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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

A Scientific Meeting was held at the University College, Cork, on Wednesday–Friday, 22–24 June 1994, when the following papers were presented.

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Long-term follow-up of coeliac disease in children. By M. ARDIFF[1], M. HURLEY[2] and S. SUGRUE[1], [1]*Dublin Institute of Technology, Kevin Street, Dublin 8* and [2]*Our Lady's Hospital for Sick Children, Crumlin, Dublin 12, Republic of Ireland*

Life-long strict compliance with a gluten-free diet is considered essential in the management of coeliac disease (Holmes *et al.* 1989). For those diagnosed at an early age, education will have been directed at parents and carers. As children with coeliac disease grow up and develop increasing responsibility for their dietary intake and food choices, education appropriate for age and development is important to ensure compliance.

A group of patients with coeliac disease (*n* 125) was identified, who had been diagnosed 5 to 15 years previously, from biopsy reports (*n* 40) and hospital records (*n* 85) from Our Lady's Hospital for Sick Children, Crumlin. Assessment of their current medical follow-up, compliance and understanding of the gluten-free diet was carried out by means of a postal questionnaire. This resulted in sixty-nine analysable responses. The responses represented patients with the age range 5.1-22.8 years (mean 13.69, SD 4.63). Mean age at diagnosis was 2.95 (SD 2.84) years of these patients. 66%(44) claimed strict maintenance of their diet, 21%(14) reported regularly breaking the diet and 13%(9) responded that they kept poorly to the diet.

The most common reasons cited for non-compliance of the diet included difficulty in maintaining the diet, especially during social occasions, 16%(10) with gluten-containing confectionery and cakes being consumed, 10%(6) found the diet too expensive. Other reasons given included ingestion of communion hosts, alcohol, social occasions and boredom with the diet.

When asked to choose gluten-containing foods from a list of eight everyday foods, 11%(7) correctly chose the three gluten-containing foods; 42%(26) chose two correctly, but 36%(23) could not identify any of the gluten-containing foods correctly.

Of those responding to the question, 61%(33) have attended a follow-up clinic in the last 3 years; 13%(7) have not attended follow-up. Despite medical follow-up, 47%(29) have only seen a dietitian once and 13%(8) have never seen a dietitian before. As coeliac disease is treated with diet, one would expect the dietitian to play a major role in treatment, but this is apparently not the case in practise. These figures are of concern as dietary compliance is considered necessary to avoid long-term complications such as intestinal carcinoma (Holmes *et al.* 1989).

Various factors were taken into account when interpreting the results of this study and were shown not to confound the results in any way. These factors included: membership of the Coeliac Society of Ireland, whether a parent or child responded and the attitude of the subject to coeliac disease in general. A more comprehensive assessment of the understanding of coeliac disease and compliance with gluten-free diet is necessary in order to develop appropriate and effective education strategies for these patients, who require follow-up education as they grow up.

Holmes, G.K.T., Prior, P., Lane, M.R., Pope, D. & Allan, R.N. (1989). *Gut* **30**, 333-338.

Dietary compliance in adults with coeliac disease. By N.M. CODD, M.GOGGINS and N.P. KENNEDY, *Unit of Nutrition and Dietetic Studies, Department of Clinical Medicine, Trinity Centre, St James's Hospital, Dublin 8, Republic of Ireland.*

Coeliac disease results from intolerance to dietary gluten, a state which is thought to be permanent. Treatment of the condition requires the dietary exclusion of gluten for life. Several studies report poor compliance with dietary management (Valletta & Mastella, 1990; Ljungman & Myrdal, 1993), evidenced by insufficient clinical response, lack of morphological improvement in jejunal biopsy or persistence of serum antibodies against α-gliadin or endomysial antigens.

The objectives of the present study of coeliac patients were to assess patients' understanding of the prescribed diet and of the importance of adhering to it. In addition, factors contributing to poor compliance were explored.

A self-administered questionnaire consisting of twenty-one questions was designed, piloted and posted to 107 patients with a biopsy-proven diagnosis of coeliac disease, attending a special outpatient clinic in St James's Hospital, Dublin. Following a postal reminder, ninety-one questionnaires were returned (85% response rate). All questions were answered by all respondents.

The mean age of the respondents was 43 (range 20-80) years, with a gender distribution of sixty-one females to twenty-two males. Mean time since diagnosis was 15 (range 0.5-50) years. The majority of patients (84%) were seen at least once a year (42% at least every 6 months) by their general practitioner or in the outpatient clinic, although, surprisingly, 51% had been seen by a dietitian only once since the coeliac disease was diagnosed (20% were reviewed by a dietitian at least every 6 months). A majority (65%) of respondents felt that the "gluten-free" diet should never be relaxed, whereas some felt that they could relax the diet during social occasions (16%), when away from home (10%) or when asymptomatic (10%). This appeared to be at variance with the large minority of patients (42%) who reported not adhering strictly to their prescribed diet (10% "often included gluten" in their diets). The pathological consequences of poor compliance were appreciated to a variable degree, most realising that diarrhoea could result (91%), but fewer aware of the possibility of weight loss (86%), anaemia (69%), food malabsorption (60%) or metabolic bone disease (27%). Factors reported to contribute to the difficulty in adhering to the diet included the expense (97%), problems during social occasions (91%) and difficulty obtaining gluten-free products (55%). Neither time since diagnosis nor socioeconomic group were significant factors in determining reported difficulty with dietary adherence.

In conclusion, a considerable degree of poor dietary compliance appears to exist in this population of adult coeliac patients. Factors contributing to this may include insufficient patient education and infrequent dietetic follow-up in addition to the factors reported by the subjects relating to expense, inconvenience and insufficient availability of gluten-free products.

Valletta, E.A. & Mastella, G. (1990). *Digestion* **47**, 20 -23.

Ljungman, G. & Myrdal, U. (1993). *Acta Pediatrica Scandinavica* **82**, 235-238.

Nutritional status and comparison of prescribed v. actual nutritional intakes of nasogastrically fed neurosurgical patients. By M. DOHERTY[1], M. HAYES[2] and M. MOLONEY[1], [1]*Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, and* [2]*Department of Nutrition and Dietetics, Beaumont Hospital, Dublin 9, Republic of Ireland.*

Neurosurgical patients are critically ill and have increased requirements for nutrients (Clifton *et al.* 1984; Kaufman *et al.* 1987, Young *et al.* 1987). Aggressive nutritional support is needed in the clinical management of critically ill patients in order to prevent or minimize the negative consequences associated with protein-energy malnutrition (Rapp *et al.* 1983; Young *et al.* 1987).

In the present study seventeen (eleven male, six female) critically ill nasogastrically fed neurosurgical patients were studied. The mean age was 51.1 (SD 22.5) years . Mean Glasgow Coma Score was 7.2 (SD 2.7). Nutritional status was evaluated at the commencement of feeding and during feeding. Anthropometrical and biochemical data indicated that these patients were critically ill (mid-arm circumference < 25th centile, mean serum albumin, 31.7 g/l). Prescribed intakes of both protein and energy were 1.2-1.5 g/kg body weight per d and 167-209 kJ/kg body weight per d respectively. Actual energy intake was 56.1 (SD 25) % of prescribed amounts. These reduced energy intakes resulted in low nutrient intakes. Fluid and energy intakes (prescribed and actual) are shown in the Table.

		mean/patient per d	SD
Volume intake (ml)	Prescribed	1758	460
	Actual	1036	640
Energy intake (kJ)	Prescribed	9824	3347
	Actual	5690	3694

Interruptions in feeding therapy accounted for a loss of 31.6% of prescribed feeding hours. The main reasons for interruptions were gastrointestinal intolerance (8.4%), medical and surgical procedures (7.7%), clinical complications of patients (7.6%), mechanical delivery problems (3.5%), and fluid restriction (2.2%). There was a delay (1-5 d) in the commencement of feeding in 43% of patients, mainly due to clinical contraindications (e.g. absence of bowel sounds).

The problems associated with early commencement and interruption of feeding neurosurgical patients need further exploration. Actual nutritional intakes could possibly be improved by the initiation of feeding via nasoduodenal, nasojejunal, or percutaneous endoscopic gastrostomy (PEG) routes, or by total parenteral nutrition (TPN). The current practice of fluid restriction may need to be reassessed (i.e. volume increased) to allow for more aggressive nutritional support. This should result in improved clinical outcome, reduced hospital stay and better cost-effectiveness in the clinical care of these patients.

Clifton, G.L., Robertson, C.S., Grossman, R.G., Hodge, S., Foltz, R. and Garza, C. (1984). *Journal of Neurosurgery* **60**, 687-696.

Kaufman, H.H., Bretaudiere, J.P., Rowlands, B.J., Stein, D.K., Bernstein, D.P., Wagner, K.A. and Gildenberg, P.L. (1987). *Neurosurgery* **20**, 254-265.

Rapp, R.P., Young, B., Twyman, D., Bivins, B.A., Haack, D., Tibbs, P.A. and Bean, J.R. (1983). *Journal of Neurosurgery* **58**, 906-912.

Young, B., Ott, B., Twyman, D., Norton, J., Rapp, R., Tibbs, P., Haack, D., Bivins, B. and Dempsey, R. (1987) *Journal of Neurosurgery* **67**, 668-676.

A survey of nutrition interest among general practitioners. By B.L. MALLON and N.P. KENNEDY, *Unit of Nutrition and Dietetic Studies, Department of Clinical Medicine, Trinity Centre, St James's Hospital, Dublin 8, Republic of Ireland.*

It is acknowledged that nutrition plays an essential role in health and in the practice of medicine. However, the considerable advances in nutrition knowledge in recent decades have not been matched by an increased emphasis on nutrition in undergraduate medical education (Judd, 1988). General practitioners receive little postgraduate nutrition training despite their potentially important position, as primary care physicians, in promoting healthy eating and in using therapeutic nutrition strategies in patient management.

The objectives of this study were to assess general practitioners' awareness of nutrition as a discipline, their perception of the role of nutrition in general practice and their interest in nutrition education. A survey of general practitioners in the Eastern Health Board area was carried out in September 1993 using a postal self-administered questionnaire. One hundred general practitioners were selected randomly from the Irish College of General Practitioners' register. A final questionnaire response rate of 56% (53) was achieved. All but five of the thirty-three questions were answered by all respondents. The awareness of nutrition-related societies ranged from 4% (European Society for Parenteral and Enteral Nutrition) to 19% (The Nutrition Society). Perception of the role of nutrition in general practice was illustrated by the findings that 96% felt that nutrition was important in preventative health care, 77% believing it to be important in general practice. These opinions seemed to be contradicted by a low reported frequency of assessing patients diets (40% carry out a dietary history only once per week on average and 36% once per month). However, 49% were unhappy with their nutrition knowledge (28% felt their knowledge of nutrition was adequate, 23% were undecided), 98% believed that doctors in general should know more about nutrition and only 51% reported having access to dietetic services. In relation to further nutrition education, 85% were interested, 75% stating their willingness to attend lectures and seminars. The topics of greatest appeal were obesity (83%), diabetes mellitus (74%), cardiovascular disease (74%), nutrition in the elderly (74%) and nutrition in infants (66%).

In conclusion, a substantial interest in nutrition was apparent among the respondent general practitioners. If this is representative of the wider population of general practitioners, efforts to promote awareness of nutritional issues and to improve the nutrition knowledge of general practitioners would be well received.

Judd, P.A. (1988). *Journal of Human Nutrition and Dietetics* **1**, 145-150.

Qualitative research by focus group discussions to identify concerns about weight among adult females. By M. KEARNEY and M. J. GIBNEY. *Unit of Nutrition and Dietetics, Department of Clinical Medicine, Trinity Health Sciences Centre, St. James's Hospital, Dublin 8, Republic of Ireland*

Qualitative research by focus group discussion is a valuable tool with which to assess a range of attitudes, beliefs and feelings about a given topic and is an adjunct to quantitative attitudinal research. It is used extensively in marketing and social sciences and is being increasingly used in nutrition research. Groups of selected subjects (*n* 8-12) participate in an open discussion format, focused by a moderator on a given problem area, in a relaxed non-directive manner. The objective is to foster involvement and interaction among the group members that will lead to spontaneous discussion and the disclosure of attitudes, opinions and information (Achterberg, 1988).

Ten groups of females (age range 28 - 40 years; secondary level education; social classes C1C2, DE, F; married with children) were recruited nationwide to investigate their attitudes about body-weight in relation to healthy eating advice. Each session began with a general discussion about health, and other topics including concern about weight, impression of diets and actions taken to change weight, were introduced. All discussions were audio-recorded for later transcription.

All groups expressed similar attitudes and beliefs about weight and its relationship to health. There was a general consensus that being overweight could affect one's health, mainly however in the short-term ('feeling well today'). Although risks of chronic disease were associated with being overweight, this appeared to be confined to extreme overweight (12-19 kg) and only for people with diseases which the women felt could be exacerbated by the excess weight (hypertension, heart disease). The women were not very concerned about their weight from a health point of view. Any concern that did exist was appearance-related and the majority tended to be more concerned about providing healthy diets for their families. However, 50% reported that they were not happy with their weight. The participants said that they did not follow diets, rather they reported 'cutting back' on quantity of food eaten. They appeared to believe that bread and potatoes were 'healthy foods', however only in limited quantities. The women seemed to be more aware of diets and healthy eating plans from popular women's magazines than from healthy eating leaflets. All dietary regimens tended to be regarded as rigid, impractical, expensive and impossible to implement in a family situation. The women seemed to associate diets and healthy eating with unusual foods and weighing food portions. The participants' concept of a 'balanced diet' was different than that of a nutritionist. They talked of 'balancing' sweets eaten at snack times with vegetables at meal times, or of trying to ensure a healthy diet for a few days, and eating freely on other days. Some participants, from social class C1C2, reported that they would like more information in healthy eating advice about 'how food works in the body', whereas others from social class DE, said they preferred 'handy tips'.

The findings suggest key measures which should be incorporated into weight-control advice for this target group. The emphasis should be on the family, rather than on the individual. The immediate benefits to health should be given more prominence than chronic diseases, the difficulty with losing weight should be recognized, prescriptive advice should be avoided and the potential for using everyday foods in weight-control diets should be highlighted.

Achterberg, C. (1988). *Journal of Nutrition Education* **20**, 244-250.

McConnell's Advertising and Laura Ready are acknowledged for their help in conducting the group discussions. Nutriscan Ltd. and the Health Promotion Unit (Dept of Health) are acknowledged for sponsorship.

An evaluation of a method for comparison of 'healthiness' of menus in work-place restaurants. By A. WISE, D. BALFOUR and R. MOODY, *The Robert Gordon University, Queen's Road, Aberdeen AB9 2PG*

Nutritional advisers to caterers need a method to measure 'healthiness' of menus which will enable the effectiveness of improvements to be tested before implementation. It is possible to consider each menu item separately, but difficult to get an overall impression of the whole menu. The number of potential meals in restaurants is very great because customers may combine dishes according to taste. One possible, relatively simple, method to compare menus is to let a computer make a large number of random meal combinations and calculate the range of potential meals. This is a simplified approach based on an idea that all possible meals are calculated (Clarke, 1989); this may number several tens of thousands and take a long time to calculate, even for a computer. The computer does not make any allowance for the cultural acceptability of certain combinations. Criteria for 'healthiness' might include the average composition and the range of potential meals for customers to choose from. Customers who are nutritionally better educated may wish for meals with lower saturated fatty acids (SFA) and non-milk extrinsic sugars (NMES) and more non-starch polysaccharides (NSP). It was decided to use the proposed method to compare two different restaurants, both of which provide lunch at work; one however is free (F is fully subsidized) and the other makes a realistic charge (C). Menus over a period of 11 d were obtained from the caterers and used randomly to construct 100 meals/d. Meals consisted of a starter, main course, vegetable, potato or rice, and sweet. Salads, snacks and beverages were excluded. In order to compare the random meals with those chosen by customers, people were asked outside the restaurants to select dishes from the menu on a computer screen. At each restaurant the response rates were 47% (*n* 387) and 45% (*n* 307) respectively. Although it had been decided to use the standard meal format to compare the restaurants, few customers chose this meal. Only twenty-one people chose a standard meal and all were at restaurant F. Customers included snack items and omitted courses. At restaurant F, 69% took a sweet course, but only 13% at restaurant C. Only 21% there took a vegetable, but 46% did so at restaurant F. The randomly generated standard meals were similar at each restaurant (shown as medians), although more 'healthy' ($P<0.001$) in restaurant C for NMES. Meals chosen by customers were lower in energy than the standard meals, especially at restaurant C. The semi-interquartile intervals as a percentage of median (non-parametric equivalent of coefficient of variation) were not very different for the standard meals at the two restaurants, but meals chosen by customers were more variable than the standards.

	Average values				Variability			
	F		C		F		C	
	Actual	Standard	Actual	Standard	Actual	Standard	Actual	Standard
Energy (MJ)	3.08	3.47	1.80	3.31	27	19	26	23
NSP (g/MJ)	2.34	2.63	2.63	2.56	40	24	38	30
SFA (% energy)	12.6	10.5	11.4	10.3	42	25	51	29
NMES (% energy)	9.9	13.0	6.2	11.1	50	25	71	40

It was concluded that the random meals generated from the menus at both restaurants were similar in both average nutritional composition and the variability of meals available. The method might be useful for estimating the potential effect of alterations to the menus, but it is clear that actual meals differ markedly especially when customers have to pay.

Clarke, M. (1989). *Journal of Human Nutrition and Dietetics* 2, 287-293.

Children's perception of their body size and some reasons for their snackfood choices. By M. GALLAGHER and M.A.T. FLYNN, Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Republic of Ireland

Studies in both Britain and America indicate that dissatisfaction with body size is common among schoolchildren as young as 10 to 12 years (Wardle & Beales, 1986; Gustafson-Larson & Terry, 1992). It is likely that these perceptions influence children's food choices. Due to a lack of information on this subject in Ireland, the present study was designed to measure senior primary-schoolchildren's perceptions about their body size and to explore some reasons for their snackfood choices.

One hundred children (mean age 11.5 years) were randomly selected from the fifth and sixth classes of two Dublin primary schools, one inner city and one suburban, ensuring equal representation of each sex from each school. Height and weight were measured and the children were asked if they were satisfied with their body size or if they wanted to be lighter/heavier, smaller/taller. Subjects categorically rated fourteen snackfoods which were commonly eaten as confirmed in a pilot study using a prototype questionnaire. These foods included seven "high fat" (i.e. 12-40% fat content) and seven "low fat" (i.e. 0 - 4% fat content) snack foods, and were rated under the following selected criteria , pleasure, health, peer pressure, parental pressure and effect on appearance.

Given in the following Table are the proportions of children found to be satisfied and dissatisfied with their body size (weight/height).

Proportions of children wanting to be.....	"Same weight"	"Lighter"	"Heavier"	"Same height"	"Smaller"	"Taller"
	% (*n*)	% (*n*)	% (*n*)	% (*n*)	% (*n*)	% (*n*)
Total group (*n* 100)	55 (55)	39 (39)	6 (6)	67 (67)	4 (4)	29 (29)
Girls (*n* 50)	54 (27)	44 (22)	2 (1)	70 (35)	4 (2)	26 (13)
Boys (*n* 50)	56 (28)	34 (17)	10 (5)	64 (32)	4 (2)	32 (16)

Using chi-square tests, no sex or school differences were found when the total group of children was examined. However, within the inner-city school, significantly more boys compared with girls (52% *v.* 20%, $P<0.05$) wanted to be taller; and significantly more of the inner-city boys compared with suburban boys (52% *v.* 16%, $P<0.01$) wanted to be taller. Examination of the weight and height centile data of the children wanting to be lighter (*n* 39) found that almost half (49%, *n* 19) were as much above average height as above average weight and, therefore, did not need to be lighter; and that only 15% (*n* 6) of these children could be described as clinically overweight (i.e. weight > 97th and height < 97th centiles). Significantly more of the children wanting to be lighter, compared with the children who were satisfied with their weight, perceived two high-fat snackfoods, namely chips (82% *v.* 54%, $P<0.01$) and peanut buttered crackers (44% *v.* 22%, $P< 0.05$), to affect appearance. In the total group of children low-fat snackfoods were found to be perceived more favourably in relation to health, parental pressure and effect on appearance. Ratings on pleasure and peer pressure, however, were similar for both low- and high-fat snackfoods. In conclusion, the findings of this study suggest that an inappropriate fear of fatness is prevalent among both male and female Irish senior primary-schoolchildren and that this weight consciousness may affect how foods are perceived.

We thank Dr Howard Johnson, EHB, Dublin, for facilitating this study in his Community Care Area, and Ms Mary Kearney, Trinity College, Dublin.

Gustafson-Larson, A.M. & Terry, R.D. (1992). Journal of the American Dietetic Association 92, 818-822.

Wardle, Y. & Beales, S. (1986). Appetite 7, 209-217.

Anthropometry, nutritional knowledge, and nutrient intakes in a group of Irish adults with essential hypertension. By M. A. T. FLYNN[1], M. A. MCGRATH[1], and D. D. SUGRUE[2]. [1]Department of Biological Sciences, Dublin Institute of Technology, Dublin 8 and [2]Department of Clinical Cardiology, Mater Misericordiae Hospital, Dublin 7, Republic of Ireland

Weight control, limited alcohol intake and a prudent diet for cardiovascular disease prevention, are recognized as important aspects of the management of hypertensive subjects (WHO/ISH 1993). The present study investigates the use of such non-pharmacological treatment in a group of Irish adults with essential hypertension.

Thirty subjects (fourteen females and sixteen males; mean age 59 years) were consecutively recruited from the cardiac outpatient clinic (75% response rate). Information on any dietetic treatment received was collected. Anthropometric measurements descriptive of overweight (body mass index (BMI) and body fat distribution (waist:hip ratio (WHR)), waist:thigh ratio (WTR) and conicity index (CI, Valdez *et al.* 1993)), were made. Alcohol intakes and nutritional knowledge on factors affecting blood pressure were assessed by an interview-assisted questionnaire. Dietary intakes were measured using the 7d dietary history method in the subgroup of subjects (n 13) not on drug therapy (which allowed estimation of sodium and potassium intakes from their 24h urinary excretions).

BMI, though commonly used, did not corrlate with indices of body fat distribution (r 0.1-0.2). The majority of the subjects (77%, n 23) were found to be overweight (BMI >25) with 30% in the obese category (i.e. >20% overweight). Where alcohol was consumed (n 17) intakes were high (see Table). Only one-third of the subjects (n 10) were found to have ever received dietary advice, while very few (n 2) were currently being treated by a dietitian. Given in the following Table is a comparison, using unpaired t tests, of the anthropometric indices, the weekly alcohol intakes for drinkers only (units) and the nutritional knowledge score (percentage of correct answers) of those subjects who had been given dietary advice compared with those who had not.

	BMI		WHR		WTR		CI		Knowledge		Alcohol		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Total group (n 30)	28.3	4.6	0.9	0.2	1.7	0.2	1.3	0.1	68	20	23	16.5	*
Advised (n 10)	29.5	4.4	0.9	0.1	1.7	0.1	1.3	0.1	66	22	19	8.8	†
Not advised (n 20)	27.4	4.0	1.0	0.3	1.8	0.2	1.3	0.1	69	20	25	18.6	‡

Alcohol drinkers only *n 17, †n 5, ‡n 12.

No significant differences were found from this comparison. With the exception of calcium, nutritional knowledge on dietary factors affecting blood pressure was good (i.e. >50% correct answers); however this was not reflected in the dietary intakes measured. Dietary analysis revealed high daily intakes of total fat (39(SD 8)% energy), saturated fat (17 (SD 5)%), and sodium (163 (SD56) mmol), while potassium intakes (88 (SD29) mmol), relative to sodium, were found to be inadequate.

This group of Irish adults were characteristically overweight, had high alcohol intakes, and reported dietary intakes not conducive to cardiovascular disease prevention. Despite this, the majority of these subjects had not received any formal dietetic treatment. These findings suggest that there is minimal use of non-pharmacological therapy in the management of Irish adults with essential hypertension.

Valdez, R., Seidell, J.C., Ahn, Y.I. & Weiss, K.M. (1993). International Journal of Obesity **17**, 77-82.

WHO/ISH Guidelines Subcommittee (1993). Hypertension **22**, 394-403.

The effect of very-low-, moderate- and high-fat snacks on postprandial reverse cholesterol transport in healthy volunteers. By L.O'FLAHERTY and M.J. GIBNEY, Unit of Nutrition and Dietetics, Department of Clinical Medicine, Trinity College Medical School, St. James's Hospital, Dublin 8, Republic of Ireland

Cellular and lipoprotein free cholesterol (FC) is transferred and esterified into high-density lipoprotein (HDL) by the enzyme lecithin : cholesterol acyltransferase (LCAT; *EC* 2.3.1.43) in the postprandial phase. The cholesterol ester (CE) fraction of HDL is then exchanged for chylomicron triacylglycerol (TAG) and the CE transferred to the liver via chylomicron remnants for catabolism. This process is known as reverse cholesterol transport and the present study set out to examine the effect of acute fat loads on this process.

Nine healthy volunteers consumed three test-meals : a very-low-fat snack of fruit and fruit juice (0.7g fat), a moderate-fat snack of a Mars bar and a can of coke (12.1g fat) and a high-fat snack of a cheese sandwich and a glass of full-fat milk (54.7g fat). Blood samples were taken at baseline and 4 h later. Plasma TAG, plasma net LCAT activity (μg CE synthesis / ml per h) and blood cell-plasma cholesterol flux (μg FC / ml per h) were determined, the latter two variables as described by Fielding *et al.* (1989). The results are given below.

		LCAT activity (μg / ml per h)		Cholesterol flux (μg / ml per h)		Plasma TAG (mmol / l)	
Time (h)		0	4	0	4	0	4
Fat (g) :	0.7	15.8	17.3	6.5	1.3	0.88	0.68
	12.1	15.1	28.4	-2.0	14.7	0.84	0.99
	54.7	15.9	26.2	0.7	16.0	0.75	1.04
Pooled SEM		1.94		3.31		0.03	

Repeated-measures analysis of variance showed a significant diet x repeat interaction for LCAT activity (P=0.015), cholesterol cell-plasma flux (P=0.008) and plasma TAG (P<0.0001). In effect, the very-low-fat snack did not stimulate reverse cholesterol transport while both other snacks did so to an equal extent.

Frequent ingestion of small fat loads (snacking) is known to reduce plasma cholesterol as significantly as low-fat diets (McGrath & Gibney, 1994). The results of the present study indicate that this may be mediated by frequent stimulation of postprandial reverse cholesterol transport. Fat ingestion is clearly necessary and the threshold response dose lies below 12 g fat per eating occasion.

Fielding, P.E., Jackson, E.M. & Fielding, G.J. (1989). Journal of Lipid Research **30**, 1211-1217.

McGrath, S.A., & Gibney, M.J. (1994). European Journal of Clinical Nutrition (In the Press)

This project was funded by Nutriscan Ltd. Trinity College, Dublin.

Evaluation of the antidiabetic effects of an edible mushroom (*Agaricus campestris*). By A.M. GRAY and P.R. FLATT, *Diabetes Research Group, Department of Biological and Biomedical Sciences, University of Ulster, Coleraine BT52 1SA*

Before the advent of insulin and oral hypoglycaemic agents, the major form of treatment of diabetes mellitus involved exploitation of the medicinal properties of certain plants. Over 400 plants are reputed to possess antihyperglycaemic properties but few have received scientific or medical scrutiny (WHO Expert Committee on Diabetes Mellitus, 1980). An edible mushroom (*Agaricus campestris*) has in the past been used as a traditional plant treatment for diabetes within Europe. Here the antidiabetic potential of *A. campestris* has been investigated using streptozotocin (STZ)-induced diabetic mice and genetically obese/diabetic (*ob/ob*) mice. Effects of *A. campestris* extract on insulin secretion *in vitro* were also studied using the clonal BRIN-BD11 pancreatic B-cell line.

A. campestris was incorporated into the diet (62.5g/kg) of adult male mice (21-24 weeks, *n* 6) fed *ad libitum* for 21 d. Animals were dosed by intraperitoneal injection with STZ (200mg/kg body weight) on day 5. Daily measures of body weight, food and fluid intake together with end-point determinations of plasma glucose and glycated haemoglobin were compared with those of control STZ-treated mice and normal mice fed on unsupplemented diets *ad libitum*. Compared with normal mice, administration of STZ resulted in significant ($P<0.05$) weight loss, polydipsia, elevated glycated haemoglobin and hyperglycaemia. Supplementation of the diet of STZ-treated mice with *A. campestris* countered these changes, and plasma glucose concentrations at day 21 were not significantly different from those of normal control mice. In contrast, administration of *A. campestris* in the diet of severely insulin resistant adult *ob/ob* mice (15-18 weeks, *n* 8) did not ameliorate hyperglycaemia or other symptoms of the diabetic syndrome.

The effect of aqueous extract of *A. campestris* (prepared by 15 min infusion) on insulin secretion *in vitro* was investigated using BRIN-BD11 insulin-secreting cells. During 20 min incubations in Krebs-Ringer bicarbonate buffer containing 1.1mM-glucose, *A. campestris* extract (0.25, 0.5 and 1mg/ml, *n* 6) induced a significant dose-dependent increase in insulin secretion ($P<0.01$). This effect was inhibited 65% by 400mM-diazoxide ($P<0.001$) which induces pharmacological blockade of insulin secretion by preventing inhibition of plasma membrane K^+-ATP channels. Prior exposure to *A. campestris* extract did not affect the subsequent stimulation of insulin release by 10mM L-alanine, indicating absence of toxic effects of *A. campestris* extract at the doses employed.

These results indicate that *A. campestris* possesses antihyperglycaemic properties manifest in STZ-treated mice. *A. campestris* appears to contain one or more water-soluble insulin releasing agents which merit further investigation.

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The effect of dietary fibres and phytate on calcium absorption in the rat. By M. HARRINGTON, A. FLYNN and P.A. MORRISSEY, Department of Nutrition, University College, Cork, Republic of Ireland

The effects of fibre or fibre components such as phytate on the bioavailability of Ca are poorly understood. The purpose of the present study was to investigate the effect of a range of dietary fibres and phytate on Ca absorption using a rat model. The rat is considered to be a useful model for studies on Ca bioavailability since the absorption mechanisms for Ca are similar in rats and humans and a number of dietary and physiological factors affect Ca absorption similarly in the two species.

Eighty 7-week-old male rats, Wistar strain, average weight 227 g, were randomized into ten groups of eight rats each and fed on a purified diet (AIN-76) containing cellulose (control) or food fibres (apple, orange, pea, sugarbeet, barley or wheat obtained from Sofalia in France), all at a level of 50 g/kg diet, or cellulose (50 g/kg) with added phytate at 7.5, 15.0 or 30.0 mmol/kg for 2 weeks. Each group was then given a meal (10 g of the same diet) containing (per kg) 5 g Ca as ^{47}Ca-labelled $CaCO_3$ and 0.2 g Fast Green FCF as a faecal marker. Fractional absorption of ^{47}Ca was determined by the ^{47}Sc:^{47}Ca ratio method of Brommage & Binacua (1991).

Dietary fibre	Phytate (mmol/kg diet)	Ca absorption (%) Mean	SE
Cellulose (Control)	0.00	54.5	1.9
Apple	0.11	48.4	1.6
Orange	0.05	49.2	1.1
Pea	0.08	52.9	3.6
Sugarbeet	0.08	48.1	3.3
Barley	0.75	53.1	2.7
Wheat	2.40	45.3*	2.4
Cellulose	7.50	45.9*	1.8
Cellulose	15.00	43.6*	1.8
Cellulose	30.00	40.8**	1.6

Significantly different from control (ANOVA): * $P<0.01$, ** $P<0.001$.

Ca absorption was not influenced by a range of dietary fibre types but was reduced by wheat fibre which contained significant amounts of phytate. Phytate added to cellulose-containing diets significantly reduced Ca absorption.

The effect of phytate on Ca absorption was also investigated in 4-week-old rats. Phytate (0, 1, 5, 10 and 20 mM) was incorporated into a slurry (50 g/kg) of steamed cooked rice (Milupa Baby Rice) in 10 mM $CaCl_2$ solution. Meals were extrinsically labelled with ^{47}Ca (17.5 KBeq/ml) and 0.5 ml administered orally to 28-d-old rats previously fasted for 10 h, and Ca absorption (14 h later) was determined by the method of Cashman *et al.* (1993). Ca absorption in the control was 97.5 %, and was significantly reduced, in a dose-dependent manner, relative to the control by phytate at concentrations of 5 (94.6 %), 10 (92.0 %) and 20 mM (89.6 %).

These results show that fibre *per se* may have little effect on Ca bioavailability but phytate, at concentrations found in some human foods, is inhibitory to Ca absorption.

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Cholesterol supplementation and tissue antioxidant enzyme activities in male and female hamsters. By N.C. ARMSTRONG, J.M. ALLEN and J.J.STRAIN, *Department of Biological and Biomedical Sciences, University of Ulster, Cromore Road, Coleraine, Co. Londonderry BT52 1SA*

Maintenance of normal cell functions in the presence of oxygen is very much dependent upon the ability of the tissues to protect against free-radical mediated oxidative stress. Several defence strategies have been developed by the cell to protect itself against these injurious species including the antioxidant enzymes: superoxide dismutase (SOD, *EC* 1.15.1.1.), catalase (CAT, *EC* 1.11.1.6.) and glutathione-related enzymes such as glutathione peroxidase (GSHPX, *EC* 1.11.1.9.), reductase (GR, *EC* 1.6.4.2.) and transferase (GST, *EC* 2.5.1.18.). The current study investigated the effects of cholesterol supplementation on these antioxidant enzymes in the livers of both male and female hamsters.

Male and female weanling Syrian hamsters were fed *ad libitum* on either a control diet (150 g maize oil /kg diet) or a cholesterol-supplemented diet (5 g cholesterol and 150 g maize oil/kg diet) for 10 weeks after which a blood sample was obtained by cardiac puncture and the liver was excised. Samples were stored at -70° until time of analysis. Results are presented in the Table.

	Control-fed				Cholesterol-fed				Cholesterol
	Male (*n* 8)		Female (*n* 9)		Male (*n* 7)		Female (*n* 10)		effect
Variable	Mean	SE	Mean	SE	Mean	SE	Mean	SE	(ANOVA)
Plasma cholesterol (mmol/l)	3.11	0.14	3.70	0.25	10.80	0.55	12.36	1.32	***
Hepatic Copper (μg/g dry weight)	17.12	1.07	15.13	0.65	10.66	0.63	10.00	0.51	***
SOD (U/mg protein)	17.81	0.65	16.67	0.93	14.59	0.39	13.25	0.81	***
CAT (k/mg protein)	0.41	0.03	0.44	0.05	0.35	0.05	0.39	0.04	NS
GSHPX (mU/mg protein)	566.52	31.97	538.16	30.06	431.94	41.55	514.46	15.57	**
GR (mU/mg protein)	61.88	3.26	60.11	3.48	25.30	1.03	21.42	0.87	***
GST (U/mg protein)	2.04	0.09	1.95	0.18	1.55	0.09	1.36	0.12	***

Significance: *P<0.05, **P<0.01, ***P<0.001. NS, not significant.

Two-way ANOVA showed that cholesterol supplementation significantly increased plasma cholesterol levels whilst significantly decreasing the activity of the following measured enzymes: SOD, GSHPX, GR and GST. Interestingly, hepatic copper levels were also significantly decreased in animals fed on the cholesterol-supplemented diet, a finding which has also been reported in the New Zealand White rabbit (Klevay, 1988). No significant effects were found in any of the variables with regard to sex.

It is possible that cholesterol feeding interferes with copper metabolism in the hamster; impaired copper status has been associated with a decrease in antioxidant status in other animal species (Johnson *et al.* 1992). An alternative explanation is that cholesterol may be acting as an antioxidant (Smith, 1991); under such circumstances there is a diminished need for additional antioxidant enzyme protection.

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Feeding trial, composition, oxidative stability and sensory evaluation of cultured turbot fed three commercial feeds. By E.M. SHEEHAN[1], P.J.A. SHEEHY[1] and R. FITZGERALD[2], [1]Department of Nutrition, and [2]Aquaculture Development Centre, University College Cork, Republic of Ireland

Turbot (*Scophthalmus maximus L.*) is a highly valued marine flat fish commonly found in northern waters. The European market is almost exclusively satisfied by fish captured in the wild. However, commercial farming of this species has developed rapidly since the mid-1970s, particularly in Spain and France. Wet fish or moist feeds (i.e. combinations of minced fish and dry mixtures of fishmeal, minerals and vitamins) are presently used during the entire grow-out period. Although production with moist feeds is an acceptable practice, there are potential nutritional problems which may be overcome by the use of dry pelleted diets. However, in comparison with salmonids, information is lacking on the optimal feed composition for turbot grown under Irish conditions. The objective of the present study was to investigate the growth performance and nutritional and sensory quality of turbot raised on a range of commercial pelleted feeds.

Ninety-six turbot (initial body weight about 480 g) were randomly distributed into six tanks (three groups x two replicates) and maintained on one of three feeds (Table) for a period of 18 weeks in an enclosed recirculation system. Feeds A and B were 'dedicated' turbot feeds, and feed C was a reduced-fat trout feed. Prior to the start of the trial six extra fish were removed for compositional analysis. Fish were weighed twice per month. At the end of the trial, the proximate composition, storage stability, and sensory properties of fillets were evaluated.

	Feed composition (g/kg wet weight)					Fillet Composition (g/kg wet weight)								K	
	Protein		Fat		CHO	Protein		Fat		DM			SGR	(g/cm^3)	
	mean	SE	mean	SE		mean	SE	mean	SE	mean	SE	FCR	(%/d)	mean	SE
Pretrial						181	6.00	19.2	0.40	786	2.00			1.004	0.025
A	493	2.50	183	3.00	125	197	6.70	19.8	0.60	769	7.10	1.25	0.508	1.070	0.013[a]
B	597	13.0	150	1.00	61.7	199	3.30	19.8	0.30	769	6.30	2.27	0.253	0.956	0.018[b]
C	530	7.50	48.1	0.40	245	215	8.30	19.0	0.50	754	10.4	2.81	0.278	0.949	0.021[b]

CHO, carbohydrate calculated by difference; FCR, feed conversion ratio (dry weight feed (g)/wet weight gain (g)); SGR, specific growth rate; K, condition factor; DM, dry matter. [a,b] Significantly different at $P<0.01$

Final body weights of turbot from group A (947 (SE 38)g) were significantly ($P < 0.01$) higher than those from groups B (649 (SE 37)g) and C (701 (SE 35) g). Furthermore, mean FCR for fish in group A were lower, and SGR and condition factors were higher than those from the other two groups. The proximate composition of turbot fillets did not differ significantly between groups, but fillets from groups A and C were significantly ($P<0.01$) more stable during refrigerated and frozen storage than those from group B. Despite its lower oxidative stability, a sensory panel expressed a significant preference for fresh fillets from group B. Of the feeds investigated, feed A was superior from a economic point of view because of the lower FCR, higher SGR and longer shelf-life observed. However, feed B produced a more acceptable product. Further work is continuing on the optimization of turbot feed composition and feeding practices for Irish conditions.

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Development and evaluation of a computer-based food atlas for portion size estimation. By F. HORAN[1], G. MCDERMOTT[2] and N.P. KENNEDY[1], [1]*Unit of Nutrition and Dietetic Studies, Department of Clinical Medicine, Trinity Centre, St James's Hospital, Dublin 8 and* [2]*Computer Applied Techniques Ltd, 3 St James's Terrace, Malahide, Co Dublin, Republic of Ireland*

The largest source of measurement error in dietary survey methods is probably that associated with estimation of portion size (Young *et al.* 1952). Subjects have great difficulty in correctly estimating amounts of foods, whether from recall of past consumption or from direct observation of food (Faggiano *et al.* 1992; Lansky *et al.* 1992). Tools currently used in retrospective dietary studies to help subjects quantify foods include food models, household measures and photographs.

Colour images of 200 foods showing up to three portion sizes for each food were taken using a MAVIS computer imaging workstation. Appropriate software was written to access the images comprising the computer atlas (CA), which were displayed on a 14-inch colour monitor. The use of this tool in the accurate estimation of portion size was assessed against a 3-d weighed intake (WI). Results obtained using a photographic atlas (PA), depicting one portion size per food (Lee & Cunningham, 1990), were also assessed against a WI to enable comparison with the CA results. Thirty-two subjects (eighteen females, fourteen males) weighed their food for two 3-d periods. On the fourth day a 3-d dietary history was elicited, using either the CA or the PA. There was an interval of 4-6 weeks between each WI. Food portion weights estimated by each subject using the CA and the PA were compared with the corresponding weighed portions in each WI record. Percentage over- and underestimation of portions were calculated if at least five portion estimates were available. Differences between weighed and estimated portions were expressed as percentages of the corresponding weighed portions (% error). Using the CA, sixteen foods were underestimated and twenty-seven overestimated by 20 - 50 % compared with thirty-one and seventeen respectively using the PA. Similarly, using the CA, no foods were underestimated and four were overestimated by more than 50% compared with three and fourteen respectively using the PA. The proportion of portion estimates in each % error category were significantly different between the CA and PA (see Table).

% Error	Computer atlas (CA)	Photographic atlas (PA)
± 0 - 20	53%	34%
± 20 - 50	37%	41%
± 50 -100	9%	16%
± > 100	1%	9%

Percentage of estimates using CA and PA differ significantly ($P < 0.01$) in each % error category using χ^2 test.

Subjects had difficulty estimating portion sizes using both CA and PA, with a greater tendency overall to overestimate than underestimate. Errors in portion estimates were larger using the PA than the CA. This may be due to a single portion size being used for each food in the PA, rather than a range. Image size and food presentation in the images may also play a role.

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Dietary fish-oil supplementation inhibits the expression of major histocompatibility complex (MHC) class II molecules and adhesion molecules on human monocytes. By D. A. HUGHES, Z. PIPER, A. C. PINDER and E. K. LUND, Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA

There is growing interest in the potential use of *n*-3 polyunsaturated fatty acid-rich fish-oil in the treatment of disorders involving overreactive immune responses, such as rheumatoid arthritis and multiple sclerosis. However, the mechanisms by which fish-oil may depress immune responses remain unclear. Specific immune responses are initiated by the presentation of antigen to helper T-lymphocytes on the surface of an antigen-presenting cell (APC). A pre-requisite for this function of APC is the cell surface expression of MHC class II molecules, aided by the presence of adhesion molecules. Elevated expression of MHC class II molecule on synovial fluid monocyte populations in patients with rheumatoid arthritis has been reported (Gadd *et al.* 1992). We have observed a reduced expression of class II molecules on human blood monocytes *in vitro* in the presence of eicosapentaenoic acid (EPA), one of the major fatty acids found in fish-oil, and it has recently been shown that a fish-oil-rich diet can suppress the expression of class II molecules on peritoneal cells from *Listeria*-infected mice (Huang *et al.* 1992). In the present study the effects of dietary fish-oil supplementation on the cell surface expression of class II molecules and adhesion molecules by human blood monocytes was investigated.

Purified monocytes were obtained from six healthy volunteers before and following a 3-week dietary supplementation with fish-oil (EPA-Forte), at a dose of 930 mg EPA and 630 mg docosahexaenoic acid/d. Six other volunteers who did not receive supplementation acted as controls. The monocytes were immunofluorescently stained with monoclonal antibodies raised against the MHC class II molecules (HLA-DR, -DP and -DQ), and the adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and leucocyte function associated antigen-1 (LFA-1). Both the percentage of monocytes expressing each of these molecules and the intensity of expression of each molecule was quantified by flow cytometry. The monocytes were examined both immediately after blood sampling and following incubation in serum-free culture medium for 24 h at 37° in the presence of interferon-gamma (IFN-g; 400 U/ml) to stimulate upregulation of MHC class II molecule expression by the monocytes.

Comparing pre- and post-supplementation samples, there was a significant reduction in the intensity of expression of all the monocyte surface molecules examined following fish-oil supplementation ($P<0.025$ in each case; Wilcoxon matched-pairs signed-ranks test), although there was no change in the percentage of monocytes expressing each molecule. Following incubation with IFN-g, there was a similar inhibition of surface molecule expression (with the exception of HLA-DQ) in monocytes obtained from the fish-oil-supplemented group and, additionally, there was a reduction in the percentage of monocytes expressing both HLA-DR and -DP molecules ($P<0.025$). No significant changes were observed in the control group.

These findings suggest that dietary supplementation with fish-oil may depress immune reactivity by inhibiting the expression of surface molecules involved in antigen-presenting cell function. It should be noted that the observed changes in surface molecule expression were obtained with doses of fish-oil considerably lower than those used in the clinical trials (>5 g fish oil/d) which have shown a beneficial effect in patients with rheumatoid arthritis.

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Effect of copper deficiency on cytokine activities in mice. By H. JIN, B.M. HANNIGAN and J.J. STRAIN, Department of Biological and Biomedical Sciences, University of Ulster, Coleraine BT52 1SA

Copper is a trace metal essential for immune function (Beach *et al.* 1982). Cu deficiency is characterized by recurrent infection and sepsis in humans and leads to an increased susceptibility to bacterial infections and decreased resistance to tumour challenge in animals. Many previous studies suggested that both non-specific and specific defences (humoral and cell-mediated immunity mechanisms) were compromised to a greater or lesser extent in Cu-deficient mice (Danks, 1988). The macrophage-derived cytokines, tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6), are important mediators in host immunity and the present investigation set out to study the effect of Cu deficiency on these cytokine activities in mice.

Three-week-old male weanling Balb/c mice were divided into three groups. Two groups were fed *ad libitum* on purified diet that contained either adequate (+Cu, 5.03 mg/kg) or deficient (-Cu, 0.99 mg/kg) levels of Cu. A third group of mice remained on the certified rodent chow (chow control group). All mice were provided with deionized water *ad libitum*. Cu status was monitored by liver Cu levels after 6, 7 and 10-week treatment periods. Resident peritoneal macrophages were obtained from mice 10 weeks following treatment for the production of TNF-α and IL-6. Serum caeruloplasmin (Cp; EC 1.16.3.1) and liver cytochrome c oxidase (CCO; EC 1.9.3.1) were also measured.

Characteristics	-Cu group		+Cu group		Control group	
	Mean	SD	Mean	SD	Mean	SD
n	7		8		8	
Body wt (g)	29.1^{a}	3.0	26.5^{a}	3.6	23.2^{b}	2.3
Cp (units/l)	23^{a}	9	45^{b}	13	29^{a}	11
Liver Cu (μg/g)	2.97^{a}	0.14	3.55^{ab}	1.04	4.32^{b}	1.31
CCO (units/ mg protein)	0.36^{a}	0.12	0.55^{b}	0.15	0.51^{b}	0.10
TNF-α (unit/l)	ND		ND		ND	
IL-6 (pg/l)	21.01^{a}	15.35	26.96^{a}	22.88	52.11^{b}	18.05

One-way analysis of variance.

^{a,b}Groups with different superscripts are significantly different ($P<0.05$; least significant difference test).

ND, not detectable.

A mild Cu deficiency was indicated by the significantly decreased Cp and CCO activities and liver Cu in the -Cu group compared with the +Cu (or control group) after 10 weeks on the diet. Treatment had no effect on measured TNF-α activity as resident macrophages did not produce detectable TNF-α activity in either Cu-deficient or

-adequate mice, while significant amounts of IL-6 were produced by macrophages from all groups of mice.

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Total reflection X-ray fluorescence: a sensitive multi-element technique for the analysis of nutritional and toxic elements. By PETER H. EVANS[1], STEPHEN J. HASWELL[2], DAVID BARCLAY[2] and CHRISTOPHER J. BATES[1], *[1]MRC Dunn Nutrition Unit, Milton Road, Cambridge B4 1XJ and [2]School of Chemistry, University of Hull HU6 7RX*

We have utilized the novel multi-element analytical technique of total reflection X-ray fluorescence (TXRF; Prange, 1989) in a preliminary study of its applicability to various biological materials, namely blood plasma and serum, and urine. The technique permits the simultaneous analysis of many cationic and also anionic elements of nutritional and toxicological interest. Analysis is performed using a TXRF spectrometer by measurement of the intensity of the element-characteristic fluorescent X-rays following ultra-low-angle irradiation of the sample material with a molybdenum X-ray tube.

Validation of the technique to determine analytical sensitivity, reproducibility and accuracy was undertaken by the use of reference material samples of blood plasma and serum, and urine. In addition, comparative assays utilizing atomic absorption spectroscopy and standard addition procedures, were performed. In brief, a 10 µl sample, together with 10 µl internal standard, typically 10 ppm vanadium solution, were placed onto a pre-siliconized quartz support reflector disc, and dried in a filtered air cabinet prior to analysis. Results for selected elements as compared with the published values for the Versieck reference human pooled serum sample are presented.

	Human serum		
Element	TXRF	Reference Value (RV)	TXRF/RV
	(µg/g)	(µg/g)	
Se	1.04	1.05	0.99
Zn	9.9	9.6	1.03
Cu	11.3	11.1	1.02
Fe	26.8	25.9	1.04
Br	48.2	48.8	0.99
	(ng/g)	(ng/g)	
Co	3.5	3.6	0.97
Mn	7.3	7.7	0.95
Ni	2.4	2.5	0.96
Cd	1.9	2.0	0.95
Hg	3.2	6.6	0.49

The results indicate that TXRF offers a sensitive, accurate, and operationally simple technique for the direct simultaneous analysis of elements of nutritional and toxicological interest.

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An assessment of ultrafiltratable iron using a continuous flow *in vitro* method. By A.M.MINIHANE and S.J.FAIRWEATHER-TAIT, Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA

A continuous flow *in vitro* assay was developed to determine the percentage ultrafiltratable iron from ^{59}Fe extrinsically labelled food digests (Minihane *et al* 1993). This method was devised to provide a rapid, reliable, reproducible and relatively inexpensive method of predicting iron availability from individual foods and meals *in vivo* and as a qualitative and semi-quantitative means of assessing the effect of individual dietary constituents on iron absorption. The assay was set up to simulate *in vivo* conditions in the small intestine more closely than the *in vitro* equilibrium dialysis system (Miller *et al* 1981) as the products of ultrafiltration are constantly removed.

A solution of ^{59}Fe-labelled food (10 g--> 100 g with water) was incubated with pepsin (E.C. 3.4.23.1), at pH 2, for 2 h at 37°. The resultant gastric digest was mixed with a bile/pancreatin solution and the pH adjusted to 7. Ultrafiltration was carried out under a pressure of 50 psi in an Amicon stirred cell (Amicon product No. 6003, Model CDS 10, Amicon Ltd, Stonehouse, Glos. GL10 2BJ), using a filtration membrane with a MW cut off of 1000 (Amicon Diaflo) for 2 h. The ultrafiltrate was removed from the system immediately after diffusion and distilled water used to maintain the pressure and therefore the volume within the system. The ^{59}Fe contents of the original food solution, retentate and ultrafiltrate were determined and percentage ultrafiltratable iron calculated.

The effects of a number of organic acids and polyphenols on ultrafiltratable iron in two infant products were determined.

Weaning Food (Milupa Vegetable Casserole)		Infant Formula (Cow & Gate Plus)	
Dietary variable	% Ultrafiltratability	Dietary variable	% Ultrafiltratability
-	7.88	-	9.53
+ 2.5 mM citric acid	10.36	+ 50 mg citric acid	17.25
+ 5.0 mM citric acid	13.59	+ 50 mg ascorbic acid	11.52
+ 10.0 mM citric acid	22.03	+ 50 mg tannic acid	0.64
+ 25.0 mM citric acid	33.36	+ 50 mg catechin	12.84
+ 50.0 mM citric acid	41.61	+ 50 mg caffeic acid	20.74
+ 0.1 mM tannic acid	0.37	+ 50 mg citric acid	
+ 0.5 mM tannic acid	0.34	+ 50 mg tannic acid	2.14
+ 1.0 mM tannic acid	0.25	+ 50 mg ascorbic acid	
+ 2.0 mM tannic acid	0.29	+ 50 mg tannic acid	2.39

The addition of only 0.1 mM tannic acid to the weaning food significantly reduced the percentage ultrafiltratable iron by 95%. Citric acid was found to be a more potent enhancer of iron ultrafiltratability than ascorbic acid; 50 mg citric acid increased the percentage ultrafiltratable iron in infant formula by 81%. When comparisons with the equilibrium dialysis system were made, the continuous flow assay showed greater sensitivity over the range of additions of citric acid and tannic acid used. The method was further evaluated by analysing foods used previously in our laboratory in animal and human absorption studies and comparing *in vitro* percentage ultrafiltratability with *in vivo* percentage absorption values.

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High-performance liquid chromatographic determination of plasma pyridoxal-5-phosphate by use of the cyanide derivative. By A.L. BAILEY, S. SOUTHON, A.J.A. WRIGHT, P.M. FINGLAS and S. MAISEY, Institute of Food Research, Norwich NR 4 7UA

Plasma pyridoxal-5-phosphate (PLP) is the primary form of circulating vitamin B_6 and comprises 70-90% of total vitamin B_6 in plasma (Leklem,1990). Measurement of plasma PLP is frequently used to evaluate vitamin B_6 status. Many current methods are based on the activation of the apo-enzymes, tyrosine apodecarboxylase (EC 4.1.1.25) or apotryptophanase (EC 4.1.99.1), by PLP. These enzymes are non-specific and react to pyridoxamine phosphate as well as PLP, and suffer from problems of lengthy enzyme preparation or clean up, instability and inconsistency. Following HPLC separation, B_6 vitamers can be detected fluorimetrically in food sample extracts but the low concentration (about 40 nmol/l) found in plasma makes detection difficult without large sample volumes. HPLC separation of all B_6 vitamers in blood samples has often been too time-consuming for routine, high-throughput analyses.

PLP concentrations in small volumes (100 µl) of human plasma were determined using an HPLC method based on the production of a fluorescent cyanide derivative (Southon *et al.* 1994). As part of a larger micronutrient study, blood samples were collected from fifty one adolescent, one hundred and thirty three adult, seventy four elderly (65-74 years) and forty five aged (>74 years) subjects in the Norwich area. PLP concentrations found in these subjects, and the percentage of subjects with plasma PLP levels indicative of inadequate vitamin B_6 status (<34.4 nmol/l or 8.5 ng/ml), are shown below.

PLP (nmol/l)	Adolescents (n 51)		Adults (n 133)		Elderly (n 74)		Aged (n 45)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Male	38.3	3.34	42.7	2.14	40.3	5.31	64.1*	8.44
(range)	(23.5 - 77.8)		(15.0 - 85.1)		(14.2 - 165.1)		(25.0 -127.8)	
% < 34.4 nmol/l	50.0		35.4		50.0		25.0	
Female	46.6	3.39	48.6	5.79	46.5	7.99	54.8	5.25
(range)	(25.1 - 105.7)		(13.4 - 384.8)		(15.3 - 268.6)		(21.1 - 132.9)	
% < 34.4 nmol/l	27.3		45.6		59.5		27.6	

*There was a small increase in mean PLP with age but this reached significance ($p < 0.05$) for aged males only .

Percentages of subjects with PLP levels below the 34.4 nmol/l cut-off would appear to indicate that large proportions of the apparently healthy population within this community are at serious risk of vitamin B_6 deficiency. There was no relationship between vitamin B_6 intake (5-7d weighed food intake) and status. However the 34.4 nmol/l cut-off was placed arbitrarily at the lower end of reference ranges for normal healthy male subjects aged 18 to 90 years (Rose *et al.* 1976) and was described by the authors as classifying 30% of their unsupplemented subjects as having marginal or inadequate B_6 status. It is clear that further work is necessary to determine fully the biochemical significance of low plasma PLP levels.

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Factors influencing the determination of folate in foods. By C.A. MARTIN, L. O' MAHONY and P.J.A. SHEEHY, Department of Nutrition, University College, Cork, Republic of Ireland

Folate concentrations in foods are usually expressed in terms of "free" and "total" folate because of the difficulty in measuring the individual monoglutamate and polyglutamates. Typically, food folates are extracted by boiling or autoclaving in the presence of ascorbate, subjected to a deconjugation treatment using human plasma (HP), hog kidney (HK) or chicken pancreas (CP) conjugase, and measured by microbiological or radioimmuno-assays. However, the present lack of harmonization in methodology between laboratories could lead to inaccuracies in the compilation of food folate composition data (Finglas *et al.* 1993). The present study focused on factors which can influence the measurement of food folate concentrations, including their thermal and pH-stability, the influence of conjugase type on polyglutamate deconjugation, and the role of the calibrant in the microbiological assay.

The thermal stability of folic acid (FA), folinic acid (5-CHO THFA), 5-methyl tetrahydrofolic acid (5-CH_3 THFA), 7,8 dihydrofolic acid (DHFA) and 5,6,7,8 tetrahydrofolic acid (THFA) was determined by incubation in Universal buffer, pH 4.0-9.0 at 37° and 70° for a period of 4 h. After 4 h at 37°, the concentrations of all forms were greater than 80% of their initial values. However, at 70°, the concentrations of FA and 5-CHO THFA remaining at pH 4.0-6.0 were slightly lower (about 80% of initial values). The stability of 5-CH_3 THFA, DHFA and THFA was markedly reduced at higher pH (> 7.0), even in the presence of ascorbic acid (0.5 g/l). The concentrations of these compounds remaining after 4 h were less than 30% of their initial values.

Free and polyglutamyl folate concentrations determined in a variety of foods are shown in the Table. Polyglutamyl folate concentrations of marmite, brewers yeast, cabbage and orange juice differed significantly depending on the source of conjugase.

Food	Polyglutamyl folate (μg/g)						Free folate (μg/g)		
	HP (*n* 6)		HK (*n* 6)		CP (*n* 6)		HP (*n* 2)	HK (*n* 2)	CP (*n* 2)
	Mean	SE	Mean	SE	Mean	SE	Mean	Mean	Mean
Marmite	7.27a	1.18	9.86a	1.66	5.81b	1.10	14.6	21.4	16.0
Brewers yeast	3.10a	0.25	8.87b	1.54	3.76c	0.18	0.67	1.20	0.75
Cabbage	0.21a	0.04	0.44b	0.05	0.54b	0.10	0.06	0.05	0.13
Orange juice	0.12a	0.02	0.01b	0.01	0.05c	0.01	0.07	0.16	0.03
Green beans	0.08	0.03	0.10	0.04	0.07	0.02	0.28	0.45	0.30
Beef liver	0.43	0.08	0.78	0.19	0.50	0.10	1.70	3.30	1.85

a,b,c Mean values within a row with unlike superscripts were significantly different, $P < 0.05$.

The growth response of *L. rhamnosus* to 5-CHO THFA, 5-CH_3 THFA, DHFA and THFA at concentrations typical of those used in the microbiological assay (0-1.4 ng /4 ml medium) was 58.2, 43.6, 26.1 and 14.9% respectively, of the response to FA. This represents a serious limitation of the microbiological assay when samples contain complex mixtures of folates. The results indicate that careful optimization of extraction pH, temperature and time, conjugase type and method of standard-curve calibration are needed in order to generate reliable food folate data.

Finglas, P.M., Fuare, U. & Southgate, D.A.T. (1993). Food Chemistry **46**, 199-213.

Supplies of antioxidant nutrients across western Europe are strongly and inversely related to heart disease (CHD) death rates. By M. C. BELLIZZI, M. F. FRANKLIN, G. G. DUTHIE and W.P.T. JAMES, *Division of Biochemical Sciences, Rowett Research Institute, Aberdeen AB2 9SB*

Increasing experimental and clinical evidence suggests that antioxidant nutrients limit the processes involved in the pathogenesis of CHD (Steinberg, 1991). Moreover, several epidemiological studies have reported significant inverse associations between circulatory diseases and plasma concentrations or intakes of antioxidant nutrients such as vitamin E, vitamin C and β-carotene (Hennekens & Gaziano, 1993).

We used food disappearance data compiled by the Food and Agriculture Organisation to calculate supplies of the vitamin E homologues, vitamin C and β-carotene in nineteen European and five other developed countries for the periods 1975-77, 1980-82 and 1985-87 and related them to data from the World Health Organisation on premature mortality from CHD in men below 65 years during 1985-87. The nutrient supplies were calculated using the German, US and British food composition tables with adjustments for the inedible portions. Correlations between CHD death rates for 1985-87 and nutrient and food supplies in western Europe (Table) for 1985-87 did not differ appreciably from those calculated for the other 3-year supply periods.

Antioxidant	Correlation coefficient	Antioxidant-rich foods	Correlation coefficient
Total vitamin E	-0.386	Vegetables	-0.653
α-Tocopherol	-0.753	Vegetable oils	-0.623
γ-Tocopherol	-0.001	Sunflower-seed oil	-0.585
Vitamin C	-0.609	Fruits	-0.337
β-Carotene	-0.495	Nuts and seeds	-0.481

For reference probability levels for the correlation coefficients: $P = 0.05$, r 0.48; $P = 0.01$, r 0.61; $P = 0.001$, r 0.72.

Total vitamin E supply was inversely associated with CHD across western Europe. The relationship was much stronger with the α-homologue, but weaker with the other vitamin E homologues. In most of the countries, more than half of the dietary α-tocopherol is obtained from vegetable oils; sunflower-seed oil being the major source. Vitamin C and β-carotene were also inversely related to CHD. Supplies of the main food sources of these antioxidants gave strong to moderate inverse associations.

When α-tocopherol, vitamin C and β-carotene supplies from 1970 to 1987 were related longitudinally with the CHD death rates for the corresponding years, then out of the twenty-four countries twenty, fourteen and eighteen respectively displayed inverse trends. These conclusions, which supported the cross-country correlations, were only marginally affected by the introduction of lag periods between supplies and CHD.

The associations between CHD and the food and nutrient supplies provide evidence consistent with a protective effect of nutritional antioxidants. The analyses highlight the importance of distinguishing between the different vitamin E homologues.

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Plasma retinol, α-tocopherol and carotenoids in the cystic fibrosis population at Cork Regional Hospital. By C. O'SULLIVAN CORBETT[1], C. SHORTT[2], A.M. FANNING[2], P.J.A. SHEEHY[1], J.B. WATSON[2] and P.A. MORRISSEY[1], [1]Department of Nutrition, University College Cork and [2]Cork Regional Hospital, Cork, Republic of Ireland.

Plasma retinol, α–tocopherol and carotenoids (Congden *et al* 1981), are below normal levels in cystic fibrosis (CF). A short study of ten CF patients at Cork Regional Hospital was performed by Pierce (1990) and low plasma retinol, α–tocopherol and total carotenoids were found in all ten patients.

In the present study plasma retinol, α-tocopherol and six carotenoids were measured in seventy five of the 106 CF patients at Cork Regional Hospital. These were compared with twenty controls matched for age and sex. These results are shown in the Table below.

	CF group plasma (μmol/L)		Control group plasma (μmol/L)		
	Mean	SD	Mean	SD	Significance
Nutrient					*P* value
Retinol	1.155	0.461	1.407	0.545	$p<0.05$
α-Tocopherol	15.33	7.919	21.21	5.691	$p<0.01$
Lutein	0.053	0.046	0.250	0.121	$p<0.001$
Zeaxanthin	0.035	0.047	0.138	0.077	$p<0.001$
β-Cryptoxanthin	0.021	0.023	0.271	0.102	$p<0.001$
Lycopene	0.026	0.055	0.158	0.122	$p<0.001$
α-Carotene	0.004	0.013	0.060	0.046	$p<0.001$
β-Carotene	0.039	0.051	0.528	0.321	$p<0.001$
Total carotenoids	0.178	0.133	1.405	0.423	$p<0.001$
E/Lipid (molar ratio)	0.003	0.002	0.004	0.001	N S

N S, not significant.

Although variation in plasma values for the above nutrients was high, the CF patients had significantly lower plasma retinol, α-tocopherol and carotenoids than controls. Because plasma lipids passively influence plasma tocopherol levels, plasma tocopherol was also expressed relative to lipid. Vitamin E: lipid (cholesterol, plus triacylglycerols) molar ratio was used.The CF group ratio was not significantly different from the control.group. This reflects the significantly lower plasma cholesterol in the CF group when compared with the controls. All CF patients are routinely supplemented with vitamin A and vitamin E. However, non-compliance is common and may account for lower plasma retinol levels. The patients are not presently receiving β–carotene supplements but are advised to eat a "normal" high-energy diet which should provide adequate β–carotene in the healthy population. This, however, does not appear to be adequate in CF.

The support of the Cystic Fibrosis Association and Cow & Gate Nutricia Ireland is gratefully acknoledged.

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The correlation between intake of lutein, lycopene and β-carotene from vegetables and fruits and concentrations in blood plasma. By K.J. SCOTT[1], D. I. THURNHAM[2], D. J. HART[1] and S. BINGHAM[3]. [1]AFRC Institute of Food Research, Norwich NR4 7UA, [2]University of Ulster, Coleraine BT52 1SA and [3]MRC Dunn Clinical Nutrition Centre Cambridge CB2 2DH

Epidemiological studies have demonstrated an inverse relationship between the intake of fruits and vegetables and the incidence of certain cancers (Block *et al* 1992). Antioxidant vitamins including the carotenoids may have a critical role in the prevention of oxidative processes involved in the induction of disease states.

The present study investigated whether there was an association between the intake of carotenoids from fruits and vegetables and their concentration in blood plasma. Seasonal weighed (4 d) intake and blood levels were measured in thirty-two women aged between 50 and 65 years of age. Additional data was available for another thirty four women which did not coincide with all four seasonal time points.

There were large inter-and intra-individual variations in dietary and plasma carotenoid levels. The significance of the diet-blood correlation was dependent on season. For the thirty-two subjects the correlation of mean daily intake with mean plasma levels was significant for lutein (r 0.670) and lycopene (r 0.572) but not for β-carotene. With sixty-six subjects there were similar correlations for lutein and lycopene, however there was also a correlation (albeit weaker than for lutein and lycopene) for β-carotene (r 0.344).

With thirty-two subjects there was an inverse correlation between body mass index (BMI) and β-carotene plasma concentrations (r -0.484) but not with intake. There was no correlation between BMI and lutein or lycopene intake or plasma levels.

It is suggested that plasma lutein may be a useful biological indicator of vegetable intake.

Correlation of carotenoid intake with plasma carotenoids (n 32)

Season		Winter	Spring	Summer	Autumn	Mean*	All Data†
Lutein	*P*	0.142	0.001	0.008	0.004	<0.001	0.001
	r		(0.520)	(0.429)	(0.468)	(0.670)	(0.282)
Lycopene	*P*	0.029	0.035	0.415	0.001	<0.001	<0.001
	r	(0.348)	(0.333)		(0.559)	(0.572)	(0.424)
β-carotene	*P*	0.303	0.025	0.088	0.642	0.135	0.040
	r		(0.359)				(0.158)

* Mean daily intake, mean plasma concentration of each subject.
† All individual intake and blood data from four seasons.

Mean plasma concentrations of lutein, lycopene and β-carotene of eight smokers were respectively 7,19, and 19% lower than those of forty-one non-smokers; corresponding levels of intake of these carotenoids were 23,32 and 7% lower. Average daily total fat intake was 15% higher in smokers.

The intake data were derived from a study to evaluate methods of assessing food intake (S. Bingham, MRC Cambridge).

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Antioxidant activity of astaxanthin in chicken embryo fibroblasts. By S.M. LAWLOR and N.M. O'BRIEN, Department of Nutrition, University College Cork, Republic of Ireland

Recent epidemiological studies in humans have suggested that high fruit and vegetable intakes and elevated serum β-carotene aid in the prevention of certain types of cancer and some chronic diseases (Ziegler, 1991). Most studies have focused on β-carotene as the active micronutrient with little regard for the possible role of other carotenoids. Astaxanthin, found primarily in crustaceans, is known to have very good antioxidant properties in *in vitro* systems including rat liver microsomes (Palozza & Krinsky, 1992). Little work has been done on the antioxidant properties of astaxanthin in cellular model systems. In the present study the ability of astaxanthin to protect against oxidative stress in a cell culture model was assessed.

Primary cultures of chicken embryo fibroblasts (CEF) were cultured in an air-CO_2 (95:5, v/v) atmosphere at 37° in HAM's F10 medium. The cells were oxidatively stressed by exposure to paraquat (PQ; 0.25 mM). Two experimental procedures were followed. In the first procedure, (shown in Table) the incubation time with PQ and astaxanthin (0.1 - 10 nM) was 18 h. With the second procedure the CEF were grown in astaxanthin-enriched media (0.1 - 10 nM) and subsequently exposed to PQ for 18 h. Activities of the antioxidant enzymes superoxide dismutase (SOD; *EC* 1.15.1.1)), catalase (CAT; *EC* 1.11.1.6) and glutathione peroxidase (GSH-Px; *EC* 1.11.1.9) were measured as indices of oxidative stress.

	SOD (U/mg protein)		CAT (U/mg protein)		GSH-Px (U/mg protein)	
Astaxanthin (nM)†	Mean	SE	Mean	SE	Mean	SE
Control‡	6.19*	1.05	11.61*	0.68	14.09*	0.95
0	13.80	0.72	23.25	1.52	6.83	0.25
0.1	10.67*	0.46	15.31*	0.94	12.70*	0.57
0.5	11.04*	0.44	18.64*	0.92	-	-
1.0	11.45*	0.49	10.99*	0.93	15.36*	0.81
10.0	9.25*	0.52	15.14*	0.82	15.19*	0.72

*Significantly different from PQ-treated cells with no astaxanthin: $P<0.05$ (unpaired t test)
†Cells were incubated with PQ and astaxanthin for 18 h.
‡Control cells containing no PQ or astaxanthin.

CEF incubated with 0.25 mM-PQ for 18 h exhibited increased SOD and CAT activities and decreased GSH-Px activity compared with control ($P<0.05$). No cytotoxicity, as indicated by lactate dehydrogenase (*EC* 1.1.1.27) release, was observed at 0.25 mM-PQ. Incorporation of astaxanthin into the PQ-treated CEF, either by incubating the cells with astaxanthin (0.1 - 10 nM) for 18 h, as shown above, or by growing the cells in astaxanthin-enriched media (0.1 - 10 nM) resulted in a decrease in both SOD and CAT activity and GSH-Px activity was returned to its control value (data not shown). Protection against PQ-induced oxidative stress was observed at all levels of astaxanthin tested. Astaxanthin, therefore, appears to be a very effective antioxidant in our model.

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DNA damage in cultured human T-lymphocytic cells as detected by the 'comet assay' : effect of increased cellular β-carotene and α-tocopherol. By S.B. ASTLEY, A.C. PINDER, and S. SOUTHON. *Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA*

DNA is susceptible to free-radical damage, including single-strand breaks, which may contribute to carcinogenesis. Single-strand breaks (a measure of DNA integrity) can be detected using alkaline electrophoresis of cells embedded in agarose. After staining with a suitable fluorescent DNA-dye, undamaged DNA is observed as a brightly fluorescent core. Strands formed by breaks move from the core, in the direction of the anode, forming an image described as a comet (Green *et al.* 1992).

The comets were digitized with a low-light-level video camera, and quantitative analysis performed by image processing software developed in-house. Head and tail components were automatically located and separated, and then quantified by a number of different approaches, including tail ratio (i.e. integrated intensity of tail: integrated intensity of head + tail) and tail moment (tail ratio x distance between centres of gravity of head and tail).

In the present study, the effect of increased β-carotene and α-tocopherol concentrations on DNA damage following treatment with H_2O_2 (5 min at 0.05 mM) was investigated in cultured human lymphocytic cells (MOLT-17). Under normal culture conditions, these cells have no detectable levels of either vitamin. Fat-soluble vitamins were introduced to the culture medium using liposomes (50 µl /ml medium) (Grolier *et al.* 1992). Cells were incubated overnight with: (1) no supplementation, (2) liposomes containing no vitamin, (3) liposomes containing α-tocopherol (3.5 µg/ml phosphate-buffered-saline (PBS)), (4) liposomes containing β-carotene (5.8 µg/ml PBS).

	Group (G)				Significance of variance ratio (F)		
Treatment (T)	1	2	3	4	G	T	G x T
- H_2O_2	0.361^{ab}	0.339^{a}	0.362^{ab}	0.394^{bc}	<0.001	<0.001	<0.001
+ H_2O_2	0.558^{d}	0.594^{d}	0.419^{c}	0.387^{abc}	<0.001	<0.001	<0.001

Pooled SEM= 0.019; LSD= 0.053 ^{abcd} values not sharing a common superscript are significantly different (P <0.05). Data (Fluorescence $_{total}$ = tail / (tail + head)) were analysed by two-way analysis of variance with variables group (G), treatment (T), and group/treatment interaction (GxT).

Preliminary data show no adverse influence of the presence of liposomes and significantly less DNA strand breaks in cells incubated with both β-carotene and α-tocopherol compared to the appropriate controls.

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UVA-light-induced oxidative stress: an investigation of potentially protective dietary constituents in an in vitro cell culture model system. By I.O'CONNOR and N.M.O'BRIEN, Department of Nutrition, University College Cork, Republic of Ireland

UV-light exposure has been implicated in skin degeneration including erythema, premature ageing and photocarcinogenesis. Oxidative damage due to UV-exposure has been proposed as the potential mechanism for UV-induced effects (Black, 1987). Many dietary constituents may exert antioxidant effects at cellular level. Little or no work has been conducted on the modulation of UV-induced skin degeneration by antioxidants. In the present study, we have developed and validated an in vitro model using controlled levels of UVA exposure to induce oxidative stress in rat kidney cells. The ability of α-tocopherol to protect against oxidative stress in our cell culture model was assessed.

Rat kidney cells were cultured in Dulbecco's modified Eagle's medium and maintained in a humidified atmosphere at 37°, 5% CO_2. The growth medium was supplemented with α-tocopherol at the indicated concentrations. Oxidative stress was induced by exposing the cells to UVA light at a dose intensity of 5.6 mW/cm^2 for a 4-h period. Lipid peroxidation, as indicated by thiobarbituric acid reactive substances (TBARS), and the activities of the antioxidant enzymes, catalase (CAT, *EC* 1.11.1.6) and superoxide dismutase (SOD, *EC* 1.15.1.1) were measured as indices of oxidative stress.

α-Tocopherol (nmol/l)	CAT (U/mg protein)		SOD (U/mg protein)		TBARS (nmol MDA/mg protein)	
	Mean	SE	Mean	SE	Mean	SE
Control †	6.28	0.18	9.55	0.87	7.88	0.94
0	3.83 *	0.97	0.83 *	0.00	12.39 *	1.43
10	4.03 *	0.18	0.97 *	0.07	11.38 *	0.34
100	4.34 *	0.25	1.67 *	0.32	8.05	0.59
500	4.81 *	0.47	8.13	0.96	7.54	1.23
1000	5.89	0.18	9.51	0.48	7.18	0.26

MDA, malondialdehyde.

* Significantly different from control cells: unpaired *t* test $P<0.05$.

†Control cells not exposed to UVA light and not supplemented with α-tocopherol.

Rat kidney cells grown in unsupplemented medium and exposed to UVA light exhibited a decrease in CAT and SOD activities ($P<0.05$) compared with control. An increase in lipid peroxidation ($P<0.05$), as indicated by increased TBARS, was also observed when cells were exposed to UVA in unsupplemented medium. No cytotoxicity, as indicated by lactate dehydrogenase (*EC* 1.1.1.27) release, was observed. Incorporation of α-tocopherol (1000 nmol/l) into UVA-exposed rat kidney cells returned CAT and SOD activities to control values. At lower levels of α-tocopherol enrichment (100 nmol/l) lipid peroxidation was decreased and returned to control level. With increasing concentrations of α-tocopherol in the supplemented media, the level of α-tocopherol in the cells increased, as determined by HPLC, indicating that α-tocopherol was incorporated into the cells. These results suggest that α-tocopherol may play a role in the protection of cells against UVA-light-induced oxidative stress.

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Children with a high milk consumption are more likely to have micronutrient intakes which meet reference nutrient intakes. By C.H.S RUXTON[1], T.R. KIRK[1], N.R. BELTON[3] and M.A.M. HOLMES[2], [1]Department of Dietetics and Nutrition and [2]Department of Management and Social Sciences, Queen Margaret College, Edinburgh EH12 8TS and [3]Department of Child Life and Health, University of Edinburgh, Edinburgh EH9 1UW

In a recent paper (Ruxton *et al.* 1993*a*), 54% of 7 to 8-year-old children had vitamin A intakes below the reference nutrient intake (RNI; Department of Health, 1991), 41% had folate intakes below RNI and 53% had iron intakes below RNI. Unpublished results from the same study have shown that the corresponding figures for selenium and zinc are 100% and 73% respectively. The present paper investigates whether drinking milk enables children to meet RNI for these micronutrients.

Dietary data on 136 7 to 8-year-olds were collected in 1990/91 using a 7 d weighed inventory. Details on methodology and the social class profile of the sample are reported elsewhere (Ruxton *et al.* 1993*b*). Data were analysed using COMP-EAT 4 and SPSS for Windows computer programs.

Milk contributed 10% of energy intake, 28% of mean daily vitamin A intake, 2% of iron intake, 11% of selenium intake, 20% of zinc intake and 11% folate intake and the mean volume of milk consumed was 2.11 litres/week. Children were classified as either low, medium or high milk drinkers. There were no significant differences in the proportion of children drinking full-fat compared with reduced-fat milks between the three groups. Intakes of vitamin A, iron, zinc, selenium and folate were expressed as a percentage of RNI and a comparison was made between the high and low milk drinking groups using a two-tailed Student's *t* test. Results are shown in the Table.

	%Reference nutrient intake			
Nutrient	Low (*n* 21)	Medium (*n* 88)	High (*n* 27)	*P*<
Vitamin A	91	103	128	0.005
Iron	92	107	115	0.01
Selenium	78	84	98	NS
Zinc	73	85	109	0.0001
Folate	92	112	134	0.001

Low, < 1 litre/week; Medium, 1 to 2.99 litres/week; High, ≥ 3 litres/week; *P* relates to difference between Low and High groups. NS, not significant.

Percentage energy from fat was 37% in both the low and medium groups and 38% in the high group but the difference was not significant. However, there were significantly higher intakes of energy, vitamin A, iron, zinc and folate in the high compared with the low milk-drinking groups. This suggests that either milk drinking is associated with a higher intake of micronutrients or that another factor such as social class or a high intake of breakfast cereals influenced the findings. This was examined further using multiple regression analysis which showed that intakes of vitamin A ($P<0.05$), zinc ($P<0.0001$) and selenium ($P<0.01$) were positively associated with the volume of milk consumed. Intakes of folate were positively associated with both the volume of milk consumed ($P<0.0001$) and a high intake of breakfast cereals ($P<0.01$) while intakes of iron ($P<0.05$) were positively associated with only a high intake of breakfast cereals. There were no significant associations between these micronutrients and social class. Thus, it is concluded that a high consumption of milk was associated with greater intakes of vitamin A, zinc and selenium in this sample of children. Percentage energy from fat was not increased when a high intake of milk was consumed.

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The effect of fortification on daily micronutrient intakes of breakfast cereal consumers in Great Britain. By H. McNULTY, J. EATON-EVANS, G. WOULAHAN and J.J. STRAIN, Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA

Recent studies have shown that the consumption of fortified breakfast cereals is associated with increased intakes of a number of micronutrients amongst British teenagers (Crawley, 1993) and in both adults and children in the Irish Republic (Sommerville & O'Reagan, 1993). It is not clear however whether the association is directly due to the fortified cereals, the milk consumed with them or the result of an overall dietary pattern in which breakfast cereal consumption is a feature. In this study, the direct effect of fortification on daily micronutrient intakes was examined in British adults aged 16-64 years (1085 males, 1108 females) by analysing the data collected in the Dietary and Nutritional Survey of British adults (DNSBA) conducted in 1986 and 1987 (Gregory et al. 1990).

The micronutrient intakes of consumers of fortified breakfast cereals, 48% of males (*n* 527) and 49% of females (*n* 543), were compared with the corresponding value which would result had these products not been fortified (Pre-Fortification). The latter values were calculated from raw material composition and actual pre-fortification published values where available from older editions of food tables (Paul & Southgate, 1978). Data were transformed where appropriate before statistical analysis (paired *t* test). The results in the table show that pre-fortification intakes (excluding supplements) were lower than actual (DNSBA) intakes for each micronutrient examined ($P<0.001$ in all cases).

	Males (*n* 527)				Females (*n* 543)			
	DNSBA		Pre-Fortification		DNSBA		Pre-Fortification	
Micronutrient	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Thiamin (mg/d)	1.91	0.54	1.59	0.43	1.40	0.37	1.17	0.31
Riboflavin (mg/d)	2.34	0.68	1.96	0.56	1.83	0.57	1.51	0.48
Niacin (mg/d)	42.4	10.2	38.4	9.4	31.1	7.8	28.1	7.1
Vitamin B6 (mg/d)	2.59	0.75	2.31	0.70	1.75	0.47	1.53	0.40
Folic acid (μg/d)	312	92	305	89	224	69	216	65
Vitamin B12 (μg/d)	7.1	5.1	6.9	5.1	5.5	4.1	5.3	4.1
Vitamin C (mg/d)	72	39	70	39	66	39	64	38
Vitamin D (μg/d)	3.68	2.42	3.18	2.34	2.77	1.63	2.34	1.61
Iron (mg/d)	15.2	5.3	13.2	3.5	12.0	4.9	10.0	2.9

These data show that, in those who ate breakfast cereals, fortification made a significant contribution to daily micronutrient intakes. Had the analysis been based on current fortification levels, the contribution of cereals and other fortified foods would undoubtedly have been far greater in the case of folic acid. The potential of fortification to make a valuable and direct contribution to micronutrient intakes demonstrated in this study may be particularly important in those on marginal diets or where intakes far in excess of those provided in the national diet are being recommended (Department of Health, 1992).

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Sommerville, J. & O'Reagan, M. (1993). *Journal of Human Nutrition and Dietetics* 6, 223-228.

Seasonal fluctuations in vitamin A status and health indicators in Gambian infants. By C.A. NORTHROP-CLEWES[1], P.G.LUNN[2] and R.M.DOWNES,[3] [1]Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA [2] MRC Dunn Nutrition Centre, Cambridge CB4 1XJ and[3]MRC Dunn Nutrition Centre, Keneba, The Gambia

Keneba, a rural farming village in the West Kiang District of The Gambia, has a rainy season peaking in August followed by little or no rain between late September and early June. The main staple crops grown in this rainy season are stored during the rest of the year resulting in seasonal fluctuations in energy intake. Even more marked than this is the seasonal intake of carotenoid-rich foods, which are available to villagers between April and June (mango season) and to a lesser extent during the rainy season (July to September) in the form of green leaves used to make sauces (Villard & Bates, 1987). Recent studies in this region of Gambia have shown that infants suffer pronounced growth faltering during the second half of their first year of life (Lunn *et al.* 1991). Their growth failure is associated with increased intestinal permeability (i.e.impaired mucosal function and integrity) and elevated levels of plasma acute-phase proteins.

Vitamin A is known to have a role in maintaining the integrity of epithelial tissues such as the gut mucosa and in the body's response to inflammatory stress. The marked seasonal fluctuation in the availability of vitamin A, as ß-carotene, has prompted a re-examination of the data to assess whether this dietary component is of importance in Gambian infants. A group of 120 infants, aged between 3 and 15 months (mean age 8.2 months) from the Keneba area were studied longitudinally at monthly intervals for 18 months, giving a total of 997 clinic visits. Anthropometric measurements and a lactulose/mannitol (L/M) intestinal permeability test of gut integrity were performed at each visit and a finger-prick sample of blood was taken (Lunn *et al.* 1991). In addition, diarrhoea morbidity data were collected by weekly home visits. The plasma was assayed for the immunoglobulins IgA, IgM and IgG and the acute-phase proteins, albumin, α-1-antitrypsin (AAT) and α-1-antichymotrypsin (ACT).

The Figure below shows several health indicators which are at or near normal values (– – –) at least between April and June and significantly different from all other months coinciding with an increased intake of ß-carotene:

Month	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
L/M					–*0.33–		–0.30–						
ACT**							–0.62–0.67–						
AAT**				–––1.33–						–––1.38–			
IgG**	–––––				10.21–				–9.37––				
IgM**	–––––––––								1.19–1.44–				

* Numbers indicate range of means during the period shown.
** g/l.

Albumin, however showed a progressive fall through the year,while IgA showed transient increases between March and August and in the latter part of the year. Weight z-scores increments declined to a nadir in August corresponding with the rainy season.

The results suggest that vitamin A status may influence some health indices but that other factors e.g. a declining energy supply associated with increased work output immediately before the rains, and increased infection rates during the rains undoubtedly also play a role in influencing infant health and growth.

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Plasma, platelet and erythrocyte α-tocopherol concentrations as static measures of vitamin E status in rats. By G.A. LYNAM, P.J.A. SHEEHY and P.A. MORRISSEY, Department of Nutrition, University College, Cork, Republic of Ireland

Accurate assessment of the relationship between vitamin E and chronic disease risk depends on the availability of reliable indicators of vitamin E status. Vitamin E status is usually determined by measuring static variables such as plasma α-tocopherol concentration or the α-tocopherol:lipid ratio of plasma. However, platelet α-tocopherol may be a better indicator of status since this measure does not vary significantly with changes in plasma lipids (Vatassery *et al.* 1983). Functional tests of peroxidative indices (e.g. erythrocyte haemolysis, erythrocyte malondialdehyde or hydrocarbon exhalation) can be criticized on the grounds that factors other than α-tocopherol also influence the peroxidative process. The objective of this study was to determine the relative merits of plasma, platelet and erythrocyte α-tocopherol concentrations as static indicators of the α-tocopherol concentrations of other tissues.

Fifty-four male weanling Sprague-Dawley rats (six per group) were placed in individual cages and fed on a basal vitamin E-deficient diet supplemented with either 0, 20, 50 or 100 mg *Dl*-α-tocopheryl acetate/kg. After 1 or 2 months, rats were fasted overnight and anaesthetized with urethane. Blood was drawn from the heart into heparinized tubes and plasma, platelets and erythrocytes were isolated. Tissues were frozen in liquid nitrogen and stored at -20°. α-Tocopherol concentrations were determined by HPLC. The correlation coefficients (r) between α-tocopherol concentrations of diets, blood components and tissues are shown in the Table.

	Dietary α-tocopherol				Plasma α-tocopherol				Platelet α-tocopherol			
	Month 1		Month 2		Month 1		Month 2		Month 1		Month 2	
Tissue	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Plasma	0.89	<0.01	0.67	<0.01	--	--	--	--	0.62	<0.01	0.48	0.05
Platelets	0.57	0.01	0.36	0.10	0.62	<0.01	0.48	0.05	--	--	--	--
Erythrocyte	0.72	<0.01	0.10	0.67	0.57	<0.01	0.13	0.60	0.76	<0.01	0.24	0.13
Liver	0.75	<0.01	0.82	<0.01	0.72	<0.01	0.76	<0.01	0.63	<0.01	0.53	0.03
Lung	0.89	<0.01	0.72	<0.01	0.84	<0.01	0.83	<0.01	0.42	0.08	0.60	<0.01
Heart	0.92	<0.01	0.89	<0.01	0.87	<0.01	0.60	0.02	0.77	<0.01	0.37	0.11
Brain	0.93	<0.01	0.81	<0.01	0.86	<0.01	0.35	0.14	0.69	<0.01	0.14	0.57
Muscle	0.84	<0.01	0.68	<0.01	0.74	<0.01	0.70	<0.01	0.68	<0.01	0.34	0.13
Adipose	0.81	<0.01	0.38	0.17	0.85	<0.01	0.21	0.52	0.33	0.17	0.06	0.85

At 1 month, both diet and plasma α-tocopherol concentrations were strongly correlated to those of platelets, erythrocytes, and other tissues. Platelet α-tocopherol concentrations were strongly related to those of erythrocytes, liver, heart, brain and muscle. After 2 months, diet and plasma α-tocopherol concentrations were still good predictors of α-tocopherol concentrations in other tissues. However, platelet α-tocopherol was less useful as an α-tocopherol status indicator at this point. Erythrocyte α-tocopherol concentrations were good indicators of tissue α-tocopherol levels at 1 month ($0.52 \leq r \leq 0.76$), but not at 2 months ($0.06 \leq r \leq 0.74$) (data not shown).

Vatassery, G.T., Krezowski, A.M. & Eckfeldt, J.H. (1983). American Journal of Clinical Nutrition 37, 1020-1024.

Relationships between adipose tissue and plasma antioxidant nutrients in Scottish men. By S. McGRATH[1], S. HIRREL[1], D.I. THURNHAM[1], K. CARRUTHERS[2] and R.A. RIEMERSMA[2]. [1]Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA and [2]Cardiovascular Research Unit, University of Edinburgh EH8 9XF.

There is considerable interest in the possibility that low levels of antioxidants predispose to coronary heart disease. Low levels of plasma α-tocopherol were related to increased risk of new angina in Scottish men (Riemersma *et al.* 1991) but a recent multi-centre case-control study (Kardinaal *et al.* 1993) showed no association between α-tocopherol levels in adipose tissue and the risk of acute myocardial infarction. The determinants of the amount of α-tocopherol and other fat-soluble antioxidants in adipose tissue are not clear. We measured antioxidant levels in plasma (fasting) by HPLC (Thurnham *et al.* 1988) and in subcutaneous fat biopsies in a random population sample of 100 apparantly-healthy Edinburgh men (<65 yr). Measurements on the first 54 biopsies were made using the method followed in the study of Kardinaal *et al.* (1993) but the remaining 46 samples were analysed by a modified method in which the saponified lipid extract was extracted twice using isopropyl ether and the internal standard was increased 10-fold to improve precision in measuring recovery. Medians and centiles (25, 75) for the plasma and biopsy concentrations were calculated for micronutrients measured by the two methods and Pearson correlation coefficients (*r*) are shown in the Table.

	Original method				Modified method			
	n	Plasma (μmol/l)	Adipose tissue* (μmol/g)	r	n	Plasma (μmol/l)	Adipose tissue* (μmol/g)	r
α-Tocopherol	54	32.47 29.05-35.07	108.51 75.4-149.3	0.055	46	30.08 25.14-37.00	270.72 160.8-355.5	0.177
τ-Tocopherol	52	2.25 1.78-2.73	14.93 9.69-20.91	0.203	46	2.49 1.46-3.02	36.46 21.02-52.53	0.204
Lutein	44	0.283 0.231-0.357	0.615 0.455-0.791	0.120	46	0.279 0.194-0.339	1.887 1.388-2.888	0.377**
α-Cryptoxanthin	47	0.073 0.049-0.113	0.100 0.050-0.211	0.122	46	0.058 0.036-0.093	0.418 0.278-0.658	0.198
ß-Cryptoxanthin	47	0.115 0.078-0.244	0.140 0.068-0.230	0.046	46	0.091 0.055-0.191	0.595 0.337-0.900	0.224
Lycopene	53	0.688 0.425-0.909	0.199 0.119-0.291	0.003	46	0.602 0.361-0.888	0.713 0.438-1.132	0.246
α-Carotene	53	0.114 0.062-0.190	0.055 0.055-0.190	0.037	46	0.086 0.043-0.133	0.308 0.245-0.467	0.212
ß-Carotene	54	0.319 0.233-0.595	0.175 0.175-0.538	-0.060	46	0.268 0.197-0.460	0.634 0.363-1.349	0.359**

*Concentrations extracted by modified method greater than original method $P<0.001$ (Mann-Whitney 'U' test)
**$P<0.02$ (Pearson correlation coefficients)

There were no significant differences between the concentrations of the plasma micronutrients in the two groups of subjects but the adipose tissue levels were two to three times higher when extracted by the modifed method. In addition *r* values for plasma and tissue correlations were all higher for the modified than the original method (except τ-tocopherol) and reached significance for lutein and ß-carotene ($P<0.02$).

These preliminary results suggest that carotene or vitamin E concentrations in biopsy tissue may offer no advantages over plasma as markers of status.

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Thurnham, D.I., Smith, E. & Flora, P.S. (1988). Clinical Chemistry **34**; 377-381.

Stability of fat-soluble vitamins in whole blood. By U.J.McLOONE[1], L.M.EDMOND[1], S.OAKES[2], C.GREENWOOD[2] and D.I.THURNHAM[1], [1]Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA and [2]Institute of Public Health, Robinson Way, Cambridge CB2 2SR

The European Prospective Investigation into Cancer (EPIC) aims to identify the factors that help protect against cancer and cardiovascular disease. It is hoped to recruit a total of 250000 people from seven collaborating European states. At the time of recruitment blood samples will be taken and lifestyle questionnaires will be completed. To ensure that samples collected at different centres were not being altered by small differences in times of collection, a study was done to compare blood vitamin measurements on samples processed by HPLC (Thurnham *et al.* 1988) at 2, 6 and 24 hours post collection. 60 mls of blood from thirty-one subjects (fourteen male, seventeen female) was collected. Citrated blood (3x10 ml) was kept at room temperature for 20 minutes and then placed at 4°. Clotted blood (3x10 ml) was stored at room temperature for 2 hours when a 10 ml sample of both bloods was processed to give plasma and serum, loaded into straws (0.05 ml) and stored at -70° prior to transferring to liquid nitrogen. The remaining blood was then stored at 4° for later processing for 6 or 24 hours as above.

	2 h Mean (SD) (min-max)	6 h Mean (SD) (min-max)	24 h Mean (SD) (min-max)
N	31	27	31
Retinol (μmol/l)	1.48 (0.30) (0.97-2.03)	1.40 (0.30) (0.94-2.00)	1.42 (0.31) (0.90-2.06)
α- Tocopherol (μmol/l)	22.16 (5.25) (14.59-40.48)	21.48 (5.56) (13.69-40.36)	21.49 (5.28) (15.24-40.91)
Lutein (μmol/l)	0.22 (0.07) (0.12-0.39)	0.20 (0.06) (0.08-0.39)	0.21 (0.07) (0.09-0.39)
β-Cryptoxanthin (μmol/l)	0.17 (0.12) (0.02-0.44)	0.16 (0.11) (0.01-0.34)	0.16 (0.11) (0.01-0.37)
Lycopene (μmol/l)	0.52 (0.31) (0.05-1.31)	0.50 (0.31) (0.04-1.39)	0.49 (0.29) (0.06-1.16)
β-Carotene (μmol/l)	0.44 (0.27) (0.14-1.27)	0.46 (0.31) (0.14-1.43)	0.44 (0.30) (0.14-1.42)

Arithmetic means and standard deviations (SD) are shown in the Table. No significant differences between any of the groups for respective nutrients were observed. Furthermore Pearson's correlations between results obtained for 2 and 6, 2 and 24 and 6 and 24 hours showed correlation coefficients >0.9 (P<0.001) for all values listed. In addition to the data shown γ-tocopherol and the peak eluting before β-cryptoxanthin were measured. γ-Tocopherol behaves in all aspects similarly to α-tocopherol. However the pre-cryptoxanthin peak was more variable and when correlated at the three time points, coefficients varying between 0.7 and 0.9 were obtained.

In conclusion samples stored up to 24 hours at 4° appear to show no deterioration in the major plasma carotenoids, γ-tocopherol, α-tocopherol and retinol.

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Thurnham, D.I., Smith, E., & Flora, P.S. (1988). Clinical Chemistry **34**: 377-381.

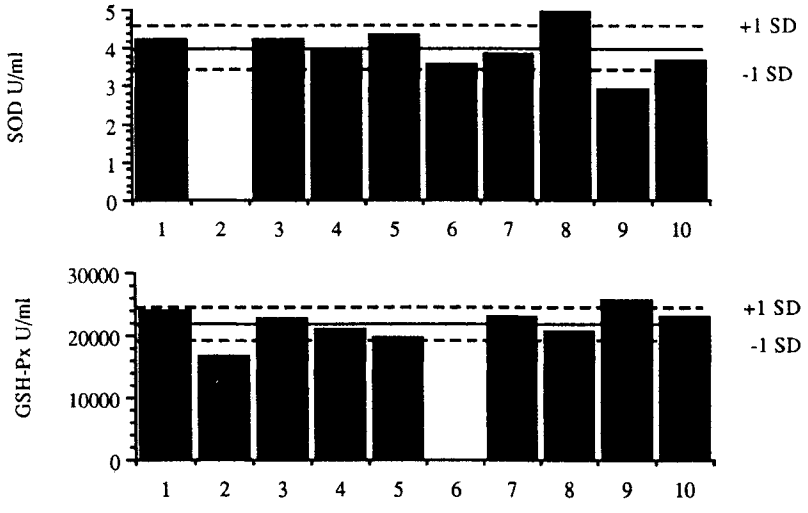
EC-FLAIR 'reference assays' for whole-blood glutathione peroxidase (GSH-Px) and erythrocyte superoxide dismutase (SOD): results of an inter-laboratory trial. By EC-Food Linked Agro-Industrial Research (EC-FLAIR) Concerted Action No. 10; Rapporteurs, J. L. BELSTEN & A. J. A. WRIGHT, Institute of Food Research, Norwich NR4 7UA

Both laboratories and government agencies would find it useful to collate and interpret information on erythrocyte Cu-SOD (EC 1.15.1.1.) and whole-blood Se-GSH-Px (EC 1.11.1.9.) activity not only as indirect methods for assessing functional Cu and Se status but also for assessing antioxidant activity *per se*. However, a major concern is the lack of comparability between assays.

A wide range of SOD and GSH-Px assays have been proposed and employed. SOD activity varies between each unique assay system depending on both the chosen electron donor and detector reaction, and on the chemical concentration of assay components. GSH-Px assays have been modified by varying the buffer, pH and temperature of reaction, and, since the enzyme cannot be assayed at saturating substrate concentrations, a slight variation in the concentration of any assay component is reflected in changed activity. After preliminary trials, using 'in-house' procedures, demonstrated between ten-fold (GSH-Px) and thousand-fold (SOD) differences in assay results, ten laboratories agreed to assay (5 replicates) commercially available samples for SOD and GSH-Px (Ransod® Calibration & Ransel® Control samples; Randox Laboratories, Crumlin, Co. Antrim, U.K.)

Using a commercially available assay kit (Ransod®, Cat. No. SD 125; Randox Laboratories, Crumlin, Co. Antrim, U.K.) as a FLAIR SOD 'reference' assay, the coefficient of variation between the ten participating laboratories (1-10 as shown below) was 14 % and 'within-assay' variation was 6 %. Likewise, using a FLAIR GSH-PX 'reference' assay procedure based upon the enzyme-linked procedure of Paglia & Valentine (1967), but modified by the addition of dithiothreitol for enzyme stabilization (Prohaska *et al.* 1977) and the use of *t* -butyl hydroperoxide as the peroxide source (Carmagnol *et al.* 1983), the coefficient of 'between-assay' variation between laboratories was 12.2 % and the coefficient of 'within-assay' (repeatability) variation was 2.7 %.

Both the FLAIR SOD and the FLAIR GSH-Px procedures are already in use in leading European laboratories where information on a 'standardized reference population' is currently being collected.



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Riboflavin depletion enhances the transit of enterocytes along the villi. By E.A. WILLIAMS, R.D.E. RUMSEY and H.J. POWERS. *University Department of Paediatrics, Sheffield Children's Hospital and Department of Biomedical Science, University of Sheffield, S10 2TH*

In an attempt to explain the disturbances in iron metabolism seen in riboflavin deficiency (Powers *et al.* 1993) we have concentrated on the structural changes in the small intestine of riboflavin deficient (RD) rats.

Weanling Wistar rats fed on a diet depleted in riboflavin were matched by weight to a control group fed on a complete diet. Blood samples were collected regularly and used to monitor riboflavin status throughout the 8-week experiment. Seventeen hours prior to sacrifice the rats were given an intraperitoneal injection of Bromodeoxyuridine (50mg/kg). Bromodeoxyuridine, a thymidine analogue, is incorporated into the DNA of replicating cells and can be detected using a monoclonal antibody staining technique. Using this technique we measured the rate of transit of labelled cells from the proliferating compartment onto and along the villi.

There was found to be a significant increase in the rate of transit of enterocytes along the villi of the RD group compared with the weight matched (WM) group ($P<0.001$). There was also a significant increase in the length of the villi in the RD group ($P<0.001$), which confirms what has previously been reported (Williams *et al.* 1993). Despite the increased length of villi, the cohort of newly replicated cells had covered a significantly greater percentage of the villi length in the RD group than the WM group.

Variable	Riboflavin deficient (*n* 12)		Weight matched (*n* 12)	
	Mean	SEM	Mean	SEM
Villus height (μm)	557.1	8.38	468.4***	9.97
Leading edge (μm)†	122.2	9.20	59.3***	4.43
Rate(μm/h)‡	7.2	0.54	3.5***	0.25
% Transit §	22	1.7	12***	0.9

*** Significantly different from RD (Mann Whitney U) $P<0.001$.

† Distance of the leading edge (ie the cohort of cells furthest from the crypt:villus junction) from the base of the villus.

‡ Rate of transit of the leading edge (ie distance travelled from stem cell/time).

§ Distance travelled by the leading edge as a percentage of the total villus height.

The 2-fold increase in the rate of transit of enterocytes along the villi may account for the increased rate of iron loss seen in riboflavin depletion. We propose that an increased rate of transit of cells leads to a functionally immature population of enterocytes on the villi.

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Influence of dietary long-chain *n*-3 fatty acids with and without additional vitamin E on aortic fatty streak formation. By LUCIE POLLARD and T.A.B. SANDERS, *Department of Nutrition and Dietetics, King's College, University of London, Campden Hill Road, London W8 7AH*

We previously reported that long chain *n*-3 fatty acids increase the requirement for vitamin E in rats (Pollard & Sanders, 1993) and that they increase the susceptibility of human low density lipoprotein (LDL) to oxidation *in vitro* (Pollard *et al*, 1994). LDL oxidation is believed to lead to conversion of macrophages into foam cells which constitute fatty streaks in arteries. The present study was designed to test whether aortic streak formation was increased by *n*-3 fatty acids in hamsters and whether this effect could be prevented by additional vitamin E. Male Golden Syrian hamsters were fed on three semi-synthetic diets which contained in g/kg: casein 200, fat 200, sucrose 100, starch 387, mineral mix 40, vitamin mix 20, cellulose powder 50, methionine 2, cholesterol 1. The *n*-3 diets both contained 8.3 g 20:5*n*-3 and 6.0 g 22:6*n*-3 which was replaced by 18:1*n*-9 in the control diet. The control diet contained 0.4 mg vitamin E/g polyunsaturated fatty acids (PUFA) and the *n*-3 diets contained either 0.4 mg vitamin E/g PUFA or 3.2 mg vitamin E/g PUFA. After 17 weeks on the diets blood was collected by cardiac puncture for the determination of LDL cholesterol and vitamin E and the aorta was dissected, stained with Oil Red O for lipid deposits, and examined by light microscopy.

	Control			*n*-3 low E			*n*-3 high E		
	n	Mean	SE	*n*	Mean	SE	*n*	Mean	SE
% Aorta covered	16	4[a]	1.85	14	16[b]	6.5	15	7	0.9
LDL cholesterol (mmol/l)	9	0.45[a]	0.07	7	1.19[b]	0.31	9	1.12[b]	0.11
LDL α-tocopherol:cholesterol	9	13.6[a]	1.91	6	6.3[b]	1.34	9	7.3[b]	0.79

[a,b] Values within a row with different superscripts are significantly different $P<0.05$.

Plasma LDL cholesterol concentrations were elevated by both *n*-3 diets compared with the control diet. Plasma α-tocopherol concentrations were greater in the vitamin E- supplemented group but the LDL α-tocopherol:cholesterol ratio was lower in the animals fed on the *n*-3 diets regardless of vitamin E intake. The proportion of aorta covered with lipid deposits was significantly higher in the animals fed on the *n*-3 fatty acids without additional vitamin E compared with the controls, however this was not so in the animals fed on the *n*-3 fatty acids with additional vitamin E. These results show that *n*-3 fatty acids in the hamster increase LDL cholesterol and in the absence of additional vitamin E increase fatty streak formation in the thoracic aorta.

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Increased postprandial lipaemia with a diet rich in oleic acid is decreased by *n*-3 fatty acids in healthy young men. By FRANCESCA OAKLEY[1], T.A.B. SANDERS[1], D. CROOK[2], M.F. OLIVER[2] and G.J. MILLER[3] , *[1]Department of Nutrition and Dietetics, King's College, University of London, Campden Hill Road, London W8 7AH, [2]The Wynn Institute for Metabolic Research, 21 Wellington Road, London NW8 9SQ and [3]MRC Epidemiology and Medical Care Unit, Wolfson Institute of Preventive Medicine, Medical College of St Bartholomew's Hospital, Charterhouse Square, London EC1M 6BQ*

There is a school of thought that believes that diets low in saturated fatty acids and rich in oleic acid are beneficial with regard to risk of cardiovascular disease. However, it is uncertain what influence such diets have on postprandial lipaemia which has been linked to the activation of clotting factor VII, a potent risk factor for fatal coronary heart disease (Meade *et al.*1993). Sanders *et al.* (1989) showed that olive oil led to a greater degree of postprandial lipaemia compared with fish oil containing *n*-3 fatty acids. We report a study of postprandial lipaemia in nine healthy male volunteers who received three isocaloric diets in random order for 3 week periods in a metabolic unit. Two diets high in oleic acid with an additional 2% energy supplied by linoleic or *n*-3 fatty acids (20:5*n*-3 and 22:6*n*-3) were compared with a diet containing a high proportion of saturated fatty acids. When analysed, the diets were shown to contain approximately 30% energy as fat and the same proportions of linolenic acid and *trans* fatty acids. The percentage of energy derived from saturated, monounsaturated, *n*-6 polyunsaturated fatty acids and *n*-3 polyunsaturated fatty acids were as follows: the high- saturated fat diet 15.0, 10.7, 3.4 and 0.4; the high- mono + linoleic diet 8.5, 15.7, 5.0 and 0.4; high- mono + *n*-3 diet 8.5, 15.5, 3.4 and 2.4. At the end of each period a fasting blood sample was obtained and the subjects were given a test meal providing 85 g fat of a composition similar to that in the experimental diet and further blood samples were obtained at 1,2,4 and 6 h after the test meal. The plasma triacylglycerol concentrations in mmol/l were as follows:

	High- saturated		High- mono + linoleic		High- mono + *n*-3	
	Mean	SE	Mean	SE	Mean	SE
Fasting	0.92[a]	0.084	0.91[a]	0.101	0.70[b]	0.086
1 h	1.57[a]	0.163	2.00[b]	0.238	1.40[a]	0.104
2 h	1.90[a]	0.161	2.69[b]	0.400	1.64[c]	0.174
4 h	1.73	0.215	2.57[a]	0.570	1.36[b]	0.195
6 h	1.33[a]	0.097	1.39[a]	0.310	0.74[b]	0.090

[a,b,c] Values with different superscripts in the same row are significantly different, $P<0.05$.

The high-mono + *n*-3 diet resulted in lower fasting triacylglycerol concentrations and less postprandial lipaemia than the other two diets. The high-mono + linoleic diet led to increased postprandial lipaemia compared with the saturated fat diet. Our results show that diets rich in oleic acid increase postprandial lipaemia compared with diets rich in saturated fatty acids but that this can be abrogated by an increased intake of $C_{20\text{-}22}$ *n*-3 fatty acids.

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The effect of low dose fish-oil supplementation on cytokines and leucocyte function in healthy female volunteers. By J.M.W. WALLACE, E. TURLEY, W.S. GILMORE and J.J. STRAIN, *Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA*

Epidemiological studies have suggested that increased dietary fish is correlated with a reduced risk of coronary heart disease and consequently many studies have centred on the effects of fish and fish-oil on vascular composition and function (Goodnight, 1993). Diets enriched with fish-oil reduce the production of tumor necrosis factor (TNF) and interleukin-1 (IL-1) by stimulated human monocytes (Endress *et al*. 1989). Fish-oil supplementation has also been shown to reduce neutrophil chemotaxis, a measure of leucocyte activation (Loustarinen *et al.* 1992). In the present study we have measured the effect of low dose fish-oil supplementation on plasma platelet-derived growth factor (PDGF) concentration. We have also assessed polymorphonuclear neutrophil (PMN) activation by measuring intracellular and extracellular peroxidase (*EC* 1.11.1.7) levels.

Thirty-three healthy female volunteers (aged 18-28 years) were recruited to take part in the double blind study. For 4 weeks the subjects added 2.4g encapsulated fish-oil, either with (*n* 16) or without (*n* 17) vitamin E, (kindly provided by Seven Seas) to their otherwise unchanged diets. Venous blood samples were taken before (PRE) and after (POST) the 4-week supplementation and again after a 9-week washout (WASH) period. Plasma PDGF and extracellular myeloperoxidase (MPO; *EC* 1.11.1.7) were measured using immunoassays. Intracellular mean peroxidase index (MPXI) a measure of the peroxidase staining intensity of leucocytes was measured using a Technicon haematology analyser.

	Fish oil (*n* 16)						Fish oil + vit. E (*n* 17)					
	Pre		Post		Wash		Pre		Post		Wash	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
PDGF (ng/l)	3878	366	2461**	254	2181**	222	3458	333	2481*	343	2297**	244
MPXI	-3.9	2.3	1.0**	2.0	-1.2	1.7	-5.4	1.8	-1.2***	1.7	-3.8	1.4
MPO (*ug*/l)	70.1	9.8	34.8**	3.4	39.0**	5.4	60.4	11.7	34.6*	7.4	54.9	9.4

Significantly different from PRE (Student's *t* test) * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Supplementation of the diet with both fish-oil or fish-oil with vitamin E caused a significant drop in plasma PDGF levels. This is important as PDGF is a major serum mitogen for smooth muscle cells. We observed a persistent suppression of PDGF levels as late as 9 weeks after the end of supplementation; other investigators have also reported the long persistence of biochemical changes associated with *n*-3 fatty acid supplementation (Endress *et al*. 1989). Both supplements caused a significant increase in intracellular peroxidase and a significant decrease in extracellular peroxidase, indicating that fish-oil supplementation may alter peroxidase release from leucocytes.

From this investigation it is possible to conclude that the beneficial effects of low dose fish-oil supplementation on mortality from ischaemic heart disease might be partly mediated by an effect of *n*-3 polyunsaturated fatty acids on plasma PDGF concentration and on neutrophil function.

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Eicosapentaenoic acid inhibits the expression of major histocompatibility complex (MHC) class II molecules and adhesion molecules on human monocytes *in vitro*. By D. A. HUGHES, A. C. PINDER and S. SOUTHON, Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA

Polyunsaturated fatty acid (PUFA)-rich diets are associated with suppression of the immune system and there is growing evidence that dietary PUFA supplementation, particularly fish-oil, is of value in the treatment of disorders involving an overreactive immune response, such as rheumatoid arthritis. One of the major fatty acids found in fish-oil is the *n*-3 PUFA, eicosapentaenoic acid (EPA). *In vitro* studies have shown that EPA can inhibit the proliferation of stimulated human lymphocytes (Calder & Newsholme, 1992). However, specific immune responses are initiated by the presentation of antigen to helper T-lymphocytes on the surface of an antigen-presenting cell (APC). A pre-requisite for this function of APC is the cell surface expression of MHC class II molecules, aided by the presence of adhesion molecules. It has recently been shown that EPA can inhibit the antigen-presenting function of mouse splenocytes (Fujikawa *et al.* 1992) but, to our knowledge, the effect of EPA on human APC has not been examined. In the present study the *in vitro* effects of EPA on the cell surface expression of MHC class II molecules and adhesion molecules by human blood monocytes was investigated.

Purified monocytes, obtained from ten healthy volunteers, were incubated with or without EPA (20 μg/ml) in culture medium supplemented with 5% fetal calf serum for 48 h at 37°. In addition, parallel cultures were performed in the presence of interferon-gamma (IFN-g; 400 U/ml) to stimulate upregulation of MHC class II molecule expression by the monocytes. Following incubation, the monocytes were immunofluorescently stained with monoclonal antibodies raised against the MHC class II molecules (HLA-DR, -DP and -DQ), and the adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and leucocyte function associated antigen-1 (LFA-1). Both the percentage of monocytes expressing each of these molecules and the intensity of expression of each molecule was quantified by flow cytometry.

In the presence of EPA alone there was no significant difference in the percentages of cells expressing each of the MHC class II molecules, but there was a significant reduction in the intensity of expression of HLA-DR ($P<0.025$). There was also a reduction in both the percentage of cells expressing ICAM-1 and in the intensity of expression of this molecule ($P<0.025$, $P<0.01$ respectively). In the presence of both EPA and IFN-g there was a significant reduction in the percentage of monocytes expressing HLA-DR, -DP and ICAM-1 and the intensity of expression of all three molecules was reduced, compared with monocytes cultured in the presence of IFN-g alone ($P<0.01$ in all cases).

Since it has been shown that the percentage of MHC class II-positive monocytes and the density of these molecules on the cell surface can alter the degree of immune responsiveness of an individual (Janeway *et al.* 1984), these findings suggest that EPA may depress immune reactivity by inhibiting antigen-presenting cell function. The more striking inhibition of class II molecule expression observed in the presence of IFN-g supports the possibility that fish-oil may be beneficial in the treatment of rheumatoid arthritis, a disorder associated with elevated expression of these molecules on synovial fluid monocytes.

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A comparison of iron supplementation with dietary counselling in the prevention of iron deficiency anaemia during pregnancy. By E.A. CAHILL[1], S.F. DALY[1], P.M. MATHIAS[2] and M.J. TURNER[1]. [1]Coombe Women's Hospital, Dublin 8, and [2]Dublin Institute of Technology, Kevin Street, Dublin 8, Republic of Ireland

Oral iron supplementation is frequently associated with gastrointestinal side-effects, which can result in poor compliance (Bonnar *et al.* 1969). The effect of dietary counselling in the prevention of iron-deficiency anaemia during pregnancy is not well established.

In the present study, eighty women (mean age 27 years) were entered into a randomized controlled trial at booking visit for antenatal care (7-15 weeks gestation) at the Coombe Women's Hospital. Maternal nutrient intake was assessed by the 7-d diet history method and iron status was assessed by haemoglobin (Hb) and serum ferritin (SF); mean cell volume (MCV) and mean cell haemoglobin (MCH) were also measured. The diet group (DG; n 40) received individual dietary advice and written information, while the supplement group (SG; n 40) were prescribed 'Meterfolic' (100 mg elemental Fe as ferrous fumarate and 350 μg folic acid). Iron status and treatment compliance was checked at 28 and 36 weeks gestation. Birth weight was recorded. The prevalence of gastrointestinal symptoms was assessed by a questionnaire in the early postnatal period.

Seventy-two women completed the study (DG, n 37; SG, n 35). Statistical analysis used *t* tests and chi-square tests as appropriate. Socio-economic status, marital status, anthropometry and dietary intakes at booking were similar in the two groups. Iron indices are shown in the Table below.

	Booking		28 Weeks		36 Weeks	
	DG	SG	DG	SG	DG	SG
	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD
Hb (g/l)	122 9.3	121 9.7	114 8.9	118 10.5	115[a] 8.6	121[b] 11.2
n	40	40	38	35	36	34
Hb< 105g/l	5%	7.5%	18.4%[c]	11.4%[d]	11.1%	12.1%
SF (μg/l)	31.9 26.5	37.2 24.3	10.0[a] 9.8	20.3[b] 15.2	10.0 11.9	14.4 10.4
n	40	36	36	33	33	32
SF< 12 μg/l	28%	20%	80%[c]	35%[d]	85%	50%

a,b Significantly different $P<0.01$ and c,d significantly different $P<0.05$.

Although Hb at 36 weeks was significantly higher in the SG ($P<0.01$), there was no difference between the groups in the incidence of iron-deficiency anaemia (Hb<105g/l). This reflects the known effects of iron supplementation which mask the normal physiological dilution that occurs during pregnancy (Huber *et al*, 1988). SF at 28 weeks was significantly higher in the SG ($P<0.01$), but this was no longer the case at 36 weeks. MCH was maintained in the DG during their pregnancy. There was no difference in mean birth weights. The postnatal questionnaire found the incidence of constipation to be more prevalent in the SG (46%) when compared with the DG (4%) ($P<0.01$).

In view of the frequent side effects associated with iron supplements and the potential adverse effects of high haemoglobin levels during pregnancy (Koller *et al.* 1980), our findings that dietary counselling is effective in the prevention of iron-deficiency anaemia during pregnancy suggest that a more selective approach to the practice of iron supplementation is needed.

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Milk feeding practices during the first year of life and their relationship to iron status at age 12 months. By V.E. FREEMAN[1], H.M.V. HOEY[2] and M.J. GIBNEY[1], [1] *Department of Clinical Medicine, Trinity College Medical School, St. James's Hospital, Dublin 8, Republic of Ireland and* [2]*Department of Paediatrics, Trinity College, Dublin 2, Republic of Ireland*

Iron deficiency anaemia (haemoglobin<110 g/l) is known to have serious clinical implications for the growing child (Lozoff *et al.* 1991). Inappropriate infant feeding practices increase the risk of anaemia. The iron status of Irish infants is not known. The present study assesses the iron status of ninety-two Irish infants and explores the link between iron status and milk feeding practices. The contribution of weaning foods to iron status is not included in this analysis.

A representative sample of 121 normal, clinically well infants was recruited at birth and followed longitudinally during the first year of life. Nutritional, anthropometric and socio-economic data and illnesses were recorded. At age 12 months a venous blood sample was taken from ninety-two infants and analysed for full blood count (Coulter counter, Model STKS) and serum ferritin (SF) (RIAgnost, Behring). Milk feeding practices were recorded monthly in the period from birth to 6 months, and at 9 and 12 months. Results are shown in the Table.

Age in months...	1	2	3	4	5	6	9	12
n...	114	112	113	111	111	109	110	108
Feeding method*	Number of infants							
Breast	30	20	20	17	15	14	8	6
Formula	93	94	96	93	97	96	82	48
Cow	1	3	5	10	9	8	30	77

*A number of infants were being fed more than one type of milk

Of the ninety-two infants whose iron status was assessed, three (3.2%) had haemoglobin (Hb)<110 g/l, the acceptable lower limit of the reference range, as defined by the World Health Organisation, and nineteen (20.7%) had serum ferritin <10 μg/l, indicating low levels of storage iron. Mean Hb was 122 (SD 7.8) g/l and mean serum ferritin was 17.5 (SD 12.2) μg/l.

There was no significant difference in the mean Hb levels between the three feeding groups based on milk feeding method at age 12 months. Six infants were being fed cow's milk as their primary milk source by the age 6 months; two of these were anaemic (Hb<110g/l with other haematological variables outside the reference ranges) and four had SF<10 μg/l. Four of the five infants who continued to be breast-fed to age 12 months had SF<10 μg/l. Hb values in this group were all >115g/l. In the group who were formula-fed throughout the first year of life, four (10.8%) infants had serum ferritin ≤10 μg/l. One of these infants was fed whey-based formula for 1 month and casein-based for the remaining 11 months. The other three were fed casein-based formula for the full 12 months. It is notable that, even in this group with a continuous source of dietary iron, serum ferritin values can be <10 μg/l at age 12 months. It has been shown that, while both whey and casein proteins inhibit iron absorption, the greater inhibitory effect is caused by casein (Hurrell *et al.*1989).

We conclude that, as is well established in other countries, the early introduction of cow's milk as the primary milk source in the infant's diet predisposes to anaemia. In all feeding groups there were infants whose SF levels were lower than the reference range. Serum ferritin levels <10 μg/l are a common finding in paediatric studies of iron status (Dallman & Siimes, 1979). This is a result of the large requirement for iron in infancy, a period of rapid growth, and the low levels of easily absorbable iron which tend to be characteristic of the infant's diet. Protein and mineral (e.g. phosphorus) inhibitors of iron absorption are common to both cow's milk and formula milks. In infancy, levels of Hb and SF show physiological variation with time. This is important in the interpretation of results which assess the iron status of infant populations.

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The predictive value of plasma antioxidant activity at birth for morbidity and mortality of premature babies. By K.M. SILVERS, A.T. GIBSON and H.J. POWERS, *University Department of Paediatrics, Sheffield Childrens Hospital, S10 2TH*

We have previously reported that plasma vitamin C concentrations are very high in some babies on the day of birth (Silvers *et al* . 1993). We hypothesized that vitamin C could act as a pro-oxidant in premature babies via an inhibitory effect on the ferroxidase activity of caeruloplasmin. We have observed up to 80% inhibition of ferroxidase activity *in vitro* at ratios of vitamin C : caeruloplasmin observed in some babies.

We have investigated the association between antioxidant activity at birth and outcome in premature babies with a birthweight below 1500 g who were ventilated from birth.

Arterial blood samples were obtained within 2 h of birth. Antioxidant activity of plasma (Dmax) was measured *in vitro* as the ability of plasma to inhibit lipid peroxidation. Dmax is inversely related to antioxidant activity and is largely a measure of the ability of plasma to remove iron in a form in which it might lead to the generation of reactive oxygen species.

Dmax increases with decreasing gestational age (P <0.001) and increasing plasma vitamin C (P <0.01). Dmax values were significantly higher in fifteen babies who died, compared with thirty-seven survivors; 176 (SEM 14.7) µl *v.* 87 (SEM 7.3) µl (P <0.001, Mann Whitney U) and were significantly higher in nineteen survivors who were ventilated for at least 120 h, compared with 14 ventilated for less; 112 (SEM 10.5) µl *v.* 69 (SEM 11.6) µl (P <0.01, Mann Whitney U). Plasma vitamin C at birth was significantly higher in babies who died, compared with survivors; 134 (SEM 17.1) µmol/l *v.* 78 (SEM 5.1) µmol/l (P <0.001, Mann Whitney U) and was higher in survivors who were ventilated for more than 120 h, compared with those ventilated for less; 85 (SEM 5.4) µmol/l *v.* 71 (SEM 10.9) µmol/l, although this failed to reach significance.

Although Dmax appeared to be strongly predictive of mortality this could be partly explained by its relationship with gestational age. We therefore investigated the independent contribution of Dmax to predict mortality in fifty-four babies using logistic regression analysis. There was a significant effect of Dmax (P <0.01) and this remained significant even after correcting for gestational age and birthweight.

Antioxidant activity within 2 h of birth shows a significant relationship with survival and a prolonged ventilatory support. Antioxidant activity may be influenced by a pro-oxidant effect of high plasma vitamin C.

Silvers, K.M., Gibson, A.T. & Powers, H.J. (1993). *Proceedings of the Nutrition Society* **52**, 313A.

Effect of oral iodized poppyseed oil during pregnancy on maternal and infant thyroid hormones and iodine status. By S. FILTEAU, K. SULLIVAN, Z. ANWAR, U. ANWAR and A. TOMKINS. Centre for International Child Health, Institute of Child Health, London WC1N 1EH

Iodine deficiency during pregnancy may impair reproductive outcome and lead to impaired infant thyroid and neurological function. We conducted a study in a region of rural Bangladesh with high goitre prevalence and without an iodization programme in order to determine whether a single oral dose, 400 mg I as iodized poppyseed oil (IPSO), during pregnancy could prevent these defects. All women recruited in the first trimester (*n* 46) were given IPSO since cretinism can result from iodine deficiency at this time and, of women recruited later in pregnancy, 137 were given IPSO while 135 served as controls. Blood samples were collected from women at recruitment and from women and infants 3 months and 8-20 months postnatally; urine samples were collected at recruitment and 3 months postnatally and breast milk at 3 months. Urine and breast milk iodine were measured by an automated Sandell-Kolthoff method and serum thyroxine (T4) and thyroid stimulating hormone (TSH) with commercial radioimmunoassay kits.

Birth weight was increased slightly but significantly ($P<0.05$) by IPSO treatment from 2.42 (SD 0.42) kg to 2.53 (SD 0.41) kg and gestational age at supplementation was not a significant covariate. Reproductive performance, in terms of spontaneous abortions, stillbirths and neonatal deaths did not differ between groups. Two cretins were born to women who had received IPSO in the third trimester.

IPSO significantly increased T4 and decreased TSH in both mothers and infants at both time points and levels at each time point were highly correlated. Infant hypothyroidism (TSH>4.8 mU/l and T4<60 nmol/l) at 3 months was decreased from 10% to 1% and at 8-20 months from 14% to 2% by IPSO treatment.

Maternal urinary iodine at 3 months was increased by supplementation (0.083 μmol/l (95% CI 0.058-0.118, *n* 92) compared with 0.017 μmol/l (95% CI 0.008-0.034, *n* 51); $P<0.0001$) although the mean for both groups was less than the mean at recruitment. Breast-milk iodine was much higher than urinary iodine and was similar in both groups (IPSO: 0.38 μmol/l (95% CI 0.29-0.50, *n* 64); control: 0.35 μmol/l (95% CI 0.23-0.52, *n* 39)). This accounts for both the low maternal urinary iodine excretion and the adequate and similar urinary iodine concentrations of infants from both groups (IPSO: 0.636 μmol/l (95% CI 0.552-0.733, *n* 72); control: 0.478 μmol/l (95% CI 0.396-0.577, *n* 54)).

The results suggest that iodine deficiency during pregnancy results in infant thyroid abnormalities which cannot be repaired by adequate iodine intake from breast milk postnatally. However, IPSO does benefit the mothers since it can mitigate the drain on iodine stores resulting from the preferential concentration of iodine in breast milk. The general lack of effect of IPSO on birth outcome suggests that iodine deficiency, which was only moderate, is not a limiting factor for birth outcome in this population.

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