an analysis of variance, it was found that iron status remained a significant independent influence on non-verbal reasoning (P 0.02) and spatial ability scores (P 0.035). The influence of iron status on overall GCA scores was found to have borderline significance (P 0.055).

In conclusion, poor iron status is common amongst 11–14 year old boys in London and iron deficiency, even in the absence of clinical signs of anaemia, has a significant effect on cognitive performance in the areas of non-verbal reasoning and spatial ability. This effect is independent of factors such as social class, age and ethnic background. This finding has implications with regard to educational achievement in adolescent boys and suggests that further investigation is warranted.

Nelson M, Ash R, Mulvihill C, Peters TJ & Rogers P (2001) *Proceedings of the Nutrition Society* **60**, 59A.
Pollitt E (1993) *Annual Review of Nutrition* **13**, 521–537.

Malondialdehyde and acetaldehyde protein adducts in heart in response to acute alcohol exposure assessed by immunohistochemistry. By O. NIEMELA[1], S. PARKKILA[1], P.W. EMERY[2] and V.R. PREEDY[2], [1]*Department of Clinical Chemistry, Anatomy and Cell Biology, University of Oulu, FIN-90220 Oulu, Finland, and EP Central Hospital Laboratory, Seinajoki, FIN 60220, Finland and [2]Department of Nutrition and Dietetics, King's College London, 150 Stamford Street, London SE1 9NN*

Heart muscle disease is a pathological response to excessive alcohol ingestion. The mechanisms are unknown but recent attention has focused on the possibility that they may involve the post-translational modification of proteins by aldehydes to form adducts. Protein-adduct formation may have important implications for the pathogenesis of alcoholic cardiomyopathy because affected proteins may have altered functional properties or turnover rates or may act as neoantigens.

We measured protein adducts by immunohistochemistry in the hearts of rats given a single intraperitoneal injection of ethanol (75 mmol/kg body weight) or saline (0.15 mol/l NaCl). To increase acetaldehyde concentration further, half the rats were given an intraperitoneal injection of cyanamide (0.05 mmol/kg body weight), an aldehyde-dehydrogenase inhibitor, 30 min before the alcohol injection. Thus there were four groups of rats: [I] saline + saline; [II] cyanamide + saline; [III] saline + alcohol; [IV] cyanamide + alcohol. Rats were killed 2.5 h after the alcohol injection and hearts were dissected, fixed in formalin, embedded in paraffin and subjected to immunohistochemical staining using polyclonal antibodies to acetaldehyde- (AA) and malondialdehyde- (MDA) protein adducts (Niemela *et al.* 1994). Antisera against AA-protein adducts were raised by injecting rabbits with bovine serum albumin that had been conjugated with 1 mM acetaldehyde under reducing conditions. Antisera to MDA-protein adducts were raised by injecting rabbits with human low-density lipoprotein that had been conjugated with 0.5 mM MDA. The intensity of the staining was scored on a scale 0 (no reaction) to 5 (strong reaction) independently by two investigators (O.N. and S.P.). Mean values and results of two-way analysis of variance are shown in the Table.

Group	I	II	III	IV	Pooled SE	Anovar Alcohol	Anovar Cyanamide	Anovar Interaction
Number of rats	8	7	8	8				
MDA adduct score	0.88	1.32	1.72	1.97	0.26	P<0.01	NS	NS
AA adduct score	1.72	1.50	1.69	1.91	0.20	NS	NS	NS

NS: Not significant (P>0.05).

Alcohol caused an acute increase in MDA-adduct levels in the heart, but cyanamide pre-treatment did not significantly potentiate this. Neither alcohol nor cyanamide affected AA-adduct levels. MDA-adducts are likely to have need formed as a result of peroxidation of polyunsaturated fatty acids. These data underline the importance of free radicals in mediating the damage caused by acute alcohol ingestion.

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

Measuring changes in serum iron concentration as an indicator of dietary iron absorption. By R. CONWAY[1], J. POWELL[1], R. HIDER[2], R. THOMPSON[3] and C. GEISSLER[1], [1]Department of Nutrition and Dietetics and [2]Department of Pharmacy, King's College London, 150 Stamford Street, London SE1 9NN and [3]Gastrointestinal Laboratory, The Rayne Institute, St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH

Changes in serum iron have been measured in the past as an indicator of iron status and of absorption from iron supplements (Dietzfelbinger, 1987). However incorporation of radio- or stable Fe isotopes into Hb is preferred for measuring absorption of dietary iron. The serum iron technique is thought to be too insensitive for measuring dietary Fe uptake (Hurrell, 1997) but has not been fully studied. However Crosby and O'Neil-Cutting's (1984) use of this method with small doses of Fe (5–20 mg) encouraged us to apply the technique to dietary iron absorption in women with low iron status.

Anaemic volunteers were recruited for the study (Hb <12.0 g/dl and serum ferritin <20 µg/l). After an overnight fast they were fed test meals with 10 mg Fe added as FeCl$_3$. Blood samples were then drawn every 30 min for 4 h. The effect on the increase in serum iron of feeding meals with varying quantities of promoters and inhibitors of non-haem iron absorption was investigated. Ten volunteers were each fed four test meals, predicted to have decreasing bioavailability. The meals were composed of combinations of vegetable shepherd's pie, fruit salad, orange juice, wholemeal bread, lentils and tea.

Test meal	Meal composition	Max increase in serum Fe (µmol/l)		Area under curve	
		Mean	S.D.	Mean	S.D.
A	Pie, fruit & juice	13.6[a]	6.24	1941[a]	1221
B	Pie, bread & water	6.0[b]	2.48	959[b]	498
C	Pie with lentils, bread & water	4.7[b]	1.89	712[b]	812
D	Pie with lentils, bread & tea	2.8[c]	1.82	276[c]	396

Pairs of meals not showing the same superscript letter were significantly different ($P<0.05$) using Wilcoxon's test.

The decreasing absorption for meals A to D is observed in clearly distinguished serum iron curves and is in agreement with absorption predicted from the results of radioisotope studies which have shown vitamin C to promote Fe absorption and phytate and polyphenols to inhibit absorption (Hurrell, 1997).

To further test the validity of the method, eight volunteers were given test meals in which the 10 mg added Fe included 2–6 mg [58]Fe. Iron absorption was then estimated in the standard way, by measuring the concentration of [58]Fe in erythrocytes at baseline and again 2 weeks after consuming the meal, assuming 80% erythrocyte incorporation of absorbed Fe (Hurrell, 1997). Percentage Fe recovery was calculated from the maximum increase in serum Fe concentration following the meal and estimated total serum volume for each individual and from area under the curve (AUC). Good correlations were found between percentage Fe absorption estimated using [58]Fe and both percentage recovery (r 0.82, P 0.014) and AUC (r 0.73, P 0.041).

These results suggest that the serum iron technique may provide a valid alternative to methods involving Fe isotopes, with the advantages of being cheaper and using more easily available laboratory resources.

We gratefully acknowledge the work of Mark Sykes, Wendy Clarke, Elizabeth Tait and Hashim Mohammed, and financial support from the Food Standards Agency.

Crosby WH & O'Neil-Cutting MA (1984) Journal of the American Medical Association **251**, 1986–1987.
Dietzfelbinger H (1987) Arzneimittel-Forschung/Drug Research **37**, 107–112.
Hurrell RF (1997) European Journal of Clinical Nutrition **51** (Suppl), S4–S8.

Reticulocyte incorporation of iron: a new method for measuring iron absorption in humans. By B. SARRIA, K. IVORY, T.E. FOX, J.R. DAINTY, J. EAGLES, J. HOOGEWERFF and S.J. FAIRWEATHER-TAIT, Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA

Iron deficiency anaemia is the most common nutritional problem worldwide, mainly caused by low dietary bioavailability. A well-established method of assessing iron bioavailability is the incorporation of stable iron isotopes, used as a label for native food iron, into erythrocytes. Simultaneous intravenous administration of a different isotope provides information on erythrocyte incorporation (Barrett et al. 1992). The method generally requires high doses of isotopes to enrich the circulating red cells sufficiently.

In order to reduce the dose of isotope required, an alternative method was tested. This method is based on the high rate of utilization of administered iron isotopes by reticulocytes (young erythroid cells). About 80% of newly absorbed iron is incorporated into red blood cells within 2 weeks of administration (Bothwell et al. 1979), but reticulocytes only form 0.5–2% of the red blood cell population; thus by isolating reticulocyte-rich fractions of blood it should be possible to obtain higher isotopic enrichment in blood samples. Van den Heuvel et al. (1998) used a density separation method to obtain reticulocyte-rich cell fractions, achieving athreefold isotope enrichment. However, this method is laborious and cumbersome. The aim of the present study was to compare iron absorption data determined by the erythrocyte incorporation method with data derived from an improved reticulocyte separation method.

On day 1 of the study, 9 healthy female volunteers, aged between 30 and 40 years, were given 5 mg of [57]Fe as ferrous sulphate with ascorbic acid (1:3 molar ratio) in an oral dose and 200 µg of [58]Fe (ferrous citrate) intravenously over 90 min. On days 11, 15, 19, 23 and 27, blood samples were taken. Reticulocyte-rich samples of whole blood (1 ml) was prepared by lysing the majority of red blood cells and centrifuging the resultant solution to separate the fraction containing primarily reticulocytes. Sample preparation involved microwave digestion (whole blood), and HNO$_3$ and H$_2$O$_2$ digestion (reticulocytes). Iron was extracted in all samples using diethyl ether (Roe & Fairweather-Tait, 1999). Enrichment of oral and intravenous iron stable isotopes was measured in both reticulocyte-rich and whole blood samples using a multicollector inductively coupled mass spectrometry. True absorption of the oral dose in reticulocyte-rich blood and whole blood was calculated by correcting for red cell incorporation using results from the intravenous dose. Table 1 shows the % absorption of iron obtained using the reticulocyte separation method (R) and the erythrocyte incorporation method (E) in 9 volunteers.

	1	2	3	4	5	6	7	8	9
	R	E	R	E	R	E	R	E	R	E	R	E	R	E	R	E	R	E
	38	36	41	36	52	47	48	32	50	57	30	29	20	15	25	20	38	28

According to a Student t test (P=0.056) there is a significant difference between the methods. The Pearson correlation coefficient is 0.87. More work is required to evaluate the new method before it can be considered an alternative to the erythrocyte incorporation technique.

Barrett JFR, Whittaker PG, Williams JG & Lind T (1992) Journal of Clinical Science **83**, 213–219.
Bothwell TH, Charlton RW, Cook JD & Finch CA (1979) In: Iron Metabolism in Man, pp. 327–335. Oxford: Blackwell Scientific.
Roe M & Fairweather-Tait SJ (1999) Forms and Bioavailability of Iron in Fortified Cereal Products. Final Project Report. MAFF Project ANO541, March 1999.
Van den Heuvel E, Muys T, Pellegrom H, Bruyntjes JP, Van Dokkum W, Spanhaak S & Schaafsma G (1998) Clinical Chemistry **44**, 649–654.

Assessing potential iron bioavailability in transgenic plants that over-express phytase. By S.

PARIAGH[1], G. DRAKAKAKI[2], E. STOGER[2], E.K. LUND[1], P. CHRISTOU[2] and S.J. FAIRWEATHER-TAIT[1], [1]*Nutrition and Consumer Science Division, Institute of Food Research, Norwich Research Park, Norwich NR4 7UA and* [2]*Molecular Biotechnology Unit, John Innes Centre, Norwich NR4 7UH*

Nutritional iron deficiency anaemia affects more than 500 million people worldwide, and is primarily a consequence of low dietary iron bioavailability. Phytate, common in foods derived from plant material, reduces iron absorption; therefore, an obvious strategy to improve iron bioavailability and hence reduce the prevalence of anaemia is to enhance the expression of phytase in crop plants. Particle bombardment was used to generate maize and wheat cultivars that over-express phytase (Christou, 1995), and their potential iron bioavailability was assessed.

Seeds of wild type (WT) and transgenic (T) wheat and maize were each ground, soaked, simmered (to cook), and then subjected to a simulated gut digestion with pepsin followed by pancreatin-bile. Prior to digestion, radiolabelled ^{55}Fe (200 nmol/l) was added to each food. A two-tier chamber system containing a dialysis membrane (Glahn *et al.* 1996) was employed to allow separation of soluble iron from each digest. The level of ^{55}Fe-dialysate, in the lower chamber, was used as a measure of iron accessibility and so potential bioavailability.

Dialysable iron from maize and wheat with increased expression phytase
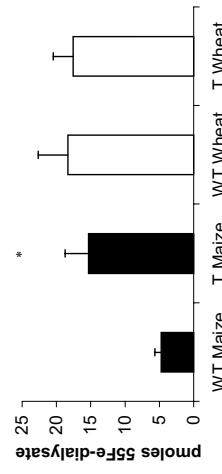
After 2 h the level of ^{55}Fe-dialysate was significantly higher (P<0.02) for transgenic maize digests, 4.75 (SE 0.91) *v.* 15.33 (SE 3.41). However, no significant difference was observed for wheat. This difference in dialysability reflects the more effective increase in expression of phytase in the maize (~10% of total soluble protein) as compared with the wheat (~1% of total soluble protein). Enhanced expression of phytase in the maize cultivars holds promise for enhancing iron bioavailability.

Christou P (1995) *Methods in Cell Biology* **50**, 375–382.
Glahn RP, Wien EM, Van Campen DR & Miller DD (1996) *Journal of Nutrition* **126**, 332–339.