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Abstracts of Original Communications

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Ascorbic acid in human tears and plasma: interrelationships and effect of vitamin C supplementation. By C.K.M CHOY[1], P. CHO[1] and I.F.F. BENZIE[2], [1]*Department of Optometry and Radiography, and* [2]*Department of Nursing and Health Sciences, The Hong Kong Polytechnic University, Kowloon, Hong Kong SAR, China*

Human tears contain ascorbic acid, and this may be important as a 'front-line' ocular defence against photo-induced oxidative damage (Choy *et al.* 2000). The relationship between tear and plasma ascorbic acid, however, has not been studied to date. Furthermore, the potential for dietary supplementation with vitamin C to increase tear ascorbic acid is not known. The aims of this study, therefore, were to determine baseline relationships between the ascorbic acid concentration of tears and plasma, and to investigate the effect of vitamin C supplementation on ascorbic acid concentration in tears and plasma.

The study was a single-blind, randomized, placebo-controlled, cross-over intervention trial. Twenty-one apparently healthy Chinese subjects (fifteen men, six women), aged 22–29 years, and with no evidence of ocular disease, were recruited with their informed consent. Subjects were randomly divided into two groups. Subjects in group 1 (n 11) followed a supplementation regimen of 1 g/d of vitamin C for 4 weeks. Group 2 received an identical, but vitamin C-free, placebo for 4 weeks. On weeks 4–6 no supplements or placebo were taken. At the beginning of week 7, subjects were crossed over onto the other treatment for the next 4 weeks. Fasting venous blood (5 ml collected into heparinized blood collection tubes) and reflex tears (120 µl induced by the yawn reflex and collected using a capillary tube method.) were collected on days 1 and 29 of each of the two supplementation periods. Immediately after tear collection, and within 15 min of plasma separation, ascorbic acid concentrations were measured using an enzyme-linked automated colourimetric method known as the FRASC assay (Benzie & Strain, 1996). A repeated measures ANOVA test was used to investigate response. A *post-hoc* test (Tukey-Kramer multiple comparisons test) was performed if a significant difference (P<0.05) was detected. Pearson's correlation was used to investigate interrelationships.

Tear ascorbic acid at baseline was around 33% that of plasma. Tear and plasma mean (SD) ascorbic acid concentrations at the first visit (baseline value) were, respectively, 17.2 (6.0), and 51.9 (13.4) µM. There was no significant correlation seen between ascorbic acid concentrations in tears and in plasma (r –0.068; P 0.771). After supplementation with 1g/d vitamin C, the ascorbic acid concentration increased significantly (P<0.001) in both plasma and tears. The increase in plasma ascorbic acid averaged 38 µM, or 73% (range <2–71 µM). The increase in tear ascorbic acid was quite large in relative terms (30%), but was small in absolute terms, averaging 5 µM (range <1–13 µM). No significant correlation was seen between the response in plasma and that in tears.

Results demonstrate that vitamin C supplementation can markedly increase fasting plasma ascorbic acid concentration, even when baseline levels are >50 µM, the threshold recommended for minimal disease risk (Gey, 1998). However, while this indicates an increased supply to the tear-producing lacrimal gland, results suggest that lacrimal gland uptake of ascorbic acid from plasma is not driven by simple diffusion, but is a controlled or saturable process. It is possible, therefore, that a mechanism exists to limit the ascorbic acid concentration of human tears, and this deserves further study.

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Choy CKM, Benzie IFF & Cho P (2000) *Investigative Ophthalmology and Visual Science* **41**, 3293–3298.
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Conjugated linoleic acid affects Th-1 derived but not Th-2 derived cytokines in healthy human volunteers. By A.P. NUGENT, E.J. NOONE, M.J. GIBNEY and H.M. ROCHE, *Unit of Nutrition, Department of Clinical Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8, Ireland*

Conjugated dieonic derivatives of linoleic acid are a mixture of geometric and positional isomers of linoleic acid. *In vitro* and animal studies demonstrate that CLA modulates the immune response, including cytokine production. Most investigations report the effect of CLA on Th-1 derived cytokines (e.g. interleukin-2 (IL-2), interleukin-6 (IL-6) and tumour necrosis factor α (TNFα); cytokines involved in the cell-mediated immune response and inflammation. CLA is reported to both increase and decrease concanavalin-A (Con A)-induced IL-2 production *in vivo* and *in vitro*, respectively (Chew *et al.* 1997; Wong *et al.* 1997; Hayek *et al.* 1999). Animal studies also suggest that CLA may reduce the production of the pro-inflammatory cytokines IL-6 and TNFα, but not interleukin-1 (IL-1) (Turek *et al.* 1998; Hayek *et al.* 1999). However, there is a paucity of information regarding the effects of CLA on human cytokine production, particularly on Th-2 derived cytokines (e.g. interleukin-4, IL-4), which are important in allergy and antibody-dependent responses.

This double-blind, placebo-controlled trial investigated the effect of two isomeric mixtures of CLA, compared to linoleic acid (control) on *ex vivo* mitogen-stimulated cytokine production by human peripheral blood mononuclear cells (PBMC). The two CLA isomer blends contained different proportions of the principal CLA isomers: *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA. Fifty-five healthy volunteers (twenty males, thirty-five females, mean age 31.5 years, mean BMI 23.9 kg/m^2) were randomly assigned to receive 3 g of (i) 80:20 *cis*-9, *trans*-10 isomer blend, (ii) 50:50 *cis*-9, *trans*-10 isomer blend, or (iii) a control fatty acid (linoleic acid) daily for 8 weeks. PBMC were isolated from whole blood drawn at weeks 0 and 8 and cultured in duplicate with 2.5% autologous serum in the presence of the T cell mitogen PHA (10 µg/ml) for 24 h. The concentrations of the cytokines IL-2, IL-4 and TNFα (pg/ml), were measured by a commercial ELISA system. Statistical analysis was completed using two-way ANOVA.

Stimulus	50:50 *cis*-9, *trans*-10 isomer (*n* 19)				80:20 *cis*-9, *trans*-10 isomer (*n* 17)				Control: linoleic acid (*n* 19)			
	Pre-		Post-		Pre-		Post-		Pre-		Post-	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
IL-2	70	18	226[a]	36	91	14	190[a]	36	101	24	246[a]	63
TNFα	1317	163	2883[a]	428	1294	128	2216[a]	245	1013	122	2432[a]	280
IL-4	31	4	41	5	28	6	36	4	29	5	36	8

[a]Denotes a significant difference between group mean values pre- and post-supplementation, P<0.02.

Each of the diets increased the production of PHA-induced IL-2 and TNFα at week 8 compared with baseline values. There was no significant difference in response between the linoleic acid (control) and the two CLA blend diets. None of the three diets had any significant effect on IL-4 production. The two CLA blend diets did appear to significantly increase the capacity of PHA-stimulated cells to produce Th-1 derived cytokines (IL-2 and TNFα) but not the Th-2 derived cytokine IL-4. Supplementation with CLA appears to modulate the production of cytokines involved in the cell-mediated immune response but not in an antibody and allergy mediated immune response.

The investigators gratefully acknowledge Loders Croklaan BV, The Netherlands who supplied the supplements.

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Reduction of tissue factor expression on monocytes during pregnancy: is this homeostasis in haemostasis? By V.A. HOLMES[1], W.S. GILMORE[1], J.M.W. WALLACE[1], P. McFAUL[2] and H.D. ALEXANDER[3], [1]*Northern Ireland Centre for Diet and Health, University of Ulster, Coleraine BT52 1SA and Departments of* [2]*Obstetrics and Gynaecology and* [3]*Haematology, Belfast City Hospital, Belfast BT9 7AB*

Thrombosis is a major cause of death and illness. The incidence of vascular thrombosis is raised during pregnancy and post-partum; pulmonary thromboembolism being a leading cause of maternal death. Previous research has examined changes in the coagulation system during pregnancy. Several coagulation factors are increased during pregnancy while the fibrinolytic system appears to be impaired (Forbes & Greer, 1991). These changes are thought to be in preparation for childbirth. Much has been documented about these changes, but there is no information on the relationship of tissue factor (TF), the main initiator of blood coagulation *in vivo*, to the risk of thrombosis in pregnancy.

Normal pregnant women (*n* 101) and non-pregnant age-matched females (*n* 39) were recruited to this longitudinal study. Informed consent was obtained and blood samples taken at 12, 20 and 35 weeks gestation. At 3 d post-partum blood samples were taken in a subsample of mothers (*n* 21) and control subjects (*n* 21). To measure the expression of TF (CD142) on monocytes, whole blood was labelled with CD142 FITC and CD14 PE and analysed by flow cytometry. Analysis was carried out on monocytes identified by both scatter characteristics and positivity for the monocyte marker CD14. Data showed a non-parametric distribution and was thus transformed logarithmically. Values are expressed as % positive cells; median (range).

Monocyte TF expression was significantly lower in pregnant women at 20 and 35 weeks gestation when measured on monocytes identified according to scatter characteristics (p=0.012) and (p=0.003), and on CD14 positive cells (*P* 0.002) and (*P* 0.034) compared with non-pregnant controls sampled at the same time. One-way analysis of variance showed an overall effect of time on TF expression in both pregnant women and non-pregnant controls ($P<0.0001$).

	Week 12		Week 20		Week 35		Birth	
	Median	(range)	Median	(range)	Median	(range)	Median	(range)
CD14 positive cells								
Pregnant	6.61	(57.67)^{a}	5.89	(48.49)^{ab}	11.47	(50.23)^{c}	13.88	(23.21)^{cd}
Control	9.32	(79.40)^{a}	8.79	(45.83)^{ab*}	16.75	(50.21)^{c*}	13.99	(23.13)^{acd}
Monocytes (scatter)								
Pregnant	4.38	(51.70)^{a}	2.98	(40.59)^{b}	6.13	(42.12)^{c}	8.85	(19.83)^{d}
Control	7.11	(64.14)^{a}	4.21	(35.11)^{ab*}	11.82	(41.72)^{ac*}	10.99	(18.03)^{acd}

Paired sample *t*-tests using Bonferroni's correction showed significant differences between timepoints, $P<0.008$, where values with different letters are significantly different from each other. Independent sample *t*-tests showed significant differences between pregnant and non-pregnant women at the different timepoints ($*P<0.05$).

In conclusion, this finding that monocyte TF is down-regulated during pregnancy is important, demonstrating another physiological change in haemostasis associated with pregnancy. As TF is the initiator of coagulation *in vivo* it is hypothesized that the down-regulation of this early marker of coagulation prevents higher incidence of thrombosis in pregnancy thus helping to maintain homeostasis in haemostasis at a time when a hypercoaguable state exists. Homocysteine, a derivative of the amino acid methionine, can induce monocyte TF *in vitro* (Khajuria & Houston, 2000). Alterations in homocysteine levels have been reported during pregnancy (Walker *et al*, 1999) and thus, future research will determine homocysteine and folate levels in our subjects to investigate whether the lower tissue factor expression in pregnancy observed in the current study is associated with changes in folate status.

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An investigation of the effects of plant sterols on lipoproteins. By H. COLGAN, E.J. NOONE, M.J. GIBNEY and H.M ROCHE, *Unit of Nutrition, Department of Clinical Medicine, Trinity Centre for Health Sciences, St. James's Hospital, James's Street, Dublin 8, Republic of Ireland*

Coronary heart disease (CHD) remains a major cause of death and disability worldwide. Several recent observational studies and meta-analyses have demonstrated that there is a linear relationship between reductions in total and low-density lipoprotein (LDL) cholesterol and lower CHD events (Holme, 1996; Law, 1999). Given the benefits, alternative methods of lowering plasma cholesterol safely have been investigated. Plant sterols (phytosterols) naturally occur in vegetable oils, seeds and nuts and have a very similar chemical structure to cholesterol but differ markedly in their synthesis, intestinal absorption and metabolic fate. Phytosterols effectively reduce serum total and LDL cholesterol, without affecting serum high-density lipoprotein (HDL) cholesterol or triacylglycerol (TAG) levels (Weststrate & Meijer, 1998; Hendriks *et al*. 1999). The average reduction in total and LDL cholesterol levels approximates 6–10% and 13%, respectively. The method of action is not fully understood; however, it is believed plant sterols directly inhibit intestinal cholesterol absorption and indirectly cause effects on hepatic and intestinal cholesterol metabolism.

While there is strong evidence that phytosterols lower cholesterol, it is not known whether they are equally effective in subjects consuming low-fat diets. Furthermore, it is not known whether increasing fruit and vegetable intake will prevent the decrease in β carotene levels observed when phytosterols are consumed. This study investigated lipoprotein metabolism and β carotene levels following consumption of 21g/d margarine (1.6g/d plant sterol) in conjunction with an advised National Cholesterol Education Programme (NCEP) Step 1 diet. Forty-eight hypercholesterolaemic individuals completed the double-blind, placebo-controlled crossover trial. An average of 18.5 (SD 3.01) g/d and 17.45 (SD 3.6) g/d of margarine A and B, respectively, was consumed. 'Margarine B' was the plant sterol-enriched spread. Repeat 3-d food diaries and repeat interviewer-assisted food frequency questionnaires monitored adherence to the Step 1 diet.

	Spread A						Spread B					
	Cholesterol (*n* 48)		TAG (*n* 48)		Glucose (*n* 48)		Cholesterol (*n* 48)		TAG (*n* 48)		Glucose (*n* 48)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Wk 0[†]	6.22	0.59	1.44	0.39	5.03	0.41	6.22	0.69	1.41	0.76	4.55	0.65
Wk 3[†]	6.11	0.65	1.54	0.59	5.02	0.49	5.89**	0.66	1.49	0.69	4.74	0.55
Wk 6[†]	6.11	0.70	1.61	0.79	4.78	0.54	6.21	0.64	1.45	0.42	4.95	0.51
Wk 9[†]	5.97	0.95	1.49	0.59	4.77	0.61	6.07	0.61	1.53	0.53	5.13	0.47

**Denotes a significant difference between mean values pre- and post- intervention, $P<0.0005$; 2-way ANOVA.
[†]All values mmol/l.

Following the intervention period on margarine B individuals achieved a 4.2% reduction in plasma total cholesterol levels ($P<0.05$) and a 6.9% reduction in LDL cholesterol levels ($P<0.05$). This decrease was greater than the reduction achieved by the NCEP Step 1 diet alone but was lower than that achieved in previous studies when individuals followed their habitual higher fat diets. The introduction of plant sterols did not affect plasma HDL cholesterol, HDL triacylglycerol or HDL phospholipid levels. Further investigations will determine the percentage response achieved β carotene levels in this study.

The investigators gratefully acknowledge Van den Berg, The Netherlands, who supplied the margarine.

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Weststrate JA & Meijer GW (1998) *European Journal of Clinical Nutrition* **52**, 334–343.

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

Conjugated linoleic acid is the term given to the positional and geometric isomers of linoleic acid (18:2*n*-6). The natural source of CLA is the lipid fraction of meat and dairy products with the *cis*-9 isomer of CLA being the principal dietary form. A number of animal studies, using isomeric blends of CLA have demonstrated that CLA exhibits potent antiatherogenic properties (Kritchevsky *et al*. 2000), improves lipid metabolism (Nicolosi *et al.* 1997) and reduces body fat (Delany *et al.* 1999). There is little information regarding the metabolic and molecular effects of the individual isomers of CLA.

Sixteen 12-week-old male *Ob/Ob* mice were randomly allocated to receive either a diet of *cis*-9, *trans*-11 CLA (*c*9,*t*11 CLA), *trans*-10, *cis*-12 CLA (*t*10,*c*12 CLA) or linoleic acid for 5 weeks. Animals were slaughtered following a 12-h overnight fast. Animals fed the *c*9,*t*11 CLA diet had significantly (*P* 0.003) lower plasma TAG concentrations than either the *t*10,*c*12 CLA or linoleic acid groups. There was no significant difference in plasma cholesterol, NEFA or glucose concentrations between groups. The *t*10,*c*12 CLA isomer significantly (*P* 0.001) reduced body weight compared to *c*9,*t*11 CLA and linoleic acid diets. The body weight of the *c*9,*t*11 CLA group was significantly (*P* 0.04) lower than the linoleic acid group. There was no significant difference in feed intake between groups. The *c*9,*t*11 CLA diet significantly (*P* 0.003) increased hepatic fatty acid synthase (FAS) mRNA expression.

	Linoleic acid (*n* 6)		*c*9,*t*11 CLA isomer (*n* 5)		*t*10,*c*12 CLA isomer (*n* 5)	
	Mean	SD	Mean	SD	Mean	SD
Cholesterol (mmol/l)	6.45	0.77	6.98	0.38	7.17	0.40
Triacylglycerol (mmol/l)	1.11	0.17	0.95[a]	0.08	1.25	0.12
Glucose (mmol/l)	12.06	1.49	10.76	1.59	12.24	1.99
NEFA (mmol/l)	0.74	0.09	0.64	0.27	0.71	0.21
Body weight (g)	52.85	6.13	46.28[b]	3.52	38.69b	4.40
FAS (FAS/GAPDH)	11.93	1.93	14.02[c]	2.01	11.12	0.97

[a] Significantly different from *trans*-10 CLA and linoleic acid diets *P* 0.003.
[b] Significantly different from linoleic acid diet *P*≤0.05.
[c] Significantly different from *trans*-10 CLA and linoleic acid diets *P* 0.03.

This investigation highlights the isomer specific effects of CLA on lipid metabolism, body weight and hepatic gene expression. The *t*10,*c*12 CLA isomer significantly inhibited body weight gain in *Ob/Ob* mice. The *c*9,*t*11 CLA isomer significantly improved plasma TAG concentrations, and increased hepatic FAS gene expression. PPARα is a transcription factor that regulates hepatic lipid metabolism. This study suggests that in the fasted state *c*9,*t*11 mediates its effect on hepatic lipid metabolism via PPARα gene expression.

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Effect of vegetables, tea and soya on endogenous *N*-nitrosation and faecal water genotoxicity during a high red meat diet in humans. By R. HUGHES[1], J.R.A. POLLOCK[2] and S.A. BINGHAM[1], [1]*Medical Research Council, Dunn Human Nutrition Unit, Welcome Trust/MRC Building, Hills Road, Cambridge CB2 2XY and* [2]*Pollock and Pool Ltd., Ladbroke Close, Reading RG5 4DX*

Many *N*-nitrosocompounds (NOC) are known carcinogens and endogenous formation represents the most potent source of human exposure to these compounds. Red meat increases colonic *N*-nitrosation (Bingham *et al.* 1996; Silvester *et al.* 1997; Hughes *et al.* 2001) and this may explain the positive epidemiological relationship between red meat intake and colorectal cancer risk. Vegetables, tea and soya have been shown to block NOC formation *in vitro* and *in vivo* (Bartsch & Frank, 1996) and are associated with protection against colorectal cancer.

To see whether these supplements affect faecal NOC excretion during a high-red-meat diet (420 g/d) eleven male volunteers were studied over a randomized series of four, 15-d dietary periods. The diets studied included the high-red-meat diet supplemented with vegetables (400 g/d as 134 g broccoli, 134 g Brussels sprouts and 134 g petits pois); tea (500 mg/d) or both vegetables and tea. Seven of these subjects completed a further dietary period to test the effects of soya (100 g/d) with the same high-meat diet. The dietary intervention was shown to affect colonic function as faecal weight increased during the vegetable and soya periods ($P<0.0001$). The dietary supplements had no effect on faecal nitrogen and ammonia excretion and faecal water genotoxicity. Soya significantly suppressed faecal apparent total NOC (ATNC) concentration as compared to the meat-only diet (*P* 0.02) but supplements of vegetables and tea had no effect.

	420 g Meat (*n* 11)		**Meat + Veg** (*n* 11)		**Meat + Tea** (*n* 11)		**Meat + Tea+ Veg** (*n* 11)		**Meat + Soya** (*n* 7)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Output (g/d)	112.7	8.6	150.1	13.4[‡]	118.4	10.8	162.3	10.5[‡]	152.6	20.2*
MTT (h)	58.7	10.8	52.0	9.6	52.2	9.0	47.6	8.0	55.1	10.8
ATNC (ng/g)	1594.1	465.8	2098.7	960.0	1196.4	216.9	2222.7	1263.6	865.1	183.7*
ATNC (μ/d)	167.4	42.5	260.8	87.2	137.6	29.0	321.9	161.4	120.4	20.6
NO_2 (μg/g)	0.63	0.12	0.53	0.06	0.34	0.10[†]	0.35	0.07	0.49	0.16
NO_2 (μg/d)	73.5	15.6	80.3	11.2	38.7	9.9	59.7	13.8	69.9	22.4

Significantly different from the meat only diet *$P<0.05$, †$P<0.01$, ‡$P<0.0001$.

Results from the present study show that increased faecal ATNC levels observed during high-red-meat diets are unaffected by vegetables and tea which are known to suppress *N*-nitrosation *in vitro* and *in vivo*. Vegetables, however, increased faecal weight and faecal weight was inversely associated with transit time on an individual basis (*r* 0.594, $P<0.0001$) so that contact between ATNC, nitrite and ammonia and the large bowel mucosa would have been reduced. Furthermore, prolonged mean transit times (MTT) were associated with elevated ATNC levels (*r* 0.42, *P* 0.002). Soya was found to have a significant suppressive effect on faecal ATNC concentration and this may contribute to the many known chemopreventive properties of soya.

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An assessment of the multivariate factors that may relate to dental caries among young Dublin schoolchildren. By A.C. GRIFFIN[1], M.A.T. FLYNN[1], E. DELAP[2], J. MCLOUGHLIN[2], S. O'HICKEY[2], M.J. GIBNEY[3] and K.M. YOUNGER[1], [1]*Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Republic of Ireland,* [2]*Department of Public and Child Dental Health, Dublin Dental Hospital, Lincoln Place, Dublin 2, Republic of Ireland and* [3]*Unit of Nutrition and Dietetics, Department of Clinical Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8, Republic of Ireland*

It is recognized that the causes of dental caries are multifactorial, encompassing dietary, socio-economic and dental hygiene variables (Navia, 1994). To date, the most accurate predictor of dental caries among Dublin schoolchildren, where domestic water supplies are fluoridated, has not been assessed. This study aimed to examine potential factors and assess their influence with respect to risk of caries, which was measured as a score of decayed, missing and filled tooth surfaces (DMFS) in young Dublin schoolchildren (*n* 130; fifty-eight boys, seventy-two girls, mean age 10.9 (0.8) years).

DMFS was measured by the same dentist, for each child, and was recorded as a DMFS≥1 while absence of caries was recorded as DMFS=0. Dietary factors were estimated as the reported amount (using food atlas and household measures) and frequency of foods recorded in two 2 d food diaries that differentiated between meals and snacks. The foods were further grouped to form twelve (one each per meal and snack) categories: sweet drinks, sweet foods, savoury foods, cheese, milk and high biological value protein foods. Social class was classified according to the highest classed occupation in the household (O'Hare, 1982). Dental hygiene habits (frequency of toothbrushing and dental examinations) and adverse dental experiences (toothache, fillings, extraction and abscesses) were reported by answering yes/no on a standardized, validated questionnaire. The mean measurement of DMFS and those variables found to be significant with caries experience (by univariate analysis) are listed in the table.

Variables significant with dental caries expressed as mean (SD) or percentage (*n*)	DMFS = 0 (*n* 88)		DMFS ≥ 1 (*n* 42)		*P* value
Mean (SD) DMFS	0	(0)	3.45	(3.9)	
Number of meals daily	2.63	(0.6)	2.38	(0.6)	<0.05
Has experienced toothache	49%	(40)	81%	(30)	≤0.001
Has had tooth extracted	8%	(7)	24%	(10)	<0.05
Has had a tooth abscess	6%	(5)	23%	(9)	<0.05
Has had fillings	38%	(33)	76%	(31)	≤0.001
Visits dentist ≥ every two years	86%	(65)	62%*	(23)	
Visits dentist < every two years	14%	(11)	38%*	(14)	
Cheese consumed as a meal (g/d)	19.1	(12)	11.3	(7.1)	<0.05
Frequency of consuming savoury foods as snacks	1.2	(0.7)	1.5	(0.7)	<0.05
Social class 1	67%	(58)	42%**	(17)	
Social class 2	16%	(14)	27%**	(11)	
Social class 3	16%	(14)	32%**	(13)	

*Significantly different from DMFS=0; χ^2=7.851, *P*<0.05. **Significantly different from DMFS≥1; χ^2=7.884, *P*<0.05.

These variables were assessed to establish if dental caries could be predicted by a forward conditional logistic regression. A model was constructed that explained 85.7% of the variance in dental caries. It proposed that visiting the dentist every 2 years or more frequently reduced the odds of having caries by 43.9 times. Toothaches and fillings were positively associated with caries. There was no significant difference in the frequency of dental visits among those children who reported experiencing aches and fillings and those who did not. Among Dublin schoolchildren, we can conclude that the mandatory dental examination of children's teeth (with follow-up as necessary), commencing when they reach 7 years of age, provides a significant protective effect against caries that negates social class, dietary and poor dental hygiene habits. This is in agreement with other European data (Bolin *et al*. 1997). Dietary factors were not predictive of caries.

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Vegetable intakes in Irish adults aged 18–64 years: analysis of the North/South Ireland Food Consumption Survey. By M.M. O'BRIEN, M. KIELY and A. FLYNN, *Irish Universities Nutrition Alliance, Department of Food Science, Food Technology and Nutrition, University College Cork, Ireland*

Substantial epidemiological evidence exists for the protective role of vegetables in the aetiology of cancer (Block et al. 1992) and cardiovascular disease (Ness & Powles, 1997). The Irish Universities Nutrition Alliance (IUNA) established a database of habitual food and drink consumption using a 7 d food diary in 1379 randomly selected adults (662 men and 717 women) who participated in the North/South Ireland Food Consumption Survey. Recently, Hannon *et al.* (2001) and O'Brien *et al.* (2001) reported that vegetables make a significant contribution to vitamin and mineral intakes in Irish adults. The current analysis describes vegetable intake from all sources including vegetables from composite dishes, recipes and manufactured foods.

	Men (*n* 662)				Women (*n* 717)				Total (*n* 1379)			
	%	Intake (g/d)			%	Intake (g/d)			%	Intake (g/d)		
	Consumers	Mean	SD	Median	Consumers	Mean	SD	Median	Consumers	Mean	SD	Median
Peas, beans & lentils	85	31[a]	33	21	78	17	19	11	82	24	27	15
Peas	67	14[a]	19	9	64	8	10	4	65	11	15	6
Baked beans	45	16[b]	26	0	37	8	14	0	41	12	21	0
Green vegetables	67	16	20	12	72	14	16	10	70	15	18	11
Broccoli	35	5	10	0	43	5	9	0	39	5	9	0
Cabbage	37	7	16	0	37	5	10	0	37	6	13	0
Green beans	13	2	5	0	13	1	4	0	13	1	5	0
Carrots	81	20	25	14	84	17	18	12	82	19	22	12
Salad vegetables	53	4	8	1	68	7	11	3	61	5	10	2
Lettuce	52	3	6	1	66	5	8	2	59	4	7	1
Tomatoes	81	26[b]	29	18	89	29	28	23	85	28	29	21
Other vegetables	97	51[b]	40	44	99	48	36	41	98	50	38	42
Mushrooms	49	6	11	0	51	5	8	0	50	5	10	0
Onions	88	15	15	11	88	12	12	9	88	13	13	10
Peppers	36	4	11	0	44	4	8	0	40	4	10	0
Cauliflower	24	3	8	0	27	3	7	0	25	3	8	0
Potatoes	100	296[a]	198	243	99	162	110	139	99	227	172	184
Total vegetables (excluding potatoes)	100	149[a]	78	138	100	132	68	121	100	140	73	129

[a] Significant differences between men and women, *P*< 0.001; [b] significant differences between men and women, *P*< 0.01.

Vegetables from composite dishes made a significant contribution (40%) to the mean daily intake of total vegetables (140g/d) for the total population. The mean daily intake values reported here are similar to the mean vegetable intake of 101–127 g reported by Lee & Cunningham (1990) and also to the mean intake of 134 g/d reported in the UK adult population (Gregory *et al*. 1990). The 95th percentile of intake of vegetables was 276 g/d (men, 288 g/d; women, 252 g/d). Intake of total vegetables was lowest in the 18–35 year (128 g) age-group and i ncreased substantially in the 36–50 year age-group (148g) and the 51-64 years (148g) for both men and women. It was found that 62% of men and 73% of women consumed less than two 80 g servings of vegetables per day. USDA (2000) currently recommends 3-5 servings of vegetables per day.

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The contribution of ready-to-eat-breakfast cereals to nutrient intake in Irish adults. By M.A. GALVIN, M. KIELY and A. FLYNN, *Irish Universities Nutrition Alliance, Department of Food Science, Food Technology and Nutrition, University College Cork, Ireland*

The consumption of ready-to-eat-breakfast cereals (RTEBC) in Irish adults and their contribution to nutrient intakes was examined using data from the North/South Ireland Food Consumption Survey. This survey collected food intake data in a representative sample of 1379 Irish adults aged 18–64 years (662 men and 717 women) using a 7 d food diary. Nutrient intakes were estimated using McCance & Widdowson's data (Holland *et al.* 1995) plus nutrient data collected on generic Irish foods.

Of the total sample, 67% were RTEBC consumers, with a mean frequency of consumption of 4.3 times per week. The mean daily intake of RTEBC in consumers was 28.6 g (men 31.1 g; women 26.5 g). Men and women consumers were classified by tertile of RTEBC consumption into low, medium and high. Nutrient intakes per 10 MJ of food energy (excluding alcohol) are shown in the table.

	Men*								Women*							
Nutrient	Non (*n* 225)		Low (*n* 146)		Medium (n 147)		High (*n* 144)		Non (*n* 225)		Low (*n* 162)		Medium (*n* 170)		High (*n* 160)	
per 10 MJ	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total fat (g)	102	16	100	12	96	14	94	15	103	15	99	16	97	15	90	15
Carbohydrate (g)	281	35	283	32	296	33	304	33	282	33	293	36	295	34	310	32
Dietary fibre (g)	22.1	6	22.5	6	22.7	7	24.8	7	22.2	6	23.4	6	24.0	6	27.0	9
Calcium (mg)	841	244	912	242	916	222	949	250	958	489	959	280	1017	248	1083	287
Iron (mg)	12.3	4	13.5	5	13.5	3	15.3	4	16.5	24	16.7	18	18.4	22	25.1	35
Thiamin (mg)	2.20	2.2	2.12	0.7	2.31	1.3	2.59	1.9	2.37	3.1	2.61	2.8	3.26	7.3	3.48	6.0
Riboflavin (mg)	1.63	0.8	1.92	0.8	2.07	0.9	2.53	2.0	2.20	3.2	2.38	2.6	2.75	4.4	3.55	5.4
Total niacin (mg)	42.5	11	44.8	11	45.5	12	45.3	9	43.4	13	46.2	15	47.5	13	52.1	16
Vitamin B6 (mg)	3.44	2.2	3.37	1.8	3.45	1.5	3.68	2.4	3.48	5.3	3.96	6.8	5.77	13.8	7.48	14.5
Total folate (μg)	260	115	281	107	299	93	337	121	283	144	325	161	357	143	442	285

*Low consumers ≤16.4 g/d (men), ≤14.3 g/d (women); medium consumers >16.4–35.7 g/d (men), >14.3–30 g/d (women); high consumers >35.7 g/d (men), >30.0 g/d (women).

Fat intake (g/10 MJ) was significantly lower ($P<0.01$) while the carbohydrate intake (g/10 MJ) was significantly higher ($P<0.01$) with higher consumption of RTEBC**.** The nutrient density of dietary fibre and micronutrients increased significantly ($P<0.01$) in men and women with increased RTEBC consumption, except for total niacin and vitamin B_6 in men. Among consumers, the contribution of RTEBC to nutrient intake (food sources only) in men (women) was: dietary fibre 8.6% (11.5%), iron 15.7% (21.6%), Vitamin D 7.9% (15.6%), thiamin 12.9% (16.0%), riboflavin 15.7% (18.4%), total niacin 8.9% (11.7%), vitamin B_6 11.3% (16.7%) and total folate 15.2% (18.3%). Although RTEBC do not contribute significantly to calcium intake overall, consumption of milk increased as RTEBC consumption increased. An additional 98 g of milk (107 g in men and 92 g in women) was consumed at a breakfast containing a RTEBC, as opposed to a breakfast that did not contain a RTEBC.

The results highlight the important contribution made by RTEBC to the diets of consumers and in particular to the diets of women in whom dietary fibre and micronutrient inadequacy was more prevalent (Galvin *et al.* 2001, O'Brien *et al.* 2001, Hannon *et al.* 2001).

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Impact of fortification on micronutrient intakes in Irish adults. By E.M. HANNON, M. KIELY and A. FLYNN, *Irish Universities Nutrition Alliance, Department of Food Science, Food Technology and Nutrition, University College Cork, Ireland*

In Ireland and the UK, the voluntary addition of vitamins and minerals to foods has been permitted for many years and a significant number of foods and beverages are now fortified. However, the impact of such fortification on micronutrient intakes has not been assessed adequately. The North/South Ireland Food Consumption Survey has established a database of habitual food and drink consumption, estimated using a 7 d food diary, in a representative sample of 1379 Irish adults aged 18–64 years. In this study the impact of voluntary fortification on intakes of vitamins and minerals was estimated. Nutrient intakes were estimated using tables of food composition (Holland *et al.* 1995) along with additional data (manufacturers' data on generic Irish foods, nutritional supplements, and on new products that were commonly consumed). The levels of added micronutrients were estimated from manufacturers' data and micronutrient data from unfortified versions of the foods.

Of the 3060 food codes that appeared in the database, seventy-seven are currently fortified, 62% of which are breakfast cereals. Thiamin, riboflavin, Fe, niacin and folic acid are the micronutrients most frequently added to foods. Fortified foods are consumed by 69% of the population. The mean daily percentage energy and the 95th percentile of energy intake from fortified foods, among consumers only, were 3.9% and 10.0%, respectively, for men and 5.0% and 13.3%, respectively, for women.

Micronutrient	Percentage contribution of fortification component to mean daily micronutrient intakes among consumers of fortified foods.	
	Men (*n* 449)	Women (*n* 508)
Folate	13	15
Riboflavin	14	15
Vitamin B_6	10	13
Iron	10	14
Thiamin	11	13
Vitamin D	8	16

The percentage of the population with mean daily intakes below the average requirement (AR) is a good estimate of the proportion of the population with inadequate intakes (Scientific Committee for Food, 1993; Food and Nutrition Board, 1997). Among consumers of fortified foods, inclusion of the fortification component reduced the proportion of the population with intakes of riboflavin less than the AR from 27% to 14% in women and from 16% to 8% in men. For 18–50 year-old women, the proportion with mean daily intakes of Fe and folate below the AR decreased from 60% to 40% and from 13% to 4%, respectively, when fortification was included. Fortification does not have a significant impact on the proportion of the population with mean daily intakes above the Tolerable Upper Intake Level for any micronutrient.

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Seasonality of vitamin D intake in 18–64 year-old Irish Adults: analysis of the North/South Ireland Food Consumption Survey. By T. R. HILL, M. M. O'BRIEN, M. KIELY, K. CASHMAN and A. FLYNN, *Irish Universities Nutrition Alliance, Department of Food Science, Food Technology and Nutrition, University College Cork, Ireland*

The North/South Ireland Food Consumption Survey established a database of habitual food and drink consumption in a representative sample (*n* 1379) of Irish adults aged 18–64 years. Food intak e data were collected using a 7 d diary and nutrient intakes were calculated using McCance & Widdowson's Composition of Foods (Holland *et al.* 1995) along with additional data (manufacturers data on generic foods, nutritional supplements and new products that are commonly consumed).

Analysis of the survey data showed that the main food groups that contributed to dietary vitamin D intakes were meat and meat products (30.6%), fish and fish products (14.3%) and eggs and egg dishes (12.3%; O'Brien *et al.* 2001). A high proportion of the Irish population has a low dietary vitamin D intake and hence is largely dependent on sunlight to maintain adequate vitamin D status. Time of year (season) is the most important determinant of vitamin D status (Stamp & Round, 1974).

In the current study, intakes of vitamin D from food sources only and from all sources including nutritional supplements were compared by season. Two seasons were defined, namely 'winter' (September–February) and 'summer' (March–August).

	Vitamin D intake (μg/d)											
	All sources						Food sources only					
	Winter			Summer			Winter			Summer		
	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median
Men												
18–35y	2.8	1.9	2.1	3.3	3.0	2.1	2.6	1.7	2.1	2.8	2.5	2.0
36–50y	4.4[a]	3.9	2.9	3.3[a]	2.2	2.5	3.4	3.2	2.5	3.1	2.0	2.5
51–64y	4.8	4.5	3.1	3.9	4.4	2.8	4.4	4.4	2.8	3.4	4.1	2.3
All	3.9	3.5	2.6	3.5	3.2	2.4	3.3	3.1	2.4	3.1	2.9	2.2
Women												
18–35y	2.9	3.2	1.8	2.8	2.9	1.8	2.0	1.5	1.6	2.0	1.7	1.6
36–50y	3.4	3.2	2.0	3.5	3.5	2.1	2.5	2.1	1.9	2.7	2.6	1.8
51–64y	4.5	4.5	3.1	4.6	5.4	2.6	3.5	3.4	2.4	3.6	4.8	2.2
All	3.4	3.6	2.1	3.5	3.8	2.1	2.5	2.3	1.8	2.6	3.0	1.8

[a] Values significantly different using Mann-Whitney *U* (*P* 0.035).

Vitamin D intakes increased with increasing age in men and women. Nutritional supplements contributed 6.2% in men and 11.4% in women of the mean daily vitamin D intake. Men aged 36–50 years had significantly higher (*P* 0.035) mean daily vitamin D intakes from all sources during winter than during summer. This trend was also present in 36–50 year-old men from food sources only, and in men aged 51–64 years from all sources and from food sources only. Otherwise, seasonality was not found to have a significant effect on vitamin D intakes. The results suggest that the seasonal variation in vitamin D status is mainly attributable to sunlight exposure rather than dietary intake. This study did not look at or consider vitamin D status measurements, but the results suggest that the seasonal variation in vitamin D status is mainly attributable to sunlight exposure rather than dietary intake.

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The contributions of meat to nutrient intakes in Irish men and women of different ages. By M. COSGROVE, M. KIELY and A. FLYNN, *Irish Universities Nutrition Alliance, Department of Food Science, Food Technology and Nutrition, University College Cork, Ireland*

The Irish Universities Nutrition Alliance (IUNA) established a database of habitual food and drink consumption using a 7 d diary in a random sample of 18 –64 year-old adults using data from the North/South Ireland Food Consumption Survey. In recent papers, it has been shown that meat and meat products were major contributors to mean daily intakes of Zn (38%), Fe (18%), Cu (15%), Mg (13%), thiamin (18%), riboflavin (18%) and vitamins A (12%), D (31%) and B_{12} (38%) in Irish adults (Hannon *et al.* 2001; O'Brien *et al.* 2001). The current study compared the relative contributions of meat, meat dishes and meat products to nutrient intakes in men and women in three age groups (18–35, 36–50 and 51–64 years). This study is based on analysis of the survey database in 958 adults (475 men, 483 women) from the Republic of Ireland. Nutrient intakes were calculated using McCance & Widdowson's Composition of Foods (Holland *et al.* 1995) and additional IUNA data.

In total, 98% of adults (*n* 942; 465 men and 477 women) were me at consumers, as they had recorded the consumption of meat in the 7 d diary. The mean daily intake of meat, meat products and meat dishes in men (232 g) was 1.5 times that in women (152 g) these figures include non-meat components of dishes and products. The intake of meat decreased with increasing age, particularly in men. The percentage contribution of meat to mean daily nutrient intakes for men and women of different ages is shown in the table.

	Men*								Women*							
	All (*n* 465)		18–35y (*n* 166)		36–50y (*n* 174)		51–64y (*n* 125)		All (*n* 477)		18–35y (*n* 161)		36–50y (*n* 201)		51–64y (*n* 115)	
Nutrients	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Energy	17	7	18	7	17	7	17	6	15	7	15	7	16	6	15	7
Protein	43	12	44	11	42	12	43	11	39	12	39	13	39	11	38	13
Total fat	26	11	27	10	25	12	27	11	21	10	21	11	22	10	21	11
Magnesium	14	6	15	6	14	6	13	6	13	6	13	7	13	6	12	6
Iron	22	10	22	10	21	10	21	10	17	10	16	11	17	10	17	9
Copper	17	11	17	10	16	11	17	14	15	12	15	12	16	13	14	11
Zinc	44	14	44	14	43	15	44	14	36	15	35	17	37	14	37	15
Total Vitamin A	13	18	12	15	12	18	14	21	13	20	14	20	14	21	11	17
Vitamin D[†]	36	22	41	22	35	22	33	22	27	22	30	23	27	21	24	23
Thiamin	23	11	24	12	22	11	23	11	19	11	19	12	20	11	19	11
Riboflavin	20	10	19	10	20	11	22	10	17	11	16	12	17	11	16	9
Vitamin B_{12}	45	20	45	19	42	20	47	21	36	22	35	22	38	22	37	20

* With the exception of vitamin A, differences between men and women were significant ($P<0.0001$).
[†] Age group had a significant (*P* 0.01) effect on the contribution of meat to mean daily vitamin D intakes.

The data show that with the exception of vitamin A, meat makes a significantly larger contribution to nutrient intakes in men than in women of all ages. As age increased, the contribution of meat to mean daily vitamin D intakes in both men and women (*P* 0.01) decreased. No other significant age-related effects were observed.

The choice of meat products (type and quantity) may affect the total intakes of a number of nutrients and may influence nutritional adequacy, which is particularly relevant in women. Further studies will look at the different types of meat.

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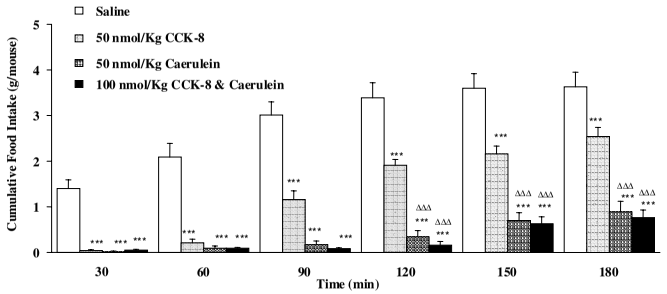
Peripheral administration of cholecystokinin octapeptide and caerulein inhibits food intake in Swiss TO mice. By Y.H.A. ABDEL-WAHAB, C.M.N. KELLY, L. McLOUGHLIN, P.R. FLATT and F.P.M. O'HARTE, *School of Biomedical Sciences, University of Ulster, Coleraine, N. Ireland BT52 1SA*

Several peptides have been suggested to play a pivotal role in the regulation of satiety. The purpose of the present study was to compare the effects of peripheral administration of the peptide hormones cholecystokinin-8 (CCK-8), caerulein (naturally occurring N-terminal analogue of CCK-8) and a combination of both on food intake in male Swiss TO mice. Mice aged 12 weeks were gradually habituated (standard breeding diet, Trouw Nutrition, Belfast) over a 3-week period to a reduced voluntary food intake of 3 h (10.00–13.00 hours) per day. A consistent daily food intake was observed for 1 week prior to beginning the experimental study. Mice (n 7–8) with an average weight of (30±1 g) were injected intraperitoneally (10.00 hours) with either saline control (0.9% w/v NaCl, 10 ml/kg), or CCK-8 and caerulein (50 nmol/kg) or a mixture of both peptides (100 nmol/kg). Food intake was monitored at 30, 60, 90, 120, 150 and 180 min post-injection. CCK-8 significantly reduced cumulative food intake by 30–97% at 30, 60, 90, 120, 150 and 180 min after administration (P<0.001) compared with saline controls (total food intake 2.5±0.2 g/mouse). Caerulein reduced (P<0.001) food intake at 120–180 min by 65–82%, proving to be significantly more potent than CCK-8 at these time points. When both peptides were injected simultaneously, food intake was significantly reduced over the entire experimental period between 30 to 180 min post-injection (P<0.001) by 79–96% compared with saline controls. CCK-8 and caerulein in combination did not give rise to a significantly more enhanced and protracted satiating effects when compared with caerulein administered alone. Food intake results are shown below.



Values are the mean (SEM) for n=8. ***P<0.001 compared with saline and $^{\Delta\Delta\Delta}P$<0.001 compared with CCK-8 at the same time points.

This study demonstrated that caerulein is a more potent inhibitor of food intake than CCK-8. Structural modification of this peptide at the N-terminus may be responsible for the enhanced satiety activity. This may be due to the increased resistance of caerulein to aminopeptidase degradation in the circulation, thus prolonging its half-life and biological activity *in vivo*.

Detection of glycated GLP-1 within the intestinal tissue of normal and diabetic animals. By M.H. MOONEY, Y.H.A. ABDEL-WAHAB, P.R. FLATT and F.P.M. O'HARTE, *School of Biomedical Sciences, University of Ulster, Coleraine, N. Ireland BT52 1SA*

Glucagon-like peptide-1(7-36) amide (GLP-1) is produced within intestinal L-cells and is released upon nutrient ingestion. GLP-1 is proposed to play a key role in the incretin effect whereby hormones released from the gut into the blood potentiate glucose-stimulated insulin release from pancreatic B-cells. Previous studies have demonstrated that GLP-1 undergoes glycation *in vitro* under hyperglycaemic conditions. The present study was undertaken to determine whether this post-translational modification occurs *in vivo* within the endocrine cells of the glucose-rich and highly vascularized environment of the small intestine of both normal and diabetic animals. Diabetic hyperglycaemia was induced in Swiss TO mice by administration of a single i.p. injection (200 mg/kg body weight) of streptozotocin (STZ) and animals were maintained for a 21 d period. Hyperglycaemia was induced in Wistar rats by the s.c. administration of hydrocortisone (40 mg/kg body weight) twice daily for 8 d. The final hyperglycaemic animals evaluated were 14–16-week-old obese hyperglycaemic *ob/ob* mice which are spontaneously diabetic with lean littermates been used as controls. The small intestines of non-fasted diabetic and age-matched control animals were excised at the end of the respective experimental periods. Following acid–ethanol extraction, separation of the extracts into non-glycated and glycated fractions was achieved by affinity chromatography using GlycoGel B columns. The concentration of immunoreactive GLP-1 in the various extracts was determined by radioimmunoassay using a fully cross-reactive side-viewing GLP-1 antiserum. The characteristics of the various animal groups are summarized below.

	n	Body weight (g)		Plasma glucose (mmol/l)		Small intestine weight (g)		Total intestinal GLP-1 content (pmol)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
STZ mice	8	26.1	1.7	52.5	7.8***	1.9	0.1***	331	43
Control mice	8	29.5	1.0	7.1	0.4	1.2	0.1	248	49
Hydrocortisone rats	8	229.8	2.5***	20.5	2.0***	5.9	0.2	1322	226
Control rats	8	351.3	4.4	6.2	0.2	5.8	0.7	1496	129
***ob/ob* mice**	8	72.3	0.4***	22.4	2.9***	2.4	0.1***	677	113
Lean mice	6	41.8	0.5	8.6	0.7	1.5	0.1	461	120

***P<0.001 compared with matching control groups.

The total intestinal GLP-1 contents of diabetic and control animals were found to be similar both in terms of total intestinal content and per g of tissue. Affinity separation of intestinal extracts demonstrated that approximately 19% of GLP-1 within the intestinal extracts of all animals were in glycated form. However, STZ-treated mice (P<0.05), hydrocortisone-treated rats (P<0.001) and *ob/ob* mice (P<0.05) were all found to possess significantly greater levels of glycated GLP-1 than control animals, corresponding to 24–71% of total GLP-1 content. The recognition that intestinal hormones are prone to glycation *in vivo* not only in diabetic but also in normal animals, suggests that such post-translational modification warrants further investigation to determine any possible significance for nutrient homeostasis and disease pathophysiology.

Biological activity of cholecystokinin octapeptide and related analogues on insulin secretion from pancreatic β-cells. By F.P.M. O'HARTE, C.M.N. KELLY and P.R. FLATT, *Diabetes Research Group, School of Biomedical Sciences, University of Ulster, Cromore Road, Coleraine, N. Ireland BT52 1SA*

Cholecystokinin (CCK) is a neuropeptide hormone found in the central nervous system and secreted from gut endocrine I-cells following nutrient absorption. CCK is involved in many physiological processes including insulin secretion, regulation of satiety, bowel motility, gastric emptying, exocrine pancreatic enzyme secretion and neurotransmission (Liddle, 1994). CCK exists in multiple molecular forms in the circulation ranging from four to fifty-eight amino acids in length but the C-terminal octapeptide (CCK-8; Asp^1-$Tyr(SO_3H)^2$-Met^3-Gly^4-Trp^5-Met^6-Asp^7-Phe^8-amide), is highly conserved between species and is the smallest molecular form believed to retain the full range of biological activities (Liddle, 1994).

This study investigated the effects of structural modification and truncation of CCK-8 on insulin secretion from cultured glucose-responsive clonal BRIN-BD11 cells. The production and characterization of this cell line has been described previously (McClenaghan *et al*. 1996). The analogues investigated included those with modifications at the Asp^1, Tyr^2, as well as N-terminally truncated forms of CCK-8. The analogues tested were native sulphated CCK-8, non-sulphated CCK-8, phosphorylated CCK-8, caerulein, Asp^1-glucitol CCK-8, CCK-7 (CCK 2-8), CCK-6 (CCK 3-8), CCK-5 (CCK 4-8) and CCK-4 (CCK 5-8). The analogues were either available commercially or were synthesized by solid phase peptide synthesis techniques using standard Fmoc chemistry. Acute (20 min) incubations of BRIN-BD11 cells at 5.6 mmol/l glucose with native sulphated CCK-8 (10^{-6}–10^{-12} mol/l) in the presence of the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX) (100 µmol/l) exerted a 1.4–2.4-fold increase of insulin secretion compared with control (5.6 mmol/l glucose alone, Students *t*-test $P<0.01$, n 8). Non-sulphated CCK-8 had significantly lower insulinotropic activity than native CCK-8 over the concentration range tested, whereas phosphorylated CCK-8 (10^{-11}–10^{-7} mol/l) was stimulatory compared to controls (1.6–2.0-fold, $P<0.01$). The N-terminally extended analogue caerulein (pGlu-Gln CCK-8), stimulated insulin release by 1.3–2.0-fold compared with control incubations ($P<0.05$). In contrast, the N-terminally modified Asp1-glucitol CCK-8 had a significantly reduced insulinotropic activity compared with native sulphated CCK-8 ($P<0.05$), which was consistent with previous findings (Abdel-Wahab *et al*. 1999). The N-terminally truncated forms of CCK-8 including CCK-7, CCK-6, CCK-5 and CCK-4 all failed to stimulate insulin release above control values.

These data indicate that amino-terminal modifications of the CCK-8 molecule can significantly affect the insulinotropic activity of CCK. Furthermore, N-terminal truncation of CCK-8 results in loss of insulin-releasing activity in this *in vitro* system. In conclusion, the present structure–function studies on CCK-8-related peptides demonstrate that the amino-terminus as well as the penultimate sulphated Tyr^2 are important features in the retention of insulin-releasing activity.

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N-terminally modified GLP-1(7-36) amide is resistant to dipeptidyl peptidase-IV degradation whilst maintaining insulinotropic action. B.D. GREEN[1], F.P.M. O'HARTE[1], P. HARRIOTT[2], B. GREER[2] and P.R. FLATT[1], [1]*Department of Biomedical Sciences, University of Ulster, Coleraine, BT52 1SA, N. Ireland and* [2]*School of Biology and Biochemistry, Queen's University of Belfast, Belfast BT9 7BL, N. Ireland*

Glucagon-like peptide-1(7-36) amide (GLP-1) is an important hormone released from the intestine upon nutrient ingestion. GLP-1 has been characterized as having potent insulin-secreting activity on the pancreatic β-cell. For this reason it is considered to be a potential treatment for type 2 diabetes mellitus (Holst *et al.* 1996). The action of GLP-1, however, is short-lived due to rapid degradation by dipeptidyl peptidase-IV (DPP-IV). This enzyme removes an N-terminal dipeptide His^7-Ala^8, rendering GLP-1 inactive. Previous studies have designed GLP-1 analogues with possible resistance to enzymatic degradation in an attempt to increase peptide half-life and prolong biological activity (O'Harte *et al*. 2000). This study examined the degradation of His^7-acetylated GLP-1 and investigated its insulinotropic action *in vitro.* His^7-acetylated GLP-1 was synthesized by solid phase peptide synthesis using Fmoc chemistry and modified using acetic anhydride. Molecular mass (M_r 3339.7) was confirmed by electrospray ionization mass spectrometry. Native and His^7-acetylated GLP-1 were incubated (37°) in the presence of either DPP-IV (1.25mU) or human plasma (7.5 µl) for 0, 4, 6 and 12 h. The extent of peptide degradation was quantified by reversed-phase HPLC analysis. Insulinotropic action was assessed by measuring insulin secretion from a glucose-responsive clonal pancreatic β-cell line (BRIN-BD11). Acute 20 min incubations were carried out at 5.6 and 16.7 mmol/l glucose in Kreb's Ringer bicarbonate buffer (pH 7.4) supplemented with peptide concentrations ranging from 10^{-6} to 10^{-12} mol/l.

After a 12 h incubation with DPP-IV, 25% of native GLP-1 remained intact compared with >99% intact for His^7-acetylated GLP-1. Following incubation with human plasma, 22% of native GLP-1 remained intact after 12 h compared with >99% intact for His^7-acetylated GLP-1. Glucose induced insulin secretion was enhanced 2.7-fold (5.6 mmol/l glucose) to 3.3-fold (16.7 mmol/l glucose) with native GLP-1. Exposure to His^7-acetylated GLP-1 enhanced insulin secretion 2.5-fold (5.6 mmol/l glucose) and 1.8-fold (16.7 mmol/l glucose). The potentiation of insulin secretion by His^7-acetylated GLP-1 did not differ from native GLP-1 in the presence of 5.6 mmol/l glucose. However, at 16.7 mmol/l glucose the insulinotropic effect of GLP-1 was reduced up to 45% by acetylation ($P<0.01$).

These results suggest that N-terminal acetylation of GLP-1 prevents DPP-IV degradation of the hormone. N-terminal acetylation does not alter the insulinotropic effect of GLP-1 at basal glucose concentration (5.6 mmol/l) but decreases its potency at high supraphysiological glucose concentration (16.7 mmol/l). Although N-terminal modification of GLP-1 protects against DPP-IV degradation further studies are required to assess whether this structural modification enhances biological activity *in vivo*.

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Plasma antioxidant status of vegetarian Taoist group in Hong Kong. By Y.T. SZETO[1], T.C.Y. KWOK[2], K.L. IP[2], S. WACHTEL GALOR[1] and I.F.F. BENZIE[1], [1]*Department of Nursing and Health Sciences, The Hong Kong Polytechnic University, Kowloon, Hong Kong SAR, China and* [2]*Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China*

Diets rich in fruits and vegetables are protective against chronic disease. Evidence is so strong that the World Cancer Research Fund recommends at least five servings of fruits and vegetables each day, and the recommended daily intake of vitamin C in the US has been revised upwards to 75 mg/d for women and 90 mg/d for men, with an additional 30 mg/d recommended for smokers. While the identity of the protective component(s) in fruits and vegetables is not yet clear, there is clear evidence that intake and plasma concentrations of vitamin C (ascorbic acid) correlate inversely with all cause mortality, and may act as biomarkers of health status (Khaw *et al*. 2001).

The aim of this study was to compare the plasma antioxidant status of vegetarians with that of age- and sex-matched members of the general population. Thirty vegetarians, (twenty-seven women, three men, mean (SD) age 44.2 (9.0) years) were recruited with their informed consent. These subjects had been vegetarian for between 5 and 55 years (mean (SD) 21.8 (12.2) years). They ate no meat or fish owing to their religious (Taoist) beliefs, but some took eggs and milk occasionally in small amounts. The control group was thirty non-Taoist members of a community health project (twenty-seven women, three men, aged 44.0 (9.2) years). Fasting, heparinized venous blood was collected and plasma separated within 2 h of collection. The plasma total antio xidant status (as the ferric reducing (antioxidant) power (FRAP) value) and ascorbic acid concentration were measured within 30 min of separation, using a modification of the FRAP assay known as FRASC (Benzie & Strain, 1999; US patent no. 6,177,260B1). Plasma was separated into aliquots and stored at –70º until assayed for α-tocopherol (total and lipid standardized), total cholesterol (TC), triacylglycerols (Tg) and uric acid. α-Tocopherol was measured by an HPLC method; uric acid, TC and Tg concentrations were measured using commercially available enzymatic methods. Differences between results obtained on the vegetarian and non-vegetarian groups were investigated using the unpaired *t*-test.

Plasma concentration Mean (SD)	Ascorbic acid (μmol/l)		α-tocopherol (μmol/l)		α-tocopherol (μmol/mmol TC+Tg)		Uric acid (μmol/l)		FRAP value (μmol/l)	
Vegetarian	90.5[a]	(21.0)	22.0[b]	(5.86)	3.76[b]	(0.57)	239[b]	(87.7)	1028	(180)
Non-vegetarian	61.8	(17.0)	27.0	(7.88)	4.23	(0.58)	306	(68.3)	1040	(178)

[a]Significantly higher than the non-vegetarian group, $P<0.0001$; [b]Significantly lower than the non-vegetarian group, $P<0.01$.

Our data show that vegetarian diets are associated with high ascorbic acid and low uric acid concentrations in fasting plasma. This combination leads to no net change in the 'total' antioxidant capacity of plasma. However, the contribution of ascorbic acid to the total antioxidant capacity of fasting plasma of the vegetarian subjects was 50% higher than in the other group. In view of uric acid's direct association with insulin resistance, hypertension and coronary heart disease risk, we suggest that the increased 'non-uric acid antioxidant capacity' may indicate improved antioxidant status. The lower α-tocopherol levels in the vegetarian group could not be explained completely by lower lipid levels, and may be related to low intake. The high ascorbic acid, however, may help conserve and recycle limited α-tocopherol supplies.

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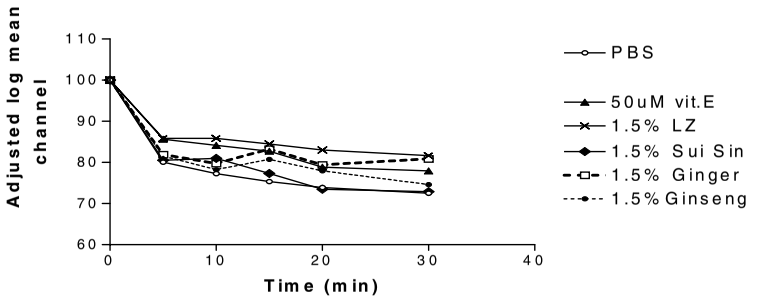
Investigation of membrane protection by antioxidant constituents of Chinese foods and herbs using probe-assisted flow cytometry. By W.Y CHUNG, C.M.N. YOW and I.F.F. BENZIE, *Department of Nursing & Health Sciences, The Hong Kong Polytechnic University, Kowloon, Hong Kong SAR, China*

Many traditional Chinese dietary agents are reported to have antioxidant properties and to be effective in both health promotion and treatment of disease. The aim of this study was to perform a preliminary investigation of the ability of some Chinese dietary agents to oppose lipid peroxidation in cell membranes using a probe-assisted flow cytometric technique (Chung & Benzie, 2000).

Trypsinized, washed HeLa cells were labelled with a lipophilic fluorescent probe (C11-fl; 5-dodecanoylaminofluorescein), incubated for 30 min with individual test agents, washed and incubated for 30 min with 300 μM cumene hydroperoxide (CH) in phosphate buffered saline (PBS). The CH is taken up by the cell membrane, and, with subsequent exposure to an iron/ascorbate mixture, induces a site-specific oxidant challenge within the membrane. Oxidation causes quenching of fluorescence. Test agents were aqueous extracts of lingzhi (*Ganoderma lucidum*), fresh ginger (*Zingiber officinale*), ginseng (*Panax ginseng*) and Sui Sin green tea (*Camellia sinensis*), all at 1.5% w/v in PBS. Forward scatter readings were taken with a Coulter Epics Elite single laser flow cytometer before and at timed intervals after initiation of oxidant challenge. D,L-α-tocopherol (50 μM) was also tested, and the total antioxidant activity, as the Ferric Reducing/Antioxidant Power (FRAP) value (Benzie & Strain, 1996) of test agents was measured.

Results showed that lingzhi (LZ), ginger and α-tocopherol protected cell membranes from oxidation. Sui Sin green tea and ginseng showed no protective effect in this test system. FRAP values (at 1.5% w/v) were 5062, 219, 6640 and 1140 μM for lingzhi, ginger, tea and ginseng, respectively, indicating no direct correlation between the total antioxidant activity and membrane protection.



Results indicate that aqueous extracts of lingzhi and ginger contain antioxidant component(s) that can slow lipid peroxidation. Their action appeared similar to that of α-tocopherol. These preliminary data suggest that some antioxidant-rich dietary agents offer no protection against lipid peroxidation, perhaps owing to matrix effects. However, others contain chain-breaking antioxidant(s), similar in action to α-tocopherol, but dispersed in aqueous medium. The nature of these component(s) remains to be established, but they may contribute to the reputed health benefits of traditional Chinese foods and medicines.

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The development of food folate extracts of yeast, spinach and egg yolk. D.J. McKILLOP[1], K.D. PENTIEVA[1], J.J. STRAIN[1], J.M. SCOTT[2], F. McCREEDY[3], J. ALEXANDER[3], K. PATTERSON[3] and H. McNULTY[1], [1]*Northern Ireland Centre for Diet and Health, University of Ulster, Coleraine, Northern Ireland,* [2]*Trinity College, Dublin, Ireland and* [3]*Loughry College, Food Centre, Cookstown, Northern Ireland*

Folate is attracting major interest in recent years as having an important role in the prevention of certain chronic diseases. However, it is becoming clear that current folate intakes are insufficient for optimal protection against disease. This could be the result of poor diet, instability during cooking and in particular the well documented lower bioavailabity of natural forms of folate in foods compared with the synthetic vitamin, folic acid. Published folate bioavailability studies involving the long-term provision of folate-rich foods are of questionable value owing to folate instability, dietary displacement and subject non-compliance.

This study explored the optimization of the extraction of natural folates from food matrices and subsequent concentration by freeze drying. The overall aim was the production of food folate extracts, which could be administered in defined amounts for comparison with equivalent amounts of folic acid in bioavailability studies. Spinach, yeast and egg yolk were selected, not only because they are considered to be rich sources of folate, but because they encompass a wide range of mono- to polyglutamate ratios considered to be of relevance to the efficiency of intestinal absorption of natural food folates. The preliminary protocol adopted was adapted from the well documented thermal extraction procedures currently used prior to the quantification of folate in food by HPLC or microbiological assay, with the modification that only reagents safe for human consumption were used. Experiments were then designed to examine the effects of pH and duration of thermal extraction on folate yield (determined by tri-enzyme treatment, α-amylase, protease and conjugase, followed by microbiological assay, *Lactobacillus casei* NCIB 10463). Analysis of results showed that the optimal folate yield in extracts of yeast, spinach and egg yolk can be achieved by thermal extraction for 10 min in a 2% ascorbate solution at pH5. Preliminary data on total folate yield and polyglutamate content based on the optimized conditions are presented.

Mean, SD of total folate (μ/100g) and percentage polyglutamate (poly) content of yeast, spinach and egg yolk extracts compared with corresponding food items

Product	Yeast			Spinach			Egg yolk		
	Mean	SD	Poly	Mean	SD	Poly	Mean	SD	Poly
Food	1625	187	98	197	21	60	178	16	0
Extract	3721	560	99.6	2300	380	50	1000	120	0

Values represent the mean of three separate measurements determined with and without conjugase.

In conclusion, we have developed a protocol for the production of concentrated extracts representing food folate sources, having a wide range of mono- to polyglutamate ratios and which can be used in human bioavailability studies. This approach may overcome the many practical difficulties in long-term studies comparing the effects of food folate with equivalent doses of folic acid.

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Can the EPIC food frequency questionnaire be used in adolescent populations? By G. LIETZ, K.L. BARTON, P. LONGBOTTOM and A.S. ANDERSON, *Centre for Public Health Nutrition Research, Department of Epidemiology and Public Health, Ninewells Hospital and Medical School, Dundee University, Dundee DD1 9SY*

Assessing the diets of adolescents can be challenging because of contemporary lifestyles, which prohibit the use of prospective methods of data collection. Although weighed records are the gold standard when used by well-motivated individuals, other dietary assessment techniques are clearly needed for those individuals who are unwilling or unable to complete such records. The aim of the present study was therefore to evaluate the EPIC food frequency questionnaire (EPIC FFQ) developed for use in adults (Bingham *et al.* 1997) as a tool for dietary assessment in Scottish adolescents. Sixty-seven school children (age 11–13 years) took part in the study which involved completing a 7 d weighed dietary record (7 d WDR) and the EPIC FFQ. Dietary data from both 7 d WDR and EPIC FFQ were analysed using Spearman rank correlation analysis on both unadjusted and energy adjusted nutrients. Limits of agreement between methods were examined using Bland-Altman plots. Fifty subjects completed both dietary assessment methods. Thirteen of these were classified as under-reporters with energy intake:basal metabolic rate <1.14 (Strain *et al.* 1994), and were subsequently excluded from the analysis. The EPIC FFQ showed higher estimates than the 7 d WDR for all nutrients. The median Spearman correlation coefficient was found to be 0.30 and increased to 0.50 after adjustment for total energy. Protein intake showed a poor correlation even after energy adjustment. The median proportion of subjects from the EPIC FFQ that appeared in the same and opposite third of intake calculated from the 7 d WDR was found to be 45.9% and 10.8%, respectively.

	7-day WDR		EPIC FFQ		Spearman's r	
	Mean (*n* 37)	SD	Mean (*n* 37)	SD	Unadjusted	Energy adjusted
Energy (MJ)	8.0	0.9	10.4	3.6	0.33*	
Protein (g)	62.1	12.6	93.5	30.1	0.30	0.31
Total fat (g)	76.5	12.4	98.0	39.8	0.52**	0.66**
Total CHO (g)	257.8	40.7	327.0	122.8	0.28	0.50**
Sugar (g)	114.2	33.8	167.1	77.5	0.33*	0.57**
Englyst-fibre (g)	10.6	5.1	17.9	7.5	0.28	0.49**

* Correlation significant at $P<0.05$ (2-tailed), ** correlation significant at $P<0.01$ (2-tailed).

Although correlation coefficients between the EPIC FFQ and the 7 d WDR on average tended to be small, the performance of the EPIC FFQ was comparable to previously evaluated FFQ (Frost Andersen *et al.* 1995; Robinson *et al.* 1999). Limits of agreement for the dietary nutrients, energy, total fat and sugar were analysed using Bland-Altman Plots. The limits of agreement were as far apart as 13.4 MJ, 120–270g for energy, fat and sugar respectively. The 95% CI for the bias for energy, total fat and sugar was 1.3–3.6 MJ, 9.8–33.2g and 29.4–76.5g, respectively. Agreement between the EPIC FFQ and the 7 d WDR for energy, total fat and sugar was low on both a group and an individual basis. This finding is contrary to the fact that significant correlation coefficients were observed for these three nutrients and stresses the importance of assessing the between method differences by using the Bland-Altman analysis. This data strongly implicates that the EPIC FFQ is not an appropriate method for estimating absolute intakes. However, the EPIC FFQ seems adequate to correctly classify consumers into low, medium and high intake groups and a modified version could therefore be used to identify adolescent population groups at risk or to evaluate their dietary change over time.

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Specificity and sensitivity of action levels for waist circumference in a representative sample of Irish adults. By S.N. McCARTHY[1], K.E. HARRINGTON[1], M.J. GIBNEY[1], M. KIELY[2] and A. FLYNN[2], *Irish Universities Nutrition Alliance, [1]Trinity College Dublin, and [2]University College Cork, Ireland*

Waist circumference is a useful tool for identifying the need for weight management (Lean *et al*. 1995) and to predict the risks for cardiovascular disease in the population (Han *et al*. 1995). Two waist circumference cut-off points have been defined as action level 1 (≥94.0 cm men; ≥80.0 cm women) and action level 2 (≥102.0 cm men; ≥88.0 cm women) which give an indication of increasing and high health risks, respectively, from being overweight. Increasing risk (action level 1) was defined as subjects with a BMI ≥25 kg/m^2 or with a high waist:height ratio (WHR) (≥0.95 men; ≥0.80 women) in those with a BMI <25 kg/m^2. High risk (action level 2) was defined as subjects with a BMI ≥30 kg/m^2 or with a high WHR in those with a BMI <30 kg/m^2. The North/South Ireland Food Consumption Survey (NSIFCS) is the first survey in Ireland to have measured waist and hip circumferences in a representative sample of adults. Analysis to date has shown that 24.1% of both men and women are at action level 1 and a further 23.5% at action level 2 (McCarthy *et al*. 2001).

The methods of Lean *et al.* (1995) were applied to these waist circumference data from the NSIFCS, to estimate the sensitivity and specificity of the proposed action levels for waist circumference, in identifying the need for weight management.

			Sensitivity %				Specificity %			
	Action level	WC (cm)	18–64y	18–35y	36–50y	51–64y	18–64y	18–35y	36–50y	51–64y
Men (*n* 491)	1	≥94	98.6	98.3	100	97.4	96.2	98.5	93.5	94
	2	≥102	96.2	100	100	91.3	96.8	97.7	94.6	98.8
Women (*n* 627)	1	≥80	98.6	95.9	99.2	100	96.4	96.5	97.5	93.8
	2	≥88	99.3	97.1	100	100	98.5	99.1	98.4	97.6

Sensitivity = true positives/(true positives + false negatives) Specificity = true negatives/(true negatives + false positives)

Sensitivity was defined as those subjects with a high BMI and subjects with a lower BMI but a high WHR who were identified correctly by the action level. Sensitivity was high, ranging from 91.3% to 100%. The subjects that had a high BMI or a high WHR with low BMI, but had a waist circumference lower than the action level (false negatives) accounted for 1.1% (*n 12*) of the total population. These false negative subjects would therefore be overlooked for weight management advice, which would account for nearly 9% of 51–64 year-old men at action level 2.

Specificity was defined as those subjects with a low BMI and subjects with a low WHR with high BMI who were correctly identified as below action level. The subjects with a low BMI or low WHR with high BMI who had a waist circumference above the action level (false positives) accounted for 3.6% (*n 41*) of the total population. Among these subjects that were misclassified at action level 1, men (2.0%, *n 10*), had BMIs of 20.6–24.97 kg/m^2 and WHRs of 0.91–0.946, while women (1.9%, *n 12*) had BMIs of 19.0–24.9 kg/m^2 and WHRs of 0.76–0.799. The specificity of action level 2 was also high. The misclassified subjects represented 2.4% of men (*n 12*) and 1.1% of women (*n 7*). Of these misclassified subjects, men had BMIs of 27.5–29.9 kg/m^2 and WHRs of 0.91–0.947 while women had BMIs of 26.6–9.9 kg/m^2 and WHRs of 0.71–0.798. Similar results were also found when age groups were examined separately. With the exception of two subjects, the misclassified men and women for each action level had BMI and WHR values close to the cut-off criteria.

Sensitivity and specificity of these waist action levels are high for the Irish population and therefore can be used to assess the need for weight management for benefit to health.

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The use of physical activity data in the identification of low energy reporters in the North/South Ireland Food Consumption Survey. By M.J. MCGOWAN, K.E. HARRINGTON and M.J. GIBNEY, *Irish Universities Nutrition Alliance, Trinity College Dublin, Republic of Ireland.*

The Goldberg cut-offs to identify low energy reporters (LER) in dietary surveys were originally calculated assuming a low physical activity level (PAL) of 1.55 among all respondents. (Goldberg *et al*, 1991). These cut-offs, however, recognised only 50% of all LER as identified by direct comparison of energy intake with energy expenditure (Black, 2000). The sensitivity of the Goldberg cut-offs increased to 74% in men and 67% in women when they were recalculated using FAO/WHO/UNU (1985) PAL values for low, medium and high physical activity groups. This lead to the recommendation to collect physical activity data in large dietary surveys to categorise men and women into low, medium and high physical activity groups for the identification of LER (Black, 2000).

This study describes the identification of the proportion of LER in the North/South Ireland Food Consumption Survey (NSIFCS) using physical activity data. Food intake data were collected using a 7-day food diary and physical activity data were collected by a physical activity questionnaire developed at the Institute of Public Health, University of Cambridge. This questionnaire collected information on activity at home, work and recreation over the previous year. MET scores (multiples of resting metabolic rate) were calculated for each activity. These scores were converted to energy equivalents (MJ/day). A resting metabolic rate was applied to time asleep and to the number of hours in a 24hr day not accounted for by the questionnaire (MJ/day). The resulting estimated total energy expenditure was divided by basal metabolic rate to calculate an estimated PAL value (ePAL) for each individual. Respondents were categorised as low, medium or high activity according to the FAO/WHO/UNU (1985) PAL values for low activity (men 1.55, women 1.56), medium activity (men 1.78, women 1.64) and high activity (men 2.10, women 1.82) and cut-offs to identify LER were calculated from these PAL values (Approach 1 in the table below). In Approach 2 subject-specific cut offs were calculated based on each individual's ePAL. LER were identified as those with an energy intake/estimated basal metabolic rate (EI/BMR$_{est}$) below the relevant cut-offs. The table also includes the proportion of LER using a cut-off of 1.05 based on a PAL value of 1.55 (Approach 3).

Cut-off		Approach 1: activity-specific			Approach 2: subject specific			Approach 3: cut-off = 1.05		
		All	Men	Women	All	Men	Women	All	Men	Women
LER's	*n*	365	141	224	328	135	193	265	98	167
		27%	22%	32%	24%	21%	27%	20%	15%	24%
Non LER's	*n*	983	503	480	1020	509	511	1083	546	537
		73%	88%	68%	74%	79%	73%	80%	85%	76%
Total		1348	644	704	1348	644	704	1348	644	704

Assuming that the 265 LER identified by Approach 3 represents 50% of the 'true' number of LER in the population, the proportion of LER identified by the activity-specific cut-offs (Approach 1) is 69% (72% of men, 67% of women). This result is in agreement with the improved sensitivity of the Goldberg cut-offs by Black (2000) when calculated for different activity categories. The lower number of LER identified by Approach 2 (using subject-specific ePAL values) than Approach 1 may be due to the wide range of ePALs in this sample (mean(SD) = 1.57(0.42)). In conclusion, the use of physical activity data in the NSIFCS to categorise respondents as low, medium and high activity increased the proportion of LER identified.

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Food choices and supplement consumption in adults from a British birth cohort. By M.K. TUMILTY[1], A.A. PAUL[1], D.C. GREENBERG[1], M.E.J. WADSWORTH[2] and C. BOLTON-SMITH[1], [1]*MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL and* [2]*MRC National Survey of Health and Development, University College and Royal Free Medical School, 1–19 Torrington Place, London WC1E 6BT*

A number of micronutrients appear to be protective against degenerative disease, hence optimizing intake is an important concept for lifelong health. Micronutrient intake can be enhanced considerably by dietary supplements, with concomitant improvement in biochemical status (Bates *et al.* 1998). The present study investigates whether healthy food choices predominate in those who consume supplements, and the influence of educational attainment on these practices. In a longitudinal study of ageing processes, the MRC National Survey of Health and Development (1946 Birth Cohort), 5 d prospective diet records were collected from 1630 members (764 men and 866 women) in 1999 at age 53 years. The diet records were coded using in-house nutrient analysis programs (Price *et al.* 1995) incorporating new foods where applicable. Dietary supplement consumption was coded using a newly created database of the 757 supplements encountered.

Over a third (35% *n* 569) of subjects reported taking supplements during the 5 d period. Logistic regression analysis, adjusting for gender, showed that significantly fewer subjects with no educational qualifications (30% of 463) took supplements than those with O levels (40% of 438) or A levels and above (36% of 635), *P* 0.014. Amongst supplement users, 51% took fish oils, 20% evening primrose or starflower oils, 15% vitamin C, 8% vitamin E, 49% multi-vitamin and/or mineral supplements, and 31% herbal or other preparations. Nearly half (*n* 277) of the supplement users took more than one type of supplement.

The percentages of men and women who reported consuming particular foods at least once over the 5 days was compared between those taking supplements and those not.

	Men			Women		
	Supplement takers (*n* 192)	Supplement non-takers (*n* 572)	*P*	Supplement takers (*n* 377)	Supplement non-takers (*n* 489)	*P*
Citrus fruit & juices (%)*	67	53	0.010	73	62	0.002
Apples & apple juice (%)	60	52	ns	67	58	0.023
Tomatoes (%)	86	80	ns	87	84	ns
Leafy green vegetables (%)	55	55	ns	61	61	ns
Brown & wholemeal bread (%)	69	57	0.011	73	68	ns
Olive oil and spread (%)	30	17	0.009	34	22	<0.001
Low fat spreads (%)	17	18	ns	21	24	ns
Skimmed milk (%)	26	17	0.010	35	27	0.017
Fatty fish (%)	37	26	0.011	38	26	<0.001
Meat pies, burgers, etc. (%)	41	42	ns	26	32	0.020
Chocolate confectionery (%)	47	46	ns	53	57	ns
Crisps, cornsnacks, etc. (%)	37	45	0.035	41	45	ns

* % of subjects consuming these foods at least once over the 5 days.
Logistic regression analysis adjusting for educational level in three categories (none, O level, A level & above); ns *P*>0.05.

After adjusting for educational level, for both men and women, citrus fruits and juices, olive oil and spreads, fatty fish and skimmed milk were all consumed by significantly more of the supplement takers than non-takers. On the other hand, there was less distinction between sweet and savoury snack foods and meat products according to supplement use. While consumers of supplements tend to be those with a healthier lifestyle (Kirk *et al.* 1999) the present results show that this is not reflected in all food choices. The net effect on health may depend on whether the micronutrient and *n*-3 and *n*-6 fatty acid intake derived from supplements is sufficiently quantitatively important to influence risk of degenerative disease.

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Assessment of the influence of energy under-reporting on food additive exposure estimates. By M.B. GILSENAN, J. LAMBE, J. KEARNEY and M.J. GIBNEY, *Institute of European Food Studies, Trinity College, Dublin 2, Republic of Ireland*

It is well documented that energy under-reporting is an inherent source of bias in all forms of dietary assessments and across a range of population sub-groups (Livingstone *et al.* 1990; Black *et al.* 1991). Under-reporting may be characterized by an under-estimation of the portion size of foods, an under-estimation of the frequency of consumption of foods and by a failure to report the intake of a food during the survey period (Becker *et al.* 1999; Krebs-Smith *et al.* 2000). At the simplest level, the procedure for estimating exposure to a food chemical (additive) is to multiply food intake by the concentration of the chemical in that food. If foods that contain the chemical of interest are under-reported, then the exposure of the chemical from those foods could potentially be under-estimated. The present study investigated the influence of including or excluding energy under-reporters on food chemical exposure estimates.

Three food additives were selected: annatto (E160b), sulphites (E220–E228), and sunset yellow (E110). Exposure to each food additive was estimated using food intake data from the North/South Ireland Food Consumption Survey (NSIFCS, 2001) and maximum legally permitted concentration levels of the additives in foods. Mean energy intake to basal metabolic rate ratios (EI:BMR) across quartiles of intakes of each additive were compared using a one-way ANOVA. For all three additives, mean EI:BMR ratios increased across quartiles of intake and the differences were significant (*P*<0.0001; see table).

	Mean (SD) EI:BMR ratios across quartiles of additive intake			
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Sunset yellow (E110)	1.24 (0.40)	1.39 (0.35)	1.45 (0.37)	1.55 (0.47)
Sulphites (E220–E228)	1.25 (0.37)	1.31 (0.36)	1.44 (0.41)	1.63 (0.41)
Annatto (E160b)	1.14 (0.36)	1.28 (0.33)	1.49 (0.35)	1.72 (0.38)

Exposure estimates of each food additive were then assessed using acceptable reporters only (defined as EI:BMR ratio ≥1.1) (Goldberg *et al.* 1991) and compared with exposure estimates using the total population (i.e. including under-reporters). The difference (%diff.) of food additive intake using total population intakes compared with intakes among acceptable reporters only, ranged from 75% at lower percentiles to 8% at upper percentiles as illustrated in the table below.

	Comparison of the 5th, 50th and 97.5th percentile additive intakes* of the total population (All) with exposure estimates of acceptable reporters only (EI:BMR ≥1.1)								
	5th%ile			50th%ile			95th%ile		
	All	EI:BMR ≥1.1	%diff.	All	EI:BMR ≥1.1	%diff.	All	EI:BMR ≥1.1	%diff.
Sunset yellow (E110)	0.001	0.004	75	0.069	0.077	10	0.417	0.433	4
Sulphites (E220–E228)	0.002	0.004	50	0.118	0.151	22	0.667	0.723	8
Annatto (E160b)	0.004	0.006	33	0.018	0.021	14	0.046	0.049	6

* (mg/kg body wt/d).

However absolute intakes were not materially different in particular at upper percentiles where exposure estimates of food additives are calculated. These preliminary analyses indicate that under-reporting in dietary surveys did not materially influence exposure estimates for these three food additives. However given that different additives may be present in different foods, this is an area which warrants further investigation.

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A longitudinal study of the relationship between serum 25-OH vitamin D and parathyroid hormone (PTH) concentrations in older women. By C. BOLTON-SMITH[1], G.D. MISHRA[3], P.A. MOLE[2], M.E.T. McMURDO[2] and C.R. PATERSON[2], [1]*Nutrition Research Group, CVEU and* [2]*Department of Medicine, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY and* [3]*MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL*

Poor vitamin D status is a risk factor for bone mineral loss and osteoporosis (DOH, 1998), which is particularly prevalent in elderly women. Uncertainly still exists regarding optimal vitamin D intake (Heaney, 1999), and the proposal that lack of change in PTH in response to vitamin D supplementation, should be the marker of vitamin D adequacy has not led to identification of a serum concentration of 25-OHD which is universally accepted as either adequate or optimal (Lips *et al.* 2001). The relationship between serum 25-OHD and PTH was investigated at three time-points in 209 healthy women (mean (SD) age 68.3 (5.7) years) who participated in a 2-year randomized, double-blind placebo-controlled trial with four parallel groups; calcium (1 g/d) plus vitamin D (10 µg/d) either alone or in combination with phylloquinone (200 µg/d), 200 µg/d phylloquinone or placebo. Outcome measures were bone mineral content and a number of biochemical variables, including serum PTH and 25-OHD. Intact PTH was assayed by chemiluninescence immunometric assay (Immulite, EURO/DPC) and 25-OHD measured by RIA (DiaSorin) (with UK NEQAS and DEQAS external quality assurance schemes, respectively) in serum collected at baseline (T_0), 12 (T_1) and 24 (T_2) months (respectively the winters, October–January, of 1998, 1999 and 2000). Dietary intake was assessed by food frequency questionnaire (New *et al.* 1997) at each time-point. Mean (SD) values are reported in the table.

	Baseline (*n* 209)	Placebo and vitamin K (*n* 110)		Vitamin D and calcium supplemented (*n* 99)	
	T_0	T_1	T_2	T_1	T_2
PTH (pmol/l)	4.9 (1.8)	5.2 (2.2)	4.9 (2.2)	4.5 (1.7)	4.4 (1.7)
Change[a] in PTH	–	0.4 (1.3)	−0.3 (1.3)	−0.4 (1.0)	−0.1 (1.2)
25-OHD (nmol/l)	59.6 (16.3)	46.9 (17.0)	47.3 (14.6)	73.5 (15.6)	72.5 (15.8)
Change[a] in 25-OHD	–	−10.4 (13.5)	0.4 (13.5)	11.4 (17.6)	−1.0 (13.5)
Diet calcium (mg/d)	1056 (248)	1069 (316)	1049 (312)	1042 (270)	1047 (250)

[a] Change from previous visit.

A repeat measure multivariate linear regression model was developed (based on Johnson & Wichern, 1982) in order to investigate the ability of serum 25-OHD to predict change in serum PTH concentrations over time. In this model, T_i log PTH (*i* 1,2) was estimated as a function of T_{i-1} log PTH, T_{i-1} 25-OHD, change in 25-OHD from T_{i-1} to T_i, age at baseline and calcium intake. The analysis was conducted using the Proc Mixed procedure of SAS (Littell *et al.* 1996). Neither age nor calcium intake were significant predictors of change in PTH. Both previous PTH and change in 25-OHD concentrations were strong independent predictors of change in PTH concentration; positive for previous PTH, coefficient × 100 (SE) 94 (3.7) $P<0.0001$, and negative for change in 25-OHD, coefficient × 100 (SE) –0.9 (0.2) $P<0.0001$. When current 25-OHD was exchanged for 'change in 25-OHD' in the model, it was not a significant independent predictor of change in PTH. The importance of change in 25-OHD for predicting the inverse change in PTH is apparently due to the role of previous 25-OHD in determining the response to supplementation with vitamin D_3: low previous 25-OHD concentrations produced a high rise, whilst a minimal increase was observed when previous values were already 'high', 50–75 nmol/l. These data support other work (for example Lips *et al.* 2001), that previous 25-OHD concentration is a major factor in determining the change in 25-OHD in response to supplementation and therefore the change in PTH levels.

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The effect of oestrogen and dietary phytoestrogens on calcium absorption in Caco-2 cells. By A. COTTER, C. JEWELL and K.D. CASHMAN, *Department of Food Science, Food Technology and Nutrition, University College Cork, Ireland*

Since oestrogen deficiency is the dominating pathogenic factor for osteoporosis in women, oestrogen therapy (HRT) remains the mainstay for prevention of bone loss in postmenopausal women. Decreased Ca absorption due to ovarian hormone deficiency is also corrected by HRT. However, fewer than one in four postmenopausal women decide to go on HRT and within 6 months over 60% of them withdraw for fear of an increased risk of malignancy and other side-effects (Taylor, 1997). Recently, phytoestrogens (PEs), which are non-steroidal plant-derived compounds that exhibit oestrogen-like activity, have been shown to prevent bone loss associated with ovarian hormone deficiency (Arjmandi *et al.* 2000). While, these compounds act directly on bone cells, their protective effect on bone may be partly due to their ability to enhance Ca absorption. Ipriflavone, a synthetic PE, which has a beneficial effect on bone, has also been shown to enhance *in vitro* Ca uptake by rat duodenal cells (Arjmandi *et al.* 2000). However, to date, the effect of PEs on Ca absorption in humans has not been investigated. Therefore, the aim of this study was to investigate the effect on Ca absorption of 17 β-oestradiol and two dietary PEs (genistein and daidzein) in human Caco-2 intestinal-like cells, which are a suitable model for predicting Ca absorption in humans.

Caco-2 cells were seeded onto permeable filter supports and allowed to differentiate resulting in a highly organized structure similar to that of the intestinal wall. The Caco-2 cell monolayers (*n* 9–17 per treatment) were then exposed to 10 nM 17 β-oestradiol, 10 nM 1,25 dihydroxyvitamin D_3 (1,25 $(OH)_2D_3$), 100 µM genistein, 100 µM daidzein or DMSO only (control) for 24 h. After exposure, transepithelial transport of ^{45}Ca and fluorescein (a marker of paracellular transport), transcellular Ca transport and transepithelial electrical resistance (TEER; an index of monolayer permeability) were studied by the method of Fleet & Wood (1994). In all studies, at least three wells were examined per treatment and experiments were repeated three times. Rates and TEER values were expressed as a percentage of control values.

		Transport rates (% of control)							
		Total transepithelial ^{45}Ca		Fluorescein		Transcellular Ca		TEER (% of control)	
Treatment	*n*	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1,25 $(OH)_2D_3$	17	117**	3	82	4	164***	27	94	2
17 β-oestradiol	17	91	5	97	8	90	24	99	1
Genistein	9	81**	3	91	4	78*	23	97	2
Daidzein	9	84*	2	89	6	76*	18	98	1
ANOVA (*P*-value)		<0.0001		0.169		<0.001		0.053	

Significantly different from control (DMSO), *$P<0.05$,**$P<0.01$, ***$P<0.001$ (Dunnett's multiple comparisons *post hoc* test).

As expected, 1,25 $(OH)_2D_3$ stimulated Ca absorption in these Caco-2 cells, by up-regulating the active, transcellular pathway. On the other hand, 17 β-oestradiol had no effect on Ca absorption. Unexpectedly, both PEs decreased Ca absorption, by reducing the transcellular transport of Ca across the epithelial monolayer. In conclusion, further studies are needed to investigate the mechanism(s) by which dietary PEs reduce Ca absorption.

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Effect of a calciuric (high sodium-high protein) diet on bone metabolism in apparently healthy postmenopausal women. By M.C. HARRINGTON, T. BENNETT, A. FLYNN and K.D. CASHMAN, *Department of Food Science, Food Technology and Nutrition, University College Cork, Ireland*

There is evidence that high Na intakes and high (animal) protein intakes adversely affect bone metabolism and may be risk factors for osteoporosis (Creedon & Cashman, 2000). Increasing Na intake and protein intake is associated with increased urinary Ca loss (calciuria). Furthermore, there is evidence that the calciuric effects of high Na and high protein intakes may be additive (Chan & Swaminathan, 1994). However, to date, there has been no study of the effect of high dietary Na and protein intake on bone metabolism in humans. Therefore, the objective of this study was to determine the effect of a high-Na, high-protein diet on metabolism of calcium and bone in postmenopausal women.

The study consisted of a randomized crossover trial of the effect of high versus low dietary Na plus animal protein intake over 4 weeks on the metabolism of calcium and bone in postmenopausal women. Eleven apparently healthy free-living postmenopausal (>5 years) women were assigned randomly to a diet high in animal protein (85 g/d) and Na (4 g/d) or a diet adequate in animal protein (60 g/d) and low in Na (1.5 g/d) for 4 weeks followed by crossover to the alternative dietary regimen for a further 4 weeks. These regimes were based on a basal diet (1.5 g Na/d, 60 g protein/d), to which appropriate quantities of animal protein and Na were added (using a modified bread) to achieve a calciuric diet (4 g Na/d, 85 g protein/d) while Ca intake will be maintained at usual intakes. Urinary Na, K, Ca, and pyridinoline (Pyr) and deoxypyridinoline (Dpyr) (markers of bone resorption), and serum osteocalcin and bone-specific alkaline phosphatase (B-Alkphase) (markers of bone formation) were measured at the end of each dietary period. Data was analysed by the appropriate analysis for a 2 × 2 crossover trial.

Diet….	Low-Na, normal protein		High-Na, high-protein		
	Mean	SE	Mean	SE	*P* value*
Urine†:					
Ca (mmol/24 h)	3.3	0.2	4.1	0.3	<0.01
Na (mmol/24 h)	69.8	7.6	138.3	10.4	<0.0001
K (mmol/24)	71.4	4.5	78.4	6.5	0.671
Pyr (nmol/24 h)	346	14	351	14	0.358
Dpyr (nmol/24 h)	73	5	73	4	0.920
Serum:					
Osteocalcin (μg/l)	25.3	3.7	23.3	3.2	0.246
B-Alkphase (U/l)	25.3	1.9	26.7	2.4	0.149

*Direct treatment effect (i.e. high-Na, high-protein) was analysed for each biochemical index by two-sample *t* tests of within-group differences. †Represents the mean content of the analyte in two consecutive 24 h urine collections.

Increasing the Na and protein content of the diet increased urinary Na and Ca excretion, but had no effect on urinary K excretion. The calciuric diet had no effect on biochemical markers of bone resorption and bone formation. Therefore, the high-Na, high-protein induced calciuria did not adversely affect bone metabolism in these postmenopausal women over 4 weeks.

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The effect of conjugated linoleic acid (CLA) on bone formation in young growing rats. By O. KELLY, C. JEWELL and K.D. CASHMAN, *Department of Food Science, Food Technology and Nutrition, University College Cork, Ireland*

Conjugated linoleic acid (CLA) has been shown to increase bone ash in experimental animals (Park *et al*. 1997), suggesting that CLA may enhance bone mineralization and protect against bone loss. However, CLA has recently been shown to reduce the rate of mineral apposition and bone formation in young growing rats, possibly as a consequence of a reduced synthesis of prostaglandin E_2 (PGE_2), a local mediator of bone metabolism (Li *et al*. 1999). Therefore, the aim of this study was to further investigate the effect of CLA on mediators of bone formation, the rate of bone formation and on bone composition in young growing rats.

In a 2 × 2 factorial design study, forty male weanling Wistar rats were fed a control diet containing 70 g/kg of added fat (soybean oil (SBO) or menhaden oil + safflower oil (MSO)) with 0 or 10 g/kg of CLA for 8 weeks. After 8 weeks on the respective diets, serums were obtained and the animals killed. Femurs and tibias were excised and analysed for various parameters.

	Dietary groups				Pooled	ANOVA (*P*-value)		
	SBO		MSO					
	CLA–	CLA+	CLA–	CLA+	SE	PUFA	CLA	PUFA × CLA
Femur:								
Ash weight (g)	0.24	0.24	0.25	0.25	0.01	0.134	0.414	0.835
Density (g/cm^3)	1.43	1.40	1.43	1.39	0.02	0.620	0.084	0.872
Length (mm)	28.13	28.23	28.21	27.99	0.02	0.593	0.727	0.456
Ca (mg/g dry weight)	274	283	294	281	9	0.391	0.848	0.118
Mg (mg/g dry weight)	4.23	4.39	4.20	4.29	0.15	0.652	0.413	0.792
Serum osteocalcin (ng/ml)	42.6	45.4	41.2	44.3	0.3	0.529	0.128	0.416
Serum IGF-I (ng/ml)	31.0	30.7	32.1	32.5	0.2	0.389	0.415	0.476

Data for *ex vivo* PGE_2 biosynthesis revealed that rats consuming SBO had a higher production (*P* 0.01) of this prostanoid (4482 pg/mg protein) in bone (tibia) organ culture compared with those given MSO (1858 pg/mg protein). Addition of CLA to either the SBO or MSO diets significantly lowered (*P* 0.04) *ex vivo* PGE_2 production (2595 and 1485 pg/mg protein, respectively). Serum insulin-like growth factor-I (IGF-I) (a modulator of bone formation), serum osteocalcin (a biochemical marker of bone formation) and femoral bone ash, density, length, Ca and Mg and body weights were unaffected by PUFA type and CLA. While Li *et al*. (1999) also reported that serum osteocalcin and bone mineral content in young growing rats were unaffected by PUFA type or CLA, *ex vivo* PGE_2 production, mineral apposition rate and bone formation rate (as assessed by bone histomorphometry) were reduced by CLA supplementation. Therefore, in our present study, we are currently examining the effect of CLA on gene expression of local factors (e.g. IGF-1, IL-1, IL-6 and TNF) controlling bone metabolism.

In conclusion, further work is needed to elucidate the molecular and cellular mechanisms of action of CLA on bone metabolism.

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The effect of oestrogen and selected phytoestrogens on osteoblast-like cell viability. By S. CUSACK, C. JEWELL and K.D. CASHMAN, *Department of Food Science, Food Technology and Nutrition, University College Cork, Ireland*

Recently, phytoestrogens (PEs), which are non-steroidal plant-derived compounds that exhibit oestrogen-like activity, have been shown to prevent bone loss associated with ovarian hormone deficiency (Anderson & Garner, 1997). The mechanism of action of these compounds on bone is unclear. Endogenous oestrogens and PEs may influence bone cells by binding to oestrogen receptors, which in turn can lead to altered molecular and cellular processes. PEs are believed to inhibit osteoclastic bone resorption, by receptor and/or non-receptor mediated mechanisms and thereby preserve bone. The effect of PEs on bone-forming osteoblasts is less clear. It has been suggested that a possible mechanism of action of PEs on osteoblasts is as an oestrogen agonist at low doses, but as an antagonist at high doses (Anderson & Garner, 1997). Therefore, the aim of this study was, firstly, to characterize two osteoblast-like cell lines (human osteosarcoma MG63 and SaOS2 cells) at a molecular level and, secondly, to test the influence of varying concentration of oestrogen and various PEs, within a physiological range, on the viability of these cells.

MG63 and SaOS2 cells were grown in Minimum Essential Medium Eagle (supplemented with 1% non-essential amino acids) and McCoy's Medium (supplemented with 2mM L-glutamine), respectively. Both media were supplemented with 10% fetal calf serum and cells maintained at 37° in a humidified 5% CO_2 environment. Both cell lines were characterized at the molecular level for expression of mRNA for oestrogen receptors (ER; α and β alkaline phosphatase, osteocalcin, osteopontin, osteonectin, collagen IA1 (markers of osteoblast lineage), using established and a novel polymerase chain reaction protocol. At confluency, cells were exposed to five PEs: genistein, daidzein, coumesterol, apigenin and ipriflavone, in the range 5–100 μM, for 24 h. 17β oestradiol (1–10 nM) was used as a control. All oestrogen-like compounds were dissolved in 100% dimethyl sulphoxide. After exposure, protein concentrations were determined in the cells using the bicinchoninic acid (BCA) assay and cell viability was assessed by the succinate dehydrogenase (MTT) and lactate dehydrogenase (LDH) cellular assays. The BCA and LDH assays were performed in triplicate, the MTT assay in duplicate.

Both cell lines exhibited mRNA for all osteoblast phenotypic markers assayed in this study. While SaOS2 cells had mRNA for the ER α and β MG63 cells only exhibited mRNA for the ER α and not the ER β. Cellular protein levels in both cell lines was unaffected by varying the concentration of 17β oestradiol or any of five PEs used in this study. Cell viability, as measured by the MTT assay, was unaffected by varying the concentration of oestrogen-like compounds in the MG63 cells. There was a reduction in cell viability (85% that of control by MTT assay) in SaOS2 cells exposed to 50 μM apigenin, genistein or ipriflavone, indicating a greater sensitivity of SaOS2 cells to these compounds. The LDH assay was found to be unsuitable for use as a viability assay in either cell line as both display very high basal levels of LDH enzyme activity.

In conclusion, varying the concentration of selected dietary PEs, in the physiological range, had little or no effect on cell viability in osteoblast-like cells in culture. Therefore, these cell models can be used in further studies of the molecular and cellular processes by which these PEs may influence bone.

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Effects of fish oil components on background and oxidant-induced sister chromatid exchange in Chinese hamster V79 cells. By L.C. O'SULLIVAN, J.A. WOODS, S.A. AHERNE and N.M. O'BRIEN, *Nutritional Sciences, Department of Food Science, Food Technology and Nutrition, University College Cork, Ireland*

Epidemiological studies suggest that diets rich in *n*-3 polyunsaturated fatty acids (PUFA) reduce the incidence of certain cancers such as breast and colon. Dietary intake of fish-oil derived *n*-3 PUFA such as eicosapentaenoic acid (20:5*n*-3; EPA) and docosahexaenoic acid (22:6*n*-3; DHA) has been shown to exhibit anti-carcinogenic effects *in vivo* and *in vitro*. The aim of the present study was to investigate whether EPA and DHA are capable of protecting against oxidant-induced DNA damage in Chinese hamster V79 fibroblast cells. In addition, squalene, a lipid molecule found in shark liver oil, was also investigated.

Chinese hamster V79 cells were cultured in Dulbecco's modified Eagle's medium supplemented with fetal bovine serum in a humidified atmosphere (CO_2: air, 5:95; v/v) at 37°. For experiments, cells were supplemented with EPA (50 μmol/l), DHA (50 μmol/l) or squalene (50 μmol/l) for 24 h. EPA and DHA were complexed to bovine serum albumin (BSA) whereas squalene was dissolved in DMSO. DNA damage was assessed using the sister chromatid exchange (SCE) assay. This assay is a sensitive indicator of genotoxicity and involves the exchange of DNA between two homologous sister chromatids. Following supplementation with EPA, DHA or squalene, test media were removed and the genotoxin hydrogen peroxide (H_2O_2; 100 μM) was added to the cells for 1 h at 37°. Again, the SCE assay was performed to assess DNA damage. SCE scores were expressed as the average number of SCE per chromosome in twenty-five well spread metaphases.

	SCE/chromosome	
	Mean	SD
Control BSA	0.24[b]	0.02
EPA	0.26[b]	0.07
DHA	0.25[b]	0.03
H_2O_2	0.39[a]	0.04
EPA + H_2O_2	0.39[a]	0.06
DHA + H_2O_2	0.37[a]	0.05
Control DMSO	0.18[b]	0.01
Squalene	0.18[b]	0.01
H_2O_2	0.39[a]	0.01
Squalene + H_2O_2	0.24[b]	0.05

[a] Significantly different from respective control ($P<0.05$). [b] Significantly different from cells treated with H_2O_2 only ($P<0.05$) ANOVA, Dunnett's test. $n\geq2$ individual experiments.

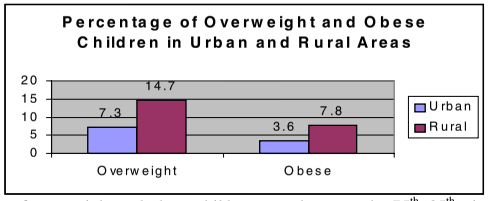
No difference was observed in the frequency of SCE (background) between controls and treated cells ($P<0.05$). V79 cells exposed to H_2O_2 alone showed a significant increase in SCE ($P<0.05$) when compared with control levels. Pre-incubation with either EPA or DHA did not significantly affect H_2O_2-induced SCE, however, pre-treatment with squalene significantly decreased the frequency of SCE induced by H_2O_2 ($P<0.05$). These results indicate that squalene was more effective than *n*-3 PUFA, EPA and DHA in protecting against H_2O_2-induced SCE.

Obesity and overweight in a cohort of Irish children aged 6-9 years: incidence and associated dietary factors. By C McGOVERN[1,2], G QUINLAN[1,2] and E. BALL[1], [1]*School of Biological Sciences, Dublin Institute of Technology, Kevin St., Dublin 8, Republic of Ireland and* [2]*Trinity College, Dublin 2, Republic of Ireland.*

An increase in the prevalence of paediatric overweight and obesity has been documented in both developed and developing countries, however there is no such data regarding the situation in Ireland. Childhood obesity is an area of concern as it can lead to a number of medical and psychosocial problems (Dietz, 1998). There are several factors involved in the aetiology of obesity, but it appears that environmental factors may play the greatest role as gene pools have not changed substantially over the past decades (Gortmaker *et al*. 1990).

Anthropometric measurements including weight, height, mid-upper arm circumference and triceps skinfold thickness, were taken on 239 schoolchildren (both urban and rural) aged 6-9 years. From these, relative weight-for-height, BMI and mid-arm muscle circumference (MAMC) were calculated. An overview of their dietary intake was assessed using a short food frequency questionnaire, completed by their parents.

The prevalence of overweight and obesity were 17.9% and 12.6% when calculated from relative weight-for-height based on Irish centile charts (Hoey *et al*. 1987), and fell to 11.3% and 5.9% respectively when calculated from UK BMI charts (Cole *et al*. 1995). This difference was found to be highly significant (P<0.000). There was no significant difference between levels of overweight/obesity and gender or age but a significant (P<0.05) association was identified between weight status and geographical area with the highest levels of overweight/obesity in rural children.



The majority of overweight and obese children were between the 75th–95th triceps percentile and between the 25th–75th MAMC percentile, indicating that excess weight was a result of increased adipose tissue. The highest levels of obesity were found in 6 year olds. The probability of an obese child becoming an obese adult increases with the duration and magnitude of obesity (Gortmaker *et al*. 1990). Therefore the likelihood of tracking appears quite high for this group.

Eighty per cent of breast-fed children were of normal weight and 64% of obese children were bottle fed as infants. Differences were found between the mean weights of overweight/obese children who were breast- and bottle-fed, with those bottle-fed weighing on average 5kg more. A high consumption of energy-dense foods was reported for the population as a whole. No statistical differences were found between the food consumption patterns of urban/rural children or boys/girls. Normal weight children had a higher daily consumption of fruit (71% *v*. 59%) and vegetables (41% *v*. 29%) than their overweight/obese counterparts who had a greater frequency of consumption for the majority of other foods i.e. breads, dairy products, crisps, cakes and minerals.

The levels of overweight and obesity found are comparable to that of other countries. International trends are difficult to compare as a wide variety of definitions of child obesity are in use. However, a new definition, based on pooled international data for BMI and linked to the adult obesity cut off point of 30kg/m^2, has been recommended (Cole *et al*. 2000). The protective role of breast-feeding is evident with lower mean weights in those breast-fed rather than bottle-fed. Poor dietary habits were reported for all children, suggesting the development of unhealthy food preferences. Lower intakes of fruit and vegetables by the overweight and obese children may be associated with a higher dietary fat intake. These children also consume a greater amount of food daily.

It appears there are a number of dietary factors which influence the development of obesity. The development of specific paediatric BMI centile charts and larger cohort studies among schoolchildren may further establish the true prevalence of paediatric overweight and obesity in Ireland. From this, the effects of any preventative measures taken can be accurately assessed.

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Incidence of obesity and overweight in a cohort of Irish children and associated physical activity factors. By G QUINLAN[1,2], C McGOVERN[1,2] and E BALL[1], [1]*School of Biological Sciences, Dublin Institute of Technology, Kevin St., Dublin 8, Republic of Ireland and* [2]*Trinity College, Dublin 2, Republic of Ireland*

The incidence of childhood overweight and obesity is increasing worldwide (WHO, 1998). Childhood obesity is associated with many adverse health risks including impaired pulmonary function, metabolic and orthopaedic disturbances as well as considerable psychosocial effects. Estimates of the prevalence of overweight and obesity among Irish children are needed in order to assess the need and urgency of public health strategy implementation. In order to design effective strategies the aetiology of childhood obesity must be thoroughly investigated. The apparent decrease in energy intake in countries where the incidence of obesity is increasing (Prentice & Jebb, 1995) would suggest that levels of physical activity play a key role in the aetiology of childhood obesity.

In addition to the anthropometric and dietary assessment methodology described by Mc Govern *et al*. (2001), a questionnaire relating to lifestyle habits of both parents and children, was completed by the parents of 239 schoolchildren aged 6–9 years. Specific objectives of this study were to investigate child physical activity levels (PAL), child participation in sedentary activities and parental weight status and PAL.

The prevalence of overweight and obesity were 11.3% and 5.9% respectively when calculated from UK BMI charts (Cole *et al*. 1995). A statistically significant association (P<0.05) was observed between weight status and location. There were 70% of overweight and 71.4% of obese children living outside Dublin, although almost half (46%) of the sample were from Dublin. A significant association between PAL and weight status was not observed. Thirty four percent of mothers and 63% of fathers reported a BMI >25, although underestimating of weights was suspected.

Activity levels were low, 16.7% of children did not participate in vigorous or moderate activity and girls were significantly less active than boys (P<0.05). Children living in Dublin were significantly more active than children in rural areas. Parental activity level was significantly associated with child activity level (P<0.01), the association being more significant for fathers' activity (P=0.001) than for mothers' activity (P=0.002). For example, 67.5% of inactive fathers had an inactive child compared with only 36.1% of active fathers. Also, 47.7% of active fathers had an inactive child, compared with 25% of inactive fathers. The majority of children (50%) travelled by car to school with 41% walking, 9% going on the bus and 0.4% cycling. Almost two thirds (64%) of the children live less than 1 mile from school.

Participation in sedentary activities (television and video games) was high. Boys and girls spent approximately equal amounts of time watching television. The association between time spent watching television and weight status was not statistically significant but a trend was observed. Twenty four percent of overweight children, including obese, spent more than 3 hours watching television on weekdays compared with 15.3% of normal weight children. Over an average weekend, 93% of obese, and 81% of overweight children watched more than three hours of television, compared with 77% of normal weight children. During the week 49.4% of children played video games and 57.6% played them at the weekend, 83% of players were male. Time spent playing video games during the week was associated with weight status (P<0.05). 50% of obese children, who played video games, played them for more than 2 hours/d, compared with 11.1% of normal weight and 7.7% of overweight (but not obese) children. However, this trend was not seen for game-play at the weekend. No association was observed between PAL and time spent playing video games.

The incidence of overweight and obesity among children sampled is a cause for concern. Further investigations are needed to more accurately explore the influence of physical activity on weight status. Parents may enhance children's activity levels by becoming more active themselves and providing a good example. Preventive intervention is clearly important as obesity tracks into adulthood and is associated with substantial morbidity and mortality. (Whitaker *et al*. 1997)

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Evaluation of the avoidance of dairy products among women with breast cancer. By J.A. MORRISSEY[1,2], N. O'GORMAN[3] and E.P. McNAMARA[1], [1]*School of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Republic of Ireland,* [2]*Trinity College Dublin, Republic of Ireland and* [3]*Department of Clinical Nutrition and Dietetics, Beaumont Hospital, PO Box 1297, Beaumont Road, Dublin 9, Republic of Ireland*

Breast cancer is the most common form of non-skin cancer to affect women in the developed world (Bingham, 2000). Countless alternative programmes and remedies have been promoted for the treatment of cancer. As attention has turned away from fat consumption as a risk factor for breast cancer, the possible role of dairy products in the aetiology of carcinogenesis has been examined (Knecht *et al*. 1996). Anecdotally, avoidance of dairy foods to prevent and cure breast cancer has gained popularity, especially since the recent successful publication of a book which strongly advocates this theory (Plant, 2000). The aim of this study was to determine the prevalence of self-imposed avoidance of dairy products among breast-cancer patients and to assess the adequacy of the present calcium and vitamin D intakes of these women. Thirty-six women with breast cancer were recruited from those attending the Oncology Day Ward of a Dublin hospital for chemotherapy or for follow-up care. All subjects completed an interview-assisted questionnaire, which included a food frequency questionnaire for calcium and vitamin D. Weight and height measurements were recorded and BMI was calculated for each subject.

Variable	Result
Following a dairy-free diet	31% (*n* 11) were following or had followed a dairy-free diet, 55% (*n* 6) of which excluded all dairy produce from the diet
Socio-demographic variables	Following the diet was independent of all socio-demographic variables
Time between diagnosis and commencement of a dairy-free diet	55% (*n* 6) began the diet more than 6 months after diagnosis with breast cancer
Reasons for following a dairy-free diet	73% (*n* 8) wished to supplement their medical treatment with "something they could do for themselves"
Source of information	36% (*n* 4) television/ radio 27% (*n* 3) relatives/ friends 27% (*n* 3) book by J. Plant (2000) 9% (*n* 1) advised by G.P.
Effect of a dairy-free diet	46% (*n* 5) felt better 46% (*n* 5) felt no different 8% (*n* 1) felt worse
Recommendation of a dairy-free diet	82% (*n* 9) would recommend the diet to others with breast cancer

Subjects following a dairy-free diet were significantly less likely to achieve the Irish recommended daily allowance (RDA) for calcium (800 mg/d) from food sources alone ($P<0.001$). Those on such a diet were also significantly less likely to meet the Irish RDA for vitamin D (0–10 µg/day; $P<0.001$).

A substantial number of women with breast cancer are excluding dairy products in an attempt to prevent a recurrence of their cancer. Exclusion of dairy foods from the diet is likely to negatively affect a patient's protein and energy status, particularly if the patient is undergoing chemotherapy. Calcium balance will also be affected, which may increase the risk of developing osteoporosis in later life. Use of alternative therapies, including special diets, by breast cancer patients is associated with a poorer quality of life and increased psychosocial distress (Burstein *et al*. 1999). Patients who insist on following a dairy-free diet should be regularly monitored by a dietitian.

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Toxic effects of oxysterols in two cell culture models. By A. O'SULLIVAN, J.A. WOODS, S.A. AHERNE and N.M. O'BRIEN, *Nutritional Sciences, Department of Food Science, Food Technology and Nutrition, University College Cork, Ireland*

Cholesterol can be oxidized to a variety of compounds called oxysterols or cholesterol oxidation products. These oxides form in the human body by endogenous oxidation and may also be derived from food, especially processed cholesterol-rich foods. Oxysterols have several biological effects including atherogenic and cytotoxic actions. The aim of the present study was to compare the cytotoxic effects of four oxysterols, namely 7β-hydroxycholesterol (7β-OHC), 25-hydroxycholesterol (25-OHC), cholesterol-5α,6α-epoxide (α-Epox) and cholesterol-5β,6β-epoxide (β-Epox) in two human cell lines.

Human colonic adenocarcinoma Caco-2 and Human hepatoma HepG2 cells were supplemented with increasing concentrations of 7β-OHC, 25-OHC, α-Epox, β-Epox (0–25 µg/ml) for 24, 48 or 96 h. Following 24 and 48 h exposure, test media were replaced with normal growth media and cells were allowed to recover for 72 and 48 h respectively. The 96 h exposure represents a constant challenge to the cells. Cytotoxicity was assessed using the neutral red uptake assay and from these results, the concentration of compound that inhibited cell viability by 50% (IC_{50}) was calculated for each oxysterol.

	IC_{50} Values (µg/ml)											
	Caco-2						HepG2					
	24 h		48 h		96 h		24 h		48 h		96 h	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
25-OHC	3.3	0.4	1.9	0.7	0.4	0.1	15.8	3.5	4.8	1.6	2.8	1.1
7β-OHC	10.5	1.5	5.4	1.2	3.6	0.5	23.0	1.4	6.4	1.6	3.9	1.0
β-Epox	15.8	2.4	10.2	2.3	4.4	1.1	>25	–	5.5	1.2	3.4	1.1
α-Epox	>25	–	>25	–	6.5	2.7	>25	–	13.7	2.5	5.9	0.7

$n \geq 3$ independent experiments.

In Caco-2 and HepG2 cells, oxysterol-induced toxicity was greater after 96 h exposure. 25-OHC was the most toxic oxysterol at all time points in both cell lines. 7β-OHC was more toxic in Caco-2 cells than HepG2 cells whereas β-Epox was more toxic in HepG2 than in Caco-2 cells. α-Epox was the least toxic of all four oxysterols investigated. In Caco-2 cells, the same trend in toxicity was observed at each time point which was: 25-OHC > 7β-OHC > β-Epox > α-Epox whereas in HepG2 cells the order of decreasing toxicity observed was 25-OHC, followed by β-Epox > 7β-OHC > α-Epox. Hence, under these conditions, our findings show that oxysterol toxicity was both concentration- and time-dependent.

Retention of a carotenoid/tocopherol mixture in human intestinal Caco-2 cells following use of Tween 40 as a delivery vehicle. By S.M. O'SULLIVAN, J.A. WOODS, S.A. AHERNE and N.M. O'BRIEN, *Nutritional Sciences, Department of Food Science, Food Technology and Nutrition, University College Cork, Republic of Ireland*

Delivery of hydrophobic carotenoids to cells can be problematic due to solubility factors and toxicity of solvent carriers. The emulsifier Tween 40 has been shown to be an effective method of delivering carotenoid mixtures to human intestinal cells (Woods & O'Brien, 2001). Tween 40 was also shown not to be toxic to these cells. The objective of the present study was to observe the retention of a carotenoid/tocopherol mixture in intestinal Caco-2 cells following use of Tween 40 as the delivery vehicle.

Human colonic adenocarcinoma Caco-2 cells were seeded at a density of 4×10^4 cells/cm^2 and grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal calf serum for 7 days. After this time the Caco-2 cells resembled colonocytes of the large bowel and had not yet differentiated into enterocyte-like cells. To monitor differentiation, dome formation and brush-border enzyme activities were measured over a 3-week period. Differentiated Caco-2 enterocytes were incubated for 24 h with a mixture of six carotenoids and two tocopherols prepared in Tween 40. The mixture contained the carotenoids β-carotene, α-carotene, canthaxanthin, astaxanthin, lutein, lycopene and α- and γ-tocopherol, each at a concentration of 5 µmol\l. Following the 24 h incubation period, test media were removed, cells were washed thoroughly and replaced with normal growth media. Cells were analysed for compound retention at 2, 6, 12 and 24 h after the test mixture was removed. Cellular carotenoid and tocopherol content was analysed by reverse phase HPLC.

	Time (h)							
	2 h		6 h		12 h		24 h	
Concentration (ng/mg protein)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
β-Carotene	1.72	1.06	1.63	1.18	1.71	1.02	1.49	1.10
α-Carotene	1.58	0.72	1.41	0.93	1.38	0.78	1.27	0.71
Canthaxanthin	10.88	0.18	7.08	4.49	7.02	3.60	5.91	3.43
Astaxanthin	12.81	3.84	10.40	5.20	9.90	3.37	7.88	3.54
Lutein	19.71	0.88	20.40	3.53	21.60	6.06	16.40	3.16
Lycopene	0.35	0.05	0.26	0.09	0.25	0.07	0.20	0.09
α-Tocopherol	8.40	2.28	7.12	3.13	7.68	2.67	6.31	2.05
γ-Tocopherol	13.15	4.63	10.91	6.20	10.40	4.78	8.33	4.00

n 2 independent experiments.

The cells efficiently took up the more polar compounds whereas the hydrophobic carotenoids, β-carotene, α-carotene and lycopene were present in the lowest amounts. All compounds were retained within the cells for up to 24 h following removal of the test mixture. In conclusion, Tween 40 is a useful, simple, rapid and non-toxic method of delivering complex mixtures of carotenoids and tocopherols to human enterocytes for the study of biological interactions of these compounds in the gut.

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Are phytosterols toxic to human intestinal Caco-2 cells? By D.M. FAHY, S.A. AHERNE and N.M. O'BRIEN, *Nutritional Sciences, Department of Food Science, Food Technology and Nutrition, University College Cork, Republic of Ireland*

Phytosterols are structurally similar to cholesterol and are found in a variety of plant and plant products including vegetable oils, seeds, nuts and some fruits and vegetables (Moghadasian, 1999). They have been reported to exhibit a range of biological effects including plasma total and low-density lipoprotein (LDL) cholesterol reduction. However, little research has been carried out on these compounds *in vitro*. The aim of the present study was to investigate the effects of four phytosterols namely β-sitosterol, stigmastanol, stigmasterol and campesterol on the viability of human intestinal cells.

Human adenocarcinoma Caco-2 cells were cultured in Dulbecco's modified Eagle's medium supplemented with fetal calf serum and maintained in a humidified atmosphere at 37º. Cells were supplemented for 24 h with concentrations of either 1.6µmol/l, 6.25 µmol/l or 12.5 µmol/l of each phytosterol. Following treatment, cytotoxicity was assessed by neutral red uptake assay (a measure of % cell viability) and lactate dehydrogenase release assay.

		% Cell viability*		% LDH release†	
	Conc. (µM)	Mean	SE	Mean	SE
β-Sitosterol	0.00	100.0	14.6	3.9	0.5
	1.60	121.4	10.9	4.8	1.1
	6.25	90.6	10.3	4.1	1.0
	12.50	64.9	5.3	4.1	0.9
Stigmastanol	0.00	100.0	9.4	5.6	0.3
	1.60	137.2	9.7	6.1	1.5
	6.25	104.6	8.6	5.6	1.3
	12.50	88.9	8.6	2.8	0.9
Stigmasterol	0.00	100.0	6.6	3.2	0.4
	1.60	119.7	7.1	3.1	0.3
	6.25	101.8	89.2	3.9	0.6
	12.50	91.5	13.7	3.9	1.2
Campesterol	0.00	100.0	13.3	3.7	1.1
	1.60	126.5	12.0	3.9	0.8
	6.25	94.4	5.3	5.1	1.5
	12.50	71.5	7.3	4.3	1.2

Statistical analysis included ANOVA and Dunnett's test, **n* 4 independent experiments, †*n* 3 independent experiments.

From these results the phytosterols had no significant effects on Caco-2 cell viability. The LDH release assay results indicated that there were no significant differences between phytosterol-treated cells and control values. In conclusion, over the concentration range tested, β-sitosterol, campesterol, stigmasterol and stigmasterol were not toxic to the human Caco-2 cell line.

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The sensory characteristics of, and consumer preference for, organic and non-organic chicken. By J.B. LAWLOR, E.M. SHEEHAN, J.P. KERRY, C.M. DELAHUNTY and P.A. MORRISSEY, *Department of Food Science, Food Technology and Nutrition, University College Cork, Ireland*

In recent years there has been a shift towards organically produced foods; this is due to a greater awareness of public health and may in part be due to concerns over animal welfare (Pearl, 1999). Today consumers are more conscious of the way in which diet is linked to a healthy lifestyle. Health is one of the major concerns among consumers in the western world and this explains why organic foods are such a strong growth sector in both Europe and the USA (Cooke, 1999). Given the choice, many people would choose organic. However, is there a taste difference between organically produced food and non-organically produced food? To answer this question the aim of our work was to investigate the sensory character of, and consumer preference for, organic and non-organic chicken. A panel of twelve trained assessors described the character of the samples using a final vocabulary of five odour, four appearance, five texture and twelve flavour terms. In parallel, 100 naïve consumers rated their preference on a nine-point hedonic scale for a reduced set of the eight following samples.

		Chicken fillets							
Cluster	*n*	Organic	Organic	Corn-fed	Free-range	Free-range	Commercial	Commercial	Commercial
1	31	**5.35**	6.00	6.55	*7.03*	6.71	6.00	6.61	*7.03*
2	16	6.19	6.63	4.75	3.50	5.44	5.63	**2.56**	*6.75*
3	20	6.00	6.75	4.95	**3.30**	6.55	5.40	*7.00*	5.10
4	17	6.06	5.12	5.35	*6.94*	6.29	5.06	**2.82**	5.00
5	16	4.00	3.75	5.31	3.63	4.69	**3.31**	*6.31*	5.94
All	100	5.52	5.65	5.38	**4.88**	5.94	5.08	5.06	*5.96*

n = number of consumers in each cluster. 'Winner' chicken fillets within each cluster are in italics and underlined, 'loser' chicken fillets are in bold.

One-way ANOVA of the sensory data found that twenty-three attributes significantly ($P<0.05$) discriminated between the chicken samples. A principal component analysis identified the main sensory characteristics of each of the chicken samples while hierarchical cluster analysis segmented the consumer population into five clusters, or groups, each homogenous in their preference. Partial least squares regression was used to relate sensory character to preference to identify the attributes that determined consumer preference. It was concluded that the sensory attributes of a chicken fillet from a commercial source including a 'cream' colour and 'strength' of flavour were the most preferred overall, while the 'processed' odour, 'firmness' of initial bite and 'oral breakdown' texture of the free-range sample were the most disliked overall. However, hierarchical cluster analysis showed that this free-range sample was the most preferred sample of clusters 1 and 4, representing 48% of the sampled consumer population, thus illustrating the heterogeneous nature of consumer preference responses.

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A comparison of the apoptotic potency of four oxysterols in the U937 cell line. By Y.C. O'CALLAGHAN, J.A. WOODS and N.M. O'BRIEN, *Nutritional Sciences, Department of Food Science and Technology, University College Cork, Republic of Ireland*

The cytotoxicity of oxysterols *in vitro* has been reported to be dependent on the structure of the oxysterol as well as the cell line involved. 7β-Hydroxycholesterol (7β-OH), oxidized at the C7 position, has been shown to be more cytotoxic to macrophage cell lines than 25-hydroxycholesterol (25-OH), which is oxidized on the side chain. 25-OH was found to be more cytotoxic in murine thymocyte cell lines. A number of studies have determined the mode of oxysterol-induced cell death to be apoptosis; however, the mechanism involved in oxysterol-induced apoptosis has not been elucidated. Glutathione, a cellular antioxidant, has been shown to be depleted in the early stages of apoptosis and it has been suggested that glutathione depletion may be the event which instigates the apoptotic process. The aim of the present study was to determine the order of apoptotic potency of the oxysterols 7β-OH, 25-OH, cholesterol 5α,6α-epoxide (α-epoxide) and cholesterol 5β,6β-epoxide (β-epoxide) in the U937 cell line and also to establish whether glutathione becomes depleted during oxysterol-induced apoptosis.

U937 cells were adjusted to a density of 1×10^5 cells/ml in RPMI 1640 medium supplemented with 25 ml/l fetal calf serum. Cells were treated with 30 μM oxysterol and incubated at 37°, air:CO_2 (95:5). Control cells were treated with an equal volume of ethanol. The concentration of glutathione was determined at 12 h. Apoptosis was assessed by staining with Hoechst 33342 and by the agarose gel electrophoresis assay at 24 and 48 h. The fluorescein diacetate–ethidium bromide assay was used to measure cellular viability.

	Control		25-OH		7β-OH		α-epoxide		β-epoxide	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Glutathione, 12 h (nmol GSH/mg Protein)	43.2	2.1	45.9	4.8	10.9**	2.4	39.4	1.4	38.5	3.8
Viable cells, 24 h (Percentage)	95.3	1.9	87.8	4.6	56.4**	7.0	94.7	2.4	92.2	1.0
Viable cells, 48 h (Percentage)	97.0	1.5	66.1*	2.8	17.3**	12.0	90.7	2.6	49.8**	3.3
Apoptotic cells, 24 h (Percentage)	4.3	0.8	8.3	2.0	11.8**	1.2	5.9	1.3	10.2	2.4
Apoptotic cells, 48 h (Percentage)	5.2	0.6	12.8	0.5	40.1**	3.6	11.8	0.1	33.0**	2.7

Mean values were significantly different from control cells: *$P<0.05$, **$P<0.01$ ($n\geq3$, ANOVA, Dunnett's test).

The oxysterols 25-OH and α-epoxide did not induce a significant level of apoptosis in this cell line. 7β-OH was found to be more cytotoxic than β-epoxide and was also a more potent inducer of apoptosis. At the 12 h timepoint 7β-OH caused a significant depletion of glutathione. Both β-epoxide and 7β-OH were shown to induce apoptosis at 48 h as they produced a ladder-like pattern on an agarose gel and apoptosis was confirmed and quantified by Hoechst staining. However, β-epoxide did not cause a depletion of glutathione. In conclusion, the oxysterols 7β-OH and β-epoxide were found to induce apotosis in the U937 cell line with 7β-OH more toxic than β-epoxide. The early depletion of glutathione may not be a necessary step in oxysterol-induced apoptosis.

No effect of zinc supplementation followed by copper supplementation on immune function and putative indices of copper status in healthy adult men. By M. BONHAM[1], J.M. O'CONNOR[1], P. WALSH[1], S.J. COULTER[1], H.D. ALEXANDER[2], C.S. DOWNES[1], B.M. HANNIGAN[1] and J.J. STRAIN[1], [1]*Northern Ireland Centre for Diet and Health (NICHE), University of Ulster, Coleraine, BT52 1SA and* [2]*Department of Haematology, Belfast City Hospital, BT9 7AD*

As a result of evidence documenting harmful effects of pharmacological doses of zinc supplementation on immune function and copper status, thirty-eight men were recruited onto a zinc supplementation trial. The aim of the study was to examine the effects of zinc supplementation, including dietary intakes, at the 'no observed adverse effect' level (NOAEL) of 40 mg/d on immune function and copper status. Subjects (*n* 19) took 30 mg zinc/d for 14 weeks followed by 3 mg copper/d for 8 weeks. The same number of subjects took placebo only for the duration of the trial. Blood samples were taken at baseline, and weeks 2, 14, 16, 18 and 22 and assessed for full blood pictures, flow cytometric analyses of lymphocyte subsets and putative indices of copper status (caeruloplasmin total protein and oxidase activity). Dietary intakes were approximately 10 mg zinc/d as assessed by 4 d dietary records.

Analysis of results by independent *t* test indicated no effect of zinc (or copper) supplementation on circulating absolute levels of peripheral blood leucocytes or on lymphocyte subsets. Putative indices of copper status were also unaltered. Independent of supplement, there appeared to be seasonal variations in selected leucocyte populations and lymphocyte subsets in the group as a whole (*n* 38) as analysed by repeated measures ANOVA and Bonferroni's correction for multiple *t* tests. Significant alterations in absolute levels of leucocytes, B cells (CD19), memory T cells (CD45RO) and expression of the interleukin-2 receptor (CD25) and the adhesion molecule ICAM-1 (CD54) were observed. Absolute levels of basophils, eosinophils, monocytes, neutrophils, CD3+, $CD3^-$(CD16+56)+, CD3+CD4+, CD3+CD8+, CD3+HLA-DR, CD3+CD45RA+, CD3+CD11a+ were unaffected.

Cell type/ surface marker (cells 10^9/ml)	Baseline (Oct/Nov) Mean SEM	Wk 2 (Nov) Mean SEM	Wk 14 (Feb) Mean SEM	Wk 16 (Feb/Mar) Mean SEM	Wk 18 (Mar/Apr) Mean SEM	Wk 22 (Apr/May) Mean SEM	*P*
Leucocytes	5.82 0.12 (*n* 37)	6.09 0.23 (*n* 37)	5.50 0.18 (*n* 37)	5.48 0.14 (*n* 37)	5.53 0.15 (*n* 37)	5.63 0.17 (*n* 37)	0.049*
CD19	0.20^{ac} 0.01 (*n* 37)	0.21^{a} 0.01 (*n* 37)	0.17^{b} 0.01 (*n* 38)	0.19^{ab} 0.01 (*n* 38)	0.18^{ab} 0.01 (*n* 38)	0.17^{b} 0.01 (*n* 37)	0.000
CD3+CD45RO+	0.79^{bc} 0.04 (*n* 35)	0.79^{c} 0.04 (*n* 37)	0.7^{ab} 0.03 (*n* 38)	0.71^{ab} 0.03 (*n* 37)	0.67^{a} 0.03 (*n* 38)	0.71^{ac} 0.03 (*n* 38)	0.002
CD3+CD25+	0.38^{ab} 0.02 (*n* 35)	0.37^{ab} 0.02 (*n* 37)	0.31^{a} 0.02 (*n* 38)	0.34^{ab} 0.04 (*n* 36)	0.33^{ab} 0.02 (*n* 38)	0.38^{b} 0.02 (*n* 38)	0.019
CD3+CD54+	0.50^{b} 0.04 (*n* 28)	0.41^{ab} 0.03 (*n* 37)	0.36^{a} 0.03 (*n* 37)	0.40^{ab} 0.04 (*n* 36)	0.42^{ab} 0.04 (*n* 36)	0.49^{b} 0.04 (*n* 38)	0.004

*Significance lost after correction for multiple *t* tests. [abc]Values with different superscript letters in the same row differ significantly from each other.

Findings indicate no adverse effects of zinc supplementation on immune function or copper status and support the established UL (upper limit) of zinc tolerance of 40 mg/d. The seasonal variations observed in peripheral leucocytes and lymphocyte subsets in the group as a whole could have implications for seasonal variability in the incidence of infectious diseases.

This work was supported by funding from the Food Standards Agency (AN0553). Thanks to Causeway Labs, Coleraine, for full blood profile analyses, Neil Dennison for his technical assistance, Professor Adrian Dunne for his statistical advice and Thomson & Joseph, Norwich, for supplying supplements.

Zinc supplementation has no effect on putative markers of copper status and lipoprotein metabolism. By L. McANENA, M.P. BONHAM, J.M. O'CONNOR, S.J. COULTER, P. WALSH, C.S. DOWNES, B.M. HANNIGAN and J.J. STRAIN, *Northern Ireland Centre for Diet and Health, University of Ulster, Cromore Road, Coleraine BT52 1SA*

Zinc supplementation has been associated with disturbances in copper status and HDL cholesterol (Black *et al.* 1988; Boukaiba *et al.* 1993). Serum copper has been reported to be depressed by just 20 mg zinc/d – the US daily tolerable upper intake level or UL is 40 mg/d while in the UK the recommended nutrient intake is 9 mg/d for adult males. The current double-blind intervention trial aimed to examine the interactions of zinc with copper status and lipoprotein metabolism by supplementing nineteen healthy men with 30 mg zinc/d for 14 weeks, followed by 3 mg copper/d for 8 weeks to counteract any potential adverse effects of zinc supplementation. Control subjects took placebo throughout. Reported mean dietary intake of zinc was 9.4mg/d. Blood samples were taken at weeks 0, 14 and 22 for assessment of putative markers of copper status (caeruloplasmin (CP) concentration and activity) and lipid profiles (HDL and LDL cholesterol and triacylglycerols).

	Week 0		Week 14		Week 22		*P*
	Placebo	30 mg Zn/d	Placebo	30 mg Zn/d	Placebo	30 mg Zn/d	value
	Mean SEM	Mean SEM	Mean SEM	Mean SEM	Mean SEM	Mean SEM	
CP Protein (g/l)	0.22 0.01 (*n* 19)	0.23 0.01 (*n* 19)	0.21 0.01 (*n* 19)	0.21 0.01 (*n* 19)	0.20 0.01 (*n* 19)	0.22 0.01 (*n* 19)	0.991
CP Oxidase activity (U/l)	543.1 17.7 (*n* 19)	554.6 19.8 (*n* 19)	561.2 17.9 (*n* 19)	554.02 20.0 (*n* 19)	594.4 23.2 (*n* 19)	563.2 22.5 (*n* 19)	0.625
Cholesterol (mmol/l)	5.23 0.30 (*n* 19)	5.12 0.28 (*n* 19)	5.15 0.32 (*n* 19)	5.12 0.32 (*n* 19)	5.05 0.28 (*n* 18)	5.13 0.32 (*n* 19)	0.873
HDL (mmol/l)	1.50 0.10 (*n* 19)	1.46 0.08 (*n* 19)	1.49 0.09 (*n* 19)	1.46 0.08 (*n* 19)	1.47 0.09 (*n* 18)	1.44 0.06 (*n* 19)	0.800
LDL (mmol/l)	3.14 0.25 (*n* 19)	3.03 0.22 (*n* 19)	2.82 0.21 (*n* 19)	3.02 0.26 (*n* 19)	2.93 0.25 (*n* 18)	3.11 0.27 (*n* 19)	0.882
Triacylglycerols (mmol/l)	1.16 0.11 (*n* 19)	1.39 0.17 (*n* 19)	1.51 0.25 (*n* 19)	1.41 0.17 (*n* 19)	1.44 0.15 (*n* 18)	1.29 0.16 (*n* 19)	0.843

Zinc supplementation, up to a total intake of 40mg/d, was found to have no significant adverse effect on any of the putative markers of copper status or lipoprotein metabolism, over a 14-week period. Copper supplementation also had no effect over a further 8-week period. These findings support recent guidelines that a UL of 40mg zinc/d should pose no risk of adverse health effects for most individuals.

This work was funded by the Foods Standards Agency (AN0553). We are also indebted to the Causeway Health and Social Services Laboratories, Coleraine, for their assistance.

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Selenium intakes in 18–64 year old Irish adults. By J.M. MURPHY, E.M. HANNON, M. KIELY, A. FLYNN and K.D. CASHMAN, *Department of Food Science, Food Technology and Nutrition, University College Cork, Ireland*

The trace mineral Se is a key component of a number of functional selenoproteins required for human health. The activity of the selenoproteins depends on an adequate Se supply from the diet. Suboptimal Se status may increase susceptibility to various chronic disorders, including cardiovascular disease and certain types of cancer (Rayman, 2000). There are currently no data on Se intakes or status of the Irish population. Therefore, the objective of the present study was to measure the intake of Se and the contribution of different food groups to Se intake in adults aged 18–64 years in Ireland. The adequacy of Se intake in the population is also assessed.

Food consumption was estimated using a 7 d food diary for a representative sample (*n* 1379, men 662; women 717) of 18–64 year-old Irish adults (The North/South Ireland Food Consumption Survey) selected randomly from the electoral register. Se intake was estimated using tables of food composition that were updated with recent food Se data (including data on the Se content of Irish foods; Murphy & Cashman, 2001).

	Men				Women			
	18–35 y (*n* 253)	36–50 y (*n* 236)	51–64 y (*n* 173)	All ages (*n* 662)	18–35 y (*n* 269)	36–50 y (*n* 286)	51–64 y (*n* 162)	All ages (*n* 717)
Se intake (μg)								
Mean	56.1[a]	63.4[b]	59.8[ab]	59.7	40.8[a]	46.9[b]	45.1[ab]	44.2
SD	21.6	24.8	27.0	24.4	17.0	20.8	17.8	18.9
Median	53.0	59.5	54.0	55.0	38.0	42.0	42.5	41.0
Percentiles								
5th	28.0	32.7	34.0	31.0	20.0	26.4	22.3	22.0
95th	95.2	110.0	107.6	104.0	71.0	81.0	74.7	76.1

Differences in mean Se intakes between men and women were significant ($P<0.001$) for all age groupings.
Significant differences ($P<0.01$) between age groupings in each sex group separately are denoted by different superscript letters.

Mean daily intake of Se (from all sources (i.e. food sources plus supplements)) for the group of Irish adults aged 18–64 years was 51.6 μg (SD 23.1). Most of the daily total Se intake (i.e. ~97%) by this group came from food sources. Mean daily intake of Se was significantly higher ($P<0.001$) in men of all ages than in women of all ages. In both men and women, mean daily Se intake was significantly lower ($P<0.01$) in the 18–35 year-old age category than in the 36–50 year-old age category. Meat and meat products (30%), bread and rolls (24%), fish and fish products (~11%), and milks and yoghurt (9%) were the main contributors to mean daily Se intake. Adequacy of Se intake in population groups was assessed using the average requirement (AR) as a cut-off value. Intakes of Se were below the AR in 45.6% of women and 17.1% of men.

In conclusion, a significant prevalence of Se intakes below the AR was observed in Irish adults. Therefore, it would seem prudent that the Se status of the Irish population be investigated at this time.

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Selenium status of a group of Irish adults: evidence of insufficiency. By J.M. MURPHY and K.D. CASHMAN, *Department of Food Science, Food Technology and Nutrition, University College Cork, Ireland*

The trace mineral Se is a key component of a number of functional selenoproteins, including the selenoenzyme glutathione peroxidase (*EC* 1.11.1.9; GSHPx), that are required for human health (Rayman, 2000). The activity of these selenoproteins depends on an adequate Se supply from the diet. Suboptimal Se status may increase susceptibility to various chronic disorders, including cardiovascular disease and certain types of cancer (Rayman, 2000). A recent study by our group has found that a relatively high proportion of Irish adults, aged 18–64 years, in particular women (~46%), have an inadequate intake of Se. Therefore, we felt it necessary to follow up these important dietary findings by examining a biomarker, or index of Se status, namely serum Se, in apparently healthy Irish adults, aged 18–67 years.

Ninety-one healthy Irish adults, aged 18–67 years, were recruited from the Cork City area. A fasting blood sample was collected from each subject and was assayed for serum Se, an index of Se status. Serums were analysed for Se by a hydride generation atomic absorption spectrophotometry method as described by Tiran *et al*. (1993).

In the present study, Se analysis of a commercially available lyophilized reference serum material (79 (5) μg/l; mean (SD)) were in good agreement with the target values (80 (70–92) μg/l; mean (range)). In the present study of apparently healthy Irish adults, aged 18–67 years, the mean serum Se concentration was 76 μg/l. There was no significant difference (P 0.63) in mean serum Se values between males (73.2 (19.4) μg/l; mean (SD), *n* 25) and females (77.2 (21.6) μg/l; mean (SD), *n* 66). Similarly, there was no significant difference (P 0.13) in mean serum Se values between postmenopausal (82.6 (20.6) μg/l; mean (SD), *n* 25) and pre-menopausal (73.9 (22.2) μg/l; mean (SD), *n* 41) women. The total group of healthy Irish adults was divided into three age-categories (i.e. 18–35 years, 36–50 years and 51–67 years) as differences in dietary Se intake have recently been reported between these age-groups in healthy Irish adults. However, in the present study, there were no significant differences (P 0.32) in mean serum Se values between the three age-categories (73.9 (20.0) μg/l, 75.2 (23.2) μg/l, and 81.4 (22.2) μg/l (mean (SD)), for 18–35 years, 36–50 years and 51–67 years old adults, respectively). An estimate of the daily dietary intake of Se can be achieved by dividing the mean Se concentrations obtained from the serum samples by a mean correlation factor, as described by Navarro *et al*. (1995). The estimated mean daily Se intake of the group of apparently healthy Irish adults, aged 18–67 years, was calculated to be 50.3 μg.

The relatively low serum Se values observed in the present study are indicative of recent low intakes of Se by this group. The estimate of daily Se intake for this group (~50 μg) are below the UK reference nutrient intake of 75 μg/d for men and 60 μg/d for women which are necessary to maximize the activity of GSHPx in plasma, an indicator of Se repletion. The plateau of serum GSHPx activity occurs at a serum Se concentration around 95 μg/l. Therefore, the current serum Se values of this group of Irish adults would not appear to be sufficient to achieve this optimal activity. Therefore, the health significance of low Se intake and marginal Se status needs to be further investigated in Irish populations.

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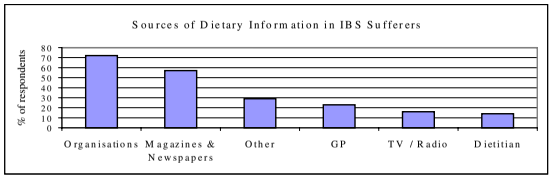
Sources of dietary information in a select group of irritable bowel syndrome sufferers. By H. KILLALA, B. MCKEVITH and C. SHORTT, *Yakult UK, 12–16 Telford Way, Acton, London, W3 7XS*

Increasingly consumers are aware that their health and quality of life can be influenced significantly by diet and thus seek dietary information avidly (Foods Standards Agency, 2001). To date, while several studies have assessed the sources and reliability of dietary information that consumers use (de Almeida *et al.* 1997; Buttriss, 1997; Margetts *et al*. 1997) few studies have evaluated the sources of information used by groups of consumers with specific dietary requirements. IBS affects the dietary choices and habits of sufferers to a considerable degree and consequently they seek nutritional advice and information eagerly. We have evaluated the impact of IBS on quality of life indices and the sources of dietary information used by a selected group of IBS sufferers.

A postal questionnaire that assessed the effect of IBS on quality of life and dietary factors was sent to 1000 members of the UK-based IBS Network in January 2001. Of the 715 questionnaires returned (response rate 72%), a total of 678 responses from individuals diagnosed with IBS were included in the final analysis.

Results suggest that IBS has a major impact on the daily life of the respondents. Over 66% reported each of the following symptoms, abdominal bloating, abdominal pain, sensation of incomplete emptying, excessive gas and diarrhoea. Some 96% reported that their quality of sleep was affected and 36% reported negative effects on vitality. Furthermore, IBS impacted on both social and work-related activities with 71% and 50% respectively indicating that these were affected. IBS appeared to have a considerable effect on dietary habits, half of the respondents indicated that they were vigilant about the amount, type and timing of the food they eat.

The respondents sought dietary information from a wide variety of sources including health professionals, TV/radio, books, alternative practitioners/clinics and the Internet. However, in contrast to general consumers, the two main sources of information that appear to influence this group are organizations and the print media. Previous studies suggest that health professionals and the broadcasting media are key sources of dietary information among consumers in the UK (de Almeida *et al*. 1997; Food Standards Agency, 2001). However, these did not appear to be major sources of dietary information in this group of individuals with IBS.



It is clear from this study that IBS has a profound effect on the quality of life with particular effects on dietary habits. The key roles that organizations and the print media play in providing dietary information to IBS sufferers should be taken into account in dietary information or health education programmes targeted at this group of individuals.

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Nurses' opinions regarding the tube-feeding of patients with advanced dementia. By A. RYAN[1,2] and E.P. MCNAMARA[2], [1]*Department of Clinical Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8, Republic of Ireland and* [2]*Department of Biological Sciences, Kevin Street, Dublin 8, Republic of Ireland*

Health professionals have conflicting views about whether or not tube-feeding ought to be initiated in patients with advanced dementia. The use of tube-feeding in these patients is controversial (Finucane *et al.* 1999). Few studies have been conducted to determine opinions that nurses might have. A questionnaire was designed to examine nurses' involvement in the decision-making process to initiate tube-feeding in these patients, to examine nurses' expectations, their willingness to initiate tube-feeding in certain scenarios, and their experiences dealing with family members of a patient with end-stage dementia. The questionnaire was piloted and distributed to 143 nurses working in five long-stay hospitals in the Dublin area. A 70% response rate was achieved (*n* 100)

Fifty-seven percent of the nurses surveyed were never involved in the decision-making process to initiate tube-feeding. The majority of nurses (58%) believed that tube-feeding could prevent aspiration pneumonia, pressure ulcers and the consequences of malnutrition and that it could improve patient survival and reduce the risk of infection. The majority (60%) disagreed that tube-feeding could improve functional status or (42%) level of comfort in a dementia patient. Almost half of nurses believe tube-feeding to have no effect on a dementia patient's quality of life. Family opinions on tube-feeding significantly affected nurses' attitudes to initiate tube-feeding in a dementia patient. Nurses placed great emphasis on the ethical principle of beneficence (*people should not have to suffer unnecessarily and so care should be provided in the patient's best interest*).

Over half (59%) the nurses surveyed reported to have experienced opposition by family members to the use of a tube-feed in a relative suffering from advanced dementia. Eighty-four per cent of nurses showed support for the greater use of advance directives to solve dilemmas that may arise when deciding whether or not to initiate tube-feeding. Nurses were asked what they regarded as the most worrying and most comforting aspect of tube-feeding for families of patients.

Most worrying aspect of tube-feeding	%	*n*
Pain and discomfort	62	37
Use of restraints to avoid extubation	18	11
Risk of aspiration pneumonia	13	8
Risk of infection	3	2
Families have no concerns about tube-feeding	3	2
Most comforting aspect of tube-feeding		
Adequate provision of food and water	77	46
Families find no comfort in the initiation of tube-feeding	13	8
Patient's life will be prolonged	8	5
Improved quality of life	2	1
Burden on nurses/carers to provide assistance with oral feeding lessened	0	0

In conclusion, most nurses were generally opposed to tube-feeding a patient with advanced dementia. Further study might question nurses with regards to their own wishes about being tube-fed if they were in a state of advanced dementia, and also include nurses' opinions on the withdrawal of tube-feeding in a dementia patient.

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Model of best practice for reduced fat food product development By B. KNOX, *Northern Ireland Centre for Diet and Health (NICHE), University of Ulster, Coleraine, BT52 1SA*

It has become increasingly important to model the food product development process given the high rate of failure of food products. Approximately 75% of new food products fail following launch (Buisson, 1995) and the success of any food product is related to efficient utilization of the product development process (Kristensen *et al.* 1998). Models for product outcomes have been developed (for example, Cooper & Kleinschmidt, 1986), but have almost exclusively examined the development of manufactured goods rather than food products. The main aim of this research has therefore been to determine factors predictive of the success or failure of new reduced-fat products and to make recommendations for best practice. A qualitative approach was adopted to obtain maximum participant compliance. Key company personnel were required to describe their experiences and provide insight into problems encountered in the innovation, development, launch and marketing of reduced-fat foods. Product developers (*n* 47) from food companies based in England and Northern Ireland (*n* 27) were consulted both individually and in small groups. Participants comprised a range of expertise including process and technical managers, chefs, marketing and sales personnel representing the meat, dairy, ready meals, snack foods and ingredients sectors. Interviews were recorded, transcribed and content analysed thematically. A database was created from the case history data, which was then reduced to binary code. The data were screened for selection and then modelled through a series of logistic regression analyses using SSPS for Windows. A total of 127 reduced-fat and standard product case histories were modelled against product outcome (success/failure) as defined by the company concerned. The results implied that successful reduced-fat products tended to be those inspired by food technologists (P 0.0077) and those for which the manufacturer rather than the retailer developed the recipe (P 0.0356). On the other hand, products inspired by fashion trends and 'me too' products (P 0.0562), products inspired by sources other than suppliers or retailers (P 0.0361), technical difficulties encountered in developing the product texture (P 0.0291) and lack of communication with the retailer (P 0.0061) were associated with product failure. The predictive strength of the model was 74–77%. The model implies that actions taken during the concept phase of the product development process and the nature of expertise employed during the process are particularly important for success. For 'best practice' in reduced-fat product development it is recommended that appropriate expertise is enlisted at the concept stage and that a food technologist is employed to ensure that the texture of the product is acceptable. Whilst it is important to liaise with retailers throughout the process, manufacturers should formulate their own product recipes and avoid following fashions and 'me too' product development.

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Health and diet from a teenager's point of view. By J.J. SITTLINGTON and B. KNOX, *Northern Ireland Centre for Diet and Health, University of Ulster, Coleraine, N. Ireland, BT52 1SA*

Very little is actually known about the health perspectives of children and teenagers. This paper explores diet and health perceptions of children aged 11–14 years. The data has been derived from a longitudinal qualitative study analysed according to the principles of grounded theory. Focused interviews took place over a period of 3 years (*n* 250) in eighteen different youth clubs based in economically deprived areas throughout Northern Ireland. The same children were interviewed on a yearly basis to determine how their perspectives changed over time. Issues explored during interview included: definitions of health, definition of a healthy diet, health behaviour, and perceptions of own health.

Spontaneous dialogue covered themes considered important to health by the teenagers, including low-fat food consumption, physical activity, the importance of personal hygiene, mental health, health and wealth, clean environment and -abstinence from smoking, drinking alcohol and the use of drugs. Although to not smoke, drink alcohol or use drugs was the most popular strategy, much emphasis was placed upon diet. For some respondents talking about 'real life' experiences appeared to make it easier to reflect on health concepts *"My uncle died a few weeks ago 'cos he was smoking too much. He died of cancer. He wasn't eating proper, he wasn't eating the right fruit"*. Much of the responsibility for the teenagers eating habits were placed upon the parents, in particular the mother who tends to do the majority of the *cooking "I eat whatever my mammy gives me, but I try to eat about one banana a day"*. This demonstrates the importance of educating the parents on healthy eating so as they can provide practical and verbal advice. As one participant states, *"Yeah (my parents advise me), I go by their advice"*. During the first 2 years of interviews, much of the children's knowledge and perspectives remained unchanged; however, as the teenagers matured into the third year (age 13–14), other themes began to emerge, including important influencing factors and sources of information that had an increasing impact on their health perspectives. This included health promotion and nutritional knowledge obtained from school and in particular from home economics classes. Knowledge of healthy eating appeared good, with most children indicating the need to avoid high fat foods and sweets, *"Sweets, fatty foods and dairy products…(avoid these)…to keep healthy"*. The consumption of fruit and vegetables and drinking water was also considered important. A small number of children spoke of the breakdown between fat, proteins and carbohydrates and children who had an interest in competitive sports spoke knowledgeably of the importance of a high carbohydrate diet and it's effect on their athletic performance. The mother's dietary habits also appeared to influence the young people's perceptions increasingly as they became older.

The qualitative approach adopted allowed for issues to be researched within the context of 'real life' experiences and provides explanations as to why children perceive health in a certain way and make the food choices that they do. The data obtained will be useful in the development of health promotion strategies targeted at young adolescents.

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A comparison of pulmonary function, smoking, alcohol intake and physical activity in first and fourth year female university students. By T. FOLEY[1,2], C. MCARTHUR[1, 2] and M. MOLONEY[1], [1]*Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Ireland and* [2]*Trinity Centre for Health Sciences, St. James Hospital, Dublin 8, Ireland*

Pulmonary function declines naturally with ageing but this decline should not occur until middle age (35–55 years; American Thoracic Society, 1991). Lifestyle habits, however, such as smoking behaviour, alcohol consumption and habitual physical activity are all related to lung function. Smoking plays a major role in accelerating decline in pulmonary function (Burchfiel *et al.* 1995). Heavy alcohol consumption has also been suggested as being a contributor to this decline (O'Heinemann, 1977). Habitual physical activity may protect against the 'normal' age-dependent deterioration in pulmonary function (Hagberg *et al.* 1998). Although university students are typically healthier than other sectors of society, it has also been shown that they are in transition from an active youth to a more sedentary adult lifestyle (Steptoe *et al.* 1997; Leslie *et al.* 1999).

In this study, anthropometric measurements (i.e. weight, height, BMI, WHR, MUAC) and pulmonary function tests (FVC, FEV_1, FEV_1/FVC%) were performed on Year 1 (*n* 30) and Year 4 (*n* 30) female Health faculty-based students. A non-assisted written questionnaire comprising information on respiratory symptoms (e.g. wheezing, asthma), smoking, alcohol consumption and physical activity level (PAL; using a questionnaire, European Commission (1998)) in relation to its intensity and duration was completed by all subjects.

A significant difference (*P* 0.008) was found in mean FEV_1/FVC% between Year 1 and Year 4 students. Smoking was prevalent in 10% of Year 1 *v.* 40% in Year 4 students. All subjects in both groups consumed alcohol. 'Sensible' levels (<15 units per week for females) of alcohol consumption were exceeded by 34% of Year 1 *v.* 44% of Year 4 students. Although a trend was found towards students reducing their PAL both on entering college and as they progressed through college, no significant difference was found in mean PAL between year groups. Significant differences between the prevalence of wheezing during the previous 12 months (10% Year 1 *v.* 33.3% Year 4, *P* 0.028) and wheezing during exercise (6.7% Year 1 *v.* 33.3% Year 4, *P* 0.01) was found. Multiple linear regression analysis showed that the higher percentage of asthmatics in Year 4 (26.7% *v.* 10% in Year 1) was a significant predictor of the lower FEV_1/FVC% (*P* 0.033) and the higher percentages of wheezing in the previous 12 months (*p* 0.000) and wheezing with exercise (*P* 0.000). The higher percentage of smok ers (Year 4) was also shown to be a significant predictor of wheezing in the previous 12 months (*P* 0.017) and wheezing during exercise (*p* 0.02). Neither alcohol, anthropometric measurements nor PAL were significantly correlated to lung function in either group.

These results suggest that smoking and asthma are key predictors of pulmonary function decline in this small group of female university students.

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Effect of soya-milk formulas on faecal microbiology and metabolism. By L. HOEY[1], I.R. ROWLAND[1], P.M. HEAVEY[1] and J.S. BROWN[2], [1]*Northern Ireland Centre for Diet and Health (NICHE), University of Ulster at Coleraine, Cromore Road, Coleraine, BT52 1SA and* [2]*Mountsandel Surgery, Mountsandel Road, Coleraine, BT52 1JB*

Diet is known to alter faecal microflora composition and also gut bacterial activity. These changes have implications for the health of the host. Studies have reported the differences between breast-fed and formula-fed infants, but no data is available on the effect of soya-milk formulas on faecal microbiology and metabolism in the developing infant. This study compares faecal microflora composition in infants fed on soya-milk formulas (SMF) and cow's-milk formulas (CMF) and metabolic activities and pH in infant and adult faecal samples.

Subjects were forty four healthy infants (twenty seven males, seventeen females) aged 4–10 months. All infants were weaned. In addition to solid foods nine infants received a SMF (six consumed SMA Wysoy, two received C&G Infasoy and one Farley's soya-milk). Ten infants received a CMF in addition to solid food (six consumed SMA formulas and four consumed C&G formulas) and twenty five infants were fed breast-milk (BM). Results are compared with those obtained from five adult omnivores aged 24–53 years. Faecal samples were collected and transported to the lab within 1 hour of being passed. The samples were homogenised in 0.9% saline to form a 20% suspension and pH determined. Ammonia concentration and enzyme activities were measured in duplicate using a spectrophotometer. Fluorescent *in situ* hybridisation (FISH) was used to identify and enumerate the bacterial groups in the faecal samples.

Table 1. Bacterial composition of infant faecal samples (log_{10} bacteria/g faeces)

	SMF (n 8)		CMF (n 10)	
	Mean	SD	Mean	SD
Total Count	10.58	0.23	10.90*	0.19
Bifidobacteria	9.49	0.66	10.53*	0.24
Bacteroides	8.53	1.49	9.72	0.59
Lactobacillus + enterococcus	7.31	1.08	7.82	1.04
Clostridia	8.04	1.22	8.84	0.44

Significantly different to SMF fed infants *$P<0.01$ (t test)

Table 2. Metabolic activities and pH in infant and adult faecal samples.

	BM (n 25) Mean (SD)	SMF (n 9) Mean (SD)	CMF (n 10) Mean (SD)	ADULTS (n 5) Mean (SD)
Ammonia Concentration (μmol/g)	7.11 (3.69)	25.74 (13.67)**	24.75 (12.72)**	26.18 (8.27)**
β-glucuronidase activity (μmol/h/g)	11.33 (9.30)†	16.04 (5.36)†	14.00 (9.79)†	43.54 (21.16)
β-glycosidase activity (μmol/h/g)	20.56 (14.64)†	22.30 (12.50)†	23.22 (12.84)†	71.46 (15.37)
pH	6.36 (0.68)	7.08 (0.49)*	6.69 (0.65)	7.39 (0.30)*

Significantly different to BM fed infants, * $P<0.01$; ** $P<0.001$ (ANOVA).
Significantly different to adults, † $P<0.001$.

The infants fed SMF had significantly lower total bacterial counts in faecal samples than the infants on CMF ($P<0.01$). The difference was reflected in all four of the bacterial groups enumerated, with the difference being highly significant ($P<0.01$) in the case of bifidobacteria. Faecal pH was significantly higher in SMF fed infants and in adults than in breast-fed infants ($P<0.01$). Faecal ammonia concentration was similar in CMF, SMF and adults, all were significantly greater than that in breast-fed infants ($P<0.001$). There were no significant differences in β-glucuronidase or β-glycosidase activity between any of the infant groups and all were lower than the mean values for adults ($P<0.001$). The results suggest that the type of infant formula consumed can have marked effects on the microbial composition of the infant gut microflora, although effects on microbial metabolism appear to be limited.

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