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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

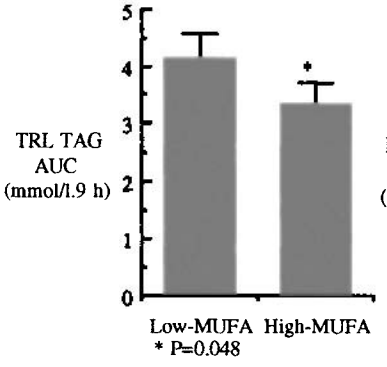
A Scientific Meeting was held at the University of Galway, on Wednesday–Saturday, 6–9 September 1995, when the following papers were presented.

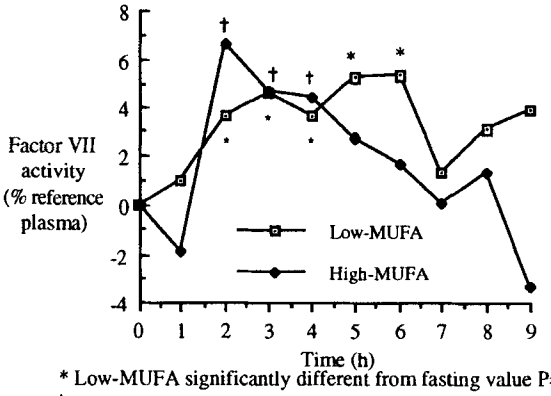
Postprandial triacylglycerolaemia and coagulation factor VII activity following meals containing varying proportions of olive oil. By H.M. ROCHE and M.J. GIBNEY, *Department of Clinical Medicine, Trinity College Medical School, St. James's Hospital, Dublin 8, Republic of Ireland*

The Mediterranean-type diet, which is rich in olive oil, has been proposed to account in part for the lower incidence of coronary heart disease (CHD) in southern Europe. However, the biochemical basis of this association remains elusive. The magnitude and duration of postprandial triacylglycerolaemia has been related to the pathogenesis and progression of atherosclerosis (Karpe *et al.* 1994). Coagulation factor VII is activated during postprandial triacylglycerolaemia and it has been proposed that meals containing less saturated fat cause least activation of factor VII during postprandial triacylglycerolaemia (Mitropolous *et al.* 1995). The present experiment investigated the effect of meals providing various levels of monounsaturated fatty acids (MUFA), derived from olive oil, on the postprandial lipaemic and thrombotic response.

The postprandial response to two meals providing an equal amount of fat (40 g) but of different MUFA levels (12 and 24% energy) was investigated in fifteen healthy, normolipaemic males. Subjects received the test meals randomly following an overnight fast and blood samples were drawn every hour for 9 h.

Postprandial plasma triacylglycerol (TAG) concentrations were significantly ($P \leq 0.0001$) elevated but there was no significant difference between meals. The area under the curve (AUC) of the postprandial TAG-rich lipoprotein (TRL) fraction was significantly ($P = 0.048$) lower following the high-MUFA meal, as illustrated in the histogram below. Coagulation factor VII activity was significantly increased ($P = 0.011$) following all meals, but rapidly returned to fasting levels following the high-MUFA meal compared with the low-MUFA meal, as illustrated below.





* Low-MUFA significantly different from fasting value P=0.05.
† High-MUFA significantly different from fasting value P=0.05.

In conclusion, these results suggest that the postprandial factor VII activity response is shortened following a MUFA-rich test meal. This may be due to reduced TRL TAG concentration or to the different composition of the TRL formed following the ingestion of greater amounts of olive oil.

Karpe, F., Steiner, G., Uffelman, K., Olivercrona, T. & Hamsten, A.. (1994). *Atherosclerosis* **106**, 83-97.

Mitropoulos, K.A., Miller, G.J., Martin, J.C., Reeves, B.E.A. & Cooper, J. (1995). *Arteriosclerosis Thrombosis* **14**, 214-222.

The effects of fish-oil supplementation on lipoprotein composition and structure. By J.D. BELL[1], M.L. BARNARD[1,2], P.D. DAGNELIE[1], E.L. THOMAS[1] and S. CUNNANE[1]. *[1] The Robert Steiner MR Unit and [2]Division of Endocrinology & Metabolism, Hammersmith Hospital, Du Cane Road, London W12*

In recent years there has been increasing interest in the effects of dietary fish oil on plasma lipid metabolism. The main effect of dietary fish oil appears to be a lowering of total plasma triacylglycerol and VLDL concentrations. Several reports have shown a significant rise in the cholesterol: triacylglycerol ratio in VLDL and LDL, suggesting smaller lipoprotein particles (Nestel, 1990). Furthermore, dietary fish oil appears to induce changes in the thermal transition of LDL particles, suggesting alteration in the physical state of lipoprotein particles (Nenster *et al*, 1991). To date however, the effects of fish oil on the physical characteristics of plasma lipoproteins have not been fully investigated.

High-resolution nuclear magnetic resonance (NMR) spectroscopy is a non-destructive technique which is finding increasing favour in metabolic studies. The purpose of the present study was to examine the effects of dietary fish oil on the physico-chemical status of plasma lipoproteins using NMR spectroscopy.

Nine healthy volunteers consumed fish oil containing 14.5g ω-3 fatty acids/d. Plasma lipoprotein fractions were isolated by sequential flotation ultracentrifugation. Total plasma triacylglycerol, cholesterol, LDL- and HDL-cholesterol concentrations were determined enzymically. Total fasting plasma and LDL eicosapentaenoic acid (EPA;C20:5*n*-3) and docosahexaenoic acid (DHA;C22:6*n*-3) concentrations were determined by GLC. ^{13}C and ^{1}H NMR was carried out on a JEOL GSX500 spectrometer.

	Total triacylglycerol*		Total cholesterol*		LDL:HDL cholesterol		Total fatty acids (g/100g) EPA		DHA	
Subjects	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	0.77	0.22	4.55	0.49	2.22	0.87	1.06	0.15	2.64	0.39
7d fish oil	0.54	0.17	4.14	0.22	1.98	0.95	14.23	2.49	5.57	0.48
Significance	$P < 0.03$		NS		NS		$P < 0.001$		$P < 0.001$	

* measured in [mmol/l]

Resonances not previously observed in ^{13}C and ^{1}H spectra of plasma and isolated lipoproteins were detected following fish-oil ingestion. The ^{13}C resonances, centred at 14.3, 127.1 and 131.6 ppm, have been assigned to specific groups ($\underline{C}H_3$-CH_2-CH=CH-, CH_3-CH_2-CH=$\underline{C}$H-CH_2-, CH_3-CH_2-$\underline{C}$H=CH-CH_2- respectively) in EPA and DHA. The new lipid resonance observed in the ^{1}H spectra of plasma (0.941 ppm) is consistent with the incorporation of these ω-3 fatty acids into lipoprotein particles. The presence of increased EPA and DHA in lipoprotein fractions was confirmed by GLC. A marked reduction in the methylene signal intensity from VLDL was also observed with fish oil. This reduction arises from a decrease in plasma triacylglycerol concentration (about 30%) and a possible reduction in the number of VLDL particles. Transverse relaxation (T_2) studies of isolated lipoprotein (VLDL and LDL) showed significant elevation in the T_2 of the -$(CH_2)_n$- and CH_3-signal from non ω-3 fatty acids. The relaxation characteristics and signal intensity of the novel ^{1}H peak point to the existence of ω-3-enriched microenvironments within lipoprotein particles. These findings suggest that incorporation of EPA and DHA into VLDL and LDL, after fish-oil ingestion, leads to significant alteration in the molecular architecture of lipoprotein particles.

Nenseter, M.S., Rustan, A.C., Lund-Katz, S., Soyland, E., Maelandsmo, G., Phillips, M.C. & Drevon, C.A. (1991). *Atherosclerosis Thrombosis* **12**, 369-379.

Nestel, P.J. (1990). *Annual Review of Nutrition* **10**, 149-147.

Lipid and carbohydrate utilization in a diet-induced model of obesity. By BEATRIZ BERRAONDO, F.I. MILAGRO, RAQUEL PEREZ, MARIA A. ZULET and J.A. MARTINEZ, *Department of Physiology and Nutrition, University of Navarra, 31008 Pamplona, SPAIN.*

Substrate utilization is influenced by food intake and diet composition (Schutz, 1993). Thus, much research has been performed in different models of diet-induced obesity involving metabolic fuel selection; however, many aspects of substrate interaction remain unclear. The aim of the present experimental trial was to study the carbohydrate and fat balance in cafeteria-fed animals by measuring several metabolic pathways through different stationary and dynamic indices. A group of Wistar female rats weighing about 160 g (4 weeks-old), after a acclimatation period, were divided in two groups: one (control) was fed on a standard laboratory diet and the other (obesity model) was fed additionally with palatable foods as described elsewhere (Rozen *et al.* 1994) for 4 weeks. The measurements that were carried out in all the experimental animals included body and liver weights, body composition (EM-SCAN Model SA-2), fat O_2 consumption (YSI Model 5300 biological oxygen monitor), CO_2 production (Torgan *et al.* 1990), enzyme activities (glucokinase: EC 2.7.1.2) and basal lipolysis through glycerol release (Martínez *et al.* 1995) by conventional validated methods.

	Control (*n* 8)		Obesity model (*n* 8)		
	Mean	SE	Mean	SE	*P*
Final body weight (BW; g)	252.0	3.40	275.0	5.30	0.0014
Liver weight (g)	6.6	0.20	8.1	0.50	0.0079
Liver glycogen (mg/g)	3.2	0.05	10.4	0.23	0.0105
Liver glucokinase (nmol/g per min)	4.3	2.24	9.5	4.30	0.0041
Fat content (%BW)	5.5	0.67	10.0	0.72	0.0004
Fat O_2 consumption* (μl O_2/g per min)	0.47	0.02	0.40	0.03	0.0470
Basal Lipolysis (μmol glycerol/100 mg)	0.13	0.01	0.09	0.01	0.0501
CO_2 production (ml/g BW per h)	1.56	0.02	1.43	0.02	0.0008

*n 5 *P indicates significance of difference groups, t test*

As expected, body and liver weights were increased in the cafeteria-fed animals compared with controls, although the relative liver weight (g/ g BW) remained unaltered. Fuel reserves such as liver glycogen and body-fat content were higher in the dietary model of obesity. Liver glucokinase, an enzyme involved in glycolysis but also in the synthesis of glycogen, was markedly increased in the obese rats and was accompanied by a lower adipose tissue O_2 consumption and whole body CO_2 production per g body weight. Moreover, basal lipolysis as measured through the glycerol release by adipocytes was reduced in the animals fed on the cafeteria diet. Also, a positive association ($P<0.05$) was found between glucokinase activity and glycogen and fat stores in the dietary groups. A reduced energy expenditure would explain the outcome of this experimental diet-induced model of obesity, but the possibility that nutritional interactions could act through other mechanisms at the level of the liver are not discounted. Also, factors such as timing, period of feeding, strain, age, etc must be considered when interpreting the development and onset of obesity.

This experiment supports the idea that several cycles involved in energy supply and thermogenesis may play a role in the metabolic integration of fuel utilization and weight control (Schutz, 1993).

Martínez, J.A., García-Calonge, M.A., Simon, E., Del Barrio, A.S. & Portillo, M.P. (1995). *Proceedings of the Nutrition Society* **54**, 8A.
Rozen, R., Brigant, R. & Apfelbaum, M. (1994). *American Journal of Clinical Nutrition* **59**, 560-565.
Schutz, Y. (1993). *International Journal of Obesity and Related Metabolic Disorders* **17**, 523-527.
Torgan, C.E., Brozinich, J.T, Willems, M.E. & Ivy, J.L. (1990). *Journal of Applied Physiology* **69**, 1987-1991.

Differences between men and women after cardiac rehabilitation: an assessment of dietary compliance, nutritional knowledge and dietary perceptions. By J.M. JULIAN[1] , M.A.T. FLYNN[1], F.RAFFERTY[2] and J.H. HORGAN[2], [1] *Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8 and* [2] *Department of Cardiology, Beaumont Hospital, Dublin 9, Republic of Ireland.*

Women need greater encouragement than men to attend cardiac rehabilitation programmes (McGee & Horgan, 1992). The present study was undertaken to test the hypothesis that, women are less likely to achieve the dietary goals of cardiac rehabilitation.

A total of forty three subjects (a response rate of 60%) were recruited from patients who had completed a cardiac rehabilitation programme in Dublin within the last 18 months. The subjects were interviewed once at which time usual dietary intakes were measured using the 7 day diet history method (Lee & Cunningham, 1990); body weight and height were measured and nutritional knowledge and dietary perceptions were assessed by interview assisted questionnaire. Under-reporters (*n* 4 women, *n* 4 men), defined as subjects reporting energy intakes less than 1.3 their BMR and who were not actively losing weight (Goldberg et al, 1991), were excluded. The groups were found to be comparable for age (mean 63.4 ranging from 45 - 76 years) and BMI (women 26.4, men 27.3). The majority of the group were married (*n* 15 women, *n* 16 men) and of socio-economic group 2 (*n* 12 women, *n* 11 men). A comparison of the mean daily macronutrient intakes of the female and male subjects is shown in the Table.

Macronutrients	Men (*n* 18)		Women (*n* 17)	
	Mean	SD	Mean	SD
Energy (MJ)	9.5*	3.4	7.3	1.6
Energy Profile:				
Protein (%)	15**	3.9	18	3.5
Total Fat (%)	40**	9.7	30	9.7
Saturated (% T. Fat)	8*	4.1	13	5.6
Monosaturated (% T. Fat)	19**	4.9	11	3.9
Polyunsaturated (%T. Fat)	13	7.4	7	3.2
Alcohol (%)	3	4.9	3	4.5
Carbohydrate (%)	43	9.3	48	8.8

*(Mean values were significantly different from those for women: *$p < 0.05$, **$p < 0.01$)*

In this particular study women were found to be more likely to achieve the dietary goal of less than 30% energy from total fat. However, men were significantly better at achieving the goal of less than 10% total energy from saturated fat. Nonetheless, when the nutritional knowledge questionnaire was examined, men and women were comparable in perceiving their dietary regimens to be mostly healthy. Given the significant differences in energy intakes micronutrient intakes were compared per 4.2 MJ. This showed the diets of women compared with men to be significantly more nutrient dense for Ca, Fe, nicotinic acid, vitamin C, vitamin B6, retinol and carotene.

Women and men were comparable with respect to nutritional knowledge and dietary perceptions except for the perception about diet and appearance where significantly more women perceived diet to have a good effect on appearance ($p = 0.05$). This study highlights that men may need greater encouragement to comply with the dietary goals of cardiac rehabilitation.

Goldberg, G.R. et al (1991). *European Journal of Clinical Nutrition 45, 569-581*
Lee, P. & Cunningham, K (1990). *Irish National Nutrition Survey, Dublin:* I.N.D.I.
McGee, H. & Horgan, J.H. (1992). *British Medical Journal 3 0 5, 283-284*

An *in vivo* study of the relationship between diet and adipose-tissue composition. By E.L. THOMAS[1], G. FROST[2], M.L. BARNARD[1,3], D.J. BRYANT[1], S.D. TAYLOR-ROBINSON[1] and J.D. BELL[1]. [1]*The Robert Steiner MRI Unit,* [2]*Department of Dietetics and* [3]*Division of Endocrinology and Metabolic Medicine, Hammersmith Hospital, Du Cane Road, London W12 0HS*

Coronary heart disease (CHD) is a leading cause of death in the UK. Adipose-tissue fatty acids independently alter plasma lipid profiles and potential risk of CHD (Wood *et al.* 1987). Screening subjects at risk of CHD is routinely performed by analysing serum cholesterol. Less attention has been directed to the contribution from adipose-tissue composition, partly due to a lack of suitable non-invasive methods. The fatty acid profile of adipose-tissue reflects long-term dietary intake and could be an alternative index of the habitual dietary fatty acid intake over the previous 2-3 years. Magnetic resonance spectroscopy (MRS) allows human biochemistry to be studied non-invasively and has been successfully applied to the study of lipids in humans (Moonen *et al.* 1988). Individual fatty acids cannot be resolved but levels of poly- and monounsaturated fatty acids may be determined. We used *in vivo* ^{13}C MRS to investigate the long-term effects of different diets on adipose-tissue composition in healthy volunteers. Three age- and sex-matched groups: vegans, omnivores, ovo-lacto vegetarians were studied. Each volunteer provided: blood samples for serum lipid analysis; 7 d dietary diary (Bingham *et al.* 1994); anthropometrics for BMI and percentage body fat; *in vivo* ^{13}C MR spectra of thigh subcutaneous fat were acquired at a magnetic field strength of 1.5 Tesla.

	LDL-c (mmol/l)		% Dietary Poly		% P in AT		BMI (kg/m^2)	
Diet Type	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Vegan (*n* 38)	2.46**	0.20	37.9**	1.6	3.5**	0.1	21.8**	0.5
Vegetarian (*n* 11)	2.65*	0.16	20.2††	3.3	2.6††	0.1	22.6	1.4
Omnivore (*n* 39)	3.41	0.23	20.5	1.2	2.3	0.01	23.6	0.5

LDL-c, low-density-lipoprotein cholesterol; P, polyunsaturated fat (refers to carbons in the -CH=**C**H-CH$_2$-**C**H=CH- positions)

Significantly different from omnivores:, $*P < 0.05$, $**P < 0.01$

Significantly different from vegetarians: $^{\dagger\dagger}P < 0.01$

No significant differences in total energy intake or the percentage total dietary fat intake were found between the three groups. Vegans had a significantly lower intake of saturated fatty acids in their diet than the omnivores or vegetarians ($P < 0.01$) assessed from their dietary records. By ^{13}C MRS we showed the adipose-tissue of vegans to have significantly more unsaturated and polyunsaturated fatty acid carbons than either omnivores or vegetarians. No significant differences in adipose-tissue composition were found between the vegetarians and omnivores. Significant correlations were found between dietary saturated fat intake and MRS measurement of both saturated (r 0.55, $P < 0.01$) and polyunsaturated (r -0.64, $P < 0.01$) fatty acid content of adipose-tissue.

In vivo assessment of adipose-tissue composition showed marked elevation in the levels of polyunsaturated fatty acids in vegans compared with omnivores and vegetarians. This is an important finding, as there is an inverse relationship between the polyunsaturated fatty acid content of adipose-tissue and risk of CHD (Kinsella *et al.* 1990). ^{13}C MRS offers a unique opportunity to study dietary influences on adipose-tissue lipid composition non-invasively, which may be useful for serial epidemiological studies in determining the interactions between diet, adipose-tissue and serum lipids.

Bingham, S.A.,Gill, C., Welch, A., Day, K., Cassidy, A., Khaw, K.T., Sneyd, M.J., Key, T.J.A., Roe, L. & Day, N.E. (1994). *British Journal of Nutrition* **72**, 619-643.

Kinsella, J.E., Lokesh, B. & Stone, R.A. (1990). *American Journal of Clinical Nutrition* **52**, 1-21.

Moonen, C.T.W., Dimand, R.J. & Cox, K.L. (1988). *Magnetic Resonance in Medicine* **6**, 140-157.

Wood, D.A., Riemersma, R.A., Butler, S., Thompson, M., Macintyre, C., Elton, R. & Oliver, M.F. (1987). *Lancet* **i**,177-182.

Analysis of body-fat distribution by magnetic resonance imaging and impact on the metabolic risk factor profile. By M.L. BARNARD[1,2], E.L. THOMAS[1], J.E. SCHWIESO[1], J.V. HAJNAL[1], G. FROST[3], N. SAEED[1], S.R. BLOOM[2] and J.D.BELL[1], *[1]The Robert Steiner MR Unit, [2]Division of Endocrinology and Metabolism and [3]Department of Dietetics, Hammersmith Hospital, Du Cane Road, London W12 0HS*

Abdominal obesity appears to be a major risk factor for coronary heart disease (CHD) and non-insulin dependent diabetes mellitus (NIDDM). This has been linked to the rapid turnover of internal visceral fat, producing non-esterified fatty acids (NEFA) which directly drive hepatic lipoprotein synthesis and gluconeogenesis. Previous studies have used the waist:hip ratio as an index of abdominal obesity. Magnetic resonance imaging (MRI) techniques have recently been developed to quantify adipose tissue (Seidell *et al.* 1990; Ross *et al.* 1992) and provide a unique opportunity to determine the effect of visceral fat on the metabolic profile.

Nine women were studied and subdivided by BMI: A > 28 kg/m^2 (*n* 6); B < 28 kg/m^2 (*n* 3). MR images were acquired at 1.0T using a rapid T_1 weighted spin echo sequence. Volunteers were scanned from head to toe in under 25 min, acquiring transverse images as the subject was moved through the magnet. Images were analysed quantitatively using a threshold and contour following programme. ^{13}C MR spectra (MRS) were acquired from the thigh. Anthropometric measurements were made and fasting serum was analysed.

MRI measurement of percentage total body fat (mean 35.7 (SEM 3.0) %) showed excellent agreement with anthropometric estimation from skinfold thickness (mean 34.6 (SEM 2.9) %) (*r* 0.96, $P<0.001$). MRI also allowed the separate quantification of fat compartments. There was a strong correlation between visceral fat and both serum triacylglycerols (*r* 0.87, $P<0.002$) and NEFA (*r* 0.67, $P<0.02$). The subjects with high BMI had significantly higher serum cholesterol, triacylglycerols, NEFA and glucose concentrations. No differences were detected between saturated (mean 20.3 (SEM 1.4) %) and unsaturated (mean 79.7 (SEM 1.5) %) fatty acid content of adipose tissue between subject groups, indicating no difference in type of dietary fat.

	Group A (BMI > 28 kg/m^2)		Group B (BMI < 28 kg/m^2)	
	Mean	SEM	Mean	SEM
Total fat (litres)	50.4	4.9	19.9*	2.3
Subcutaneous fat (litres)	42.6	4.1	17.2*	2.3
Visceral fat (litres)	4.7	0.5	1.4*	0.2
Serum glucose (mmol/l)	5.20	0.19	4.60*	0.11
Serum NEFA (mmol/l)	0.74	0.08	0.35*	0.09
Serum cholesterol (mmol/l)	5.40	0.34	4.42*	0.21
Serum triacylglycerols (mmol/l)	2.33	0.47	0.82*	0.1

*Mean values were significantly different from those for group A, $P<0.05$ (analysed by t-test).

This study shows that body-fat distribution analysis by MRI is a rapid technique which compares extremely well with conventional anthropometric methods. Furthermore this technique allows the absolute quantification of visceral fat volumes, which in this study showed a direct correlation with serum lipids. The future combination of MRI and MRS will enable an integrated approach for assessing the health implications of both the distribution and composition of body fat. The application of these techniques will provide key information on the aetiology of CHD and NIDDM and will determine the protective effect of diet or exercise against visceral fat deposition.

Ross, R., Leger, L., Morris, D., de Guise, J. & Guardo, R. (1992). *Journal of Applied Physiology* **72**, 787-795.
Seidell, J.C., Bakker, C.J.G. & van der Kooy, K. (1990). *American Journal of Clinical Nutrition* **51**, 953-957.

Underfeeding by reduction in fat or carbohydrate intake: effects on energy expenditure, macronutrient oxidation and subsequent food intake in lean men. By P.M. HEAVEY[1,2], A.P.M. McKENNA[1,2], G.R. GOLDBERG[1], P.R. MURGATROYD[1] and A.M. PRENTICE[1], [1] *Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH and* [2] *School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA*

This study investigated the effect of mild underfeeding on macronutrient oxidation and subsequent voluntary energy intake (EI). Nine men (mean weight 70.3 (SD 9.5) kg; BMI 22.8 (SD 2.9) kg/m^2) were each studied three times. On each occasion a stabilization day during which EI (1.35 x measured BMR) and physical activity were controlled was immediately followed by 2 d in a whole-body indirect calorimeter. On day 1 subjects received a fixed EI comprising a control diet (40:48:12 % energy from fat: carbohydrate (CHO):protein) or underfeeding diets which were manipulated to have 15% less energy than control, removed as CHO (CHOred: 47:39:14 % energy from fat:CHO:protein) or as fat (FATred: 29:57:14 % energy from fat:CHO:protein). Control EI was calculated to match an anticipated energy expenditure (EE) of 1.46 x BMR. On day 2 *ad lib.* access to foods of the same composition as the control was allowed. A fixed activity protocol was followed on both days. The study was approved by the Dunn Nutrition Unit Ethical Committee. The data (MJ/d) presented in the Table were analysed by paired *t* -test.

	Day 1 (fixed EI)						Day 2 (*ad lib.* EI)					
	Control		CHOred		FATred		Control		CHOred		FATred	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Intake												
Energy	10.0	0.9	8.5***	0.7	8.5***	0.7	10.7	1.3	11.9*	1.6	11.5	1.5
CHO	4.8	0.4	3.3***	0.3	4.8	0.4	5.1	0.6	5.7*	0.7	5.5	0.7
Fat	3.9	0.4	4.0	0.4	2.5***	0.2	4.2	0.5	4.6*	0.6	4.5	0.6
Expenditure												
Energy	10.2	0.8	9.9*	0.8	9.9*	0.6	10.4	0.7	10.4	0.8	10.3	0.6
CHO	4.3	0.8	3.2**	0.5	4.3	0.6	4.8	0.5	4.3	0.7	4.7	0.5
Fat	4.9	1.0	5.5*	0.9	4.4*	0.6	4.4	0.9	4.8	1.2	4.4	0.8

Significantly different from control: *P<0.05, **P<0.01 ***P<0.001.

On Day 1 on the control treatment mean EE was 1.52 (SD 0.08) x BMR. The dietary manipulations caused autoregulatory changes in fat and CHO oxidation. There was a significant effect of CHO underfeeding on subsequent ad *lib.* intake and a trend towards increased EI after fat underfeeding. In our opinion one subject's EI after the control treatment was atypically high (12 MJ, 1.9 x BMR). With his data excluded, mean *ad lib.* EI (MJ) were: control 10.52 (SD 1.32), CHOred 12.10 (SD 1.47) (P = 0.001 *v.* control) and FATred 11.74 (SD 1.40) (P = 0.044 *v.* control). Mean overall energy balance values in eight subjects (48 h intake minus expenditure) were -0.14, 0.34, 0.04 MJ on control, CHOred and FATred respectively. In this study voluntary EI compensated for the energy imbalance imposed by removing energy as fat or CHO. In an earlier study (Shetty *et al.* 1994) we observed no differences in *ad-lib.* EI after manipulations of CHO or fat at a constant energy intake.

Shetty, P. S., Prentice, A. M., Goldberg, G. R., Murgatroyd, P. R., McKenna, A. P. M., Stubbs, R. J. & Volschenk, P. A. (1994). *American Journal of Clinical Nutrition* **60**, 534-543.

Glycation of insulin and proinsulin occurs in the pancreatic islets of lean and spontaneously obese-diabetic mice. By F.P.M. O'HARTE, Y.H.A. ABDEL-WAHAB, C.R. BARNETT and P.R. FLATT, *School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA*

It has been demonstrated that the non-enzymic glycosylation (glycation) of insulin occurs under hyperglycaemic conditions *in vitro* (O'Harte *et al.* 1994). The present study was undertaken to monitor glycation of insulin and proinsulin in the isolated islets and pancreas of 12-18-week old lean and obese hyperglycaemic (ob/ob) mice. Isolated islets or pancreatic tissue were extracted following homogenization in a Waring blender with ice-cold acid-ethanol (0.7M HCl in 750 ml/L ethanol, 5.0 ml/g wet weight). The acidified ethanol was removed by dialysis (2000 Da cut-off) against saline (9g NaCl/L) at 4° for 18 h. Glycated and non-glycated immunoreactive insulin (IRI) fractions were separated by Glyco-Gel B (Pierce) affinity chromatography and quantified using an insulin radioimmunoassay (RIA) which cross-reacts 100% with proinsulin. Increases in percentage glycated IRI were observed in collagenase-isolated islets from ob/ob mice (10.4 (SE 0.4)%, n 5) v. lean controls (4.0 (SE 0.8)%, n 7; $P<0.001$). The plasma glucose concentrations in these groups were 22.4 (SE 2.9) mmol/l, and 8.6 (SE 0.7) mmol/l respectively ($P<0.001$). Moreover islets from lean mice which were cultured in RPMI-1640 medium containing 33.3 mmol glucose/l for 24 h had a significantly ($P<0.001$) higher percentage glycated IRI (18.3 (SE 1.4)%, n 5) compared to islets in 5.6 mmol glucose/l (3.7 (SE 0.4)%, n 5).

The contribution of proinsulin to the IRI pool was determined in a subsequent experiment. Whole pancreas from lean (n 8) or hyperglycaemic ob/ob mice (n 5) was snap-frozen in liquid N_2, pooled, and extracted in acid-ethanol. Briefly, IRI from each extract was concentrated on a C-18 Sep-Pak cartridge (Waters) and glycated and non-glycated material was separated on Glyco-Gel B and applied to a 250x4.6 mm Vydac 218TP54 (C-18) column. The mobile phase consisted of solvent A: 50 mmol/l phosphoric acid, 20 mmol/l triethylamine, 50 mmol/l sodium perchlorate, adjusted to pH 3.0 with NaOH, and solvent B: acetonitrile-water (90:10 v/v). The concentration of solvent B was raised from 29% (v/v) to 31% over 30 min, held at 31% for 20 min, raised to 34% over 5 min, held at 34% for a further 25 min and finally raised to 36% over 70 min, using linear gradients. Fractions (150 x 1.0 ml) were collected at a flow rate of 1.0 ml/min. Samples were lyophilized to approximately 50 µl and reconstituted to 1.0 ml with RIA buffer containing 1 g bovine serum albumin/L. Fractions (22-52) corresponding to insulin and (55-85) corresponding to proinsulin were pooled and the IRI content determined by RIA.

	Total pancreatic IRI (glycated + non-glycated) (ng/ml*)	Total pancreatic proinsulin (glycated + non-glycated) (ng/ml*)	Total glycated IRI (ng/ml*)	Glycated insulin (ng/ml*)	Glycated proinsulin (ng/ml*)
Lean	135.3	15.9	9.5	6.9	2.6
Obese	570.9	57.1	11.8	8.5	3.3

*Results are expressed in ng per ml of pooled eluent from C-18 RP-HPLC column.

As shown in the Table, total pancreatic IRI content (insulin plus proinsulin) of obese mouse pancreatic extracts was 4.2-fold higher than that of lean controls. The contribution of proinsulin to the total IRI was approximately 10-12%, corresponding closely to findings in acid-ethanol extracts of rat pancreatic tissue (Leahy, 1993). The contribution of proinsulin to total glycated IRI was approximately 27-28%. These data indicate that glycation of insulin and proinsulin occurs within the pancreatic islets of Langerhans in this insulin-resistant animal model of diabetes. The observed glycation of insulin and proinsulin in diabetes may be of relevance to the insulin resistance and increased levels of proinsulin and split products reported in NIDDM (Kahn *et al.* 1994).

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Energy expenditure and carbohydrate flux in type 1 (insulin-dependent) diabetics. By M. TOTTON[1,2], P.R. MURGATROYD[1], G.R. GOLDBERG[1] and A.M. PRENTICE[1], [1]*Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH and* [2]*School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA*

There is very little information about energy and macronutrient metabolism in type 1 diabetics. The aim of insulin administration is to normalize blood glucose, however the extent to which carbohydrate (CHO) utilization is normalized has not been extensively studied. We have used whole-body indirect calorimetry to measure energy expenditure and storage and oxidation of CHO over the range of physical activities which constitute a typical sedentary day. The subjects were six well-controlled male diabetic patients (weight 69.5 (SD 10.6) kg; BMI 21.9 (SD 2.6) kg/m^2; age 34 (SD 10) years) and normoglycaemic controls individually pair-matched for weight, BMI and age. The study was approved by the Dunn Nutrition Unit and Cambridge Health Authority Ethical Committees.

Throughout the study, diabetics kept to their usual insulin regimen and pattern of meals and snacks. The composition, amount and timing of foods was of their own choosing. Each control was fed according to the regimen selected by his diabetic pair. On the first day of the study subjects reported for breakfast and then remained in the metabolic suite of the Dunn Clinical Nutrition Centre. All meals were taken in the Centre and no vigorous physical activity was allowed. Subjects entered a whole-body calorimeter at 20.00 hours where they remained for the following 37 h, and all followed the same protocol which included a period of cycling and one of stepping exercise. Blood glucose was monitored regularly. The period in the calorimeter from 09.00 hours on the first morning was used to calculate the 24 h values (MJ) shown in the Table. Protein oxidation was calculated from urinary nitrogen excretion. Rates of CHO and fat oxidation were calculated from non-protein oxygen consumption and carbon dioxide production.

	Diabetic Group		Control Group	
	Mean	SD	Mean	SD
Energy expenditure	9.8	1.3	9.9	1.4
Energy intake	7.7	1.8	7.7	1.8
BMR	7.8	1.1	7.4	1.3
CHO oxidation	3.9	0.8	4.1	0.7
Fat oxidation	4.6	1.1	4.3	1.6
Protein oxidation	1.3	0.2	1.5	0.5

There were no significant differences in 24 h energy expenditure or balance. BMR measurements in diabetics were, in all but one case, higher than in their matched controls although the difference was not significant. Physical activity levels (24h energy expenditure expressed as a multiple of BMR), were significantly lower in the diabetics: 1.25 (SD 0.06) *v.* 1.34 (SD 0.075), $P<0.05$.

Although all subjects were close to CHO balance, post-meal CHO oxidation was significantly suppressed in the diabetics relative to the controls after both breakfast (08.30 to 10.30 hours): 237 *v.* 337 kJ, $P<0.035$ and the evening meal (19.00 to 20.00 hours): 134 *v.* 205 kJ $P<0.05$.

The diabetics had no physiological control over their insulin levels. Although their pattern of CHO oxidation was displaced, overall they achieved the same CHO balance as their controls.

The effect of intensive dietary counselling on the nutritional status of patients with head and neck cancer undergoing radiotherapy. By F. NÍ CHOILEÁIN[1,2] M. MORIARTY[2], M.J. GIBNEY[1] and M. MOLONEY[1,3], *[1]Department of Clinical Medicine, Trinity Centre for Health Sciences, St James's Hospital, James's Street, Dublin 8, [2]St Luke's and St Anne's Hospital, Radiotherapy and Clinical Oncology Centre, Highfield Road, Rathgar, Dublin 6 and [3]Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8.*

Patients with head and neck cancer are particularly prone to malnutrition; it has been estimated that more than 80% of these patients have a significant weight loss during treatment (Chencharick & Mossman, 1983). A comparative prospective study was designed to assess the value of intensive dietary counselling and oral supplementation in the management of patients with head and neck cancer receiving radiotherapy. The results are presented.

A total of seventy-nine patients were recruited consecutively from St Luke's and St Anne's Hospital. Ethical approval was given by St Luke's Hospital Ethics Committee. Forty-one of these patients entered the intervention group (IG) who received intensive dietary counselling and advice regarding supplementation before commencing radiotherapy and at regular intervals during and after treatment. Thirty-eight patients entered the standard management group (SMG) and did not receive specific dietary advice from a dietitian. Serial 7d diet history, anthropometric and biochemical measurements were conducted at regular intervals throughout the 2-month trial period. The data were analysed using repeated measures ANOVA. The results for weight, energy and protein intake are shown in the table, and for each of these variables there was a significant time-by-treatment interaction (P <0.0001).

	Baseline		Week 2		Week 4		Week 6		Week 8		P
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Weight (kg):											
IG	64.9	13.3	65.1	13.4	64.3	13.3	64.8	12.9	65.9	9.9	0.0001
SMG	74.2	17.3	72.5	17.7	72.1	17.3	68.8	15.7	69.3	15.8	
Energy (kJ / kg):											
IG	118	32	139	37	133	48	140	49	152	48	0.0001
SMG	124	36	112	32	102	36	108	36	118	33	
Protein (g/kg):											
IG	0.90	0.26	1.11	0.41	1.12	0.41	1.16	0.36	1.12	0.29	0.0001
SMG	0.91	0.29	0.92	0.34	0.82	0.36	0.91	0.35	1.0	0.35	

P indicates sigificance of difference between IG and SMG.over time.

Energy and protein intakes were significantly greater in patients receiving intensive dietary counselling when compared with the SMG. The counselled group had experienced significantly less (P < 0.0001) weight loss than the standard management group. This was also clinically significant as the former group experienced a weight gain of 1.5% compared with a weight loss of 6.6% in the SMG despite their higher initial body weight.

The results of this study suggest that intensive dietary counselling may allow patients with head and neck cancer to maintain their weight,and energy and protein intakes thus preventing further nutritional depletion during radiotherapy treatment. In order to achieve net weight gain during radiotherapy more aggressive methods of nutritional support such as gastrostomy or nasoenteral feeding may be required.

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Development of a food protein atlas as an educational tool in dietary counselling of renal patients. By S. M. DOYLE and M. MOLONEY, *Department of Biological Sciences, Dublin Institute of Technology, Kevin St., Dublin 8, Ireland.*

Nutrition education is essential for renal patients (Caggiula & Milas, 1993). Monitoring dietary intake of protein forms an integral part of the overall management of patients with renal disease. A food atlas can be used to depict average portion sizes or exchanges of food. It can be used to educate people and also to quantify foods in dietary histories (Howat *et al.* 1994). The primary aim of the present study was to design and develop a food atlas in terms of single protein-foods (as protein exchanges) which could be used by a professional in the dietary education and counselling of renal patients. A second aim was to evaluate the potential benefits of such an atlas.

By means of a questionnaire, dietitians involved in the dietary management of renal patients were requested to state the key protein foods they wished to be included in the food atlas. The food atlas was divided into nine different sections namely: meat and poultry; fish; beans, peas and lentils; cheese and dairy products; nuts; breakfast cereals; pasta, rice and potatoes; bread and biscuits, and finally, photographs of the tableware used in order to aid interpretation of scale and measure. Each food item was labelled with a corresponding weight and protein exchange value (e.g 2g or 7g).

In an attempt to evaluate the potential use of the atlas, a small pilot study was undertaken. Twenty-five randomly chosen males and females took part in the study. The aim was to compare the potential advantages of the food atlas against household measures which is currently the most common method of teaching protein exchanges to renal patients in Ireland. The participants also had to judge portion sizes without receiving instruction.

	MEAN % CORRECT ANSWERS					
	Macaroni	*Sweetcorn*	*Chicken*	*Bread*	*Cheese*	*Rice krispies*
NO INSTRUCTION	52	52	28	48	56	28
HOUSEHOLD	80	56	76	64	76	64
FOOD ATLAS	80	88	88	92	100	92

Advantages of using such an atlas are that it is easy to transport and is user friendly, it can be used to instruct patients on 'amorphous' foods and to instruct illiterate patients. It needs little explanation, slides can be easily developed for group discussions, and extra-large prints can be developed for the visually impaired. It avoids the use of scales which can become tedious and it is a relatively cheap tool.

There is a need for educational training in estimating food portion size (Howat *et al.* 1994). Therefore the use of a food protein atlas in dietary education and counselling of renal patients could lead to the patient regarding eating behaviour as a habitual process, requiring as little intervention as possible.

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Cytotoxic effects of cholesterol oxides and the potentially protective properties of α-tocopherol and β-carotene in rat kidney cells in culture. By A.M. WILSON, R.M. SISK and N.M.O'BRIEN. *Department of Nutrition, University College Cork, Republic of Ireland*

Autoxidation of cholesterol in biological systems and the cytotoxicity of a number of cholesterol oxidation products (COP) have received considerable attention (Sevanian & McLeod, 1987). COP have been described as being cytotoxic, atherogenic, mutagenic and carcinogenic (Bosinger *et al.* 1993). In the present study, we have utilized an *in vitro* model to demonstrate the cytotoxicity of various cholesterol oxides. The ability of α-tocopherol and β-carotene to protect against COP-induced toxicity in our cell-culture model was assessed.

Rat kidney cells were cultured in Dulbecco's modified Eagle's medium and maintained in a humidified atmosphere at 37°c and enriched with CO_2 (50 ml / L). The growth medium was supplemented with cholesterol, 7-ketocholesterol, cholestan-3β-5α-6β-triol or 25-hydroxycholesterol in the presence or absence of α-tocopherol or β-carotene, both at the concentrations shown in the table. The cells were exposed to the cholesterol oxides and the antioxidants for 24 h. The cytotoxicity was determined by the neutral-red-uptake assay (modified by Hunter *et al.* 1987). The results are expressed as percentage cell viability compared with an appropriate control, with values greater than 100% corresponding to a stimulation of cell growth and values less than 100% corresponding to an inhibition.

	Supplementation									
	Control		Cholesterol (40 μM)		7-keto-cholesterol (30 μM)		Cholestan 3β-5α-6β-triol (15 μM)		25-hydroxy cholesterol (40 μM)	
α-Tocopherol concn (nM)	Mean %viability	SE	Mean % viability	SE	Mean % viability	SE	Mean % viability	SE	Mean % viability	SE
0	100.0	3.1	104.0	3.7	61.7	4.0	43.8	6.0	42.3	2.2
100	90.9	2.1	102.9	5.3	72.2	4.1	29.7	1.9	56.7	3.8
250	111.0	3.8	96.3	4.3	72.5	5.8	30.4	4.6	53.7	5.0
500	102.9	7.8	98.1	5.4	78.1	5.3	40.0	3.7	59.9	8.2
750	98.6	8.2	86.6	3.2	87.2	4.2	30.0	1.4	60.7	3.3
1000	90.7	3.9	93.8	4.5	76.0	5.2	47.7	6.9	68.9	5.9
LSD (P<0.05)	NS		NS		6.4		2.2		6.3	

Statistical analysis LSD, least significant difference, was by one-way ANOVA, NS: not significant, *n* 4 for all treatments

The addition of cholesterol to the growth medium did not influence the viability of the cells. However, when the growth medium was supplemented with 7-ketocholesterol, 25-hydroxycholesterol or cholestan-3β-5α-6β-triol, cytotoxicity was observed. When the cells were cultured in the presence of α-tocopherol, there were significant reductions ($p<0.05$) in the cytotoxicity induced by 25-hydroxycholesterol or 7-ketocholesterol. α-Tocopherol (at concentrations less than 1000 nM) did not have a protective effect when the cells were incubated with cholestan-3β-5α-6β triol. The addition of β-carotene to the growth medium in the presence of the COP failed to reduce significantly the level of cytotoxicity (results not shown). These results demonstrate that COP are cytotoxic in this *in vitro* model and α-tocopherol may modulate their toxicity in certain cases.

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Protective effect of carotenoids against UVA-light-induced oxidative stress in rat kidney cells in culture. By I. O'CONNOR, J. YOUNG and N.M. O'BRIEN, *Department of Nutrition, University College Cork, Republic of Ireland*

Considerable research interest exists in the potential antioxidant activity of dietary carotenoids. We assessed the ability of the carotenoids β-carotene, lutein and astaxanthin to protect against oxidative stress in our cell culture model.

Rat kidney cells were cultured in Dulbecco's modified Eagle's medium and maintained in a humidified atmosphere at 37° and CO_2 (50 ml/l). The growth medium was supplemented with β-carotene (0-1000 nmol/l), lutein (0-1000 nmol/l) or astaxanthin (0-10 nmol/l). Oxidative stress was induced by exposing the cells to UVA light at a dose intensity of 5.6 mW/cm^2 for a 4 h period. Lipid peroxidation, as indicated by thiobarbituric acid reactive substances (TBARS), and the activities of the antioxidant enzymes catalase (CAT, *EC* 1.11.1.6) and superoxide dismutase (SOD, *EC* 1.15.1.1), were measured as indices of oxidative stress.

	CAT (U/mg protein)		SOD (U/mg protein)		TBARS (nmol MDA/mg protein)	
	Mean	SE	Mean	SE	Mean	SE
β-Carotene (nmol/l)						
Control †	8.59	0.37	10.28	0.64	3.68	0.23
0	4.76*	0.02	3.39*	1.23	7.04*	0.15
10	5.43*	0.56	4.02*	0.73	6.64*	0.45
100	6.32*	0.09	6.89*	1.09	4.39	0.25
500	7.11*	0.64	9.30	1.48	4.49	0.05
1000	7.85	0.21	9.80	1.52	4.22	0.46
LSD (P<0.05)	1.12		3.22		0.87	
Astaxanthin (nmol/l)						
Control †	7.58	0.25	9.78	0.04	4.88	0.49
0	3.84*	0.56	3.81*	1.15	9.30*	0.81
0.1	5.82*	0.53	5.90*	0.79	8.97*	1.23
1	6.08*	0.73	7.16*	0.07	5.43	0.51
5	7.06	0.49	7.06*	1.03	5.32	0.29
10	7.67	0.08	9.73	0.04	3.56	0.17
LSD (P<0.05)	1.42		2.04		1.94	

MDA, malondialdehyde; LSD, least significant difference.

* Significantly different from control cells (P<0.05) one-way ANOVA, n 6 for all groups.

† Control cells not exposed to UVA light and not supplemented with carotenoids.

Rat kidney cells grown in unsupplemented medium and exposed to UVA light exhibited a decrease in antioxidant enzyme activity and an increase in lipid peroxidation. No cytotoxicity, as indicated by lactate dehydrogenase (*EC* 1.1.1.27) release, was observed. Incorporation of β-carotene (1000 nmol/l) and astaxanthin (10 nmol/l) into the media of UVA-exposed cells returned CAT and SOD activities and lipid peroxidation to control levels. With increasing concentrations of the carotenoids in the supplemented media, the level in the cells increased, as determined by HPLC, indicating that carotenoids were incorporated into the cells. Results similar to those for β-carotene were observed for lutein with antioxidant enzyme activity returning to control level at 1000 nmol/l (results not shown). These results suggest that carotenoids may play a role in the protection of cells against UVA-light-induced oxidative stress, with astaxanthin offering protection at lower concentrations than β-carotene or lutein.

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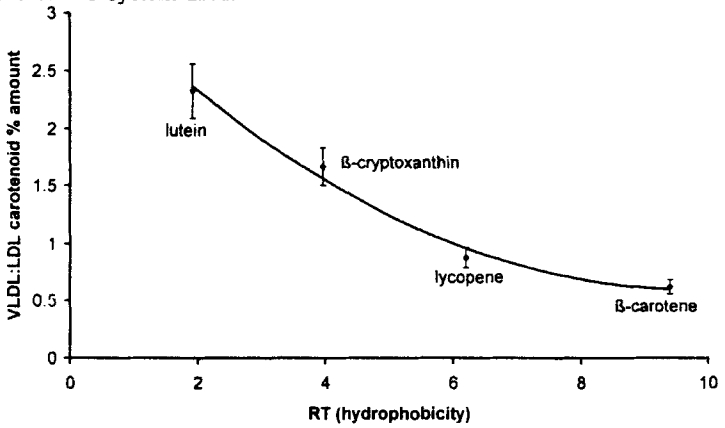
The location of a carotenoid in a lipoprotein is a function of its polarity. By L. M. EDMOND, U. J. McLOONE, D. I. THURNHAM and M. CHOPRA, *Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA*

The carotenoids are a class of fat-soluble pigments of varying polarity. They are associated with the lipoproteins which are important in the aetiology of coronary heart disease. Lipoproteins consist of protein, triacylglycerol, cholesterol and phospholipids in various proportions as well as vitamin E and trace amounts of carotenoids. Each class of lipoprotein plays a role in the transport of lipids determined by the protein they contain (Goldstein *et al.* 1983). The conversion of VLDL to LDL has the effect of reducing the triacylglycerol content from 50 to 10%, increasing the cholesterol content (free+esterified) from 22 to 48%, increasing the protein content from 7 to 22% and increasing slightly the phospholipid content from 20 to 22% (Gurr & Harwood, 1991). VLDL is the precursor of LDL so by comparing the carotenoid compositions of each it should be possible to determine with which fraction of the lipoprotein the carotenoids are associated.

Fasting plasma samples were obtained from thirty healthy, male and female volunteers. The lipoprotein fractions were separated by ultracentrifugation on a KBr gradient (Puhl *et al.* 1994) and analysed for the carotenoids and vitamin E by reverse phase HPLC (Thurnham *et al.* 1988). Mean lutein, ß-cryptoxanthin, lycopene and ß-carotene concentrations were calculated for the fractions VLDL and LDL. These four mean concentrations were then summed and each expressed as a percentage of the total. The ratio of this percentage in LDL to the percentage in VLDL was plotted against the carotenoids' hydrophobicity (fig.). The hydrophobicity value assigned to each carotenoid is its retention time (RT) on the HPLC system used.



These data show that the lipophilic carotenoid ß-carotene makes up a greater proportion of the carotenoid pool in LDL as compared to VLDL suggesting that it associates more readily with cholesterol than fatty acids, whereas for lutein, a hydrophilic carotenoid, the opposite is observed. Also, on conversion from one class of lipoprotein to another, the carotenoids will remain associated with the same lipids. This does not apply for vitamin E. These observations show that polarity is an important predictor of location in the lipoprotein. Each carotenoid would therefore be expected to reflect the body's regulation of fatty acid and cholesterol metabolism as a function of its polarity.

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The effect of *in vitro* and *in vivo* supplementation with lutein on LDL oxidation *in vitro*. **By** U. M[c]LOONE[1], M. CHOPRA[1], N.R. WILLIAMS[2] and D.I. THURNHAM[1], [1] *Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA and* [2]*COAG Laboratory, Papworth Hospital, Papworth Everard, Cambridge CB3 8RE.*

It is now widely accepted that one of the mechanisms predisposing to the development of atherosclerosis (a hallmark of cardiovascular disease), is the oxidation of cholesterol-rich LDL. The role of the lipophilic antioxidants, carotenoids and vitamin E in preventing LDL oxidation is of interest, since their levels can be manipulated without clinical side-effects. Most research has focused on ß-carotene and vitamin E, but there are other important carotenoids such as lutein, lycopene and ß-cryptoxanthin, which are easily identifiable in blood and have antioxidant potential. Plasma lutein concentration is similar to or greater than that of plasma ß-carotene (Thurnham,1988), but has received relatively little attention.

In the present study we have investigated the effect of *in vitro* and *in vivo* incorporation of lutein on LDL oxidation in humans.

For *in vitro* supplementation, plasma was incubated with lutein at 37 °C before LDL isolation. Isolation of LDL was carried out as described previously (Puhl *et al.* 1994). Suscepility of LDL to oxidation was monitored by measuring diene conjugate formtion (expressed as lag phase). Lutein inhibited the Cu-initiated oxidation of LDL. The effect was concentration dependent within individuals, but the extent of inhibition between individuals was variable.

For *in vivo* supplementation, twelve healthy volunteers were given orally, with a meal either 30 mg/d lutein in sunflower oil or sunflower oil only for 2 weeks. Although there was a 4-fold increase in plasma lutein after 1 week, and a 6-fold increase after 2 weeks supplementation, there was no significant effect on *in vitro* LDL oxidation. The Table compares the effect of *in vitro* and *in vivo* lutein supplementation on LDL oxidation.

Table: Change in LDL lutein concentration and its effect on lag phase

		Difference before and after supplementation			
		Lutein (mol/mol LDL)		Lag phase (min)	
Supplementation		Mean	SD (n)	Mean	SD (n)
In vitro		7.61	4.1 (10)	17.3	7.7(10)
In vivo, 2 weeks	Placebo	0.004	0.001 (5)	-2.0	4.0 (5)
	Treatment Group	0.262	0.133 (6)	2.0	2.0 (6)

The results of our study show that although lutein has the potential to inhibit LDL oxidation *in vitro*, supplementation *in vivo* with a high dose over a longer period of time may be required before any significant effect on LDL oxidation can be observed.

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Plasma tocopherols, carotenoids and antioxidant activity of neonates. By M.E. KIELY and P.A. MORRISSEY. *Department of Nutrition, University College, Cork, Republic of Ireland.*

Reactive O species have been implicated in the aetiology of several diseases (Gey et al. 1993). It has been demonstrated that preterm and low-birth-weight infants are particularly vulnerable to damage mediated by free O radicals (Pitkanen *et al.* 1990). Investigators have suggested, therefore, that serum antioxidant activity is low in preterm infants and that it increases with gestational age (Sullivan & Newton, 1988). The present study was initiated to examine plasma antioxidant potential of term neonates and to explore the interaction between maternal and neonatal antioxidant potential.

Heparinized blood was taken from forty women at less than 20 weeks gestation. After delivery, umbilical cord blood was sampled and the plasma stored at -70° until analysis. Plasma tocopherols (α–toc, δ–toc and γ–toc), retinol and carotenoids (lutein, lycopene, zeaxanthin, β-cryptoxanthin, α and β–carotene) were analysed by HPLC. Plasma cholesterol levels were determined photometrically. Plasma antioxidant activity (Dmax) was assessed using a modification of the method of Sullivan & Newton (1988). A high Dmax implies poor antioxidant activity and relates essentially to the iron sequestering activity of plasma, i.e. caeruloplasmin and transferrin concentrations. The most important results of the tocopherol and carotenoid analyses are shown in the Table below.

	n	α-toc		α-toc:chol		γ-toc		γ–toc:chol		β-Crypt		*n*	Zeax	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD		mean	SD
Neonates	40	7.27	1.9	3.50	0.8	0.43	0.2	2.07	1.2	0.04	0.04	34	0.02	0.01
Mothers	40	19.57	3.3	4.17	0.8	2.16	1.0	4.54	2.1	0.16	0.13	34	0.05	0.04

Mean concentrations of vitamins are expressed in μmol/l. α-toc:chol and γ-toc:chol molar ratios are multiplied by 10^3 and 10^5 respectively.

Molar concentrations of maternal γ-tocopherol were significantly associated with neonatal levels (r 0.45, $P<0.01$). This correlation was strengthened when lipid ratios were considered (r 0.53, $P<0.001$). δ–Tocopherol was not detectable in cord blood. Maternal zeaxanthin was significantly associated with neonatal levels (r 0.54, $P<0.001$). Similarly,a statisically significantly correlation was found between maternal and neonatal plasma β–Cryptoxanthin levels (r 0.48, $P<0.02$). A large difference was found between maternal and neonatal plasma antioxidant activity. Mean maternal Dmax was 27.1 SD 5.4μl compared with 81.1 SD 37.2μl for cord samples. Dmax in the cord samples was unrelated to birth weight or maternal values.

This study shows that some of the essential antioxidants, tocopherols and carotenoids are present in reduced concentrations in neonatal plasma and suggests that the primary antioxidants (caeruloplasmin and apotransferrin) have reduced activity relative to maternal values.

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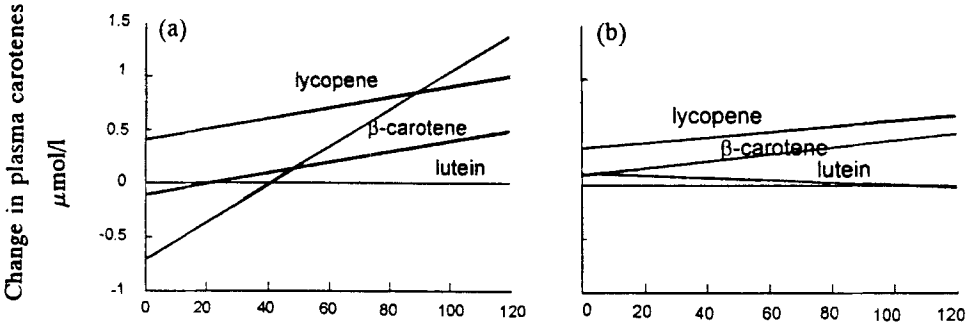
Plasma lutein, lycopene and ß-carotene levels in smokers and non-smokers following vegetable supplements. By M. E. O' NEILL[1], U.J. McLOONE[1], M. CHOPRA[1], D. I. THURNHAM[1], I. HININGER[2] and A. M. ROUSSEL[2]. [1]*Human Nutrition Research group, University of Ulster, Coleraine, BT52 1SA and* [2]*Université J. Fourier, F38000, Grenoble, France.*

Much evidence to date suggests an inverse correlation between the consumption of fruit and vegetables and the risk of diseases such as heart disease and cancer (Gey,1993) . Smokers (S) have a higher risk of coronary heart disease and normally consume less fruit and vegetables than non-smokers (NS) (Gregory *et al.*1990).

Eleven normal healthy volunteers (six males, five females) including five Smokers (two females, three males) increased fruit and vegetable intakes to obtain 18-36 mg/d of the specific carotenes ß-carotene(ß-car), lutein(lut) and lycopene(lycop), in 3 day cycles, that is, lut administered on day one, ß-car on day two and lycop on day three and continuing on this cycle over a 2 week period. Mean carotene intakes were calculated using Comp-Eat (Royal Society of Chemistry, 1993) to which data on lut and lycop were added from Hart and Scott (1995). Data on plasma carotenes and fat-soluble vitamins were obtained at the end of each week according to the methods of Thurnham *et al* (1988).

Mean increases for dietary carotenes (weeks 1 and 2) were 18, 28 and 36 mg/d for lut, ß-car and lycop respectively during supplementation. Significant increases of >3-fold were observed in plasma for the more lipophilic carotenes, ß-car and lycop between weeks 0 and 1 but no further changes occurred between weeks 1 and 2. No change however was observed in plasma lut after approximately a 10-fold increase in dietary lut over the supplemented period.



The Fig. shows the separate changes in plasma lut, lycop and ß-car in response to the changes in carotene intake in (a) NS and (b) S . Only changes in plasma ß-car from S and NS combined were significantly correlated with the changes in ß-car intake ($P<0.05$, r 0.69, n 11). The lack of significance in the individual groups is probably due to the small numbers. The regression data suggest that the absorption of lycop may be similar in S and NS but absorption of lut and ß-car appears to be depressed in S. These observations support results reported by ourselves and others, that plasma lycop does not differ between S and NS, whereas all other plasma carotenes appear to be lower in S (Thompson *et al.* 1985).

MO, UM, MC and IH are supported by AAIR2-CT93-0888.

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Evaluation of a nutrition risk scoring method. By A.M.TULLY[1], P.FLOOD[2] & N.P.KENNEDY[1], *Unit of Nutrition and Dietetic Studies, Department of Clinical Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8 and [2]Department of Clinical Nutrition, St. James's Hospital, Dublin 8, Republic of Ireland*[1]

It is increasingly recognized that a substantial proportion of hospital inpatients are malnourished or are at risk of developing nutrient deficiencies during their hospital stay (Bernstein *et al.* 1993; Mc Whirter and Pennington, 1994). A number of screening tools exist to identify such patients on admission to hospital, one of which was developed in Birmingham (Reilly *et al.* 1995). The purpose of the present study was to evaluate the Birmingham nutrition risk score (NRS, based on a questionnaire) in a sample of patients admitted to St. James's Hospital, Dublin.

All patients admitted to one surgical ward (*n* 24) and to one medical ward (*n* 37) over a period of 1 month were studied. Those unable to communicate verbally were excluded. Patients were classified as being at low risk, moderate risk or high risk of malnutrition. The classification of patients into these categories with the NRS was compared with the classification achieved using the usual method of assessing nutritional status in this hospital, comprising anthropometric assessment (actual and usual body weight, height, mid-arm circumference, skinfold thikness at four sites and grip strength) and biochemical indicators of nutritional status (albumin, total protein, transferrin and haemoglobin).

	Risk by standard nutritional assessment			
Risk by NRS	Low	Moderate	High	Total
Low	19	18	2	39
Moderate	0	6	0	6
High	0	0	16	16
Total	19	24	18	61

67.2% agreement between methods, with kappa (κ) statistic of 0.52, $p<0.001$.

The Birmingham NRS method yielded results which compared well with standard nutritional assessment in identifying well nourished (all ninteen correctly classified) and severly malnourished (sixteen out of eighteen correctly classified, 89%) patients. However, most of the mildly and moderately malnourished patients were incorrectly classified as low risk using the NRS method, in which the subjective questions relating to loss of appetite or of body weight are directed towards recent events rather than a gradual change over a long period. The two misclassified "high risk" patients had poor awareness of weight loss, which explained their lower scores using the NRS method. Better performance of the NRS might be achievable by minor adaptation of the NRS classification criteria, perhaps by the addition of a further objective variable such as mid-upper arm circumference, or mid-arm muscle circumference. An additional benefit likely to accrue from the routine use of this type of admission screening is the promotion of awareness, among healthcare personnel, of the need to assess nutritional risk of hospital inpatients.

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Waist circumference identifies cardiovascular risk factors. By THANG S. HAN[1], EDITH M. VAN LEER[2], JACOB C. SEIDELL[2] and MICHAEL E. J. LEAN[1], [1]*University Department of Human Nutrition, Glasgow Royal Infirmary, Glasgow G31 2ER, and* [2]*Department of Chronic Disease and Environmental Epidemiology, Postbus 1, 3720 BA Bilthoven, The Netherlands*

The relative risks of cardiovascular risk factors were determined in random sample of 2183 men and 2698 women aged 20-59 years living in The Netherlands. Subjects were divided into three groups on the basis of their waist circumference (Lean *et al.* 1995): ≤94 cm in men, ≤80 cm in women (below 'action level 1'), 94-≤102 cm in men, 80-≤88 cm in women (between 'action levels' 1 and 2), and ≥102 cm in men, ≥88 cm in women (above 'action level 2').

Measurements of height, weight, waist and hip circumferences (World Health Organization (WHO), 1989), total plasma cholesterol, HDL-cholesterol, blood pressure and lifestyle (alcohol consumption, cigarette smoking, physical activity and education levels) were made. Hypercholesterolaemia was defined as ≥6.5 mmol/l (WHO, 1988), low HDL-cholesterol as ≤0.9 mmol/l (European Atherosclerosis Society, 1987), and hypertension as treated and/or ≥160 mmHg systolic and/or ≥95 mmHg diastolic blood pressure (WHO, 1988).

	Prevalence (%), and age and lifestyle adjusted odds ratios (OR) of cardiovascular risk factors											
Waist	Hypercholesterolaemia			Low HDL-cholesterol			Hypertension			One or more risk factors		
(cm)	Prev	OR	95% CI	Prev	OR	95% CI	Prev	OR	95% CI	Prev	OR	95% CI
Men												
<94	10.3	1.00	_____	19.0	1.00	_____	4.2	1.00	_____	29.4	1.00	_____
94-<102	17.7	1.38	1.02, 1.87	32.8	2.37	1.85, 3.04	11.5	1.98	1.33, 2.95	51.1	2.23	1.78, 2.78
≥102	27.2	2.29	1.67, 3.14	44.4	3.64	2.75, 4.80	21.6	4.03	2.72, 5.96	69.9	4.57	3.48, 5.99
Women												
<80	9.4	1.00	_____	4.4	1.00	_____	2.7	1.00	_____	15.5	1.00	_____
80-<88	20.2	1.51	1.14, 2.00	6.1	1.54	1.00, 2.38	7.6	1.84	1.17, 2.88	30.4	1.64	1.30, 2.08
≥88	22.8	1.42	1.06, 1.89	14.0	3.80	2.59, 5.59	18.4	4.23	2.83, 6.33	44.3	2.55	2.02, 3.23

The Table shows that compared with subjects with waist circumference below 'action level 1', those with waist between 'action levels' 1 and 2 had age and lifestyle adjusted odds ratios (logistic regression analysis) significantly higher for cardiovascular risk factors. Those with waist above 'action level 2' had further increases in all relative risks, except for plasma cholesterol in women which covaried with age and waist circumference.

These results support the proposed 'action levels' of waist circumference for health promotion, to identify those at greatest risk of cardiovascular disease and in need of weight management.

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An investigation of the oils used in fast-food outlets in Galway city. By B. KELLY, S. FRIEL, G. NOLAN and C. KELLEHER, *National Nutrition Surveillance Centre, Department of Health Promotion, University College Galway, Republic of Ireland*

Accurate estimations of fat intake depend on knowledge of the type and source of fats in commercially available foods. Increasingly, meals are purchased outside the home and this needs to be considered in population-based surveys of diet, particularly where the food-frequency method is used. One meal type is so-called fast food. While there is increased interest in the relationship between diet and health there has also been an an increased consumption of fast foods, popularly believed to be high in fat, particularly saturated fat. There is evidence that vendors of such fast foods now include healthier foodstuffs in their menu choices. The present study was undertaken to investigate the degree to which this new consumer interest in healthier choices was reflected in the food preparation techniques used in fast-food outlets in Galway city.

A census survey of thirty-two Galway city fast-food outlets was conducted by a sole investigator. The criteria for inclusion in the study were threefold : chips/french fries had to be sold, the chips/french fries had to be cooked on the premises and the chips/french fries had to be sold for take-away purposes. Proprietors were asked which oils were used for chip making, how often the oil was changed and nutrition information of the oil.

There was a 100% response rate. Of the thirty-two establishments, thirty used vegetable oil and the other two used beef fat. Thirteen different brands of vegetable oil and one type of beef fat were used. Prices varied between the four most commonly used vegetable oils but were not a deciding factor in choice. The frequency of oil change varied from once per day to once per week but on average the oils were changed once in every 4 d.

Frequency of Chip-Pan oil change	Number of Outlets
Everyday	3
2 days	2
3 days	4
3.5 days	4
4 days	1
5/6 days	2
Every 7 days	5
"depends"	11

Only three of the fourteen oil containers had a nutritional analysis of the contents on the label though a more detailed analysis was elicited subsequently from the manufacturers. Content of saturated and unsaturated fats varied but the majority were predominantly unsaturated. The re-use of vegetable oil, involving repeated subjection to high cooking temperatures, may change the chemical structure from unsaturated into saturated fatty acids, so that recommendations on re-use should be included in product information.

Evaluation of the Nutrition Education at Primary School (NEAPS) programme developed by the Departments of Health and Education, the Health Promotion Unit and the North West Health Board. By S. FRIEL, G. NOLAN and C. KELLEHER, *National Nutrition Surveillance Centre, Department of Health Promotion, University College Galway, Republic of Ireland*

The Hearty Heart Nutrition Education programme, under the auspices of the Departments of Health and Education was implemented over a 2-year period of 1993-4 with the objective of designing suitable educational materials for Irish schoolchildren aged 8-10 years old. Development of this pilot nutrition education programme was undertaken in order to build awareness of the benefits of healthy eating, induce positive behaviour change towards them and to increase the children's knowledge about healthy eating.

A total of eight schools (453 children) was chosen for participation in the 10-week programme of lessons. Four were in the North Western Health Board area and classified as rural; the remaining four belonged to the urban part of Eastern Health Board catchment area. Certain schools were categorized as disadvantaged due to a shortage of available funds, priority of subjects and varying levels of literacy. Three reference schools (368 children) were also selected where no intervention took place.

A food-pairing questionnaire, validated from the Minnesota Health Project, was used to measure the effectiveness of the programme in three areas; behaviour, preference and knowledge in relation to healthy food. The children were asked to identify, for each of eighteen food pairs, which food they ate most often, which they preferred and which food they thought was the healthier choice. The questionnaire was given to the children both before the programme and upon its completion.

Students *t* test and ANOVA statistical methods were used to analyse for differences between the groupings; intervention, control, urban, rural, disadvantaged and advantaged schools.

Of the intervention children 78% responded to the pre-test questionnaire and 74% post-test. Significant differences were found in the intervention children's behaviour and preference levels after the NEAPS programme ($P < 0.01$ in both sections). Knowledge levels of these children also improved, although not significantly.

	Pre-Test Score	Post-Test Score	*P* value
Behaviour	25.36	24.20	< 0.01
Preference	27.42	26.25	< 0.01
Knowledge	20.50	19.68	ns

Rurally located children appeared to benefit more from the programme regarding their behaviour and preferences ($P < 0.01$). Although the urban children showed a positive change in their behaviour and preference it was not statistically significant. Locality did not significantly affect the influence of the programme on the children's knowledge levels. The NEAPS programme appeared to be more effective in the advantaged schools than the disadvantaged ($P < 0.01$ for each section behaviour, preference and knowledge).

Process evaluation of a community nutrition post in order to examine the implementation of the Nutrition Health Promotion Framework for Action. By M. O'DONNELL[1] and M. LYONS[2]
[1]*Western Health Board* and [2]*External Evaluator, Dublin, Republic of Ireland.*

As part of the Nutrition Health Promotion Framework for Action (Department of Health, 1991), a pilot Community Nutrition project was established in the Western Health Board in February 1992 and ran until July 1994.

A process evaluation was carried out by the Community Nutrition Service in order to re-examine the implementation of the Framework for Action. General aims of the pilot project were to develop, implement and evaluate a community based healthy food choice programme, and to promote healthy eating and lifestyle guidelines as stated in the Framework for Action policy document. Specific objectives were to provide training and educational programmes for health professionals; to provide information for existing groups and to network with these groups. A strategic plan was completed on an annual basis with quarterly reviews. Nutrition education needs assessments were completed for health professional groups.

During the project, process evaluation of educational methods used in sessions such as talks, workshops, exhibitions and the type of promotional activities used in national campaigns were carried out. Evaluation forms were completed by participants after sessions. In addition, sessions and activities were rated by the nutritionist on an observational scale of 1 - 5, poor to excellent.

Results of the evaluation revealed that the project has run well, building up a comprehensive community profile, liasing with health professionals to further increase their nutrition knowledge and networking with other organizations, food retailers and interest groups.

	Sessions*		
Target Groups	1992	1993	1994***
Health Professionals	23	28	12
Voluntary Groups	19	17	2
Community Groups	12	17	5
Mentally Handicapped Services	15	1	5
General Public	10	3	1
Private Sector	9	14	1
Schools and Colleges	10	4	2
Media	1992	1993	1994
Articles published	33	11	17
Photographs published	9	1	6
Radio **	90	200	85

* A session represents less than a three hour presentation
** Recorded in minutes
*** Represents summary of activities January to July inclusive 1994

Some projects have been more successful than others, due to excellent organization and co-operation/support of the health board, the many community groups, local businesses, food agencies, food retailers and the media. Other findings were the need for a nutrition and food policy within the Western Health Board to support the implementation process, and computerization and secretarial support to assist the running of the project.

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Evaluation of a pilot community nutrition programme in the Western Health Board. By G. NOLAN[1], S. FRIEL[1], C. KELLEHER[1] and M.O'DONNELL[2], *[1]National Nutrition Surveillance Centre, Department of Health Promotion, University College Galway and [2]Community Nutritionist, Western Health Board, Merlin Park Hospital, Galway, Republic of Ireland*

The post of community nutritionist was established in the Western Health Board in 1991. This initiative was part of the Framework for Action, a 5-year plan by the Health Promotion Unit, Department of Health, to improve the nutritional status of the Irish population.

The general objective of the present study was to evaluate the effectiveness of the role of the community nutritionist in the Western Health Board. More specific objectives were to quantify the level of knowledge of nutrition, to assess the attitudes to nutrition of target groups and to evaluate nutrition education strategies or approaches used by the community nutritionist. A comprehensive process evaluation was also conducted by the community nutritionist herself.

A baseline survey was conducted on a representative sample (10 %, n 168) of health professionals and organizations targeted by the community nutritionist across the three counties covered by the Western Health Board. A postal questionnaire was devised and piloted. The final response rate was 63% and comprised general practitioners (11.5%), public health nurses (11.5%), teachers (34%) and others (43%).

The results showed that overall awareness of the community nutritionist was high, though only 19% had, to date, had contact with the service. The remainder have been targeted by the community nutritionist for intervention, the effectiveness of which will form the second stage of this evaluation project. On the whole, nutrition knowledge of the respondents was good, particularly among the pharmacist group.

Profession	% correct answers
Public health nurse	78
General practitioner	67
Teacher	56
Pharmacist	89
Other	78

The majority of respondents were most interested in having more information on two subjects: healthy eating and nutrition and weight control. On average 80% of respondents felt that nutrition education was part of their professional role. Individual professions differed in their opinions as to what the role of the community nutritionist should include and what nutritional targets should be covered; however most were in favour of her involvement in clinical work in GP clinics. Respondents tended to favour workshops rather than lectures . On the whole, those who had a course with the community nutritionist felt more able to provide nutrition education to the public. Results of this stage of the evaluation imply that the community nutrition service merits continuation and further expansion.

Validation of methodology employed in the diet and lifestyle survey of Irish and West Virginian women. By B. KELLY, S. FRIEL, G. NOLAN and C. KELLEHER, *National Nutrition Surveillance Centre, Department of Health Promotion, University College Galway, Republic of Ireland*

Ireland and West Virginia in the United States of America have very similar demographic characteristics. We wished to compare diet and lifestyle behaviours in the two areas. Members of two women's organisations, Irish Countrywoman's Association and the Extension Homemakers Association of West Virginia were recruited to participate in a comparative study. A food-frequency questionnaire was the investigation instrument used. A random sample of 400 people in each country were sent the food-frequency questionnaire. The method chosen for validation of the food-frequency questionnaire was a 7 d food diary which included colour photographs of foods commonly eaten. A 10% sub-sample (forty women) were asked to fill in the food diary.

The present paper deals with the validation of the use of photographs in the food diary. A food diary is a method of measuring the subjects' food intake over a specified period of time. Coloured photographs, showing pictures of small, medium and large servings of some common breakfast, lunch and dinner foodstuffs were included in these particular diaries. Each serving size was of a pre-defined weight though the weight was not shown on the photograph. In order to determine if the photographs correctly represented the amount of food for each portion size, a sample of ten people were asked to participate in a 3 d weighed intake study. Each participant was provided with a 3 d food diary, a set of food pictures and a weighing scale. They were asked to record every food item they ate over 3 d and to quantify it using two methods: to weigh it using the scales provided, and to choose a photograph best representing the food item. Upon return of the diaries, two were discarded due to non-compliance.

Non-parametric statistical techniques were employed to determine whether differences existed between the size of food portions suggested by the photographs and the actual weighed records. Initially comparisons were made, for each respondent, between their set of recorded weights and corresponding photographs. Seven of the eight respondents correctly completed the experiment and demonstrated no significant difference in the photograph portion size selected and the actual food weight. Each individual's mean weights were compared using the sign test. No significant difference was found between the mean weighed intake and that suggested by the photographs.

Respondent	Mean weight by photographs (g)	Mean weight by weighing (g)
1	110.83	88.33
2	57.50	245.00
3	101.25	136.50
4	107.27	95.45
5	110.42	108.33
6	80.00	175.00
7	122.35	143.00
8	130.00	100.00

P value 0.72367

The results indicate that the food photographs adequately represent small, medium and large portion sizes as actually eaten and can be confidently included in the food diaries to be used in the diet and lifestyle survey.

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The use of food photographs as a tool for quantifying food and nutrient intake by 24 h recall. By P.J. ROBSON and M.B.E. LIVINGSTONE, *Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA*

Food photographs are useful for helping subjects to describe food quantity under controlled conditions, where recall of amounts actually consumed is not required (Lucas *et al.* 1995). However studies which rely on subjects estimating food quantity are usually based on recall procedures. The aim of the present study was to evaluate the errors made by subjects when using photographs to quantify amounts of food known to have been eaten 24 h earlier and the subsequent impact on assessment of nutrient intake.

Thirty adults (fifteen male, fifteen female) aged between 18 and 36 years volunteered to eat breakfast, lunch and dinner in the metabolic suite on two non-consecutive days. Different menus were served on the 2 d. At each meal the subjects served themselves as much food as they wanted from pre-weighed individual serving dishes and were permitted to return for more helpings if they wished. When they had finished eating, the remaining foods and leftovers were weighed. On the day following each observation, subjects quantified all foods which they had eaten in terms of fractions or multiples of the amounts shown in single-portion-size colour photographs. The table-ware previously used by the subjects in the metabolic suite was the same as that shown in the photographs. Nutrient contents of weighed and estimated food portions were calculated using computerised food tables. Errors in estimation of food and nutrient intakes were expressed as percentages of weighed intakes.

All subjects completed the procedure for day 1 and twenty-seven (90%) completed day 2. On day 1, ten foods were overestimated at group level and six were underestimated. Cheddar cheese was the most difficult food to estimate accurately with individual errors ranging from -39% to +285%. At least one fifth of the subjects made errors in excess of ±50% for ten foods. Only orange juice was estimated within ±10% of the amounts consumed by more than half of the subjects. On day 2, seven foods were overestimated and ten were underestimated. At group level, mean percentage errors ranged from +25% for muesli to -26% for coleslaw. Breakfast cereals tended towards overestimation but there was no evidence to suggest that any other visual characteristic could be used to predict error patterns. The calculated nutrient contents of the weighed and estimated quantities were then divided into thirds and the percentages of subjects whose estimated and weighed food intakes fell into the same, adjacent and opposite tertiles were determined.

	Percentage of subjects					
	Day 1 (*n* 30)			Day 2 (*n* 27)		
Nutrient	Same	Adjacent	Opposite	Same	Adjacent	Opposite
Energy (MJ)	66.7	33.3	0	77.8	18.6	3.6
Protein (g)	60.0	40.0	0	77.8	22.2	0
Fat (g)	70.0	30.0	0	70.4	25.9	3.7
Carbohydrate (g)	80.0	20.0	0	77.8	22.2	0
Calcium (mg)	66.7	33.3	0	66.7	22.2	11.1
Iron (mg)	63.3	36.7	0	77.8	22.2	0
Ascorbic acid (mg)	80.0	20.0	0	70.4	29.6	0
Thiamin (mg)	63.3	36.7	0	63.0	37.0	0
Riboflavin (mg)	70.0	30.0	0	63.0	29.6	7.4
Total folate (mg)	66.7	33.3	0	70.4	29.6	0

These results suggest that despite large errors in quantifying specific foods, food photographs may be useful for ranking subjects according to nutrient intake in recall situations.

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***Trans* fatty acid status of Irish food products.** By D.A. CRONIN and J. O'NEILL, *Department of Food Science, Agriculture Faculty, University College Dublin, Republic of Ireland*

The impact of dietary *trans* fatty acids derived from partially hydrogenated fats on the aetiology of coronary heart disease is controversial with recent large scale studies coming to apparently conflicting conclusions (Willett *et al*, 1993; Aro *et al*, 1995). In order to assess the intake of these components in the Irish diet detailed analytical studies are being undertaken on the fat component of a wide range of food products. Fat samples extracted using petroleum ether, chloroform and chloroform-methanol (2:1), depending on the product, were converted to their fatty acid methyl esters (FAME) by heating with H_2SO_4 in methanol (20 ml/l). Gas chromatographic analysis on a Silar 10C glass SCOT capillary column (30m x 0.6 mm i.d.) was used to determine the fatty acid composition of the FAME while *trans* fatty acid contents of the latter were measured in chlorobromomethane solutions (10, 20 and 50 ml/l) at 10.3 μm using a double-beam infrared spectrophotometer against methyl oleate in the reference cell.

		Trans fatty acid (%)	
Product	***n***	**Mean**	**Range**
Dairy spreads	14	19.2	11.8-31.2
Non-dairy spreads	19	11.2	1-22.3
Hard margarines	8	31.0	20.5-42.1
Cooking fats and shortenings	11	19.9	<1-42.0
Potato chip fat	25	6.8	<1-27.6
Biscuits	36	15.4	<1-49.2
Cakes	22	16.2	<1-38.6
Fruit-filled pies	7	12.4	<1-33.6

The tabulated data clearly highlight the ubiquitous occurrence of *trans* fatty acids as significant components of the fats in the product range examined with some such as hard margarines showing very high levels, and others, including cooking fats, shortenings, confectionery products and potato chip fats from a range of outlets around the country, showing highly variable levels. In general, high *trans* content (>30%) was strongly associated with the presence of hydrogenated marine oil in fat formulations.

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Waist circumference indicates the need for weight management. By THANG S. HAN[1], CAROLINE E. MORRISON[2] and MICHAEL E. J. LEAN[1], [1]*University Departments of Human Nutrition and* [2]*Public Health, Glasgow Royal Infirmary, Glasgow G31 2ER*

The risk of metabolic abnormalities and related diseases including cardiovascular disease and type 2 diabetes has been associated with increases in body fatness (indicated by BMI), and central fat distribution (reflected by waist:hip ratio) (Lapidus *et al.* 1988; Larsson *et al.* 1988). The present study aimed to test the hypothesis that a single measurement, waist circumference, might be used to identify people at health risk both from being overweight and from having a central fat distribution.

Height, weight, waist and hip circumferences (World Health Organization, 1989) were measured in a community-derived random sample of 904 men and 1014 women, and a second validation sample of eighty-six men and 202 women.

	Waist circumference				Sensitivity	Specificity
	(cm)	(in)	False Positive	False Negative	(%)	(%)
Determination sample						
Men						
'Action Level 1'	94	37.0	8/340	10/288	96.8	98.2
'Action Level 2'	102	40.0	12/541	4/105	97.9	97.8
Women						
'Action Level 1'	80	31.5	7/328	14/337	96.5	98.3
'Action Level 2'	88	34.5	2/471	2/151	99.2	99.6
Validation sample						
Men						
'Action Level 1'	94	37.0	0/38	2/32	94.1	100
'Action Level 2'	102	40.0	0/49	0/15	100	100
Women						
'Action Level 1'	80	31.5	3/98	0/63	100	97.1
'Action Level 2'	88	34.5	1/120	0/28	100	99.2

The Table shows that waist circumference ≥94 cm (37 in) for men or ≥80 cm (31.5 in) for women identified individuals with high BMI ≥25 kg/m^2 and those with lower BMI but high waist:hip ratio (≥0.95 for men, ≥0.80 women) with a sensitivity of >96%, and specificity >97.5% as defined by Sturmans (1984). Waist circumference ≥102 cm (40 in) for men or ≥88 cm (34.5 in) for women categorized individuals with BMI ≥30 kg/m^2 and those with lower BMI but high waist:hip ratio with a sensitivity of >96% and specificity >98%, misclassifying only about 2% of the entire sample.

Waist circumference could be employed in health promotion programmes to identify individuals who should seek, and be offered, weight management: the present study suggests 'action level 1' ≥94 cm for men and ≥80 cm for women for no further weight gain, and 'action level 2' ≥102 cm for men and ≥ 88 cm for women for weight reduction.

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Body-fat distribution and interaction with coronary heart disease risk. By M.L. BARNARD[1,2], G. FROST[3], E.L. THOMAS[1], J.E. SCHWIESO[1], J.D. BELL[1], J.V. HAJNAL[1], N. SAEED[1], O.M. ABU-MUHANA[1,2] and S.R. BLOOM[2], *[1]The Robert Steiner MR Unit, [2]Division of Endocrinology and Metabolism and [3]Department of Dietetics, Hammersmith Hospital, Du Cane Road, London W12 0HS*

Coronary heart disease (CHD) is linked to insulin resistance and the high insulin levels may directly affect cell proliferation in atherosclerotic plaque formation. Abdominal obesity is a major risk factor for CHD, associated with insulin resistance and dyslipidaemia. This has been linked to the release of free fatty acids (FFA) from the internal visceral fat directly into the portal vein. These FFA may drive hepatic lipoprotein synthesis and gluconeogenesis, diminish hepatic insulin clearance and induce resistance to insulin-controlled glucose uptake in muscle (Vague & Raccah, 1992). The waist:hip ratio (WHR) has been used as a surrogate measure of visceral fat. Magnetic resonance imaging (MRI) has been applied to analyse body fat and measure separate fat depots (Ross *et al.* 1992; Barnard *et al.* 1994). The present study directly quantified abdominal fat by MRI, to determine the relationship between visceral fat and cellular insulin resistance.

Male volunteers (two Caucasian, two Asian) were examined before coronary artery bypass grafting. Transverse MR images of the whole abdomen were acquired using a rapid T_1 weighted spin-echo sequence at 1.0T. Visceral adipose-tissue volume was normalized to body size (height and obesity) by dividing by the BMI (visceral fat index). At operation, a pre-sternal fat biopsy was taken for analysis of cellular insulin-stimulated glucose uptake. Isolated adipocytes were incubated in 10 g/l albumin buffer with or without insulin. 2-Deoxy-[U-^{14}C]-D-glucose was added and radioactivity associated with the adipocytes was determined by liquid scintillation counting. Results are expressed as percentage increase in glucose uptake (attmol/cell per min) with insulin stimulation, so that insulin resistance results in a reduced glucose uptake.

Subject		Stimulated insulin sensitivity*	Visceral fat index (litre/kg per m^2)†	BMI (kg/m^2)	Waist:hip ratio
1	Caucasian	133 %	0.17	28.3	1.04
2	Asian	44 %	0.17	23.4	0.91
3	Asian	36 %	0.22	24.0	0.87
4	Caucasian	26 %	0.26	28.9	1.02

*Percentage increase in glucose uptake after insulin added to isolated adipocytes (attmol/cell per min).

†Visceral fat index: absolute visceral fat volumes normalized to BMI.

A direct relationship was found between increasing insulin resistance and increasing visceral fat (Kendall's coefficient 0.92, $P<0.05$). This was not found on examining overall obesity (BMI). A difference in body-fat distribution between Asian and Caucasian subjects was suggested by a significant difference in the WHR (mean 0.89 (SEM 0.01) *v.* mean 1.03 (SEM 0.01), $P<0.02$). However, the WHR did not reflect either the visceral fat index or the absolute measurement of visceral fat volumes across the whole abdomen or in a central abdominal slice.

This study suggests a direct relationship between visceral fat quantification and cellular insulin resistance. These findings support current hypotheses which link risk of CHD to body-fat distribution. The poor association between the WHR and visceral fat quantification may reflect the mixed ethnic group of volunteers, with differences in body-fat distribution. However, the MRI measurement of visceral fat proved rapid and reliable. This safe, non-invasive MRI method of visceral fat analysis may therefore become the "gold standard" research tool for future studies aimed at reducing the risk of CHD.

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The relationship between women's birth weight and their current intra-abdominal fat-mass. By THANG S. HAN[1]*, GERALDINE McNEILL[1] and DORIS M. CAMPBELL[2], *Departments of [1]Medicine & Therapeutics and [2]Obstetrics & Gynaecology, Aberdeen University, Aberdeen AB9 2ZD. *Present address: University Department of Human Nutrition, Glasgow Royal Infirmary, Glasgow G31 2ER*

Reduced growth early in life has been found to be associated with increased risk of developing type 2 diabetes and cardiovascular disease later in life (Barker, 1992). A relation between birth weight and intra-abdominal fat mass deposition has been suggested by studies of waist:hip ratio and birth weight in men (Law *et al.* 1992). However, waist:hip ratio is an indicator of the trunk:limb fat-mass ratio rather than of intra-abdominal fat-mass *per se*.

We have recently found that waist circumference was more closely related to intra-abdominal fat-mass (r^2 79.2%; P <0.001) than waist:hip ratio (r^2 35.5%; P <0.05) measured by magnetic resonance imaging (Han *et al.* 1995); we therefore investigated the relationship between intra-abdominal fat-mass and birth weight in women by comparing waist circumference in forty-six women, mean age 32 (SD 8) years, weight 66 (SD 13) kg and waist circumference 80 (SD 14) cm, with birth weight 3.1 (SD 0.4) kg obtained from obstetric records.

As there was a significant relationship between current body weight and birth weight (r 0.39; P = 0.008), body weight and birth weight were used as independent variables in multiple regression analysis to predict waist circumference (dependent variable). A significant inverse relationship between birth weight and waist circumference was observed (regression coefficient of birth weight: -6.2; 95% CI: -11.0, -1.4; P = 0.01). The same variables used in a previous study of men (Law *et al.* 1992) were analysed in the present study of women showed no significant relationship between waist:hip ratio and birth weight, adjusted for BMI (regression coefficient of birth weight: -1.2; 95% CI: -5.7, 3.3; P = 0.60).

The results of the present study suggest that early nutrition may influence the risk of metabolic abnormalities and related diseases through increased intra-abdominal fat-mass deposition in women.

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Non-invasive characterization of neonatal adipose-tissue by ^{13}C magnetic resonance spectroscopy.

By E.L. THOMAS[1], D. HANRAHAN [2], D. AZZOPARDI [2], M.L. BARNARD [1,3], D.J. BRYANT [1], G. FROST[4] and J.D. BELL[1], *The Robert Steiner [1]MR Unit, Departments of [2]Paediatrics, [3]Endocrinology and [4]Dietetics, Hammersmith Hospital, Du Cane Road, London, W12 0HS*

Examination of the fatty acid composition of subcutaneous adipose-tissue of infants has so far required tissue samples obtained by biopsy or at post-mortem for analysis by GLC. It has therefore not been possible to perform serial, longitudinal studies of adipose-tissue composition of healthy infants. It is important to study neonatal adipose-tissue fatty acids, as they provide a source of lipid precursors, destined for membrane development for various organs, such as the liver or brain (Farquharson *et al.* 1992). The quantity and composition of neonatal adipose-tissue may, therefore, have implications for normal development. The aims of the present study were to use ^{13}C magnetic resonance spectroscopy (MRS) to study adipose-tissue composition of healthy infants and their mothers.

Term (gestational age of 38 - 41 weeks) and preterm (30 - 34 weeks) infants were studied on day 1 after birth. Six term infants (fully breast-fed) were studied again after 6 weeks. *In vivo* ^{13}C MR spectra were collected from the adipose-tissue of the left thigh from the mother and from the upper thigh and buttocks of the neonates.

	% Saturated		% Unsaturated		% Poly†		% Mono‡	
Subject	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Mother (*n* 6)	89.6**	0.3	10.4**	0.3	2.8**	0.2	7.7**	0.2
Full-term (*n* 12)	92.7	0.3	7.3	0.3	1.9	0.1	5.4	0.3
6-weeks old (*n* 6)	90.7*	0.4	9.3*	0.4	2.9**	0.2	6.4	0.4
Preterm (*n* 6)	93.9	0.6	6.1	0.6	2.4	0.4	3.75*	0.6

†Poly refers to carbons in -CH=C**H**-CH_2-C**H**=CH- positions.

‡Mono refers to carbon in -C**H**=C**H** and -C**H**=CH-CH_2-CH=C**H**- positions.

Significantly different from full-term infants: $*P < 0.05$, $**P < 0.01$

Using ^{13}C MRS, we showed differences between newborn infants and mothers and showed the influence of maturity on adipose-tissue composition of newborn infants. Newborn infants had significantly more saturated fatty acids than their mothers. Infants adipose-tissue at 6 weeks showed an increase in unsaturated fatty acids; these changes predominantly reflect the effects of diet. Differences between the spectra of preterm and full-term newborn infants arise from gestational age rather than diet. Accretion of unsaturated fatty acids during the third trimester of pregnancy is particularly rapid (Clandinin *et al.* 1981). Only a few additional weeks of intrauterine development will result in the different fatty acid composition of adipose-tissue. These differences can be clearly observed by *in vivo* MRS.

Our results suggest that ^{13}C MRS offers a unique opportunity to study non-invasively neonatal lipid metabolism during development and disease. It should also enable the *in vivo* study of long-term dietary influences on lipid composition and aid in determining the effect of maternal diet on neonatal and infant adipose-tissue composition.

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The effect of dietary α-tocopherol, curcumin and eugenol on lipid peroxidation in rat tissues. By I. O'CONNOR, A. O'SULLIVAN and N.M. O'BRIEN, *Department of Nutrition, University College Cork, Republic of Ireland*

Concern over synthetic antioxidants has led to increased interest in evaluating the antioxidant potential of natural plant phenolic compounds. Some spice components commonly used in Asian diets, have been shown to possess antioxidant properties (Pulla Reddy & Lokesh, 1994a, b). In the present study we have compared the antioxidant activities of α-tocopherol, curcumin (a component of the spice turmeric) and eugenol (an essential oil from cloves).

Sixty male Wistar rats, average weight 119 g, were randomized by weight into six groups (n 10). The rats were fed *ad libitum* on a control purified diet (AIN-76) or the control diet supplemented with α-tocopherol (0.2 g/kg), curcumin (5 and 10 g/kg) or eugenol (0.25 and 0.5 g/kg). At the end of the 8-week feeding period, the animals were sacrificed and the liver, lung, heart and spleen excised. Basal and Fe-ascorbate-induced levels of lipid peroxidation, as indicated by thiobarbituric acid reactive substances (TBARS) were measured. Activities of the antioxidant enzymes, catalase (CAT, *EC* 1.11.1.6) and superoxide dismutase (SOD, *EC* 1.15.1.1), were also determined. Basal TBARS levels, which are an indication of *in vivo* lipid peroxidation, are shown in the Table.

TBARS (nmol malonaldehyde/mg protein)

	Control		α-Tocopherol (g/kg diet) 0.2		Curcumin (g/kg diet) 5		Curcumin (g/kg diet) 10		Eugenol (g/kg diet) 0.25		Eugenol (g/kg diet) 0.5		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	LSD
Liver	0.63	0.01	0.53*	0.02	0.54*	0.05	0.53*	0.02	0.62	0.02	0.49*	0.02	0.08
Lung	0.35	0.01	0.30*	0.01	0.36	0.01	0.36	0.01	0.35	0.02	0.35	0.04	0.05
Heart	0.57	0.01	0.36*	0.04	0.58	0.01	0.45*	0.04	0.61	0.01	0.58	0.02	0.08
Spleen	0.69	0.02	0.71	0.01	0.63	0.04	0.64	0.02	0.74	0.03	0.72	0.03	0.08

LSD, least significant difference. * Significantly different from control: (one-way ANOVA): $P<0.05$.

Food intake and growth rate were not affected by dietary α-tocopherol, curcumin or eugenol supplementation. Dietary α-tocopherol and curcumin (10 g/kg diet) significantly reduced basal levels of TBARS in the liver and heart and also decreased susceptibility of tissues to Fe-ascorbate-induced lipid peroxidation (results not shown). Eugenol (0.5 g/kg diet) exerted a protective effect only in the liver. The activities of CAT and SOD were higher in organs of rats fed a curcumin-(10 g/kg diet) supplemented diet in comparison with control or eugenol-supplemented diets (results not shown). These results suggest that certain natural phenolic compounds, such as curcumin, may play a role in decreasing lipid peroxidation *in vivo*. However, in this study lower levels of α-tocopherol inhibited lipid peroxidation in the tissues, indicating that it is a more efficient antioxidant.

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An investigation of the intracellular location of cholesterol oxidation products in Swiss albino 3T3 cells in culture. By R.M. SISK, A.M. WILSON and N.M.O'BRIEN, *Department of Nutrition, University College Cork, Republic of Ireland.*

Cholesterol is involved in many essential physiological functions including the maintenance of cellular membranes. However, cholesterol is subject to oxidation under conditions such as exposure of foods to elevated temperatures, yielding cholesterol oxidized products (COP). These COP influence sterol biosynthesis, cell growth and proliferation, and have been implicated in the development of atherosclerotic lesions (Peng *et al.* 1991).

In the present study, differential centrifugation techniques were used to determine the intracellular location of cholesterol and COP in the Swiss albino embryo murine cell-line (3T3). 3T3 cells were cultured in Dulbecco's modified Eagle's medium and maintained in a humidifed atmosphere at 37°c and CO_2 (50ml / L). The growth medium was supplemented with 20 µmol / L of cholesterol, 7-ketocholesterol (7-keto) or 25-hydroxycholesterol (25-OH) for a 24 h period. Pellets enriched in nuclear (P1), mitochondrial (P2) and microsomal (P3) fractions were obtained by centrifugation at 600 ***g***, 1500 ***g*** and 100, 000 ***g*** respectively. The cytosolic fraction present in the supernatant fraction (S3) was also retained for analysis following centrifugation at 100, 000 ***g***. The purity of the fractions was verified using particular marker enzymes.(Hubbard & Ma, 1983). The samples were analysed for cholesterol and COP by GC and were compared with a standard.

Cholesterol and COP measured in the fractions*

	Cholesterol	Epoxide	25-OH	7-keto
	Mean SE	Mean SE	Mean SE	Mean SE
Control†				
P1	0.139±0.12	-	-	-
P2	0.218±0.11	-	-	-
P3	0.454±0.08	0.031±0.00	-	-
S3	0.178±0.01	0.070±0.01	-	-
Cholesterol (20 µM)				
P1	1.091±0.01	0.054±0.04	-	-
P2	1.621±0.78	-	0.075±0.02	-
P3	1.126±0.48	-	-	-
S3	0.542±0.05	-	-	-
7-Keto (20 µM)				
P1	0.039±0.01	-	-	0.094±0.02
P2	0.163±0.05	-	-	0.047±0.02
P3	0.324±0.08	-	-	0.026±0.00
S3	0.170±0.04	-	-	0.081±0.02
25-OH (20 µM)				
P1	0.578±0.03	0.043±0.00	0.451±0.07	-
P2	0.176±0.00	0.208±0.03	0.036±0.01	-
P3	0.220±0.06	0.166±0.01	0.094±0.01	-
S3	0.154±0.01	0.016±0.02	0.009±0.00	-

*Compound measured by gas chromatography and expressed as µg / mg protein.
†Control cells cultured with growth medium alone. ***n*** **8** for all treatments.

Levels of cholesterol appeared in all fractions of the control cells, while β-epoxide was present in P3 and S3 fractions. When cells were cultured with cholesterol, the concentration in all fractions increased. Cells supplemented with 7-keto were found to incorporate the oxide in all fractions. Both 25-OH and β–epoxide were observed in all fractions of those cells treated with 25-OH. The results suggest that 7-keto and 25-OH were taken up by the cells and that 25-OH was incorporated predominantly in the nuclear fraction.

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The effect of oxalate and citrate on calcium absorption in the rat. By T. BENNETT, K. CASHMAN and A. FLYNN, *Department of Nutrition, University College, Cork, Republic of Ireland*

The aim of the present study was to investigate the effect of oxalate and citrate on Ca absorption using a rat model which has been shown to be useful for studies on Ca bioavailability (Cashman & Flynn, 1994; Harrington *et al.*. 1994).

Forty 7-week-old male rats, Wistar strain, average weight 201 g, were randomized into five groups of eight rats each and fed on a purified diet (AIN-76) for 2 weeks. Each group was then given a meal (10 g of same diet) containing (per kg) oxalic acid or citric acid at 0 (control), 129 or 258 mmol acid and 5 g Ca as $^{47}CaCO_3$. ^{47}Ca was determined in quantitative daily faecal collections over 7 d, and fractional absorption of ^{47}Ca was estimated by extrapolation of the linear portion of the plot of log ^{47}Ca retention *v.* time back to the time of isotope administration (Cashman & Flynn, 1994). The effect of both acids on Ca absorption was also investigated in 4-week-old rats. Oxalic acid or citric acid (0, 5, 10 and 20 mM) were added to a 10 mM-$CaCl_2$ solution which was then labelled with ^{47}Ca (17.5 kBq/ml) and 0.5 ml administered orally to the 4-week-old rats, previously fasted overnight. Animals were killed 14 h later and ^{47}Ca absorption determined (Cashman & Flynn, 1993).

	9-Week-old rats						
	Control	Oxalic acid		Citric acid			
Acid (mmol/kg)...	0	129	258	129	258	Pooled SEM	LSD(P<0.05)
Ca absorption (%)	34.4	21.3*	20.5*	35.4	31.0	1.7	4.9

	4-Week-old rats					
Acid (mM)...	0 (control)	5	10	20	Pooled SEM	LSD(P<0.05)
Ca absorption (%):						
Oxalic acid	92.6	85.5	82.9*	81.3*	2.7	7.9
Citric acid	89.6	84.2	85.1	88.8	2.1	5.6

LSD, least significant difference
Significantly different from control (one-way ANOVA): *P<0.05.

Increasing the meal oxalate concentration from 0 to both 129 and 258 mmol/kg (representing an increase in the oxalate:Ca molar ratio from 0:1 to 1:1 and 2:1 respectively) significantly reduced Ca absorption in 9-week-old rats. Similarly, oxalate concentrations of 10 and 20 mM significantly reduced Ca absorption in 4-week-old rats. Increasing citrate concentration had no effect on Ca absorption in either 9-week-old or 4-week-old rats.

These findings suggest that citrate has little effect on Ca absorption whereas oxalate, at oxalate:Ca molar ratios found in some human foods, is inhibitory to Ca absorption.

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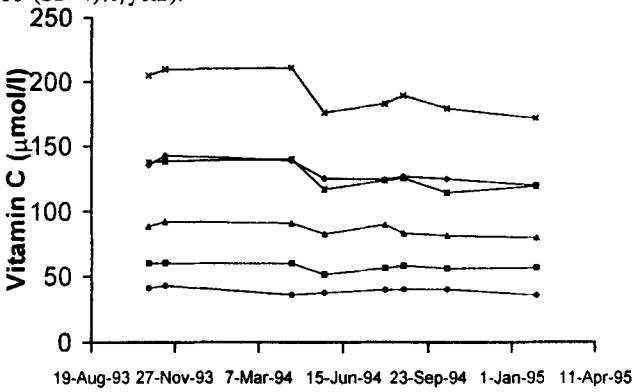
The stability of plasma vitamin C during storage in cryo-bio straws in liquid nitrogen and in metaphosphoric acid at -70° over a 16-month period. By L.M.EDMOND[1], D.I.THURNHAM[1] and S.A.BINGHAM[2]. [1]*Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA and* [2]*Dunn Clinical Nutrition Centre, Hills Road Cambridge CB2 2DH*

Blood is being collected and stored in liquid N_2 for many years as part of the European Prospective Investigation into Cancer (EPIC). The containers being used are soft plastic straws (BICF, Paris) which have not previously been used to store blood products. These data were acquired as part of a larger project to monitor a range of oxidation-sensitive biomarkers during storage.

A bank of six citrated plasma samples apportioned into 0.5 ml straws was set up in August 1993 and stored in liquid N_2. In addition portions of metaphosphoric acid (MPA)-stabilized serum (one part serum to two parts 100g/l MPA) were prepared and stored at -70°. One of the samples was taken from a smoker, two from females (one on vitamin supplements) and two were spiked with vitamin C.

On removal from $N_{2(l)}$ the plasma was stabilized with MPA as above. Ascorbate was measured using electrochemical detection following reverse-phase chromatography through a 100 mm, 3 μm, Spherisorb ODS2 column (modification of that of Heiliger, 1980). The mobile phase contained 0.1 M sodium acetate, 1 M octylamine, 200 mg/l Na_2EDTA and 100 mg/l DL-homocysteine. The pH was adjusted to 5.5 using 2 M NaOH. Run time was 3 min.

Over 16 months, 8 measurements for each of the six samples under both conditions were performed in duplicate with the system calibrated on the day of each run. The inter- and intra-batch precision of the assay were 5 and 1% respectively. The mean rate of deterioration of ascorbate over the 16 month-period was less for plasma stored in liquid N_2 (10 (SD 3)%/year), shown in the figure than for MPA at -70° (16 (SD 4)%/year).



In addition, we analysed plasma ascorbate in an age- and sex-stratified subsample (n 199) from the first 2000 volunteers in the Norfolk sample of the EPIC study. The mean concentration was 41.7 (SD 17) μmol/l which compares with 55.1 (SD 20) μmol/l for ascorbate measured within 2 weeks of collection on MPA-stabilized plasma. The 20% difference in these measurements, 12 months apart, can be accounted for by a 10% deterioration and a possible 10% methodological variation as the method used on the fresh samples measures both ascorbate and dehydroascorbate (Vuilleumier & Keck,1989). However the two sets of measurements were highly correlated (p=0.0001, r 0.874) indicating that useful results for ascorbate can still be produced after this period of $N_{2(l)}$ storage.

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Evaluation of the biological activity of monoglycated human insulin using the euglycaemic hyperinsulinaemic clamp technique in man. By A.C. BOYD[1], S.J. HUNTER[2], F.P.M. O'HARTE[1], I. WIGGAM[2], C.N. ENNIS[2], R. GAMBLE[2], B. SHERIDAN[2], C.R. BARNETT[1], H. McNULTY[1], P.M. BELL[2] and P.R. FLATT[1], *[1]School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA and [2]Sir George E. Clark Metabolic Unit, Royal Victoria Hospital, Belfast BT12 6BA*

Non-enzymic glycosylation (glycation) is a common post-translational modification of both structural and functional proteins, the extent of which increases as a consequence of long-term hyperglycaemia encountered in diabetes mellitus. Glycation of insulin has been shown to occur in pancreatic ß-cells isolated from animal models of diabetes. It has been postulated that glycation of proteins may alter their biological potency. Thus, the aim of the present study was to examine the biological activity of glycated (GI) and non-glycated (NGI) human insulin in man.

Monoglycated insulin was prepared *in vitro* under hyperglycaemic conditions. Briefly, Humulin S (Eli Lilly; 2.4 U/ml), glucose (220 mM) and 1000 molar excess of $NaBH_3CN$ were incubated for 24 h at 37°, purified by reverse-phase HPLC (O'Harte *et al.* 1994) and subjected to sterility and pyrogen testing. GI (glucitol adduct on B-chain Phe[1]) and NGI action was assessed in eight healthy male volunteers aged 20 - 22 years (BMI 22.18 (SE 0.83) kg/m^2), using the euglycaemic hyperinsulinaemic clamp and [3 ^{3}H]glucose isotope dilution technique. Subjects were admitted at 08.00 hours on two separate occasions following an overnight fast (12 h), and consecutive 2 h low and high insulin doses were infused (NGI 0.4 and 2.0 mU/kg per min; GI 0.47 and 2.35 mU/kg per min).

	GI infusion rate				NGI infusion rate			
	Low		High		Low		High	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Insulin-mediated glucose uptake (μmol/kg per min)	22.8	2.3	63.7	2.0	26.6	2.3	59.3	2.2
Endogenous glucose production (μmol/kg per min)	5.1	1.0	4.9	0.6	4.7	0.7	4.5	0.8
Metabolic clearance rate (ml/min per kg)	19.8	1.4	15.8	1.1	22.3	2.0	16.3	1.8
Plasma glucagon (ng/l)	90.9	8.2	75.9	7.3	84.5	7.4	81.9	6.6

No significant differences between GI and NGI insulin values (n 8) were observed (Student's paired t test).

The Table illustrates that both insulin-mediated glucose uptake and metabolic clearance rate of GI and NGI displayed no significant difference during the insulin infusions, despite the use of 20% more GI. Endogenous glucose production and circulating glucagon levels were suppressed to a similar degree during GI and NGI infusions.

This study suggests that glycation of human insulin at the Phe[1] position of the B-chain impairs peripheral and/or hepatic insulin action in man.

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Evaluation of Nutrifil, a nutritionally complete supplementary food, with respect to acceptability and weight gain in malnourished children in Katale Refugee Camp, Zaire. By P.M.MATHIAS and D.G.BYRNE, *Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Republic of Ireland.*

Supplementary feeding programmes play an important role in maintaining the health and nutritional status of displaced persons in humanitarian emergencies. Nutritionally complete supplementary foods are frequently used to avoid the logistical problems involved in providing a wide variety of foodstuffs. Nutrifil (Danecreek Ltd., Dublin, Ireland) is a newly developed supplementary food, consisting of a blend of pre-gelatinized wheat flour, fat-filled milk, sugar, and a vitamin/mineral premix. The nutritional composition per kg of Nutrifil is shown in the Table below.

Energy	17.9 MJ 4170 Kcal	Vitamin A (retinol) Vitamin C	5500 μg 560 mg	Niacin Vitamin B6	70 mg 6.0 mg	Potassium Magnesium	8600 mg 1200 mg
Protein	149 g	Vitamin D_3	800 μg	Folic Acid	1440 μg	Zinc	45 mg
Fat	107 g	Vitamin E	470 mg	Vitamin B_{12}	13 μg	Copper	5.0 mg
Carbohydrate	650 g	Thiamin (B_1)	5.0 mg	Iron	50 mg	Iodine	850 μg
Sodium	1700 mg	Riboflavin (B_2)	9.0 mg	Calcium	4700 mg	Selenium	250 μg

The formulation, which complies with the Food and Agricultural Organisation Codex Alimentarius standards, was designed to ensure that as little as a 2.10 MJ (500 Kcal) ration provides up to 100% of reference nutrient intakes (RNI) for vitamins and minerals for children under 5 years of age (Department of Health, 1991). It also corresponds to a recommended nutrient profile for use in supplementary feeding programmes (Golden *et al* 1995). Nutrifil was incorporated into the feeding regime at a therapeutic feeding centre in Katale, Zaire, during the recent Rwandan refugee crisis. A limited field trial was undertaken to assess the acceptability of Nutrifil and its effect on weight gain in children treated at the centre. Nutrifil was prepared by mixing with water to a caloric density of 4.18 KJ/ml. Intakes and weight gain were recorded over 7 days in 20 malnourished children aged 12-60 months (mean 38 months), with an average weight of 11.7 (SD, 3.1) kg and a mean weight for age of 79 (SD,15)%. The mean total daily energy intake was 6.1 (SD, 0.37) MJ. Nutrifil provided 78% of total intake with other supplementary foods, BP5 (a high energy biscuit) and Nutriset Weaning Food (a soya flour-containing dry cereal blend), contributing 16% and 6%, respectively. Children showed a strong taste preference for Nutrifil. Mean body weight after 7 days was 12.6 (SD, 3.3) kg, significantly higher than the mean body weight at day 1 ($P<0.005$). The mean rate of weight gain was 11.0 (SD, 4.9) g/kg body weight/day, which indicated children were achieving rapid "catch-up" growth (Ashworth, 1967). Weight gain was significantly correlated with Nutrifil intake (r 0.94, $P<0.001$). The results of this study suggest that Nutrifil has a good potential as an acceptable and effective nutritional product for nutrition rehabilitation.

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The effect of oral and intramuscular iodine supplementation on goitre and thyroid hormone status of Tanzanian schoolchildren. By F.J. MALONE[1], J.J. STRAIN[1], A.M. GRAY[1], N.C. ROLLINS[2], J.A. DODGE[2], K. MTEBE[3] and N. BANGU[3], [1]Human Nutrition Research Group, University of Ulster, Coleraine BT 52 1SA, [2]Queen's University of Belfast, BT12 6BA and [3]Sokoine University of Agriculture, PO Box 3006, Morogoro, Tanzania

Although the iodization of edible salt can be an effective and low cost method of preventing I deficiency, it is often unsuccessful because of unfavourable climatic, economical or geographical factors. Single intramuscular injections of iodized oil may provide enough I for 2 or 3 years but have serious advantages compared with the less frequently used oral method of administration. The aim of the present study was to determine which method of I supplementation, oral or intramuscular, is most effective at reducing goitre and improving thyroid function over a 3-month period.

A total of 270 schoolchildren, aged 7-17 years, from the village of Maskat in the Morogoro region of Tanzania were randomly allocated by age and sex into three groups: a control group (A), given an oral placebo capsule, a group given an oral supplement of iodized oil (480 mg I)(B) and a group given an intramuscular injection of iodized oil (480 mg I) (C). The supplements were kindly supplied by Laboratoire Guerbet, France. Personal details, anthropometric measurements and clinical data were collected at baseline. A 2 ml blood sample was taken pre-supplementation (May) and 3-months post-supplementation (August). A total of 69% of children had goitre at baseline which indicated that there was I deficiency in this area.

		Serum T3 (nmol/l)		Serum T4 (nmol/l)		Serum TSH (μmol/l)	
Group	n	Mean	SD	Mean	SD	Mean	SD
A: May	63	2.55	0.66	114.40	35.32	2.13	1.12
August	63	2.63	0.53	107.15	23.83	2.50*	1.69
B: May	73	2.51	0.63	107.43	30.36	2.28	1.08
August	73	2.68*	0.59	107.52	22.72	2.08	1.11
C: May	73	2.43	0.49	106.61	23.42	2.06	1.04
August	73	2.45	0.48	110.48	33.16	1.74**	1.01

Significantly different from baseline measurement (paired *t* test): *$P<0.05$, **$P<0.01$.

In the placebo group serum thyroid stimulating hormone (TSH) concentration increased during the 3-month period. This suggests the presence of a dietary goitrogen. Oral supplementation resulted in reduction of goitre size. There was no significant change, however, in either thyroxine (T4) or TSH concentrations whereas tri-iodothyronine (T3) was significantly increased with oral supplementation. Intramuscular supplementation improved thyroid function, indicated by a significant decrease in mean serum TSH concentration. A significant increase in goitre size, however, was also recorded in this group.

Oral I supplementation appeared to give a more rapid beneficial effect during the 3-month period than intramuscular injection. This method has other advantages owing to cheap and easy administration, absence of necessity for trained personnel and elimination of disease transmission.

Copper supplementation and bone-mineral density in middle-aged women. By J. EATON-EVANS[1], E.M. McILRATH[2], W.E. JACKSON[3], H. McCARTNEY[1] and J.J. STRAIN[1], [1]*Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA,* [2] *The Royal Hospitals Trust, Belfast BT12 6BA and* [3]*Skegoneill Health Centre, Belfast BT15 3LL*

Cu deficiency in animals and pre-term infants has been shown to result in thin, light bones with increased fragility. The mechanism involved is thought to be a deficiency of the Cu-containing enzyme lysyl oxidase (*EC* 1.4.3.13) which in turn causes a defect in the cross-linking of bone collagen (Danks, 1988; Strain, 1988). Osteoporosis is a defect in both the quality and quantity of bone.

In the present study, seventy-three women, aged 45 - 56 years, were recruited from a health centre in north Belfast and were given either a supplement of 3 mg Cu as an amino acid chelate or a placebo to take daily for 2 years. At the beginning and end of the study, bone mineral density (BMD) of the lumbar vertebrae (L2-4) was measured by computed tomography (CT)-scan and random blood samples were taken for putative measurements of Cu status including erythrocyte superoxide dismutase (SOD; *EC* 1.15.1.1) using Ransod kits (Randox, Crumlin, Co. Antrim) and haemolysed erythrocyte Cu using atomic absorption spectrophotometry. The women were seen every 3 months to monitor compliance with supplementation.

The initial and final BMD of the seventy-three women was 121.0 (SD 30.5) mg/cm^3 and 116.5 (SD 30.8) mg/cm^3 respectively (paired *t* test, P=0.02). Twenty-four women took the Cu supplement, thirty-two women the placebo and seventeen women were non-compliers taking neither the Cu supplement nor the placebo for the total period of the study. There were no differences between the 3 groups of women with respect to age, body mass index, dietary intake and smoking and drinking habits although the non-compliers were more likely to have husbands in non-manual work and have fewer children.

Over the study women who took the copper supplement did not lose BMD (initial 124.6 (SD 32.1) mg/cm^3 and final 123.8 (SD 36.3) mg/cm^3) while the women who took the placebo lost BMD (initial 120.7 (SD 29.2) mg/cm^3 and final 113.2 (SD 26.6) mg/cm^3, P=0.01). The regression equations for final BMD from initial BMD were:

Cu- supplemented: final BMD =1.035 (SD 0.097) x initial BMD -5.22 (SD 12.43), r 0.92,
placebo: final BMD=0.769 (SD 0.089) x initial BMD + 20.42 (SD 11.04), r 0.84.

The slopes of the regression lines were significantly different (P=0.05). There was no significant difference in the initial and final BMD of the seventeen non-compliers (initial 116.4 (SD 31.6) mg/cm^3 and final 112.6 (SD 29.6) mg/cm^3) and these women were not included in the analyses for the regression lines.

Although there was no effect on biochemical measurements (including erythrocyte SOD and erythrocyte Cu), Cu supplementation appeared to reduce the rate of loss of BMD in these women over the study period.

We gratefully acknowledge support from the Howard Foundation for this study.

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Excretion of urinary pyridinium crosslinks in healthy young adults: inter- and intra-individual variations and effects of sodium. By F. GINTY, K. CASHMAN and A. FLYNN, *Department of Nutrition, University College Cork, Ireland*

Recent advances have resulted in the development of assays for circulating and urinary markers that reflect specifically either bone formation or bone resorption. Urinary pyridinium crosslinks of collagen, pyridinoline (Pyr) and deoxypyridinoline (Dpyr), show promise as specific and sensitive biochemical indices of bone resorption (Eyre, 1992). The objectives of the present study were to examine the inter- and intraindividual variations in the excretion of the crosslinks in healthy young adults. A correlation study was also carried out between urinary crosslinks and urinary Na to establish a dietary effect on the excretion of crosslinks.

Eighteen adults (eleven female, seven male) with a mean age of 24 years, with no history of bone disease and no intake of medication that could affect bone and cartilage metabolism, were recruited. Fasting morning void urine samples were collected for five consecutive days and samples stored at -20° until required for analysis. Urinary Na was determined by flame photometry and urinary creatinine was measured using a colorimetric kit method. The measurement of the urinary pyridinium crosslinks was carried out by a three-step procedure: each urine sample (0.25 ml) was hydrolysed with an equal volume of 12 M-HCl at 110° for 18 h, the crosslinks were then extracted by CF1 cellulose chromatography and finally were measured by fluorometry after reversed-phase HPLC (Eyre *et al.* 1984). The values of urinary Pyr and Dpyr were expressed relative to urinary creatinine.

	Female		Male		*P* value*
	Mean	SD	Mean	SD	
Creatinine (mmol/l)	12.8	0.5	17.0	2.4	<0.01
Pyr (nmol/mmol creatinine)	51.5	7.3	54.3	18.9	NS
Dpyr (nmol/mmol creatinine)	11.1	1.3	12.5	4.3	NS
Na (mmol/mmol creatinine)	4.61	1.05	5.62	1.52	NS

* Comparison of means between males and females by unpaired Student's *t* test.

Intraindividual coefficients of variation for Pyr ranged from 14 to 66 % and for Dpyr ranged from 3 to 67 %. Mean daily excretion of Pyr and Dpyr for individuals ranged from 35 to 73 and from 6 to 15 mmol/mmol creatinine respectively. No sex-related differences in crosslink excretion were found in the group. Urinary Pyr and Dpyr correlated strongly with each other (r 0.85, $P<0.001$). Both crosslinks were significantly correlated with urinary Na with r values of 0.219 ($P<0.05$) for Pyr and 0.249 ($P<0.01$) for Dpyr. These correlations suggest a direct relationship between Na intake and urinary crosslink excretion and consequently, bone resorption.

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The effect of dietary casein on calcium absorption from a purified diet in the rat. By T. BENNETT, M. HARRINGTON, K. CASHMAN and A. FLYNN, *Department of Nutrition, University College, Cork, Republic of Ireland*

The purpose of the present study was to investigate the effect of dietary casein content and meal casein content on Ca absorption using a rat model which has been shown to be useful for studies on Ca bioavailability (Cashman & Flynn, 1994; Harrington *et al.* 1994).

Two studies were carried out using 7-week-old male rats, Wistar strain, average weight 201 g. In study A, thirty-two rats were randomized into four groups of eight rats each and fed on a purified diet (AIN-76) containing casein at a level of 200 g/kg diet for 2 weeks. Each group was then given a meal (10 g) containing (per kg) 0, 100, 200 or 300 g casein, 5 g Ca as $^{47}CaCO_3$ and 0.2 g Fast Green FCF as a faecal marker. In study B, seventy-two rats were randomized into three groups of twenty-four rats each and fed on a purified diet (AIN-76) containing casein at levels of 200, 350, or 500 g/kg diet for 2 weeks. Each group was further randomized into three groups of eight rats each and given a ^{47}Ca-labelled meal (10 g), as above, containing (per kg) 200, 350 or 500 g casein. Fractional absorption of ^{47}Ca in both studies was determined by the ^{47}Sc:^{47}Ca ratio method of Brommage & Binacua (1991).

Study A		Study B		
Meal casein (g/kg)	Ca absorption (%)	Dietary casein (g/kg)	Meal casein (g/kg)	Ca absorption (%)
0	44.2	200	200	39.0
100	42.3		350	44.4
200	43.5		500	59.6
300	47.8			
		350	200	36.6
			350	35.8
			500	41.8
		500	200	29.5
			350	29.8
			500	39.5
Pooled SEM	2.6			2.3
Least significant difference ($P<0.05$)	7.0			6.6
Significance (P) of:				
Meal casein				<0.001
Dietary casein				<0.001
Meal x dietary interaction				0.03

In study A, increasing meal casein concentration from 0 to 300 g/kg had no significant effect on Ca absorption (one-way ANOVA). In study B, Ca absorption was significantly affected by the casein content of the meal and of the diet with a significant interaction between these two variables (two-way ANOVA). Increasing dietary casein intake reduced the efficiency of Ca absorption and this reduction was greater for high-casein meals. Increasing meal casein content increased the efficiency of Ca absorption overall but this increase was more marked in animals fed on diets containing casein at 200 g/kg. These results indicate that Ca absorption is enhanced by high-casein meals, especially when the absorptive capacity of the adaptive vitamin-D-dependent route is high. At high dietary casein intakes adaptation results in a reduction in the efficiency of Ca absorption by the vitamin-D-dependent route.

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

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Influence of sulphydryl content of meats on the *in vitro* dialysability of iron. By B. MULVIHILL and P.A. MORRISSEY, *Department of Nutrition, University College, Cork, Republic of Ireland*

Meat is a known enhancer of non-haem Fe bioavailability from foods. The exact mechanism by which the enhancement occurs is unclear. It has been proposed that the component in meat (meat factor) promoting the absorption of non-haem Fe is a polypeptide chain, rich in cysteine residues (Kirwan *et al.* 1993). The present study was designed to compare the sulphydryl-group effect of tissues from different species, on *in vitro* dialysability of Fe.

Samples (containing 4 g protein) from a number of species were homogenized in water and 0.05 mmol $FeCl_3$ was added to the homogenate. The volume was adjusted to 100 ml with water. The pH was reduced to 2.0 with HCl. Distilled water was used as a control. Egg albumin was used as the control protein. The sulphydryl (SH) content was determined by the method of Ellman (1959). A 10 ml sample of homogenate was used for the *in vitro* dialysability of ferrous and total Fe, which was determined by the method of Kapsokefalou & Miller (1991). This method involves a two-stage simulated gastrointestinal digestion using pepsin (*EC* 3.4.23.1) and pancreatin, followed by determination of dialysable Fe.

		% Dialysable ferrous Fe		% Dialysable total Fe	
	µmol SH / 10 ml homogenate	Mean	SE	Mean	SE
Control	-	1.23	0.08	1.89	0.27
Egg albumin	16.21	2.83*	0.15	6.05	0.13
Lamb's heart	20.25	7.83	0.69	12.86	0.41
Pork	23.74	11.19	0.12	13.09	0.95
Lamb's kidney	26.15	-	-	11.24	0.19
Beef	29.53	12.31	0.87	14.32	0.19
Chicken leg	30.90	5.17	0.21	18.88	1.22
Chicken breast	31.52	21.30	0.06	27.31	0.27
Lamb	32.07	22.27	0.30	24.22	0.48
Lamb's liver	34.41	21.67	0.11	24.84	0.14

* not significantly different from control, $P<0.05$ (students t-test)

When compared with egg albumin, all tissues significantly enhanced ferrous Fe dialysability. Chicken breast, lamb and lamb's liver dialysed the highest percentage of ferrous and total Fe and these tissues contained the highest levels of SH groups (r 0.461). In the case of lamb's kidney, only ferric Fe was dialysed.

The presence of the SH blocking agent, N-ethylmaleimide (NEM) (0-20 µmol), significantly reduced ferrous Fe dialysability from lamb's liver. Complete inhibition of ferrous Fe dialysability occurred with 30-40 µmol NEM addition. These present results further suggest that the free SH content of meat plays an important role in enhancing Fe bioavailability in foods.

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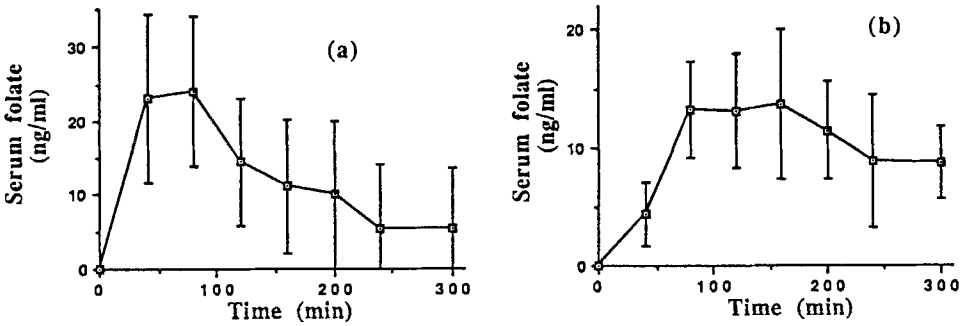
Bioavailability of pteroylglutamic acid in bread in human subjects. By M. CANTWELL[1], J. McPARTLIN[1], M.A.T. FLYNN[3], M. GOGGINS[1], D. WEIR[1] and J. M. SCOTT[2], *Departments of [1]Clinical Medicine and [2]Biochemistry, Trinity College, Dublin and [3]Department of Biological Sciences, Dublin Institute of Technology, Republic of Ireland*

In response to the findings of a number of studies establishing the relationship between folate intake and the incidence of neural tube defects there is broad international agreement on the need to increase the amount of folate in the diet of women of child-bearing age. The most effective means of supplying additional folate is by fortification of staple foods such as bread.

In the present study the comparative bioavailability of folic acid in bread and isotonic saline was assessed by the appearance of folate in serum following an oral dose. A cross-over study was designed in which ten healthy subjects (eight females, two males, aged 21-43) were pre-supplemented with folic acid tablets (800 μg/d for 5 days) before the test. A baseline blood sample was taken before the consumption of folic acid (1 mg) either dissolved in isotonic saline (Dioralyte, Rorer, 200 ml) or impregnated and dried into commercially-available white bread (60 g). Blood samples were taken at regular intervals over a 5h period post-consumption. Subjects were maintained on the supplementation regime for an additional 5d before consumption of the alternate test meal and blood-sampling. Total serum folate was assayed by microplate microbiological (Lactobacillus *casei*) assay. The Fig. shows the mean and standard deviations of the serum folate response to both isotonic saline (a) and bread (b) in the ten volunteers, the values being adjusted to a pre-dose baseline of zero.



Repeated measures analysis demonstrated that there was significant variation between individuals following the ingestion of folic acid in either bread or isotonic saline (P <0.0001). Meal-by-repeat interaction demonstrated significantly different responses of serum folate with time between both test meals (P <0.0001). Areas under the curve (AUC) were determined by the trapezium rule for each subject response to both oral regimes. Paired t test analysis for the ten subjects showed the bioavailability of bread as determined by AUC was signficantly less than that for isotonic saline (bread 5043.44 (SD 1430), saline 6298.74 (SD 2093), P =0.0019, CI 95%: -1909, -602.0).

The bioavailability of folic acid in bread (80.0% compared with saline) reported here is much greater than that reported by Colman et al. (1975). Nevertheless, this experimental design did not account for probable losses due to processing as folic acid was added after baking. Therefore, reduced bioavailability as well as losses due to baking should be accounted for in planning fortification of flour with folic acid.

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Changes in folate status in response to pteroylglutamic (folic) acid supplements, folic acid-fortified foods, food folate and dietary advice. By GERALDINE J. CUSKELLY[1], HELENE McNULTY[1] and JOHN M. SCOTT[2], [1]*University of Ulster, Coleraine BT52 1SA and* [2]*Department of Biochemistry, Trinity College Dublin, Ireland*

Following the results of a major study confirming the protective effect of folic acid (the synthetic form of the vitamin folate) in preventing neural tube defects (NTD; MRC Vitamin Study Group, 1991), the Department of Health (1992) has published new recommendations. Women of child-bearing age with no previous history of NTD are advised to take an extra 400 μg folic acid/d which it is claimed may be achieved by taking a folic acid supplement and/or increasing consumption of either folic acid-fortified foods or natural food folate sources. It is unknown, however, whether these routes of intervention are equally effective in optimizing folate status. The objective of the present study was to compare the response of folate status to each of these approaches.

Following recruitment, non-pregnant female subjects (*n* 62) were instructed to exclude folic acid-fortified foods from their diets for an equilibration period of 3 months, after which time they were randomized into groups as follows: I, supplements (400 μg folic acid/d); II, fortified foods (an additional 400 μg folic acid/d); III, dietary folate (an additional 400 μg food folate/d); IV, dietary advice (advice from a dietitian to consume folate-rich foods) and V, control (no supplements or fortified foods). Red cell folate was measured pre- and post-intervention by microbiological assay. Dietary intakes were estimated using the diet history method supplemented with a food-frequency questionnaire. Responses were assessed in those who completed the study (*n* 46), following transformation of data.

	I		II		III		IV		V	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Red cell folate (μg/l)										
n	8		6		9		9		8	
Pre-intervention	349	64	326	46	378	88	319	65	325	77
Post-intervention	495**	126	498**	135	394	107	375	102	326	58
% change (pre *v* post)	41.7	30.8	51.9	26.4	6.1	24.0	18.0	25.8	3.3	15.8
Folate/folic acid intake (μg/d)										
n	8		6		10		7		9	
Pre-intervention	204	36	186	35	216	66	175	40	183	53
Post-intervention	593***	38	407**	76	426***	124	268*	67	209	63
(of which folic acid)	400		269	93	0		51	53	0	
% change (pre *v* post)	190.7	16.9	127.2	65.1	108.6	76.3	58.9	48.3	20.9	39.0

Differences between pre- and post-intervention: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (Student's paired *t* test).

As expected, there was no change either in intake or folate status (red cell folate) in the control group V. By contrast, an additional intake of 400 μg folic acid/d increased intake and status whether taken as supplements (I) or in fortified foods (II). While the dietary folate group (for which food was purchased to increase folate intake by 400 μg/d) showed a significant increase in intake, it was not accompanied by an increase in status. Likewise, those given dietary advice showed an increase in intake but not in status. The contrast between groups I and II compared with groups III and IV is almost certainly due to the well known increased bioavailability of synthetic folic acid over food folates. Consequently, increased intakes of folic acid either as supplements (I) or as fortified foods (II) seem to be very effective in changing folate status. It is unlikely that all women will take a folic acid supplement throughout their reproductive years, and therefore unless a wider range of fortified foods is made available, public health measures to reduce the incidence of NTD are likely to be ineffective.

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Analysis of the readership, belief and usage of food label information amongst Irish shoppers. By JOAN K. MAHON[1,2] and MICHAEL J. GIBNEY[2], *[1]Department of Nutrition and Dietetics, St Luke's General Hospital, Kilkenny and [2]Unit of Nutrition and Dietetic Studies, Department of Clinical Medicine, Trinity Centre for Health Sciences, St James's Hospital, James's Street, Dublin 8, Republic of Ireland.*

Current European Union food legislation regulates ingredient lists and nutritional labelling while legislation on nutrition claims is pending. Such legislation is intended to help consumers choose a healthier diet. The purpose of the present study was to examine the relevance of food labelling to Irish shoppers. The results of interviews with 1045 Irish shoppers randomly recruited from supermarkets in three or more towns per county in the south east of Ireland are given below.

	Food ingredient	Nutrition claims	Nutrition labels
% who read:			
Often/very often	29	65	19
Seldom/never	43	21	64
% who use to choose food:			
Often/very often	26	48	15
Seldom/never	52	33	70
% who believe:			
Yes	58	52	60
No	21	22	25

Just over half the shoppers believed the information shown on food labels while their use of labels in choosing food items was in the order of nutrition claims, food ingredients and nutrition labels.

The subjects were given three comparative tests using food labels to make choices. In the first test, subjects were asked to choose which of two spreadable fats, one a full-fat spread high in saturates, the other a low-fat spread high in monounsaturates, was in their opinion the healthier option. Fifty percent chose the healthier option. In the second and third tests, subjects were asked to choose between two food labels taken from breakfast cereals, which of the two products was higher in Fe and which of the two was higher in fibre. In the case of Fe, 29% chose the correct product, while in the case of fibre, 24% were correct in their choice.

In choosing the food higher in Fe and higher in fibre, the subjects could make their selection using numeric values per 100 g, numeric values per serving, or two graphic formats (high/medium/low or percentage RDA per serving). Only 11% said they used graphic formats, while 52% used numeric values per 100 g and 18% used numeric values per serving: 19% were unable to use any label format.

These findings suggest that the use of food labels to communicate nutrition messages to Irish shoppers is of limited value, and indicate that nutrition claims may be a preferred strategy for consumers.

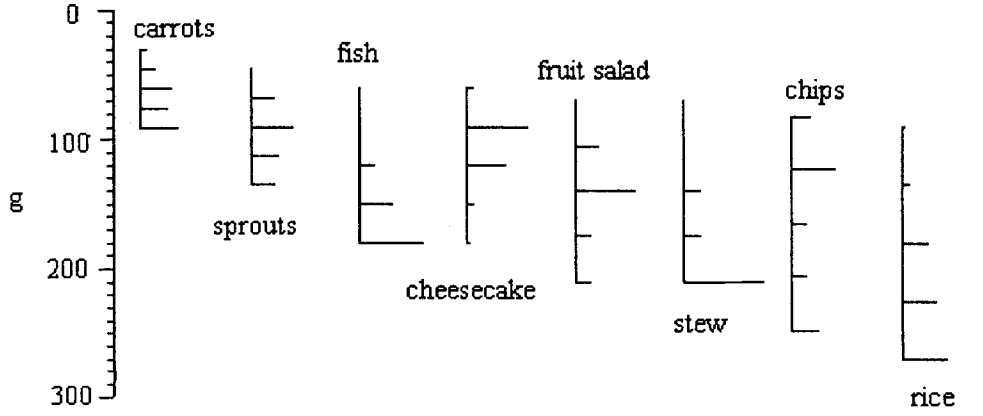
This work was funded by Nutriscan Ltd. and the Irish American Partnership.

How do caterers decide on portion sizes? By Y. KINGHORN, A. WISE and G. BLWYDDIN. *The Robert Gordon University, Queen's Road, Aberdeen AB9 2PG*

Many people derive a significant proportion of their diet from outside the home so it is important to study the attitudes of caterers to nutrition. It is not known how caterers determine the amount of each food they serve. The present study was designed to ask caterers what factors influence them. Establishments (*n* 50) in Aberdeen were selected for inclusion if they served traditional fare; fast-food outlets, ethnic restaurants and contract caterers were excluded. Most (*n* 45) agreed to participate and a personal interview was arranged with the head chef. The caterers associate the University with a course in hospitality management, so that there was no likely bias towards nutrition in the interview. The first questions concerned the establishment size, type of meals served and chef's education. Chefs were then asked to score seven attitude statements related to factors that influence them in deciding on a portion size (1:low-7:high). One of the factors was the nutritional content of the food and this was scored highly by the chefs; the Table shows the number of chefs giving each score.

Attitude Score	1	2	3	4	5	6	7
Cost of food	0	0	0	3	2	2	38
Time of year	13	2	2	2	9	4	13
Time of day	22	3	0	3	5	6	6
Food is hot or cold	11	1	0	2	2	5	24
Personal experience	1	1	0	4	1	8	30
Text books	32	3	3	1	3	0	3
Nutritional content	1	2	1	0	5	5	31

The chefs were then shown, in random order, five colour photographs (100 x 150 mm) of eight foods and asked what portion resembled the one they would serve. The distribution of the portion weights is shown by the length of horizontal lines in the figure.



The chefs were then shown a list of foods and asked which they would serve in a 'healthy' menu. Answers considered more correct according to current thinking were scored 1 and a maximum score of 15 was possible. Their knowledge appeared relatively good with 79% scoring 13 or above. However, when asked to list Government dietary guidelines unprompted, 65% could give no answer. Responses were accepted if they were as simply stated as to reduce saturated fat and increase dietary fibre, but here the knowledge was poor. It was concluded that chefs included the 'nutritional content' in deciding portion weights, that the weights chosen differed among the chefs, who apparently knew which foods are considered 'healthier'. It is important to study in more depth what caterers mean by the 'nutritional content' of food and design education towards them, taking into account their knowledge and attitudes.

The criteria by which two groups of consumers classified high, medium and low fat-containing foods. By FIONA O'BROIN and ANNE E. de LOOY, *Department of Dietetics and Nutrition, Queen Margaret College, Clerwood Terrace, Edinburgh, EH12 8TS*

A potential barrier to reducing fat intake is an inability to identify and select foods with different fat contents (Mela, 1994). The present study aimed to assess consumers' ability to categorize foods from photographs according to their percentage fat content and to describe the criteria they used to make their assumption.

Thirty female college students (aged 20-40 years) were recruited. Fifteen were attending a nutrition course (NC) and fifteen were attending non nutrition-related courses (NNC). Nutrition education has been previously demonstrated to affect the ability of groups to classify foods (Towler & Shepherd, 1990). Subjects were asked to assign twenty-five common foods into three groups representing high-fat foods (>53% fat energy), medium-fat foods (17.6-52.9% fat energy) and low-fat foods (<17.5% fat energy) (Coronary Prevention Group, 1988). The criteria by which they categorized a subset of twelve foods were determined by a semi-structured interview.

The mean scores, out of a total of 25, of correct classification were 16.6 (NC) and 13.0 (NNC) ($P<0.001$). Thirteen criteria were identified and the Table shows the frequency of citing the most popular criteria within each group (figures in brackets indicate the percentage of the group using that criterion).

	NC	NNC
Low fat	Nutritional knowledge (25%)	Nutrition label (24%)
	Nutrition label (22%)	Media influence (21%)
	Considered nutrients (20%)	Considered nutrients (19%)
Medium fat	Ingredient knowledge (25%)	Media influence (18%)
	Considered nutrients (19%)	Comparison with another food (17%)
	Comparison with another food (12%)	Ingredient knowledge (15%)
High fat	Cooking method (29%)	Cooking method (32%)
	Ingredients (22%)	Appearance (21%)
	Nutritional knowledge (17%)	Taste (13%)

Different criteria were cited by each group within each fat banding. This was unexpected and may be a reflection of the foods displayed within each band. The criteria which differentiated between the more successful categorization of foods , as shown by the scores of the NC, were, in rank order: use of ingredient knowledge, considered nutrient composition, the nutrition label and nutritional knowledge.

It is suggested that more work be undertaken to identify the criteria used by different groups of consumers to identify high-, medium- and low-fat foods so that health education can be more effectively targeted. However, it would appear that a knowledge of ingredients and the nutrient composition of foods may help the consumer to differentiate between foods of differing fat composition.

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Mela, D. (1994). *Nutrition and Food Science* 3, *19-21*.

Towler, G. & Shepherd, R. (1990). *Journal of Human Nutrition and Dietetics* 3, 255-264.

Social status differences in biscuit, cake and confectionery consumption in north Glasgow. By W.L. WRIEDEN[1], M.K. McCLUSKEY[2] and C. BOLTON-SMITH[2], [1]*School of Food and Accommodation Management, University of Dundee, DD1 4HT and* [2]*Cardiovascular Epidemiology Unit, Ninewells Hospital and Medical School, Dundee DD1 9SY*

As part of the 1992 World Health Organisation MONICA study (Wrieden *et al.* 1994) the dietary habits of over 2000 men and women aged 25-74 years in north Glasgow were studied using a food-frequency questionnaire (FFQ). The FFQ included questions on the weekly frequency of consumption of all major types of foods, including the confectionery items, plain biscuits (including digestives), sweet biscuits and cakes (including scones and buns), and chocolates and sweets (including chocolate bars). Preliminary investigations suggested that there were age, sex and social class differences in the frequency of consumption of these items but that these were complicated by the larger proportion of people of higher social status (as measured by employment, education or housing status) in the younger age groups. The following results relate to two distinct groups of the sample further divided by sex and age. Subjects who reported having or having had a non-manual job, an education beyond school and were living in owner-occupied accommodation were termed the higher social group (HSG) and those with manual jobs, no post-school education and who rented local authority housing were termed the lower social group (LSG). The percentages of people in the different consumption groups, less than once a week and rarely or never (<1), 1-3 times per week (1-3) and 4 or more per week (≥4) are given.

Frequency	Plain biscuits			Sweet biscuits/cakes			Chocolates and sweets		
(times/week)	<1	1-3	≥4	<1	1-3	≥4	<1	1-3	≥4
Men(25-50 years)									
HSG(n 70)	40.0	37.1	22.9	38.0	36.6	25.4	19.7	54.9	25.4
LSG(n 131)	41.2	36.8	22.1	42.0	38.2	19.8	45.3	33.6	21.2***
Men(51-74 years)									
HSG(n 27)	33.3	51.9	14.8	25.9	48.1	25.9	32.1	50.0	17.9
LSG(n 236)	35.5	31.4	33.1	50.2	28.7	21.1*	58.5	27.1	14.4*
Women(25-50 years)									
HSG(n 81)	43.9	25.6	30.5	37.0	34.6	28.4	18.3	36.6	45.1
LSG(n 109)	43.8	42.0	14.3**	33.0	40.4	26.6	24.1	49.1	26.8*
Women(51-74 years)									
HSG(n 22)	34.8	43.5	21.7	27.3	54.5	18.2	39.1	39.1	21.7
LSG(n 174)	25.9	37.4	36.8	35.0	46.3	18.6	43.3	40.4	16.3

Significantly different from HSG using chi-squared test,*$P<0.05$ **$P<0.01$ ***$P<0.001$.

The Table shows that young women in the HSG had double the percentage of frequent (≥4/w) plain biscuit eaters and chocolate and sweet eaters than those in the LSG. Similarly in men of both age groups there was a higher percentage of chocolate and sweet eaters (≥1/w) amongst those in the HSG. In addition, in the older men there was a higher proportion of sweet biscuit and cake eaters in the HSG. These results, like those of the British Adult survey of 1986-1987 (Ministry of Agriculture, Fisheries and Food, 1994), suggest that confectionery consumption is more prevalent in the relatively affluent sectors of the community, that is, in the social groups who tend to have the 'healthier' diets (Wrieden *et al.* 1994).

C.B-S acknowledges financial support from Mars Inc.

Ministry of Agriculture Fisheries and Food (1994). *The Dietary and Nutritional Survey of British Adults-Further Analysis.* London: H.M. Stationery Office

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The potential role of starch-rich foods in achieving national targets for dietary fat reduction. By S. L. BURKILL, J.P. LANDMAN, P. GRAY and T.R. KIRK. *Department of Dietetics and Nutrition, Queen Margaret College, Edinburgh EH12 8TS*

The present study aims to investigate if simple advice to increase consumption of starch-rich foods and provision of one such food, without any other dietary intervention will result in a replacement of energy derived from fat with energy from starch.

To test this hypothesis, subjects were provided with ready-to-eat breakfast cereal and asked to increase their consumption by approximately 420 g per week for 12 weeks. The breakfast cereal was to be accompanied by semi-skimmed or skimmed milk. No other dietary advice was given. Screening with a food frequency questionnaire (Paisley, *in the press*) identified students with dietary fat energy $\geq 35\%$, and who ate three portions or less of breakfast cereal per week at baseline. Students recruited to the study were matched for age, sex and smoking, and randomly assigned to an intervention group (*n* 24, one male and twenty-four females, mean age 19.8 (SEM 0.4) years) and a control group (*n* 25, one male and twenty-four females, mean age 19.8 (SEM 0.4) years). At baseline, 4 weeks and 12 weeks, 7 d weighed records checked compliance, measured energy, fat, carbohydrate (CHO) and nutrient intake and distribution of eating occasions throughout the day. The following variables were assessed: weight, BMI, percentage body fat, and plasma total cholesterol, HDL- and LDL- cholesterol and triacylglycerol levels. Data were analysed using COMP-EAT 4 and SPSS for Windows computer programs. Significant differences between the intervention and control group were determined by a paired *t* test. Results for energy and percentage energy from fat, CHO, protein and starch at 4 weeks are shown in the Table.

	Baseline				Week 4				Changes in nutrient intake			
	Intervention		Control		Intervention		Control		Intervention		Control	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Energy (kcal)	1784	57.3	1822	67.6	1790	68.7	1847	88.0	+6.0	60.0	+25.0	62.9
Energy (kJ)	7457	239.3	7615	282.6	7483	287.2	7721	367.7	+26	250.8	+106	262.8
% energy:												
Fat	34.0†	1.1	36.6	0.9	28.9***	1.1	36.7	0.7	-5.1‡	1.1	+0.1	1.0
CHO	45.9	1.4	46.2	0.8	51.2***	1.3	45.8	0.8	+5.3‡	0.7	-0.4	1.1
Protein	12.4*	0.4	13.3	0.3	13.1	0.4	13.6	0.4	+0.7	0.4	+0.3	0.4
Starch	26.1	1.0	26.1	0.9	31.7**	1.0	27.1	0.8	+5.6‡	0.9	+1.0	0.8

Significantly different from baseline: * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Significantly different from control group: ‡ $P< 0.001$

The Table shows that there was no significant difference in total energy, fat and CHO between the intervention and control groups at baseline. At week 4 of the intervention period a significant reduction in percentage energy from fat (-5.1%) was achieved in the experimental group. This corresponded to a significant increase in percentage energy from carbohydrate and starch and no significant differences in total energy, indicating replacement of fat energy by carbohydrate and starch energy. The nutrient intakes of the control group did not vary significantly.

The findings at week 4 indicate that a significant reduction in the percentage energy derived from fat may be achieved by advice to encourage an increase in consumption of starch-rich foods, such as breakfast cereal, in the diet, rather than concentrating on advice to reduce fat. Results at 12 weeks will be presented in due course, and will indicate if these changes can be sustained.

Supported by the Kellogg Company of Great Britain.

Paisley, C. (in the press) *Journal of Human Nutrition and Dietetics*

Changes in pattern of nutrient intake with advancing age in a healthy Irish population

By MOIRA HURSON and SHEILA SUGRUE. *Irish Nutrition and Dietetic Institute, 17 Rathfarnham Road, Dublin 6W, Ireland*

Advancing age is generally associated with a decline in energy intake (Munro 1981). One of the main problems occurring as a consequence of reduced intake may be a failure to meet recommended intakes for certain macro- and micronutrients. This may place elderly individuals (>60 years) at risk of becoming deficient in one or more nutrients. In Ireland there have been relatively few direct dietary intake studies providing information on the nutrient intake of elderly people. The aim of the present study was to evaluate macro- and micronutrient intakes of elderly people (>60 years) participating in the 1990 Irish National Nutritional Survey and compare findings with those of a younger age group (25-40 years). A total of 657 adults were interviewed; in this sample there were 107 individuals between the ages of 25 and 40 years and 163 over 60 years of age. A 7d diet history using a photographic atlas as an aid was completed (Lee & Cunningham, 1990). The results are summarized in the following Table.

	Men >60 (*n*79)		Men 25-40 (*n*85)		Women >60 (*n*84)		Women 25-40 (*n*122)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body mass index	25.6	3.8	26	3.2	26.4	4.9	24.2	4.7
Energy (MJ/d)	9.55**	3.09	12.7	3.3	7.07	2.39	7.7	2.9
Protein (g/d)	84.42	26.95	112	32	67.0	16.75	72	24
Fat (g/d)	86.12	34.79	123	39	64.08	26.39	73	31
Carbohydrate (g/d)	293.50	104.03	363	105	227.2	87.64	230	100
Alcohol (g/d)	9.82	14.93	16.7	16.8	1.59	3.41	5.9	12.6
Vitamin C (mg/d)	60.3**	34.7	78.7	38	55.09**	35.88	76.4	48.9
Vitamin A (μg/d) R.E.	701.6**	1094	1381	1239	606.44**	945.62	1372	1491
Vitamin B_{12}(μg/d)	4.65**	4.79	6.5	4.6	4.13	4.19	5.5	6.0
Vitamin D (μg/d)	1.90	1.75	2.0	1.3	1.85	1.54	1.6	1.5
Vitamin E (mg/d)	3.65**	1.59	5.0	2.0	2.9	1.07	3.6	1.6
Iron (mg/d)	11.38**	4.11	15.0	4.0	9.2	3.13	10.8	4.4
Zinc (mg/d)	11.27**	4.31	14.4	4.2	8.43	2.04	9.4	3.3
Total folate(μg/d)	188.4**	78.2	244	87	165.5	61.67	182	82
Calcium (mg/d)	966.1**	444.3	1361	588	774	296.59	891	472
Vitamin B_6 (mg/d)	1.64	0.61	2.4	1.0	1.24	0.41	1.5	1.5

Significantly different from younger age group of the same sex; **$p < 0.01$

For the elderly, macronutrient intakes were adequate and in keeping with the recommended guidelines (Department of Health, 1991). However, energy intakes of older individuals were lower than younger but only elderly females had an energy intake below recommendations (Department of Health, 1991). BMI for all age groups were high. The lower energy intakes recorded for older individuals may be responsible for their lower intake levels of micronutrients compared to the younger. Energy does not account for the difference observed in intakes of vitamin A and C between younger and older women. These low energy intakes for elderly women, paralleled with a high BMI raises the possibility of limited physical activity or underreporting. Increases in micronutrient intake with emphasis on fruit and vegetables consumption may improve vitamin and mineral status. In order to allow for a higher energy intake in the females without increasing weight, an increase in physical activity would also be desirable.

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Macronutrient intake in South Asian and Italian women in the West of Scotland. By ANNIE S. ANDERSON[1], M. E. J. LEAN[1], H. BUSH[2], H. BRADBY[2] and R. WILLIAMS[2], [1]*University of Glasgow, Department of Human Nutrition, Glasgow Royal Infirmary, Alexandra Parade, Glasgow G31 2ER, and* [2]*MRC Medical Sociology Unit, 6 Lilybank Gardens, Glasgow, G12 8RZ*

In cardio-protective terms the traditional diets of South Asians and Italians are considered healthy. However following migration to England and Wales, (McKeigue *et al.* 1989) rates of coronary heart disease (CHD) increase in first-generation South Asians. CHD rates also increase in Italian migrants but, in contrast to South Asians, they remain lower than the average for England and Wales (Marmot *et al.* 1984).

To explore differences in diet-related risk factors for CHD between South Asian and Italian women living in Scotland, 7 d weighed dietary records were completed by groups of women of 1st and 2nd (or subsequent) generation South Asian origin (1 SA and 2 SA), 1st and 2nd (or subsequent) generation Italian origin (1 IT and 2 IT) and the general population (GP). Nutrient intake was estimated using compositional data from *McCance and Widdowson's The Composition of Foods* (Holland *et al.* 1991), supplements (Wharton *et al.* 1983; Tan *et al.* 1985; Holland *et al.* 1992), and unpublished data (Kassam-Khamis personal communication 1995). Trained interviewers conducted a structured sociological interview which explored issues related to food choice, and they took anthropometric measurements.

Statistical analysis using ANOVA and the Scheffé test showed that significantly higher intakes of energy, percentage food energy from fat, percentage food energy from saturated fat and lower intakes of percentage food energy from protein were found in 1st generation South Asians compared with 1st generation Italians. No significant differences were detected between the groups for percentage food energy from carbohydrate or BMI.

		Food energy (MJ)		% Food energy (carbohydrate)		% Food energy (fat)		% Food energy (saturated fat)		% Food energy (protein)	
	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
GP	35	6.97	2.1	45.6	4.8	39.1	5.3	13.5	2.7	15.1	2.9
1 SA	35	7.96	1.6	44.5	6.6	42.4	6.2	15.1	3.6	13.1	1.6
2 SA	37	7.78	2.5	46.3	5.5	39.8	6.0	13.1	3.3	13.7	1.9
1 IT	30	5.92	1.2	47.4	5.0	35.7	5.8	12.1	2.9	16.9	3.0
2 IT	38	6.80	1.5	45.9	4.6	38.3	5.2	13.8	2.9	15.7	3.1
Significance		$P < 0.05$		NS		$P < 0.05$		$P < 0.05$		$P < 0.01$	

The results demonstrate a clear convergence from 1st to subsequent generation of both South Asians and Italians in fat intakes, towards those of the general population. Fat and saturated fat intakes of 1st generation Italian migrants are low, which would be in keeping with relative protection against CHD.

Funded by ESRC grant number X209252023

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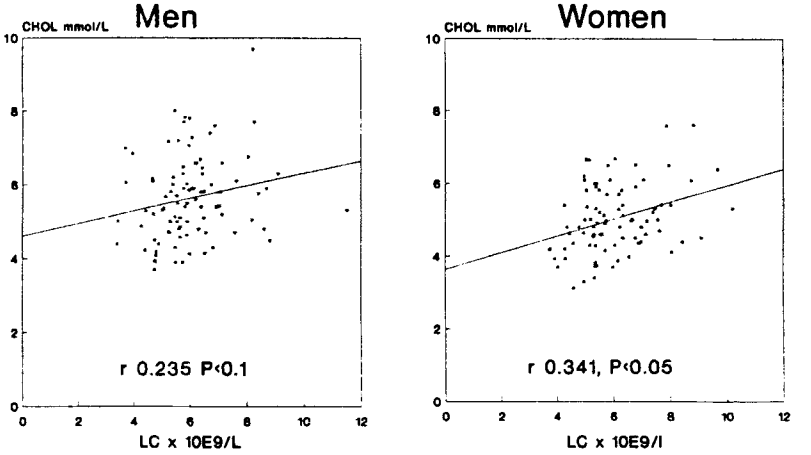
Blood cholesterol and health in persons aged 25-45 years. By U.J. McLOONE, M. CHOPRA, M.E. O'NEILL, B. McPEAKE, and D.I. THURNHAM, *Human Nutrition Research Group, University of Ulster, Coleraine, BT52 ISA.*

An EC-supported study is currently investigating the hypothesis that carotenes play a role in reducing oxidation damage to human tissue components. As part of this study forty male and forty female volunteers who were healthy, non-smokers and aged 25-45 years, were recruited in five different European centres (Coleraine, Cork, Grenoble, Madrid and Zeist). In Coleraine, 102 persons (fifty-three men and forty-nine women), mainly from within the university, including academic staff and tradespeople, were screened for suitability. Exclusion criteria included; post-menopausal women, anyone on prescribed medication apart from oral contraceptives, serum retinol <1.0 μmol/l, heavy drinkers and an abnormal lipid profile (cholesterol (chol) >6.8 mmol/l or triacylglycerol (TGL) >4.0 mmol/l or LDL:HDL-chol ratio >5 in conjuction with TGL >2.3). Nine men and one woman were excluded on the basis of chol, one man (no women) on the basis of TGL and four men (no women) on the basis of LDL:HDL chol.

Analysis of the screening data of those enrolled against those excluded found no significant differences in haematological profiles, liver function tests or plasma retinol between the two groups. If anything, plasma retinol was greater in those subjects who were excluded. For this abstract we examined the data more carefully to consider whether in selecting "healthy" people, it was justifable to excluded on the basis of elevated blood lipids.



The Fig. shows the distribution of chol values plotted against total leucocyte count (LC). In addition we examined LC against the other markers of lipid profile. TGL showed similar results to chol (men r 0.261, NS: women r 0.331, $P<0.05$) while the LDL:HDL-chol ratio was not related to LC.

An increase in LC is an early marker of disease (Sipe,1985). Both chol and TGL were correlated with LC in women and the slopes of the regression lines in the Fig. are very similar suggesting that chol (and also TGL, not shown) would be significantly related to LC with larger numbers of men. The variance in the chol or TGL data explained by LC is approximately 10% in both sexes suggesting that LC has little influence on chol or TGL levels in this age group, however the positive correlation between the lipids and LC suggests that it is justifiable to exclude persons with a high chol (or TGL) in spite of the relatively young age range. High lipid values appear to be associated with increased risk of ill health.

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