Proceedings of the Nutrition Society

Abstracts of Original Communications

A Scientific Meeting was held at the University College, Cork, Republic of Ireland on 27–30 June 2000, when the following papers were presented.

All abstracts are prepared as camera-ready material.

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Contemporary beliefs and attitudes to weaning. By A.S. ANDERSON[1], C. GUTHRIE[2], E.M. ALDER[2], F. WILLIAMS[2], J.S. FORSYTH[3] and P. HOWIE[4] [1]*Centre for Applied Nutrition Research, Matthew Building, University of Dundee, Dundee DD1 4HT,* [2]*Department of Epidemiology and Public Health,* [3]*Tayside Institute for Child Health and* [4]*Department of Obstetrics and Gynaecology, Ninewells Hospital and Medical School, Dundee DD1 9SY*

Nutrition in the early years of life is a major determinant of growth and development and also influences adult health. Wilson *et al.* (1998) have demonstrated that infants introduced to solid food early (before 4 months), have higher levels of morphometric features characteristic of cardiovascular risk such as increased body fat and body mass index. In addition, they found that the early introduction of solids was associated with more wheezing and respiratory illness. These findings support current recommendations by the Department of Health (1994) that the majority of infants should not be given solid foods before the age of 4 months. However, in Scotland in 1995, 22% of infants had received solids by 2 months and 64% by 3 months (Foster *et al.* 1997) and it is not clear why parents reject current guidelines.

The present study was undertaken to identify a broad range of attitudes, beliefs and knowledge that influence the timing of introduction to solid food. Five focus group discussions were undertaken with new mothers to explore early feeding behaviour, stimuli to changing feeding habits and subsequent responses. The group communications were taped and transcribed. Two researchers read the scripts and independently identified common themes and areas of confusion or uncertainty relating to weaning.

In total, twenty-two primiparous and seven multiparous mothers (mean age 27.0 (SD 4.8) years) participated in the discussions concerning their baby's (mean age 13.0 (SD 4.2) weeks) feeding habits. Almost 40% of the participants had already introduced solid food to their infants (mean age of introduction 11.6 weeks (range 2–16 weeks)).

The form and route of solid feeding varied widely from teaspoons of baby rice added to bottles to two-course meals on temperature-sensitive plates. Mothers believed that the introduction of solids was baby-led, as shown by some physical characteristic or behavioural action of the infant. A common stimulus to thinking about changing feeding practices was when babies had been described as "hungry babies" as if these infants were somehow exceptional in their nutritional requirements. All mothers were aware of current recommendations to avoid the introduction of solid food until 4 months but few knew why this should be and concepts of long-term ill health were difficult to conceptualize when their babies were content and happy. The conflict between rigid current feeding guidelines and general advice from supportive health professionals to be "flexible" in baby-care created some confusion over the importance of good weaning practices.

In conclusion, these data describe a range of factors which influence current weaning practice. We are carrying out a prospective study using The Theory of Planned Behaviour in order to identify the relative importance of these issues and how they might be utilized in the design of an effective intervention programme to delay weaning until 4 months of age.

Department of Health (1994) *Weaning and the Weaning Diet. Committee on Medical Aspects of Food Policy.* London: HMSO.

Foster KD, Lader D & Cheeseborough S (1997) *Infant Feeding 1995.* London: HM Stationery Office.

Wilson AC, Forsyth JS, Greene S, Irvine L, Hau C & Howie P (1998) *British Medical Journal* **316**, 21–25.

Children's understanding of fruits and vegetables – implications for nutrition education. By C. HIGGINS[1], M.M. HETHERINGTON[1], A.S. ANDERSON[2], L.E.G. PORTEOUS[2], E. FOSTER[3] and A.J. ADAMSON[3], [1]*Psychology Department,* [2]*Centre for Applied Nutrition Research, University of Dundee, Perth Road, Dundee DD1 4HN,* [3]*Human Nutrition Research Centre, University of Newcastle, Newcastle upon Tyne NE1 7RU*

Current dietary guidelines recommend an increased consumption of fruits and vegetables in the general population (WHO, 1991; Health Education Authority, 1997). Educational attempts to promote increased consumption have focused on a "5 fruit and vegetables a day" message (Anderson *et al.* 1998). However, the use of this approach with schoolchildren has not been fully explored.

The work reported here forms part of an ongoing study to develop an effective school-based nutrition education intervention aimed at increasing consumption of fruits and vegetables in primary school-aged children. As part of baseline measurement, attitudes, subjective norms (social pressure), intention and understanding about fruits and vegetables were assessed using concepts based on The Theory of Planned Behaviour (Ajzen, 1988).

Sixty-eight children aged 5–6 (P2) and seventy-one children aged 10–11 (P7) completed assessments using semi-structured one-to-one interviews, simple card sort techniques and taste assessments using facial hedonic scales. All tasks were designed to avoid literacy and schematic development biases. The results demonstrated that the P7 sample generally performed better than the P2 sample on photographic categorization of fruits and vegetables, with one third of the younger age group being unable to categorize turnips, onions, radishes, courgettes, tomatoes, raisins and mango. A similar proportion in the older group was unable to categorize radish, courgettes and tomatoes. In the younger age group, less than half of the sample were able to judge the fruit and vegetable content (defined as "none", "some", "lots") of tomato-flavoured crisps, apple pie and carrots but this task was performed well by the majority of the older children. However, when the older age group was asked to identify "5 portions of fruits and vegetables" from a selection of portions of eighteen common food items, less than a quarter were able to fully achieve this task.

In assessments of subjective norms (in relation to "the school nurse"), more than two-thirds of the younger children knew that they would be recommended to eat more orange juice, carrots, apples, grapes and bananas but around a third did not know whether they should also be eating more biscuits, confectionery, crisps and cola drinks. These results were matched by "intention to eat" scores where 72–91% of children said they intended to eat more of the foods recommended for higher consumption but 48–56% reported they also intended to eat more of the non-fruit and non-vegetable snack items not generally recommended for increased consumption. Detailed dietary data collected pre- and post-intervention will assess whether children really carry out these intentions over the next academic year.

In conclusion, nutrition education for children needs to elaborate on fruit and vegetable definition in addition to "portion counts". Information on the relative values of confectionery, crisps and other food options should also be discussed.

Ajzen I (1988) *Attitudes, Personality and Behavior.* Chicago: Dorsey Press.

Anderson AS, Cox DN, McKellar S, Reynolds J, Lean MEJ & Mela D (1998) *British Journal of Nutrition* **80**, 133–140.

Health Education Authority, in association with the Ministry of Agriculture, Fisheries and Food and the Department of Health (1997) *Eight Guidelines for a Healthy Diet.* London: HMSO.

World Health Organisation (1991) *Diet, Nutrition and the Prevention of Chronic Diseases.* Technical Report Series 797. Geneva: World Health Organisation.

Body image concerns and slimming practices of inner-city Dublin women. By H.R. CASEY[1], M.A.T. FLYNN[1], K.M. YOUNGER[1], M.J. GIBNEY[2], M. McDONNELL[3] and W. SHANNON[3], [1]*Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Ireland,* [2]*Department of Clinical Medicine, Trinity College, Dublin 2, Ireland,* [3]*Department of General Practice, Royal College of Surgeons in Ireland, Dublin 2, Ireland*

The prevalence of overweight and obesity is increasing in all age groups in developed countries (Prentice & Jebb, 1995). In spite of this, preoccupation with body image has never been greater, particularly among women. This is not confined to the overweight and obese as several studies report normal-weight individuals engaging in weight loss practices (Biener & Heaton, 1995). This study examined the self-perception of and satisfaction with body weight and shape among a group of inner-city Dublin women. The slimming practices employed by the female volunteers and the effect of socio-economic class (SEC) on all outcomes measured were also assessed. One hundred and thirty-four non-pregnant female volunteers mean age 32.3 (SD 10.5) years with no history of chronic disease completed the study. These women were recruited from four inner-city G.P. practices. The subjects were interviewed once using a standardized, validated, interview-assisted, multiple-choice questionnaire designed for the study. A standardized drawing was used to enable the female volunteers to identify their self-perceived body shape. Height, weight, waist and hip measurements were recorded and used to estimate body mass index (kg/m^{2}) and waist-hip ratio (WHR).

The female volunteers in each socio-economic class were comparable in terms of age, BMI and WHR. The self-perception of body shape was accurate among the female volunteers in each socio-economic class with 98%(52) of those with an abdominal body fat distribution, or 'apple' shape, correctly identifying this. Ninety-six percent (78) of the female volunteers with a gluteal-femoral body fat distribution, or 'pear' shape, recognised this. Almost two-thirds (78) of the female volunteers were satisfied with their body shape. Of the female volunteers surveyed, 22.4%(30) were found to be overweight and 13.4%(18) were in the obese BMI range (Table). Although not significant, there was a trend towards a higher rate of overweight and obesity in the lower socio-economic classes.

| | Actual weight | | Self-perceived weight | |
	(%)	(n)	(%)	(n)
Underweight	6.7	(9)	9.7	(13)
Normal weight	57.5	(77)	29.9	(40)*
Overweight	22.4	(30)	44.0	(59)*
Obese	13.4	(18)	16.4	(22)

*Statistically highly significant difference (P=0.000).

A highly significant disparity existed between actual weight and self-perceived weight, with subjects tending to perceive themselves as being heavier than they actually were (Table). This overestimation of body weight existed in all socio-economic classes. Over four-fifths (110) of the female volunteers reported feeling dissatisfied with their body weight. Of those who reported never feeling dissatisfaction with body weight, significantly more were of high socio-economic status (SEC 1) (28.1%(16) vs. 11.1%(3) in SEC 2 and 10%(5) in SEC 3, P=0.047). Of the 110 female volunteers who were dissatisfied with their body weight 92%(101) wanted to be lighter even though 50%(55) of them were of normal weight. Eighty-two percent (110) of the female volunteers had previously tried to lose weight. Over three-quarters of these women reported exercising or going on a diet in an attempt to lose weight. Although not significant, unsafe slimming practices such as the use of diet pills, laxatives, vomiting and smoking were used more by women of low socio-economic status. Concern with body weight was pervasive among the female volunteers in all body weight and age ranges.

Biener L & Heaton A (1995) *American Journal of Public Health* **85**, 714–717.
Prentice AM & Jebb SA (1995) *British Medical Journal* **311**, 437–439.

Nutritional knowledge, food preparation knowledge and cooking confidence of 187 schoolchildren. By S.A. REVILL[1], A.J. ADAMSON[1], A.S. ANDERSON[2], R. STACY[1], J. HOOPER[1], H. TAYLOR[1] and P.J. MOYNIHAN[1], [1]*Human Nutrition Research Centre, University of Newcastle, Newcastle upon Tyne NE1 4LP,* [2]*Centre for Applied Nutrition Research, University of Dundee, Dundee DD1 4HT*

The demise of food preparation teaching in schools is likely to have the worst impact on those from lower income backgrounds because the lack of food preparation skills has a more detrimental effect on low-income families (National Food Alliance, 1997).

As part of baseline measurements in a controlled study of nutrition education intervention (after-school food club), knowledge of nutrition and food preparation and perceived cooking confidence were assessed by questionnaire in children from deprived social backgrounds. The questionnaire was adapted from one previously applied to adults from low-income backgrounds and was pilot-tested in children aged 11 years to ensure validity and reliability (Anderson AS *et al.*, unpublished results). Nutrition knowledge was assessed by asking subjects to select the healthiest choice from a list of options (e.g. different potato dishes), and how many portions of fruit and vegetables health experts recommended per day. For each correct answer they received one point (total score 0–8). The children were also asked to select the proportions of meat, potatoes and vegetables that composed the healthiest meal. Cooking knowledge was assessed by asking the children to list the main ingredients in four common dishes for which they received a score of 0–15 (one point for each correct ingredient). Subjects were asked the cooking times of five popular foods for which they received a score of 0–5. Cooking confidence was assessed by asking the children if they could make nine specified dishes all by themselves (3 points), with a little help (2 points), with a lot of help (1 point) or not at all (0 points). Points were summed to give a cooking confidence score of 0–27. Data were entered into SPSS, mean scores (with SE) were derived and differences between sexes determined using a *t*-test. Relationship between Townsend material deprivation scores (Townsend *et al.* 1987) and nutrition knowledge and cooking scores were determined using Pearson's correlation.

Two hundred children from year seven (aged 11–12 years) from ten schools in deprived areas of Tyne and Wear were recruited into the study and 187 (70 boys and 117 girls) completed the assessment.

| | n | Ingredients score (maximum 15) | | Cooking times score (maximum 5) | | Cooking confidence score (maximum 27) | | Nutrition knowledge score (maximum 8) | |
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
All	187	8.4	0.2	1.7	0.1	17.0	0.4	5.3	0.1
Girls	117	9.3	0.3	1.7	0.1	17.8	0.5	5.5	0.2
Boys	70	7.0	0.3	1.7	0.1	15.8	0.8	5.0	0.2
P value		0.000		0.8		0.04		0.1	

Overall 64% of boys and 69% of girls correctly identified the healthiest balance of meat, potatoes and vegetables. A small, but significant, relationship was found between nutrition knowledge score and Townsend score (r −0.27, P=0.001). No significant relationship was found between overall food preparation knowledge or confidence and Townsend score.

This study has shown that, in children from deprived social backgrounds, food preparation knowledge and confidence are higher amongst girls than boys and that nutrition knowledge decreases with increasing social deprivation.

National Food Alliance (1997) *Myths about Food and Low Income.* London: National Food Alliance.
Townsend P, Philmore P & Beattie A (1987) *Health and Deprivation:Inequality and the North.* London: Croom Helm.

Survey on Lifestyle, Attitudes and Nutrition, SLÁN: sociodemographic profile of food pyramid compliance. By J. HARRINGTON, S. FRIEL, G. NOLAN and C. KELLEHER, *National Nutrition Surveillance Centre, Centre for Health Promotion Studies, National University of Ireland, Galway, Republic of Ireland*

A sample of 6539 adults, randomly selected from the electoral register, returned a self-administered postal questionnaire. Based upon the food frequency questionnaire used in the EPIC (Bingham, 1997) study, the SLÁN questionnaire included 149 food items arranged into the main food groups consumed in the Irish diet. Data were analysed based upon the recommended servings from the food pyramid. In line with international dietary guidelines, the Irish food pyramid, recommends daily consumption of a number of servings from four of the five shelves. It is recommended that foodstuffs from the top shelf be eaten sparingly.

Overall, 52% of respondents consumed less than the recommended three servings from the milk, cheese and yoghurt shelf. These respondents were more likely to be female and significantly more likely (*P*<0.001) to be of lower social class. Significantly more males and females over 55 years consumed less than three servings, compared to their middle-aged counterparts. A significantly higher percentage of females aged between 18 and 34 years consumed less than three servings compared to males of the same age. Significantly more older males living alone (*P*<0.001) consumed less than the recommended three servings.

The recommended three servings from the milk, cheese and yoghurt shelf were consumed by 22% of respondents, with no significant difference across age and gender. Significantly more respondents from social classes 1/2 and urban dwellers consumed the recommended number of servings compared to those in lower social classes and rural locations.

A total of 26% of respondents consumed more than the recommended three servings from the dairy shelf of the food pyramid. These respondents were significantly more likely (*P*<0.001) to be younger males and females compared to their older counterparts. Rural dwellers were significantly more likely (*P*<0.05) to consume more than recommended three servings compared to urban dwellers.

Overall 22% of respondents consumed less than the recommended two servings from the meat, fish and poultry shelf. These respondents were significantly more likely (*P*<0.05) to be female than male. Respondents aged 55 years and over were significantly more likely to consume less than the recommended two servings compared to their younger counterparts. Females aged between 18 and 34 years from social classes 1/2 were significantly more likely (*P*<0.001) to consume less than the recommended two servings compared with females or males of the same ages in the lower social classes. Urban dwellers were significantly more likely (*P*<0.05) to under-consume products from the meat, fish and poultry shelf compared with rural dwellers.

A total 38% reported consuming the recommended two servings from the meat, fish and poultry shelf. These respondents were significantly more likely to be male than female and to be aged between 35 and 54 years compared to their younger or older counterparts.

Twenty-four percent of respondents consumed more than the recommended number of servings from the meat shelf of the food pyramid. These respondents were significantly more likely to be male than female. A significantly higher percentage of males aged between 18 and 34 years consumed more than the recommended two servings compared to their female counterparts. Rural dwellers and respondents from social classes 5/6 were significantly more likely (*P*<0.01) to consume more than the recommended number of servings from this shelf.

In conclusion, there was a low compliance with the milk, cheese and yoghurt shelf by females; males and females over 55 years from social classes 5/6, and older males living alone. Additionally, males and females over 55 years and females from social classes 1/2 aged between 18 and 34 years under-consumed foods from the meat, fish and poultry shelf. Under-consumption from these shelves has implications for calcium and iron status, leading to concerns about osteoporosis and iron deficiency.

Bingham SA, Gill C, Welch A, Cassidy A, Runswick SA, Oakes S, Lubin R, Thurnham DI, Key TJ, Roe L, Khaw KT & Day NE (1997) *International Journal of Epidemiology* **26**, Suppl. 1, S137–S151.

A database of vegetarian convenience foods. By R.L. REID and A.F. HACKETT, *Liverpool John Moores University, School of Education, Community and Social Science, IM Marsh Campus, Barkhill Road, Liverpool L17 6BD*

There has been a rapid increase in the number of vegetarian convenience foods catering for both vegetarians and meat reducers, many of whom appear to perceive meat-free food to be healthier. A Food Commission Report (1995) found that vegetarian products are not necessarily healthy, with at least 50% of the energy in seventeen out of twenty-one products coming from fat, including in some cases relatively unhealthy hydrogenated fat. The studies of Nathan (1995) in children and Robinson (1998) in adults suggest a new era of vegetarianism for some who are heavily dependent on vegetarian convenience foods. Hence what is known about vegetarianism in terms of health is likely to be outdated and new studies are required to investigate the impact of modern vegetarianism. However, despite the information contained in the food tables and its supplement: vegetable dishes (Holland *et al.* 1992*a*) there is a lack of information on the growing number of vegetarian convenience foods.

Using Microsoft Access, a database of vegetarian convenience foods has been developed. Data was acquired by writing to the manufacturers and from packet information. The database so far contains a list of 1231 vegetarian products, which are either manufactured, handled as wholesale products or produced for retailers for 101 different companies. The range of products is large and increasing and there is great variability within types, as the examples in the Table demonstrate.

Product	No.	Total fat Mean (g/100g)	Total fat Range (g/100g)	Energy from fat (%)	Saturated fat Mean (g/100g)	Saturated fat Range (g/100g)	Energy from saturated fat (%)	Meat products* total fat (g/100g)	Meat products* energy from fat (%)
Burgers	67	10.3	0.2–24.0	2.0–50.0	2.0	0.5–6.6	3.0–34.6	17.3	58.9
Sausages	56	9.9	3.5–18.3	30.6–55.4	3.6	0.5–8.0	4.4–24.2	17.3	58.7
Pizza	44	11.3	5.4–16.8	25.2–57.9	4.0	2.4–5.4	11.2–18.6	10.7	38.5
Lasagne	30	3.9	1.5–7.1	16.9–49.5	1.8	0.8–3.1	7.1–21.6	3.8	33.5
Mince	14	4.6	0.8–18.5	2.9–46.8	0.5	0.5–0.5	4.9–4.9	15.2	59.7

*Data on selected meat products from Holland *et al.* (1992*b*).

Many of the products contained in the database are high in fat, but there is a wide variation in the fat content, ranging from 2% to 57.9%. Data on the amount of total fat is available for 331 products; in 210 of these products there is between 33% and 50% of energy coming from fat, and a further 104 products provide more than 50% of energy from fat. Furthermore, saturated fat provides more than 10% of energy in 89 products and more than 15% in 73 of the 203 products for which data have been gathered. It is apparent that many of these vegetarian products contain a similar, and in some cases a greater, proportion of energy from fat than their meat-containing counterparts. Whilst vegetarian convenience foods may appear attractive in terms of health as well as for ease and speed of preparation, they are not necessarily of superior nutritional value compared with meat-containing equivalents. For example, simply swapping meat for meat replacements will not guarantee a lower-fat diet.

Food Commission Report (1995) *The Food Magazine* **31**, 27–29.

Holland B, Welch AA & Buss DH (1992*a*) *Second Supplement to McCance and Widdowson's The Composition of Foods*, 5th ed: *Vegetable Dishes*. Cambridge: Royal Society of Chemistry.

Holland B, Welch AA, Unwin ID, Buss DH & Southgate DAT (1992*b*) *McCance and Widdowson's The Composition of Foods*, 5th ed. Cambridge: Royal Society of Chemistry.

Nathan I (1995) The dietary intake and growth of vegetarian children (aged 7–11) compared with matched omnivores in NW England. PhD Thesis, LJMU, Liverpool.

Robinson FC (1998) Changing to a self selected vegetarian diet: Two studies of diet and selected physical and lifestyle parameters. PhD Thesis, LJMU, Liverpool.

Who are the vegetarians? By C. JAGGERS, J. CADE, D. GREENWOOD and A. GREENHALGH, *Nutrition Epidemiology Group, Division of Public Health Medicine, Nuffield Institute for Health, 71–75 Clarendon Road, Leeds LS2 9PL*

Over the last few years vegetarianism has been on the increase, although there is much confusion over the true definition of a vegetarian diet and many do not consider fish or poultry to be meat. A vegetarian lifestyle has been shown to reduce cancer mortality by 40% (Thorogood *et al.* 1994) and to have a beneficial effect in reducing premature mortality. In this study we aimed to define the vegetarian diet in today's society and to investigate the characteristics of the subjects who choose to follow this lifestyle.

The sample consisted of 35 374 subjects who were taking part in the UK Women's Cohort Study. This is a prospective cohort study looking at the relationship between diet and the occurrence of certain chronic diseases. These subjects have completed a food frequency questionnaire (FFQ) of 217 food and drink items. The study also looked at lifestyle habits such as alcohol intake, smoking, physical activity, socio-economic factors and self and family medical history.

Subjects were grouped according to whether they defined themselves as vegetarian or vegan (combined here as vegetarians) or not (grouped here as meat-eaters).

Characteristic	Vegetarian (n 9044)	Meat-eater (n 23 581)	P value
Age (mean)	49	53	< 0.001
BMI (mean)	23.4	24.9	< 0.001
Have a professional job (%)	28.8	22.9	< 0.001
Age when finished full time education (mean)	18.7	17.9	< 0.001
Smoker (%)	10.4	11.5	< 0.001
Drink alcohol more than once/d (%)	47.4	54.0	< 0.001
Vigorous activity (mean h/week)	3.2	2.9	< 0.001
Diet product use (mean portion/d)	0.89	0.91	0.336
Low & very low fat spread use (mean portion/d)	0.36	0.43	< 0.001
Skimmed milk use (%)	26.5	24.9	< 0.001
Use dietary/food supplements (%)	64.3	55.3	< 0.001
Meat and fish intake (mean portion/d)	0.20	1.40	< 0.001

The vegetarians tended to be slightly younger, with a higher status job and social class and to have stayed at school for longer than meat-eaters. They also had a healthier lifestyle with only 10.4% smoking daily compared to 11.5% for the meat-eaters, drank alcohol less often and did more vigorous exercise (mean 3.2 h/week *v.* 2.9 h/week meat-eaters). The vegetarian group had a high use of dietary supplements with 64% using them compared to 55% of the meat-eaters. Defining vegetarian status by reported food consumption from the FFQ, instead of self-definition, we found that 22% of the cohort never ate red meat or poultry and 12% never ate any meat (including fish). Of those that had previously defined themselves as vegetarian or vegan only 42% never ate meat or fish according to the FFQ.

The vegetarians were significantly different in the majority of their characteristics from meat-eaters. However, the definition of vegetarianism is not straightforward, since only half the self-reported vegetarians never ate meat or fish.

Thorogood M, Mann J, Appleby P & McPherson K (1994) *British Medical Journal* **308**, 1667–1670.

Dietary interventions in general dental practice: an unexplored opportunity for promoting dietary change in low income communities? By K. L. BARTON[1], A. S. ANDERSON[1], C. M. PINE[2], M. G. PATERSON[2] and G. BURNSIDE[2], [1]*Centre for Applied Nutrition Research, University of Dundee, Matthew Building, Perth Road, Dundee DD1 4HT* [2]*Dental Public Health & Health Psychology, University of Dundee Dental School, Park Place Dundee DD14HR*

It is widely recognised that a diet rich in starchy carbohydrates, fruits and vegetables and low in fats (especially saturated fats) is likely to reduce or delay the development of the major causes of morbidity and mortality in the UK (Department of Health, 1991, 1999). Achieving dietary change in the entire population presents a major public health challenge (Scottish Office, 1996; Department of Health, 1999) and particularly so in low-income communities. Dietary education interventions can be an effective way of encouraging individuals to change their food intake, and whilst there is some evidence of success in general medical practice (Roe *et al.* 1997), little work has been done on current practice and opportunities for dietary intervention within general dental practice (GDP).

This study aimed to examine the feasibility of using dental practices as a setting for dietary interventions. It utilized one day of patient consultations of thirty-five general dental practitioners (using observation and video techniques) to identify common approaches to communicating oral health messages. This was followed by a semi-structured interview with the same practitioners to identify perceived barriers and opportunities to dietary interventions in GDP. To explore consumer issues patients being seen by participating dental practitioners were invited to complete a questionnaire, which examined issues around dental health, dietary habits and readiness to change dietary habits.

A total of thirty-five dentists participated in the study, of which nineteen were solely NHS funded. In total 765 patients were seen by the participating dentists, of which 735 (96.1%) consented to being observed and 700 (91.5%) to being videoed. Questionnaires were completed by 614 patients (children under secondary school age were not invited to attempt the questionnaire).

Questionnaire results indicated that 33% of patients would like some form of dietary information in the dental setting. The most preferred formats being leaflets and personal advice with posters and videos being less popular routes. From the semi-structured interviews, all dentists reported a positive attitude towards diet and health and they felt that diet was important (or very important) to overall well being and prevention of disease. They also felt there was a role for the dental team to give dietary advice. Many thought that this should usually be from a dental perspective (as opposed to general health) and in some cases only if a specific need was identified. Half the dentists were observed to give some dietary advice but only 4.8% (74% of which were children) of consultations contained any dietary communications. Advice was usually given following a clinical trigger (e.g. new caries) and usually took the form of a single statement with little interaction from patients. The dentists were not observed to follow this advice with back up materials (e.g. leaflets). These findings were consistent with those of the semi-structured interviews, which suggest that dentists provide advice "as and when required" and that they are more likely to give advice to children and their parents. The content of the dietary communications focused on dietary matters related to dental issues such as high sugar drinks, sweet food and sweets and did not extend to broader nutritional issues. Overall, a wide variety of dietary communication methods were observed (oral, leaflet, poster, diaries, colouring materials) although these varied in quantity, quality and consistency of message. From the semi-structured interviews, dentists identified a range of barriers, which they perceived as restricting the provision of dietary information in GDP these included funding, referral procedures, access to effective communication materials, staffing issues and physical space.

In conclusion, there appears to be some support from patients and dentists for dietary advice in GDP but further work is required on defining cost-effective, practical, dietary interventions and funding routes.

Department of Health (1991) *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom*. London: HMSO.
Department of Health (1999) *Saving Lives: Our Healthier Nation*. London: HMSO.
Roe L, Hunt P, Bradshaw H & Rayner M (1997) *Health promotion interventions to promote healthy eating in the general population: a review*. London: HEA.
Scottish Office Department of Health (1996) *Eating for Health: A Diet Action Plan for Scotland*. Edinburgh: HMSO.

Readership, knowledge, usage of and confidence in genetically modified food labelling amongst Irish consumers. By F. MOLONEY, J. ENNIS and K. SHEIL, *Department of Clinical Medicine, The Trinity Centre for Health Sciences, University of Dublin, Trinity College, Dublin 2, Republic of Ireland*

Within medicine, the boom in biotechnology is obvious. People readily accept it when better drugs and clearer diagnosis are seen to improve their lives. However, the application of genetic engineering to food production has proved controversial. Due to a lack of international agreement, segregation of conventional and genetically modified products is not mandatory. Labelling requirements are strongly debated. The risk-based approach of the Food and Drug Administration in the United States (US) is in contrast to the European Union, where the production technique (i.e. genetic modification) determines the legislation regarding labelling (Anon., 1999; Miller, 1999). Imports of genetically engineered products into Europe from the US do not have to follow the European labelling laws. This denies consumers the right to choose and thus has caused widespread debate (Dixon, 1999). A cross-sectional, non-randomized, non-validated study in which shoppers completed a face-to-face interview-assisted questionnaire was designed.

In total 204 adults (aged 18 years upwards) completed the questionnaire. Subjects in this study were predominately female (58.3%), mainly under 45 years of age (67.6%) and many had third level education (48.0%). Overall 87.3% of respondents mentioned that they read food labels regularly (29.4%) or sometimes (57.8%). Of these people, 53.3% read them to determine whether genetically modified ingredients are present. Recognition of foods on the Irish market which may/may not contain genetically modified ingredients was generally poor, with only two people choosing the correct answer for each of the five foods listed. A high proportion indicated that they were unsure of the correct answer for each of the various foods (range 54.7–72.6%). Increasing level of education ($P<0.05$), usage ($P<0.05$) and a positive attitude ($P<0.05$) towards the technology was associated with an increased likelihood of choosing the correct answer and a greater perceived ability to answer. Only 15.8% of respondents stated that they use genetically modified food, a result that is surprising considering that it is estimated that over 60% of processed foods contain soya, maize or their derivatives. Subjects whose purchase decision is sometimes or always influenced by the presence of genetically modified ingredients were predominately over 45 years (78.7%), were concerned by the technology (81.0%) and always read food labels to check for the presence of genetically modified ingredients (86.3%). Desire to avoid this type of food was associated with a perception that it is of more or equal harm to alcohol ($P<0.05$), cigarettes ($P<0.05$), fatty foods ($P<0.001$) and pollution ($P<0.01$). Lack of confidence in the labelling of genetically modified foods was high with 41.2% expressing no confidence in its accuracy. An overwhelming majority stated that it is necessary to label genetically modified food (92%). Approximately seven out of ten respondents perceive the current labelling as being inadequate, the main reason being due to the fact that it lacks information (78.5%). The perception of the "ideal" label for genetically modified food highlights the desire for further information with more than 70% stating it is necessary to include information on the type and amount of ingredient modified (81.1% and 76.3% respectively), the reason for modification (72.6%) as well as a clear, bright, easy to identify label to allow for ease of identification (83.7%).

Based on these results it appears that labels are an important information tool that reach much of the population. Perhaps, however, they are not utilized to their full potential as is evident by the lack of awareness about genetically modified food on Irish supermarket shelves, the level of dissatisfaction with the current labels and the perception of what information should be contained on an "ideal" label for this type of food. However, caution should be exercised in the interpretation of these results as self-reports of label usage may be over-estimated due to a social desirability bias (Guthrie *et al.* 1995). The concern expressed by much of the sample emphasises the need/importance of providing adequate, accurate and unbiased information, preferably from an independent source, to allow for better-informed purchase decisions. Further research on how this may best be achieved in order to allow the progression of modern biotechnology whilst retaining consumer confidence is necessary.

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Slimming clubs: their impact on weight management and health promotion. By J. LAPSLEY and F. RABIEE, *School of Health and Policy Studies, University of Central England, Perry Barr, Birmingham B42 2SU*

Obesity is a major public health problem in the United Kingdom (Department of Health, 1992). Despite the Government's specific health targets it is increasing amongst adult men and women (Department of Health, 1995, 1998). Links between obesity and ill health are well established (Prentice, 1993; Robinson, 1993). Problems of excess weight occur in both sexes; however, dieting is more common in women than in men. Slimming clubs have grown in popularity in recent years and attract a large number of women. No study, however, has looked at their potential role in health promotion and the question of sustainable weight loss amongst their members.

This study was set up to explore reasons why overweight women join slimming clubs, the extent to which these clubs help women to lose weight and maintain their weight loss and whether the dietary advice given is in line with current recommendations on nutrition.

Using a qualitative, phenomenological approach (Denzin & Lincoln, 1994) data were collected from sixteen women aged 22–60 who had been members of a slimming club and had lost weight or achieved their "ideal" body weight. The methods of data collection were: semi-structured/structured individual recorded interview and documentary analysis of the slimming clubs' literature. The participants were recruited voluntarily through their response to posters placed at a number of community and voluntary organizations in Erdington, Birmingham. Information was collected about reasons for joining slimming clubs, types of activities, support and dietary advice provided by the clubs, their dietary and exercise patterns, the psychological effects of being overweight and the health gains after losing weight.

All respondents agreed that the motivation and support offered by slimming clubs attracted them into becoming members. Slimming organizations offered similar activities, the main focus being upon getting weighed. Although not an activity, the respondents found the support mechanism provided by the clubs and peer group to be the most beneficial feature. On average, participants were dieting for 12 years. The mean weight loss was 4 stones (ranging from 2 to 9 stones). More than half of the women ($n=10$) managed to maintain their weight loss for up to 3 years. A few women ($n=4$) commented that paying to attend the slimming club made them determined to lose their weight. However, for the majority of the respondents ($n=8$) the cost was perceived as a barrier to long-term attendance. All the respondents thought slimming clubs provided an invaluable service, which to their knowledge was not available within the health service. The one respondent who had experience of a dietetic service mentioned it as "a poor quality service". Health problems alleviated after losing weight included high blood pressure, back and joint problems. Other benefits cited were increased confidence, improved self-esteem and a general feeling of well-being. Although most slimming clubs encourage a sensible and gradual weight loss regime, there were discrepancies between current recommendations on healthy eating and the advice provided by some of the slimming clubs, e.g. high protein and low carbohydrate diets.

In conclusion, data from this study echo the findings of Garrow (1991) that slimming clubs offer a valuable network of support to women and encourage the individual to achieve and sustain weight loss.

The findings also suggest that in line with the government's health strategy (Department of Health, 1998) collaboration between health professionals, particularly the dietetic services, and slimming clubs should be encouraged to provide appropriate nutrition information. Further research is recommended to explore the viability of schemes similar to "exercise on prescription" for slimming clubs and to examine their uptake among low-income women.

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Tracer dosage for total energy expenditure measurement using doubly labelled water. By T. PRESTON[1], A. MOSES[2], C. SLATER[3], M.D. BARBER[2] and K.C.H. FEARON[2], [1]*Isotope Biochemistry Laboratory, SUERC, East Kilbride, G75 0QF,* [2]*Department of Surgery, Royal Infirmary, Edinburgh EH3 9YW,* [3]*Department of Child Health, Yorkhill, Glasgow G3 8SJ*

It is important to optimize tracer dosage when measuring total energy expenditure (TEE) by the doubly-labelled water (DLW) method, as $H_2{}^{18}O$ is currently in short supply and its cost is high. Accurate estimation of TEE and total body water (TBW) depends greatly on the error in ^{18}O and ^2H dilution volume estimates. We have measured TEE by DLW in a group of twelve cachexic pancreatic cancer patients (Preston *et al.* 2000). To minimize wastage of costly ^{18}O tracer, total body water (TBW) was estimated by bioelectrical impedance and tracer dosage was accurately weighed for each subject with target initial enrichments in body water of ~125 ppm excess (ppme) ^2H and ^{18}O. Dose portions, pre-dose urine samples and samples (the second urine sample of the day) taken 1, 2, 3, 12, 13 and 14 d after receiving the tracer dose were analysed by rapid, automated, continuous-flow isotope ratio mass spectrometry (HYDRA; PDZ Europa, Crewe), after equilibration with standard gases (3% CO_2 in N_2; 5% H_2 in He). 'Multi-point' calculations were used to derive turnover rates and initial enrichments of each isotope, to estimate CO_2 production and TBW respectively. Schoeller's equation for estimating TEE was used as given by Goran *et al.* (1994). A resampling procedure was used to estimate the errors in TEE measurement (Wolfe, 1992).

Due to the current problems in ^{18}O supply and intermittent delivery during this study, it was not feasible to prepare a common batch of tracer for every subject. Two approaches were compared to quantify the exact amount of tracer received: absolute calibration of each tracer batch by gravimetric dilution, and gravimetric dilution and analysis of a portion of each patient's dose. Tracer batch calibration yielded an average ^2H:^{18}O distribution volume or pool space ratio of 1.0343 (SD 0.012). Diluted dose analysis yielded a pool space ratio of 1.0306 (SD 0.020). The conventional diluted dose approach was used, although it was noted that batch calibration gave improved precision.

Isotope fractionation significantly compresses the enrichment scale during H_2O/H_2 equilibration. Largely due to this, analytical errors assessed from working standards run throughout this study were poorer for ^2H analysis (SD 0.93 ppm) than for ^{18}O analysis (SD 0.42 ppm), with little variation over the range of enrichments encountered. Several observations support the contention that DLW doses would best be governed by analytical precision rather than scaled to natural variations (0.6 ppm ^2H per ppm ^{18}O observed in natural waters due to evaporation; IDEGG, 1990). In this study, we used target enrichments of 125 ppme for both ^2H and ^{18}O. Precision of TBW analysis was consistently superior with ^{18}O (SD 0.13 kg vs 0.18 kg with ^2H); measurement precision of ^{18}O elimination rate (k_O; mean R^2 0.9991) was superior to that of ^2H elimination (k_H; mean R^2 0.9980; when 10 times the ^2H dose was used in one subject, improvements in k_H (R^2 0.9995) and TBW (SD 0.10 kg) precision were noted. In their multi-laboratory analytical comparison, Roberts *et al.* (1995) observed poorer agreement in ^2H than ^{18}O analyses, as noted in the current study. Thus, a target value of 125 ppme ^{18}O in body water may be appropriate when using a multi-point DLW procedure in a 14 d study in adults with normal water turnover rate ($k_O/k_H = 1.30$; ^2H half-life, ~10 d). However, when using the equilibration method to analyse ^2H, analytical precision considerations would suggest a target value of 275 ppme ^2H, rather than 75 ppme ^2H suggested by considering natural variations alone (IDEGG, 1990). Indeed Schoeller, cited in Wolfe (1992), recommended use of a dose that give a final enrichment of 100 times background variation, which can be close to analytical error. Typical enrichments at 14 d during this protocol were ~40 ppme ^{18}O and ~50 ppme ^2H, further supporting use of a larger ^2H dose. The cost implications of this action would be trivial. Consideration should also be given to determining the amount of tracer received by each subject following gravimetric calibration of the absolute enrichment of each tracer batch.

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Total energy expenditure and measurement errors using doubly labelled water in cancer cachexia. By T. PRESTON[1], A. MOSES[2], C. SLATER[3], M.D. BARBER[2] and K.C.H. FEARON[2], [1]*Isotope Biochemistry Laboratory, SUERC, East Kilbride, G75 0QF,* [2]*Department of Surgery, Royal Infirmary, Edinburgh EH3 9YW,* [3]*Department of Child Health, Yorkhill, Glasgow G3 8SJ*

Total energy expenditure (TEE) measurements in disease are less common than in healthy subjects. Such TEE measurements would yield valuable information on the components of the energy balance equation and in assessing energy requirements. In this study we have measured TEE in a group of cachexic pancreatic cancer patients who had each lost >5% of their pre-illness weight. We have used TEE and resting energy expenditure (REE) data to derive physical activity level (PAL = TEE/REE) and have assessed errors using the doubly-labelled water (DLW) technique. The latter is important for statistical evaluation against other studies and in longitudinal intervention studies. A group of twelve pancreatic cancer patients (6M: 6F; weight 61.6 (SD 9.5) kg; BMI 21.6 (SD 3.4) kg/m^{2}; age 68 (SD 9) years) supplied a basal urine sample before receiving an oral dose of water enriched with ^2H and ^{18}O to give an initial enrichment in body water of ~125 ppm excess. The subjects continued their normal daily routine as outpatients and supplied further urine samples (the second sample of the day) 1, 2, 3, 12, 13 and 14 d after receiving the tracer dose.

Urine samples, dose portions and standard waters were equilibrated with standard gases (3% CO_2 in N_2; 5% H_2 in He) and were analysed by using continuous-flow isotope ratio mass spectrometry (HYDRA; PDZ Europa, Crewe). 'Multi-point' calculations were used to derive turnover rates and initial enrichments of each isotope, to estimate CO_2 production (rCO_2) and TBW, respectively. Schoeller's equation for estimating TEE was compared to those of Coward and Speakman in their forms as given by Goran *et al.* (1994). A resampling procedure was used to estimate the errors in TEE and TBW measurement (Wolfe, 1992).

Assuming a respiratory quotient of 0.85 and using Schoeller's equation, TEE was estimated from rCO_2 to be 7.95 (sd 1.66) MJ/d. Using REE data measured by ventilated hood indirect calorimeter, PAL averaged 1.30 (SD 0.21), similar to that reported by Gibney *et al.* (1997) in small cell lung carcinoma patients (1.39 (SD 0.24)), using a bicarbonate tracer dilution method. TEE errors estimated by the resampling procedure averaged 4.3% (0.35 (SD 0.35) MJ/d). TBW averaged 31.42 (SD 6.26) kg and TBW errors averaged 0.55% (0.17 (SD 0.28) kg). Surveying sixteen published DLW studies in free-living healthy subjects, Schoeller & Hnilicka (1996) calculated a within-subject TEE variation of 7.8% (CV) and estimated analytical variation to be 4%. Goran *et al.* (1994) reported a within-subject variability of 8.5%. Roberts *et al.* (1995) arranged a multi-laboratory analytical comparison and estimated a between-laboratory variation of 6.6% for a subject with a typical k_O:k_H ratio (k_O is ^{18}O elimination rate and k_H is ^2H elimination rate; their ratio reflects the level of energy expenditure relative to water intake). Average k_O/k_H ratio was normal at 1.301 (SD 0.074) in the current study. The three equations used to derive TEE differ principally in the way that they treat the ^2H:^{18}O distribution volume or pool space ratio. In the current study, the pool space ratio was 1.306 (SD 0.02), very close to the value of 1.297, fixed in Schoeller's equation. As a result, Speakman's equation, which uses a study mean pool space ratio, yielded very similar TEE estimates. Similarly, Coward's equations, which use measured pool spaces, yielded an almost identical mean TEE value (7.94 (SD 1.87) MJ/d), although individual measurements differed from those obtained using Schoeller's equation. Thus, TEE and DLW were measured with good precision in twelve pancreatic cancer patients and with errors that compare well with studies in healthy subjects. Modest TEE and a PAL of 1.30 reflected the sedentary lifestyle of these subjects.

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A comparison of three methods of measuring gastric emptying. By W.L. HALL[1], A. GIOUVANOUDI[2], C.J. SEAL[3], J.C. MATHERS[3], N. SPYROU[2], W.B. AMAEE[2] and L.M. MORGAN[1], [1]Centre for Nutrition and Food Safety, University of Surrey, Guildford GU2 7XH, [2]Department of Physics, University of Surrey, Guildford GU2 7XH, [3]Human Nutrition Research Centre, Department of Biological and Nutritional Sciences, University of Newcastle, Newcastle upon Tyne NE1 7RU

Methods that have been developed to measure gastric emptying have many clinical and research applications. Scintigraphic measurement is regarded as the 'gold standard' but this method is expensive, and exposes subjects to ionizing radiation, limiting it for large studies or repeated measurements on an individual. From a nutritional research viewpoint, the measurement of gastric emptying rate is important because it determines rates of nutrient absorption, postprandial hormonal, metabolic and satiety responses. In order to test some of the available gastric emptying methods, a three-way comparative study was carried out, measuring gastric emptying by electrical impedance epigastrography (EIE) (McClelland & Sutton, 1985), and two absorption methods, the paracetamol absorption test (Heading et al. 1973) and the ^{13}C-octanoic acid breath test (Ghoos et al. 1993).

Six women aged 22–31 years, BMI <25 kg/m², consumed two liquid test meals of equal volume (450 ml) and differing fat content on two separate occasions (low-fat meal 4 g fat, 1239 kJ; high-fat meal 44 g fat, 2745 kJ). Gastric emptying was measured simultaneously by the three methods. Samples of expired air were collected for 4 h after the test meal and indirect calorimetry was used to measure CO_2 output over this time period. The cumulative appearance of [13]CO_2 in expired breath was fitted to the model equations of Ghoos et al. (1993) and half-emptying time (BT_{50}) was calculated. The concentration of plasma paracetamol at intervals over the 4 h was used to calculate three parameters related to gastric emptying: time until peak concentration (T_{max}), peak concentration (C_{max}) and area under the curve (AUC). EIE uses the change in conductivity (thus change in impedance) that occurs when a non-conductive liquid meal passes through the stomach. T_{50} values (ET_{50}) were calculated from the peak of the deflection as a result of ingestion of the meal and the point at which the trace has reached the postprandial baseline.

Meal	EIE		^{13}C-octanoic acid breath test		Paracetamol absorption test					
	ET_{50} (min)†		BT_{50} (min)		T_{max} (min)		C_{max} (mmol/L)		$AUC_{(0-60\ min)}$ (mmol/L by min)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Low-fat	21	2	208	26	63	25	0.11	0.01	3.47*	0.61
High-fat	28*	2	272*	33	175**	16	0.13	0.01	1.60*	0.12

Mean values were significantly different from the low fat meal for all parameters except C_{max}, *$P<0.05$. **$P<0.01$. † n 5, whereas all other parameters are n 6.

Of all the parameters that produced significant differences between the two test meals, paracetamol T_{max} was the best discriminator, followed by paracetamol $AUC_{(0-60\ min)}$. A previous experiment (Giouvanoudi et al., 1999) found mean T_{50} values (min) of 61 (SEM 4) and 160 (SEM 38) for identical low- and high-fat liquid meals measured by scintigraphy (n 6). EIE values are shorter, as gastric secretions reduce impedance, and therefore they cannot be considered true half-emptying times. Breath test values are longer than the scintigraphic values, but there is a considerable length of time expected for the ^{13}C-octanoic acid to be digested, absorbed and oxidized before it is expired in the breath. The paracetamol T_{max} values, however, appear to be comparable to the scintigraphic T_{50}s. In conclusion, all the methods tested detected differences in gastric emptying times with the two different test meals. The paracetamol absorption method was the best discriminator between meals; measurement of its T_{max} value may be a useful estimate of the scintigraphic T_{50} of liquid meals.

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Calibration of a heart rate monitor to estimate CO_2 production rate for ^{13}C-breath tests. By C. SLATER[1], T. PRESTON[2] and L.T. WEAVER[1], [1]Department of Child Health, Yorkhill Hospitals, Glasgow G3 8SJ, [2]Isotope Biochemistry Laboratory, SUERC, East Kilbride, G75 0QF

Heart rate monitors have been used successfully in studies of energy expenditure in free-living populations. These studies have relied on individual calibration of the heart rate monitor using indirect calorimetry. The aims of this study were to calibrate carbon dioxide production rate (VCO_2) measured using a ventilated hood indirect calorimeter (GEM; NutrEn Technology, Manchester) against heart rate, measured by a heart rate monitor (Polar Vantage NV; Polar Electro Oy, Finland), and to explore a generic calibration, which can be utilized for subjects of different body size. VCO_2 is required to calculate the percentage dose recovered (PDR) in many ^{13}C breath tests (Amarri et al.1998).

Minute-by-minute measurements of heart rate (HR) and VCO_2 were made simultaneously, at rest (supine), sitting, standing and during a continuous series of increasing work loads on a bicycle ergometer with an almost steady state at each level. Measurements continued after the final load with the subject supine until both HR and VCO_2 had returned to resting values. Both HR and VCO_2 data were downloaded directly to a computer. The calibration procedure was carried out twice on each subject. VCO_2 (mmol/min/m²) was plotted against heart rate. Four calibration methods were explored: (1) linear-flex method (Spurr et al. 1988), (2) steady state mean above resting (Livingstone et al. 1992), (3) relative HR (Sneddon et al. 1985), and (4) non-linear (sigmoid) fit of smoothed HR data that included all data (i.e. both exercise and recovery phases). As there was no difference between the two calibration curves from the same subject, data were combined for the curve fitting exercise. Smoothing HR data can account for the delay observed between HR changes and VCO_2 changes and brings the exercise and recovery data onto the same line. To explore a generic calibration, VCO_2 data from both subjects were also plotted against HR, relative HR (HR/resting HR), and HR above resting HR. All calculations, including the curve fitting, were performed using a propriety spreadsheet (Microsoft Excel Version 7.0a).

Method	Subject 1 (F, age 44 y, wt 55 kg, ht 1.59 m)		Subject 2 (M, age 46 y, wt 74 kg, ht 1.83 m)		Generic calibration	
	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
1	−5.5	29.4	−0.17	28.7		
2	−7.7	28.9	−9.9	25.9		
3	−1.6	24.8	−1.3	27.8		
4a	−0.0002	13.4	0.0003	20.5	0.0001	23.4
4b	0.0002	13.4	0.0002	20.5	0.00005	20.6
4c	0.0001	13.5	0.0002	20.5	0.00006	19.5

Accuracy was defined as the difference between the mean measured VCO_2 and the mean predicted VCO_2, expressed as a percentage of the mean measured VCO_2. Precision was defined as the root mean square of the difference between the measured and predicted VCO_2 (residual) for each heart rate point, expressed as a percentage of the mean measured VCO_2. Methods 1–4a normalized VCO_2 to body surface area (Haycock et al. 1978). Method 4b normalized VCO_2 to body weight. Method 4c normalized VCO_2 to height.

There was no advantage in including a resting HR term. Using a non-linear model to fit the data gives more accurate and precise estimates of VCO_2 than linear models and makes no assumption about resting HR. Normalizing VCO_2 to body surface area, weight or height³ offer the most potential for a generic calibration. The magnitude of this error is acceptable for use in ^{13}C breath tests, when compared to the bias introduced by using resting VCO_2 when subjects are not at rest, which can be up to 100%. This method has potential for improving the accuracy of those ^{13}C breath tests where PDR is the desired end point. Further calibrations are planned on a broader range of subjects with respect to age and size.

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The effect of aspartame on stomach to caecum transit time in rats. By B.E. MURRAY and D. RUMSEY, *Department of Biomedical Science, University of Sheffield, Western Bank, Sheffield S10 2TN*

Aspartame has been shown to suppress food intake in humans, possibly by a post-ingestive mechanism (Rogers *et al.* 1995). The present investigation was designed to determine whether aspartame exerted any effect on meal transit time which might be considered as a mediator of appetite.

Adult male rats (250–300 g) were fasted overnight and fed fixed 4 ml-volume meals by tube. Each meal consisted of homogenized baked beans and 10% lactose in water. After administration of the test meal each rat was placed unrestrained in a Perspex metabole perfused with room air. The effluent gas was sampled at 10-min intervals with a hydrogen monitor and the stomach to caecum transit time (SCTT) for the meal was determined by the difference in time between feeding and the initial rise in exhaled hydrogen as unabsorbed carbohydrate in the meal arrives in the caecum (Brown *et al.* 1987).

The stomach to caecum transit times of this group of rats were determined following administration by tube of two concentrations (100 mg kg^{-1} body wt. or 500 mg kg^{-1} body wt.) of aspartame in water, or water as a control, on successive random occasions. Pre-meal intubations at a volume of 2 ml were performed at two time intervals, 30 and 120 min, before the test meal. Minimum intervals of 3 d were instituted between determinations on each animal. The values for SCTT are presented in terms of the mean and standard error of the mean for the six animals used. Statistical analysis was carried out by one-way repeated measures analysis of variance followed by the Student–Newman-Keuls multiple comparison test.

	Stomach to caecum transit time (min)			
	30 min premeal		120 min premeal	
	Mean	SEM	Mean	SEM
Standard meal	71.2	2.5	71.7	2.1
Water pre-meal	72.5	3.6	110.8**	13.4
Aspartame (100 mg kg^{-1}) pre-meal	75.0	6.8	–	–
Aspartame (500 mg kg^{-1}) pre-meal	98.3**	9.1	104.2**	10.0

**$P < 0.05$ vs standard meal

From the table it may be seen that the high dose of aspartame given 30 min prior to the standard meal significantly ($P<0.05$) delayed the passage of that meal along the gastrointestinal tract, an effect which was absent when the same volume of water was administered. The lower concentration of aspartame had no effect on SCTT. However, when aspartame at 500 mg kg^{-1} was administered 120 min before a meal, a similar delay in SCTT was observed as that which took place when water alone was given. This study therefore demonstrates that SCTT is capable of being extended by a high dose of aspartame and also by the administration of water, depending on the time interval between pre-meal and meal. These findings may help to clarify other observations which have demonstrated the effects of control drinks on appetite ratings (Black *et al.* 1993). Indeed the results support the hypothesis that any delay in the gastrointestinal transit of a meal, induced by any mechanism, influences appetite and food intake.

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A test meal suitable for measuring gastric emptying of solids by means of a [^{13}C-1]-sodium acetate breath test. By W. MEIER-AUGENSTEIN[1], H.F. KEMP[1] and T. PRESTON[2], [1]*Department of Anatomy & Physiology, OMS, University of Dundee, Dundee DD1 4HN, [2]SUERC, University of Glasgow, East Kilbride, G75 0QF*

Delayed gastric emptying is a relatively frequent complication in a variety of diseases (Chaudhuri & Fink, 1991) such as functional dyspepsia, irritable bowel syndrome (IBS), gastroesophageal reflux disease (GERD) and diabetes mellitus. It can be treated successfully by motility-promoting drugs (Fraser *et al.* 1993). However, diagnosis and therapy control is usually based on ^{99m}Tc-colloid radio-scintigraphy, a time-consuming and expensive procedure that exposes the patient to ionizing radiation. Ghoos *et al.* (1993) proposed to use a ^{13}CO$_2$-breath test as a non-radioactive alternative.

Assessing gastric emptying (GE) by ^{13}CO$_2$-breath tests is undertaken conventionally using two different tracers, [^{13}C-1]-sodium acetate (Braden *et al.* 1995; Leese *et al.* 1995) and [^{13}C-1]-octanoic acid (Ghoos *et al.* 1993) for liquid and solid meals, respectively. To aid comparisons we proposed to use the same tracer for both tests. Since octanoic acid is not readily soluble in aqueous media and many subjects, especially children, find it unpalatable, we chose to use sodium acetate. We also aimed to use a test meal with a long shelf life that could be prepared in batches, thus making the GE breath test for solids more convenient to use in a clinical setting. The flapjack seemed to meet these demands.

Subjecting a labelled flapjack to a gastric simulation showed a tracer retention of 95.2% after 1 h, which was in line with that reported for octanoic acid (Ghoos *et al.* 1993). Initial studies were then carried out to assess intra-individual variability and to see if we could distinguish between liquid and solid GE. ^{13}C recovery in the breath was plotted against time and modelled. Gastric emptying coefficient (GEC) and gastric half-emptying time ($t_{1/2}$) were taken from the original Maes model (Ghoos *et al.* 1993) and were initially not corrected for tracer retention. Encouraged by the initial results, solid GE was then measured in 22 bona-fide healthy volunteers (Table 1).

Table 1 Inter-individual study

		Solid GE	
	Mean	SE	CV (%)
	6 males, 16 females, 23–61 years		
GEC	3.93	0.22	5.6
$t_{1/2}$ [min]	108.7	12.01	11.05

Table 2 Comparison of the three techniques

	Flapjack/[^{13}C]-sodium acetate		Egg yolk/[^{13}C]-octanoic acid		Colloid/^{99m}Tc	
	Mean	SE	Mean	SE	Mean	SE
GEC	3.93	0.22	3.93	0.3		
$t_{1/2}$ [min]	78.7	8.7	72.0	22.0	79.7	20.0

Corrected for the observed acetate retention time of 30 min (Leese *et al.* 1995), our results were in excellent agreement with the published octanoic acid data (Table 2), lag-phase corrected by 66 min (Ghoos *et al.* 1993), and with the scintigraphic data reported by the same group.

The new test meal, a flapjack doped with 150 mg of [^{13}C-1]-sodium acetate, offers a convenient test for assessing gastric emptying of solids by means of a ^{13}CO$_2$-breath test. With its low variation coefficient of 11.05 % it offers a viable alternative to radio-scintigraphy thus eliminating the risk associated with radioactive tracer techniques.

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The effect of the CCKA receptor antagonist loxiglumide on appetite ratings, energy intake and gastric emptying in humans. By S.J. LONG[1], A. GIOUVANOUDI[1], A. SUTTON[2], W.B. AMAEE[1], N. SPYROU[1], P. ROGERS[3], M. D'AMATO[4], J. CALAM[5] and L.M. MORGAN[1], *[1]University of Surrey, Guildford GU2 7XH, [2]Royal Surrey County Hospital, Guildford GU2 5XX, [3]University of Bristol, Bristol BS8 1TN, [4]Rotta Research Laboratories, Milan, Italy, [5]RPMS, Hammersmith, W12 0NN*

Although fat is generally thought to be the least satiating macronutrient, intestinal lipid infusions have been shown to have short-term effects upon satiety (Welch *et al.* 1985). The hormone cholecystokinin (CCK) is released primarily in response to fat ingestion, and infusion of exogenous CCK has been shown to reduce food intake in human volunteers (Lieverse *et al.* 1995). To investigate the effect of endogenous CCK upon human appetite, an infusion of the CCKA receptor antagonist loxiglumide was used. Plasma CCK and GLP-1 response and gastric emptying rate were also assessed.

Eight healthy volunteers (seven male, one female) were recruited. Using a single blind randomized crossover design, subjects received an infusion of loxiglumide or saline. A loading dose of 30mg/kg per h of loxiglumide was administered for the first 10 min of infusion, followed by a maintenance dose of 10mg/kg per h for 3 h 20 min. At 30 min after the start of the infusion, subjects consumed a high-fat liquid preload providing a total of 3115 kJ. Gastric emptying was then assessed for 60 min using epigastric impedance epigastrography, after which time subjects were offered an *ad libitum* pasta-based test meal from which energy intake was calculated. Infusion continued throughout consumption of the pasta test meal and for a further 2 h. Blood samples were taken at regular intervals and hunger and satiety assessed using visual analogue scales every 30 min during the study period.

There were no significant differences in hunger and satiety ratings between infusions, although subjects tended to feel more hungry and less satiated after preload consumption with the loxiglumide infusion. Loxiglumide infusion significantly increased test meal energy intake compared to the saline control (loxiglumide mean 3641 SD 206 kJ, saline mean 2588 SD 265 kJ, *P*=0.008). Satiety quotient, a measure of energy consumed in relation to the change in appetite, was calculated from test meal energy intake for hunger and satiety ratings (*P*=0.04). There was a significant difference for satiety ratings between infusions (*P*=0.09). Thus subjects ate more during loxiglumide infusion to elicit the decrease in hunger and increase in satiety observed compared to the saline infusion. In addition, gastric emptying rate was shown to be significantly faster with loxiglumide infusion (*P*=0.001). Hormone analysis showed circulating CCK to be significantly higher during loxiglumide infusion (*P*=0.004), which was accompanied by significantly raised GLP-1 (*P*=0.008).

In conclusion, the blockade of peripheral CCK receptors by loxiglumide was shown to increase food intake and increase the rate of gastric emptying, providing evidence of a physiological role for this hormone in human satiety. Lower satiety quotients during the loxiglumide infusion suggest appetite response was less sensitive to energy intake with this infusion, possibly due to the increased rate of gastric emptying observed. As an increased energy intake was observed in spite of higher circulating GLP-1 levels during loxiglumide infusion, these data also suggest that CCK has a more marked effect on appetite than GLP-1 following high-fat mixed meals.

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Peripheral administration of cholecystokinin octapeptide and islet amyloid polypeptide inhibits food intake in mice. By C.M.N. KELLY, N. DUFFY, P.R. FLATT and F.P.M O'HARTE, *School of Biomedical Sciences, University of Ulster, Coleraine, N. Ireland BT52 1SA*

Both cholecystokinin octapeptide (CCK-8) and islet amyloid polypeptide (IAPP), which are released in response to nutrient stimuli, have been reported to act as postprandial satiety signals in rodents (Hirosue *et al.* 1993; Guidobono, 1998). The present study compared the peripheral effects of IAPP and CCK-8, both alone and in combination, on food intake in male Swiss TO mice. Mice aged 12 weeks were gradually habituated (standard breeding diet; Trouw Nutrition, Belfast) over a 3-week period to a reduced voluntary food intake period of 3 h (10.00–13.00 hours) per day. A consistent daily food intake was observed for 1 week before beginning the experimental study. Mice (*n*=7–8) with an average weight of 29.5 (SD 0.1) g were injected intraperitoneally (10.00 hours) with either a saline control (0.9% w/v NaCl, 10 ml/kg) or peptide (100 nmol/kg) IAPP, CCK-8 or a mixture of both peptides (100 nmol/kg). Food intake was monitored at 30, 60, 90, 120, 150 and 180 min post-injection. IAPP significantly reduced cumulative food intake by 22–59% at 30, 60, 90 and 120 min after administration (*P*<0.01–0.001) compared with controls (total food intake 2.87 (SE 0.26) g/mouse). CCK-8 reduced (*P*<0.01–0.001) food intake at 30, 60, 90 and 120 min by 46–89%, proving to be significantly more potent than IAPP (*P*<0.01–0.001) at these times. When both peptides were injected simultaneously, food intake was significantly reduced over the entire experimental period between 30–180 min post-injection (*P*<0.001) by 47–100% compared with saline controls. IAPP and CCK-8 in combination gave rise to a significantly enhanced and protracted satiating effect when compared to either peptide administered alone (*P*<0.01–0.001). Food intake results are shown below.
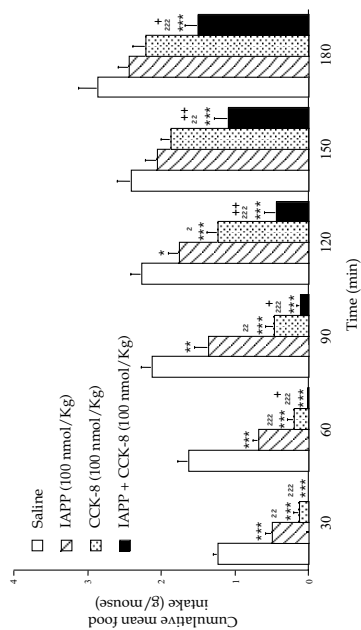
Significant differences indicated by *P<0.05, **P<0.01, ***P<0.001 compared to saline, ‡P<0.05, ‡‡ P<0.01 and ‡‡‡ P<0.001 compared to IAPP and +P<0.05, ++P<0.01 compared to CCK-8 at the same time.

These results strongly support the hypothesis that IAPP may act synergistically with the meal related signal CCK-8 to control feeding behaviour in mice. We can speculate that the mechanism of action on food intake may be different for both peptides as their combined actions have an additive effect, supporting previous findings (Morley *et al.* 1994). Together IAPP and CCK-8 may prove to be a useful treatment for moderating caloric intake in obesity and other metabolic disorders.

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Post-exercise replenishment of intramyocellular lipids. By J. DECOMBAZ[1], B. SCHMITT[2], R. KREIS[3], M. FLEITH[1], B. DECARLI[1], M. ITH[3], H. HOPPELER[2] and C. BOESCH[3], [1]Nestlé Research Centre, Nestec Ltd, Lausanne, Switzerland, [2]Institute of Anatomy and [3]MR-Centre and Department of Clinical Research, University of Bern, Switzerland

Endurance-trained subjects have a higher capacity for fat metabolism than untrained subjects. For instance, they show enhanced activity of muscle lipoprotein-lipase (Nikkilä et al. 1978) and fatty-acid transport proteins (Kiens et al. 1997), and improved clearance of circulating triacylglycerol-rich lipoproteins (Sady et al. 1986). Based on these observations, we hypothesized that postexercise repletion of intramyocellular lipids (IMCL) would be increased in trained compared with untrained subjects due to an increased uptake of blood-borne fatty acids.

We studied the influence of a high-fat (55% energy from lipids) and a low-fat (15% energy from lipids) diet on IMCL repletion kinetics following exercise. Subjects were six highly endurance-trained runners with a peak $\dot{V}O_2$ of 69.4 (SE 1.6) ml/min/kg and six untrained individuals with a $\dot{V}O_2$ value of 48.5 (SE 1.5) ml/min/kg. Both groups exercised on a treadmill for 2 h at approximately 50% of their respective peak $\dot{V}O_2$. Tibialis anterior m. IMCL levels were measured before, immediately after, and 8 and 30 h post-exercise by [1]H-MR-spectroscopy (Boesch et al. 1997).
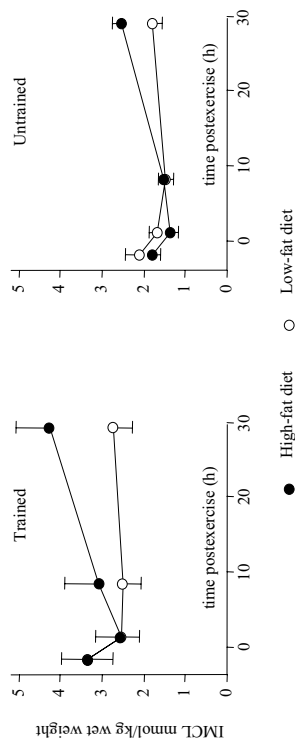
As expected, the trained subjects had a 71% greater resting IMCL content than the untrained subjects. In both groups, the percentage decrease in IMCL content was similar: 20–24% over the 2-h exercise period. With the 15% fat diet, IMCL content decreased further (in the untrained subjects) or remained constant (in the trained subjects) in the first 8 h after exercise, while with the 55% fat diet, we found a partial repletion of IMCL stores during this period. In the period between 8 and 30 h post-exercise, repletion rates were similar in trained and untrained subjects; however, they were much larger with the 55% fat diet, leading to a super-compensation under this regime. With the low-fat diet, repletion was only at 81–85% of the pre-exercise value 30 h post-exercise.

In conclusion, we found that dietary lipid concentration in the post-exercise period strongly influenced lipid repletion in muscle, whereas training status did not change recovery significantly. These data also confirm (Boesch et al. 1999) that replenishment of IMCL used during exercise can be complete within a day when fat intake is sufficient.

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Gender differences in postprandial substrate oxidation. By A.E. JONES[1], R.D. SMITH[2], C.N.M. KELLY[2], K.D.R.R. SILVA[2], M.C. NYDAHL[2], C.M. WILLIAMS[2] and S.A. WOOTTON[1], [1]Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD, [2]Hugh Sinclair Unit of Human Nutrition, University of Reading, Reading RG6 6AP

Gender differences in post-absorptive substrate oxidation have been reported, with women having lower absolute and relative rates of fat oxidation compared with men (Toth et al. 1998). In addition, we have reported that these differences may persist in the postprandial state, with a trend towards greater absolute net fat oxidation in men than in women attributed, at least in part, to a greater oxidation of exogenous fat (Jones et al. 1998). The present study examined the effect of gender on postprandial substrate oxidation in both absolute and relative terms, as the contribution to energy expenditure.

Eleven men (18–25 years; LBM 64.5 (SD 5.3) kg) and fourteen women (18–25 years; LBM 46.1 (SD 5.7) kg) consumed a controlled diet (% energy: 48.9% CHO; 38.7% fat; 10.6% protein; 1.7% alcohol) for 8 weeks. Following consumption of a standard evening meal and a 12 h overnight fast, post-absorptive energy expenditure and gaseous exchange, to estimate net substrate oxidation (Frayn, 1983), were determined by indirect calorimetry (GEM; PDZ Europa Ltd, Crewe). A test meal containing [1,1,1-^{13}C]tripalmitin (10mg/kg body weight) as a lipid–casein–glucose–sucrose emulsion was consumed (3.7 MJ; 93 g CHO; 45 g fat; 33 g protein). Hourly measurements of energy expenditure, gaseous exchange and excretion of [13]CO$_2$ on breath, to estimate exogenous fat oxidation, were performed over the next 8 h. Body composition was determined by bioelectrical impedance (Bodystat 1500; Bodystat Ltd, Isle of Man). The results are shown in the table as absolute values and in relative terms, as the contribution to energy expenditure (% EE), over the 8 h postprandial period.

	Net carbohydrate oxidation (g over 8h)				Net fat oxidation (g over 8h)				Exogenous fat oxidation (g over 8h)			
	Mean	SD	(% EE) Mean	SD	Mean	SD	(% EE) Mean	SD	Mean	SD	(% EE) Mean	SD
Men	89.1	16.0	52.4	10.9	22.3	10.9	29.2	12.3	6.8	1.3	9.1	1.5
Women	78.6	14.9	61.8*	10.8	10.1*	7.0	17.9*	11.4	4.8*	1.6	8.8	2.7

*Mean value significantly different from men, $P<0.05$ (independent t-test).

Energy expenditure over the 8 h postprandial period was significantly greater in the men (2750 (SD 294) kJ) than the women (2039 (SD 199) kJ; $P<0.001$) and was positively related to LBM (R 0.881; $P<0.01$). When provided with the same test meal as a source of energy and substrates to satisfy these energy requirements the proportion of CHO and fat oxidized differed between men and women. The absolute amount of CHO oxidized did not differ for men and women although net fat oxidation was greater in the men than in women. In relative terms, net CHO oxidation was lower in men than in women, with net fat oxidation providing a greater proportion of energy in the men than in the women. The absolute amount of exogenous fat oxidation was also greater in men than in women but in relative terms provided equivalent proportions of energy. In relative terms there was no relationship between net CHO oxidation or exogenous fat oxidation and LBM, although there was a positive correlation between net fat oxidation and LBM (R 0.443; $P<0.05$). There appear to be gender differences in postprandial substrate oxidation which cannot be explained simply by differences in body size and composition.

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Dietary saturated fat intake but not the *n*-6:*n*-3 ratio determines glucose tolerance and insulin resistance in the rat. By M.M.Sh. DUWAIHY[1] and D.J.MILLWARD[2], [1]*Department of Nutrition, King Abdul Aziz University Hospital, King Saud University, PO Box 245, Riyadh J141J, Saudi Arabia,* [2]*Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

While there is a growing consensus that dietary fat quality is the important determinant of CHD risk, with SFA increasing risk and MUFA and PUFA posing little risk (Grundy, 1999), the relative importance of the *n*-6:*n*-3 PUFA ratio is less certain, especially its influence on insulin resistance, which is thought to link dietary fat with CHD. We report here on animal studies designed to investigate the relative importance of MUFA, *n*-3 PUFA and *n*-6 PUFA, compared with SFA, as determinants of glucose tolerance and insulin sensitivity.

Thirty-six male rats (mean weight = 150 g), were fed a fixed intake (close to the maximum *ad libitum* level observed in pilot studies) of either chow or high-fat diets (40% energy, 200 g/kg) of corn-oil, olive oil, butter, fish oil/olive oil (1:1), or fish oil/butter (1:1) for 5 weeks. Overnight-fasted rats were anesthetized with urethane, the left carotid artery was catheterized, a baseline blood sample (400 μl) was taken and glucose (50 mg/100 g body weight) was rapidly injected followed by 0.5 ml saline. Further blood samples were collected at 3, 6, 9, 12 and 15 min after the glucose injection and glucose tolerance ($K_{glucose}$) was calculated as the slope of the regression of ln plasma glucose between 3 and 15 min. Insulin was measured by RIA so that insulin sensitivity could be calculated from $K_{glucose}$/insulin,AUC. The insulinogenic index (insulin/glucose, a crude measure of β cell responsiveness to glucose) was calculated at 15 min. Rats were then sacrificed and organs dissected and weighed. The sum of suprarenal mesenteric and epididymal fat pads was taken as a measure of adiposity. Fatty acid profiles were measured by GC in pooled samples of membrane phospholipids extracted from red cells and liver.

Diet	Red cell fatty acid profile (%)		Fat pad (g/100g wt)		k-value (%/min)		Insulinogenic index (mmol/l)/(μU/ml)		Insulin sensitivity k-value/AUC×10³	
	SFA	*n*-6:*n*-3 ratio	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Chow	44	19	1.62ab	0.15	3.75b	0.27	5.82ab	0.74	4.25b	0.37
Corn oil	46	28	2.16b	0.07	3.96b	0.25	4.94a	0.44	4.77b	0.36
Olive oil	41	44	2.46bc	0.15	3.77b	0.26	4.46a	0.29	4.90b	0.30
Butter	57	4	2.70c	0.26	1.99a	0.13	6.14a	0.23	2.07a	0.15
Fish oil /olive oil	41	1	1.78ab	0.12	3.79b	0.19	4.21a	0.29	4.91b	0.19
Fish oil /butter	44	1	2.06ab	0.12	3.65b	0.17	4.89a	0.29	4.36b	0.16

Different superscript letters signify $P<0.05$ by Duncan's test after one-way ANOVA.

Weight gain was greater for rats given the olive oil ($P<0.05$) and butter ($P=0.07$) diets compared with the chow-fed control groups, with heavier organ fat weights implying that differences in dietary fatness contributed to the different weight gains. Compared with the chow-fed control groups, glucose tolerance was impaired by the butter diet and this was associated with an increased insulinogenic index and impaired insulin sensitivity. However, glucose tolerance was well maintained in all other groups including the butter/fish oil group, and the heavier and more obese olive oil group. Membrane fatty acid profiles reflected dietary intakes with higher total SFA in the butter-fed group compared with all other groups and with wide variations in the *n*-6:*n*-3 ratio between all groups; but only total SFA was predictive of impaired glucose tolerance. These data clearly demonstrate that in the rat, (1) glucose tolerance and insulin resistance is only influenced by high saturated fat feeding and not by high fat MUFA- or PUFA-rich diets; (2) the *n*-6:*n*-3 ratio has no influence on glucose tolerance or insulin sensitivity, and (3) changes in body fatness need not influence glucose tolerance.

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The glycaemic index of commonly consumed Irish foods By [1,2]ALICE M. LONG and [2]MARY MOLONEY, [1]*University of Dublin, Trinity College, Dublin, Republic of Ireland and* [2]*Dublin Institute of Technology, Kevin Street, Dublin, Republic of Ireland*

Different carbohydrate foods produce different blood glucose responses despite an apparent lack of difference in macronutrient composition (Conn *et al.* 1936). The glycaemic index is defined as the incremental area under the blood glucose curve in response to a standardised carbohydrate load (Jenkins *et al.* 1981). The glycaemic index has many therapeutic uses, it can be used in the dietary management of Type I and Type II diabetes, lipid lowering diet and in the management of obesity. Foods are generally classified into low (<55) and high glycaemic index (>70). This study calculated the glycaemic index of commonly consumed Irish foods using white bread as the standard, and it also compared the results with similar foods tested in other centres.

The glycaemic index was determined after the ingestion of 50g portions of available carbohydrate. Nine subjects were recruited, three males and six females, with a mean age of 22.3 years and an average BMI of 23.6 kg/m². Capillary finger prick samples were taken at fasting, 15, 30, 60, 90 and 120 min after the consumption of the test meal. The incremental area under the curve for both the standard and the test foods were then calculated, along with the glycaemic index.

Food	Glycaemic Index (GI) Mean	SD	GI (Brand-Miller *et al.* 1996) Mean†	GI (Jenkins *et al.* 1981) Mean†
Bananas	73.3*	28.1	74.2	89
Brown bread‡	108.6	31.89	107	-
Crisps	51.65	22.19	75.6	73
Low fat milk	26.6	17.9	37.8	46
Full fat milk	31.18	16.98	37.8	49

* Mean value was significantly different from both varieties of milk: P < 0.05.
† Mean value was significantly different from all other foods tested: P < 0.05.
‡ Standard deviation not available.

The results of this study compare favourably with the results of similar foods tested in other centres (Jenkins *et al.* 1981; Brand-Miller *et al.* 1996). Various factors affect the glycaemic index of foods including food form, fat content and protein content. Subject characteristics such as sex, BMI and the menstrual cycle also affect the glycaemic index. One of the female subjects tested gave very different results when compared with other subjects. This variation was probably due to the effect of the menstrual cycle on gastrointestinal transit time, which is greatly reduced during the luteal phase of the cycle due to progesterone levels being at their highest (Wald *et al.* 1981). This slowing of gastrointestinal transit could, therefore, reduce the glycaemic index. The glycaemic index of the brown bread was twice that of the crisps. This was possibly due to the high fat content of the crisps (55%), compared with 6.72% fat content in the brown bread. Fat delays gastric emptying, which in turn lowers the glycaemic index (Wolever *et al.* 1990). Further study is warranted into determining the glycaemic index of different varieties of commonly consumed Irish foods, for example, potatoes, breads, and mixed meals such as Irish stew.

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Dietary fat quality and the sensitivity of insulin mediated pathways of glucose and lipid tolerance in genotypically uncharacterized middle-aged normal men. By A.C. FEREDAY, K.A. SLEVIN, E. AH SING, A. IRVINE, L.M. MORGAN, C.M. WILLIAMS, J.WRIGHT and D.J. MILLWARD, *Centre for Nutrition and Food Safety, University of Surrey, Guildford GU2 5XH*

We examined the insulin-mediated pathways of both glucose and lipid homeostasis to identify how dietary fat influences these relationships. We studied thirty-two sedentary, non-smoking, middle-aged men, genotypically uncharacterized, with waist:hip ratio >0.8, fasting insulin >60 μm/ml, in three dietary intervention trials. Trial 1 was a 10-week randomized cross-over design with a control or fish oil diet (2–3 g long chain n-3 PUFA, n 11; Fereday *et al.* 1998). Trials 2 and 3 compared habitual high-saturated-fat diets (>15% dietary energy) with decreased SFA intakes (8% energy) achieved by replacing SFA with either carbohydrate (n 12, Trial 2; Slevin *et al.* 1999), or MUFA (n 9, Trial 3). Insulin sensitivity was measured as $K_{glucose}$ and K_{NEFA} in the short intravenous insulin tolerance test (SIITT; Slevin *et al.* 1999). Postprandial lipaemia (PPL) was studied as AUC total TAG, apoB48, glucose, insulin, total cholesterol and NEFA, over 9 h after a standardized high-fat (80 g) breakfast (Fereday *et al.* 1998) with post-heparin measurements of LPL at 9 h and with a fat biopsy taken at 6 h for measurement of LPL mRNA. In Trials 1 and 2 we also measured the suppression of fasting glycerol concentrations and production rates and NEFA release during an insulin infusion in fasted subjects (Slevin *et al.* 2000).

Initial fasting insulin was a poor predictor of insulin sensitivity ($K_{glucose}$). This varied more widely than expected, resulting in a variability in responses to the interventions which obscured overall responses to the treatments. Thus for $K_{glucose}$, fifteen improved, five worsened and twelve varied by less than 15%, the intra-individual variation observed in our studies. However for the combined intervention groups, there were small but significant reductions in fasting NEFA and apoB-48$_{AUC}$ (reduced by 35%; $P<0.001$). Because red cell fatty acid profiles correlated with measured intakes of MUFA (r 0.42, $P=0.005$ for C18:1), and PUFA (r 0.41, $P=0.006$ for C18:2), and changed predictably with the three interventions, we analysed their relationship with outcome variables by multiple regression of log transformed data and by analysis of quartiles of variables ranked in order of the appropriate fatty acid.

In the pre-intervention group (n 32) $K_{glucose}$ was related only to C16:0 (partial correlation coefficient = -0.68, $P=0.007$), a 30% worsening between top and bottom quartile, and was weakly apparent postintervention (n 32; partial = -0.25, $P=0.17$). For all other outcome variables, in regression only n-3 PUFA emerged as significant predictors in both pre- and post-intervention subjects. Thus in the pre- and postintervention subjects, total n-3 PUFA predicted decreasing lipaemia (TAG$_{AUC}$: r -0.41, $P=0.001$, Apob48$_{AUC}$, partial = -0.35, $P=0.03$) with a 30% and 18% improvement between top and bottom quartiles for each parameter (significant for the improvement in lipaemia ($P<0.05$ ANOVA of quartiles, $P=0.06$ top versus bottom quartile). LPL activity was also predicted by total n-3 (partial = 0.287, $P=0.08$, postintervention group). HDL cholesterol also correlated with total n-3 PUFA (r 0.52, $P<0.01$) but an ANOVA of quartiles showed no significant differences.

In conclusion, although the relationships observed in these genotypically uncharacterized men were weak, the studies strengthen the growing body of evidence that dietary fat quality is a determinant of insulin resistance, with saturated fat associated with impaired glucose disposal and n-3 PUFA associated with improved lipaemia and NEFA homeostasis. However since neither LPL mRNA or activity were significantly related to total n-3 PUFA, the mechanism for this influence of n-3 PUFA on lipaemia is not established.

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The sensitivity of glucose and lipid-related targets of insulin action are unrelated in genotypically uncharacterized middle-aged normal men. By K.A. SLEVIN, A.C. FEREDAY, E. AH SING, A. IRVINE, L.M. MORGAN, C.M. WILLIAMS, J. WRIGHT and D.J. MILLWARD, *Centre for Nutrition and Food Safety, University of Surrey, Guildford GU2 5XH*

To better understand diet and cardiovascular disease risk, we examined whether impaired insulin-mediated glucose disposal predicts the impairment of insulin-mediated pathways of lipid metabolism and consequent dietary fat intolerance (postprandial hypertriglyceridaemia). Thirty-two sedentary, non-smoking, middle-aged men with a waist:hip ratio >0.8 and fasting insulin >60 μm/ml were entered in three dietary intervention trials. Trial 1 was a 10-week randomized cross-over design with a control or fish oil diet (2–3 g long-chain n-3 PUFA, n 11; Fereday *et al.* 1998). Trials 2 and 3 compared habitual high-saturated-fat diets (>15% dietary energy) with decreased SFA intakes (8% energy) achieved by replacing SFA with either carbohydrate (n 12, Trial 2; Slevin *et al.* 1999), or MUFA (n 9, Trial 3). Insulin sensitivity was measured as $K_{glucose}$ and K_{NEFA} in the short intravenous insulin tolerance test (SIITT; Slevin *et al.* 1999). Postprandial lipaemia (PPL) was studied as AUC total TAG, apoB48, glucose, insulin, total cholesterol, NEFA, over 9 h after a standardized high-fat (80g) breakfast (Fereday *et al.* 1998), with post-heparin measurements of LPL at 9 h, and with a fat biopsy taken at 6 h for measurement of LPL mRNA. In Trials 1 and 2 we also measured the suppression of fasting glycerol concentrations and production rates and NEFA release during an insulin infusion in fasted subjects infused with [1,2,3,²H5]glycerol to measure the glycerol production rate (Slevin *et al.* 2000).

We compared insulin sensitivity as $K_{glucose}$ with other variables by correlation and by analysis of quartiles of variables ranked in order of $K_{glucose}$. The intra-individual variation of $K_{glucose}$ (six young men measured on four occasions) was 15%, similar to fasting TAG (14.5%) and total cholesterol (10%). We conducted this analysis twice, on baseline/control diets (n 32) or post-dietary intervention (n 32).

In subjects at baseline/control diet $K_{glucose}$ correlated with fasting glucose, (r 0.41, $P=0.02$), glucose/insulin, (r 0.37, $P=0.04$), fasting and postprandial insulin AUC (marginally, r 0.34, $P=0.06$) although ANOVA of quartiles showed no significant differences for any of these parameters. K_{NEFA} or NEFA suppression with the low insulin infusion was unrelated but fasting NEFAaemia was weakly correlated (r 0.30, $P=0.094$). Neither HDL nor LDL cholesterol were related. With TAG$_{AUC}$ and B48$_{AUC}$, although ANOVA of quartiles showed no differences, they were both inversely correlated with $K_{glucose}$ (r -0.37, $P=0.04$; r -0.53, $P=0.002$). This may have reflected an increased TAG clearance due to increasing hyperinsulinaemia with insulin insensitivity since there was a highly significant inverse relationship with the TAG$_{AUC}$:insulin$_{AUC}$ ratio: r -0.53, $P=0.002$, ANOVA of ranks: $P=0.02$). However, neither LPL activity nor mRNA level were related to $K_{glucose}$. Analysis of all subjects after the dietary interventions showed that apart from a weak correlation with K_{NEFA} (r 0.32, $P=0.07$), there were no significant relationships of $K_{glucose}$ with any of the other measured insulin, glucose or lipid-related parameters, although as in the pre-intervention data set, mean values of TAG or B48$_{AUC}$ tended to increase with increasing sensitivity. These data show that in genotypically uncharacterized middle-aged men, the insulin sensitivity of glucose disposal as measured by the SIITT does not predict the apparent sensitivity of lipid-related targets of insulin action in a simple way.

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A comparison of changes in plasma cholesteryl ester and phospholipid fatty acid composition after a monounsaturated-enriched diet. By B.A. FIELDING[1], R.D. SMITH[2], C.N.M. KELLY[2], M.C. NYDAHL[2], K.D.R.R. SILVA[2], M.L. CLARK[1], V. ILIC[1] and C.M. WILLIAMS[2], [1]Oxford Lipid Metabolism Group, Radcliffe Infirmary, Oxford OX2 6HE, [2]Hugh Sinclair Unit of Human Nutrition, University of Reading RG6 6AP

The fatty acid composition of adipose tissue, plasma lipid fractions, red blood cell and platelet phospholipids reflect the fatty acid composition of the diet. Although significant correlations between such biomarkers and long-term dietary fat intake have been reported within whole groups, associations are not strong and are not consistent between different lipid fractions or classes of fatty acids (Ma et al. 1995; Beysen & Fielding, 1999). However, within subjects, the fatty acid composition of plasma lipid classes alters quite markedly in response to dietary change, and can be used to monitor dietary intervention where specific classes of fatty acids have been targeted (Katan et al. 1997). We have compared the use of plasma cholesteryl ester (CE) and phospholipid (PL) as biomarkers in a dietary study. We have also investigated whether the changes in biomarkers correspond to changes in plasma cholesterol concentrations.

We studied fifty-one students (twenty-six males and twenty-five females) who were participating in a dietary intervention study aimed at substituting saturated fatty acids (SFA) with monounsaturated fatty acids (MUFA) (Smith et al. 2000). After 8 weeks of a reference (SFA) diet (at time M0), the students were randomized to receive diets with the following target intakes: either a moderate-MUFA diet (% energy: 13.1 SFA, 15.2 MUFA, 5.9 PUFA) or high-MUFA diet (% energy: 10.5 SFA, 17.8 MUFA, 5.9 PUFA) for 16 weeks (finishing at time M16).

From M0 to M16, the proportion of oleic acid (18:1n-9) in fasting blood samples increased significantly in both lipid classes for the two dietary groups combined (see Table) but the proportion of palmitate (16:0) decreased only in PL. Within the high-MUFA group, there were significant increases in both CE-and PL- 18:1n-9 (P<0.05) and corresponding decreases in CE- and PL-16:0 (P<0.05). However, within the moderate-MUFA group, there were no significant changes in CE- or PL-18:1n-9, but there was a significant decrease in PL-16:0 (P<0.05).

The increases in proportions of PL- and CE- 18:1n-9 were not significantly correlated with the decreases in total and LDL cholesterol concentrations achieved in response to the MUFA diets (Smith et al. 2000).

g/100g total fatty acids		16:0		18:1 (n-9)		18:2 (n-6)	
		M0	M16	M0	M16	M0	M16
CE (n 49)	Mean	12.5	12.2	19.7	20.4*	54.8	54.0
	SE	0.27	0.31	0.31	0.39	0.45	0.59
PL (n 51)	Mean	30.6	29.5*	11.0	11.6*	25.1	24.2*
	SE	0.36	0.33	0.18	0.20	0.37	0.37

*Significantly different from M0 (Wilcoxon Signed Ranks Test)

These results suggest that PL may be a more sensitive marker of change in dietary 16:0 than CE. The changes in the composition of biomarkers and changes in plasma cholesterol concentrations may have been too modest to give rise to significant associations.

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The effect of ethanol on postprandial lipoprotein metabolism with particular reference to chylomicrons. By N. MOHD-ESA, A.J. HAWDON, K. MATTU, J.W. WRIGHT, B.A. GRIFFIN and B.J. GOULD, Centre for Nutrition Research, School of Biological Sciences, University of Surrey, Guildford GU2 7XH

When ethanol is taken with a meal, postprandial lipaemia is observed. There are no studies of changes in chylomicron (CM) metabolism in this situation. To follow the metabolism of CM and their remnants (CMR), retinyl palmitate (RP) was included in an oral-fat load test. The retinyl esters (RE) derived from RP are incorporated into the core of CM. In human subjects, apolipoprotein B-48 (apo B-48) is produced only by the intestine and one molecule of apo B-48 is present per CM (Phillips et al. 1997) where it remains associated with the surface of the particle. Theoretically both RP and apo B-48 should remain associated with CM and CMR from their secretion until their uptake by the liver. However, some studies have shown that apo B-48 and RE are not equivalent markers of postprandial intestinal lipoprotein (Lovegrove et al. 1999).

Eight volunteers attended our clinical investigation laboratory on two occasions, having fasted overnight. They ate a high-fat meal in 20 min, which included RP (170 000 IU). On one occasion they also drank the equivalent of 0.6 g ethanol/kg as Vodka. Serial blood samples were taken for 8 h. Triacylglycerol (TAG) was assayed by an automated enzymic method, RE, by HPLC (Ruotolo et al. 1992) as modified by Jackson (1996), total apo B, by immunoturbidimetry, and apo B-48, by Western blotting after separation on 3–16% SDS-PAGE following by a specific and highly sensitive immuno-detection method (Amdex) as previously described (Mohd-Esa & Gould, 2000). The apo B-48 measurement was semi-quantitative with the apo B-48 concentration being expressed as percentage of an internal standard.

The table shows incremental area under the curve for TAG, total apo B, RE and apo B-48, after ingestion of high-fat meal (control v. ethanol).

	Control		Ethanol	
	Mean	SD	Mean	SD
TAG (min.mmol.l⁻¹)	295.60*	89.71	489.90*	109.02
Total apo B (min.g.l⁻¹)	−22.59	43.40	20.22	112.30
Apo B-48 (min.%)	1158.75	1676.30	2501.75	2980.99
RE (min.ug.ml⁻¹)	192.99	135.58	238.30	224.90

Significantly different (paired t test), *P<0.01.

The TAG value demonstrated the expected increase in lipaemia due to ingestion of ethanol. The most obvious change was the increased TAG values after 4 h. Although total apo B was unchanged throughout the study in both groups of volunteers, apo B-48 remained elevated up to 8 h after ethanol but decreased to the fasting value in the control group by 6 h. RE showed a similar pattern to apo B-48 but there were no significant changes.

The increased postprandial lipaemia caused by the ingestion of ethanol with a meal is partly explained by an increase in the number of TAG-loaded CM. This is probably due to the increased competition with VLDL for lipoprotein lipase.

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The presence of ispaghula inhibits the fermentation of lactulose by human faecal bacteria *in vitro*. By M.K. KHAN and C.A. EDWARDS, *Department of Human Nutrition, Glasgow University, Yorkhill Hospitals, Glasgow, G3 8SJ*

The short-chain fatty acids (SCFA), produced by the fermentation of carbohydrates, have therapeutic effects on human colonic health (Edwards & Rowland, 1992). Most studies of fermentation have considered a single source of carbohydrate (Edwards & Parrett, 1999). However, it is likely that carbohydrates will interact when combined together. A recent study has shown a delay in fermentation (breath hydrogen production) when ispaghula and lactulose were fed together to human subjects (Washington *et al.* 1998). The effect of combining carbohydrates on the production of SCFA was not studied.

We investigated the effect of combining lactulose and ispaghula on fermentation and SCFA production in an *in vitro* fermentation model (Adiotome *et al.* 1990). A slurry of faeces (32%) from each of five human subjects was used to inoculate cultures containing different amounts of carbohydrate in a basic salts medium. Ispaghula and lactulose were combined in different ratios, making a total 100mg of substrate in each culture. In a second experiment, separate 25, 50 and 75 mg portions of lactulose and ispaghula were fermented. The fermentation was carried out anaerobically at 37° in a shaking water bath (50 strokes/min). Supernatants from cultures (centrifuged at 4° for 30 min) were used to determine the production of SCFA (Spiller *et al.* 1980). Values of net total SCFA from 8 hours fermentation of 25 mg portions of each carbohydrate were used to calculate the theoretical amount of SCFA expected from a hypothetical mixture. Results were compared by one-way ANOVA using Minitab10.5 Xtra. Results are shown as mean and SEM.
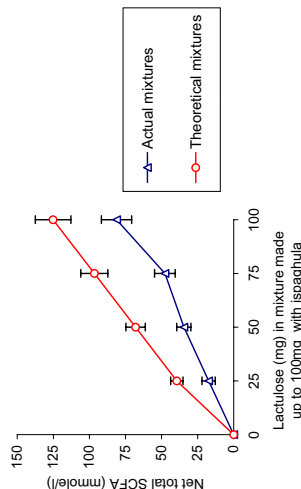
The net total SCFA in actual mixtures were significantly lower (*P*<0.02) than expected values, after correction for inhibition due to increased substrate concentration (unpublished data). Net total SCFA progressively decreased with increasing amounts of ispaghula in mixtures. The mechanism of this inhibition is unclear but may be related to the increased viscosity in cultures containing ispaghula. Fermentation of mixtures of carbohydrates may therefore be the result of an interaction between carbohydrates rather than a direct addition of the fermentation of each.

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The impact of apoE genotype on postprandial triacylglycerol responsiveness to fish oil supplementation. By A.M. MINIHANE[1], S. KHAN[2], E.C. LEIGH-FIRBANK[1], P TALMUD[3], B.A. GRIFFIN[2] and C.M. WILLIAMS[1], *[1]Hugh Sinclair Unit of Human Nutrition, University of Reading, Reading RG6 6AP, [2]Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH, [3]Centre of Cardiovascular Genetics, UCL, London WC1E 6JJ*

An elevated postprandial (PP) triacylglycerol (TG) response is now recognised as an important determinant of CHD risk (Dallongeville *et al.* 1998), and an integral component of the atherogenic lipoprotein phenotype (ALP). This pro-atherogenic phenotype, which is associated with a three-fold increased risk of CHD, is characterized by a fasting (1.5–4.0mmol/l) and PP hypertriacylglycerolidaemia, low high density lipoprotein-cholesterol (HDL-C) levels (<1.1mmol/l), and a predominance of the atherogenic low density lipoprotein (LDL) particle, LDL-3 (>40% total LDL) (Griffin & Zampelas, 1995). Although the beneficial impact of fish oil supplementation on fasting TG levels is well documented, the impact of this intervention on PP TG metabolism remains controversial. Apolipoprotein E (apoE) is a structural and functional component of several classes of lipoproteins and is an important determinant of the clearance of TG-rich particles from the circulation. In the current study the association between apoE genotype and PP TG and non-esterified fatty acid (NEFA) responsiveness to fish oil supplementation in an ALP group was investigated.

Fifty males (mean age 56 years) with an ALP profile completed a randomized crossover double-blind placebo controlled study. The study consisted of two 6-week periods of fish oil (6 g oil, providing 3.0 g long chain *n*-3 fatty acids/day) or 6 g olive oil/day, separated by a 12-week washout period. At the end of each supplementation arm a PP assessment was carried out (*t*=8 h), where following consumption of a standard breakfast (*t*=0 h, 49 g fat) and lunch (*t*=5.5 h, 31 g fat), regular blood samples were taken for analysis of TG and NEFA. At 8 h, 100 IU/kg of heparin was administered intravenously and a blood sample was collected 15 min later in order to determine lipoprotein lipase (LPL) activity.

| | All (*n* 50) | | % change* over 6 weeks | | | | | | | |
| | | | apoE2 (*n* 8) | | apoE3 (*n* 22) | | apoE4 (*n* 20) | | |
	Mean	SE	*P*[†]	Mean	SE	Mean	SE	Mean	SE	*P*[‡]
Fasting TG (mmol/l)	−35.3	5.3	0.000	−30.7	7.6	−34.9	7.0	−37.6	10.6	0.561
TG AUC	−23.3	3.0	0.000	−32.5	4.6	−18.4	4.3	−24.8	5.2	0.136
TG IAUC	−7.9	5.6	0.007	−27.7	7.0	−2.7	10.0	−5.5	8.2	0.014
Fasting NEFA (µmol/l)	−7.8	4.8	0.012	−22.1	5.9	−5.7	5.7	−4.1	9.9	0.616
NEFA AUC (270–480min)	−7.4	3.2	0.005	−18.3	6.7	−4.8	4.7	−5.7	6.3	0.608
LPL activity (nmol FA/ml/min)	14.7	14.5	0.065	47.2	29.7	2.1	9.7	17.3	33.9	0.177

*% change = % change on fish oil – % change on olive oil; TG = triacylglycerol; NEFA = non-esterified fatty acids; AUC = area under the curve (mmol/l/480min); ‡IAUC = incremental area under the curve (mmol/l/480min); [†]The statistical significance of the overall group changes; [‡]The inter-apoE group differences were analysed using ANCOVA with age as the covariate.

In the overall group (*n* 50) fish oil supplementation resulted in a significant decrease in fasting TG of 35%, in PP TG area (TG AUC) of 23%, in TG IAUC of 8%, in fasting NEFA of 8%, and in NEFA AUC of 7%. The observed 15% increase in LPL activity reached borderline significance (*P*=0.065). ApoE genotype had little impact on the responsiveness of fasting TG. However a greater reduction in PP TG responses was seen in apoE2 carriers. In particular, a significantly greater reduction in TG IAUC was seen in the apoE2 group (28%) relative to the apoE3 (3%) or apoE4 (6%) groups. TG IAUC which represents PP TG minus the fasting TG levels is thought to be more representative of changes in PP events, particularly TG clearance, than TG AUC, which is an index of both fasting and PP TG levels. A trend towards a greater increase in LPL activity, indicating increased hydrolysis of TG-rich lipoproteins, and a more effective NEFA clearance, may have contributed to the greater decrease in TG IAUC in the apoE2 subgroup.

In conclusion, fish oil supplementation resulted in the greatest attenuation in postprandial TG responses in individuals who are apoE2 carriers.

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Anthropometric results from the North/South Ireland Food Consumption Survey 2000. By S.N. McCARTHY[1], K.E. HARRINGTON[1], M.J. GIBNEY[2], M. KIELY[2], A. FLYNN[2] P.J. ROBSON[3], M.B.E. LIVINGSTONE[3] and J.J. STRAIN[3], *Irish Universities Nutrition Alliance, [1]Trinity College Dublin, Ireland, [2]University College Cork, Ireland, [3]University of Ulster, Coleraine, Northern Ireland*

Anthropometric measurements have useful applications in the determination of the nutritional status of a population. To date, there is a paucity of recent and reliable anthropometric data in Ireland. A representative sample of 1379 adults aged 18–64 years from the entire island of Ireland participated in the survey and, as part of the survey, weight, height, waist circumference, hip circumference and body composition were measured by standard procedures. Body mass index (BMI) was calculated from weight and height. Waist and hip circumferences were used to calculate the waist to hip ratio (WHR).

	All (18–64)			18–35 years			36–50 years			51–64 years		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
Weight (kg)	74.89	(15.04)	1368	72.53[a]	(14.86)	518	75.6[b]	(15.34)	519	77.44[b]	(14.35)	331
Height (m)	1.68	(0.09)	1311	1.70[a]	(0.10)	505	1.6[b]	(0.09)	498	1.6[b]	(0.09)	308
BMI (kg/m²)	26.35	(4.47)	1311	25.05[a]	(4.05)	505	26.8[b]	(4.44)	498	27.73[c]	(4.61)	308
Waist (cm)	86.97	(13.26)	1120	83.26[a]	(12.14)	442	87.7[b]	(13.48)	418	91.94[c]	(12.92)	260
Hip (cm)	102.81	(8.96)	1118	101.15[a]	(8.61)	441	103.53[b]	(8.84)	418	104.48[b]	(9.31)	259
WHR	0.84	(0.09)	1118	0.82[a]	(0.08)	441	0.85[b]	(0.09)	418	0.88[c]	(0.09)	259
Body fat (%)	27.79	(8.85)	1098	23.94[a]	(8.29)	441	29.3[b]	(7.89)	420	32.27[c]	(8.57)	237

[abc]Between each age group, the mean difference is significant $P<0.05$ (ANOVA).

The mean (and SD) anthropometric measurements for the total sample and the variations in the measurements between age groups are presented in the Table. BMI, waist circumference, WHR and body fat increased significantly with increasing age. Weight and hip circumference were significantly lower in the 18–35 year group with no significant difference between the 36–50 and 51–64 groups. Height was significantly greater at 18–35 years.

Mean weight, height and BMI have increased in both males and females since the previous similar surveys were conducted in the Republic of Ireland (Lee & Cunningham, 1990) and Northern Ireland (Barker *et al.* 1988). When BMI was classified according to the 1997 WHO recommendations (International Obesity Task Force, 1998), it was found that less than 1% of the population were underweight, 42.4% were in the normal BMI range followed by 39.1% and 17.8 % in the pre-obese and obese categories respectively. Chi-squared analysis revealed that in the normal BMI category there were significantly more females than males ($P<0.001$), whereas in the pre-obese category, there were significantly more males ($P<0.001$).

Social class did not have a significant effect on BMI; however, professional workers had the lowest BMI (25.87 kg/m²) and unskilled workers had the highest BMI (26.88 kg/m²). Location of residence had a significant effect ($P=0.02$) among 18–35 year old females, with those residing in the open country/village being of a higher BMI (25.56 kg/m²) than females residing in a city environment (23.53 kg/m²). Smokers had a lower BMI (25.17 kg/m²) than ex-smokers (27.89 kg/m²) and non smokers (26.68 kg/m²) which was significant at $P<0.001$. The BMI of ex-smokers was also significantly higher than BMI in non-smokers. This work shows that weight and BMI among other anthropometric variables are increasing in the Irish population, in accordance with current trends in Western Europe. This stresses the need to address and directly target the increasing prevalence of obesity and its associated co-morbidities.

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Dietary precursors of sulphide in the human large intestine. By E.A.M. MAGEE[1], C.J. RICHARDSON[2] and J.H. CUMMINGS[1], *[1]Department of Molecular and Cellular Pathology, Ninewells Hospital and Medical School, Dundee DD1 9SY, [2]MRC Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH*

Emerging evidence suggests that hydrogen sulphide is a bacterially derived, metabolic toxin in the inflammatory bowel disease, ulcerative colitis (UC). Sulphide is produced by anaerobic bacteria and is dependent on the availability of sulphur-containing dietary compounds that reach the colon (Pitcher *et al.* 1995). The major dietary sources of sulphur are the sulphur-containing amino acids (Saa) methionine and cysteine and inorganic sulphur anions (S(IV)). Previous work has shown increases in faecal sulphide excretion with increased meat intake and may have implications for sulphide production in UC (Magee *et al.* 2000). S(IV) compounds are widely used in the UK diet as preservatives, bleaching agents and antioxidants. The principal forms are sulphur dioxide (E220), sulphites (E221–E228) and sulphates (E514–E518). There are no good estimates of *per capita* intake of S(IV) compounds in the diet and little is known about the contribution of sulphur additives in food and drink to sulphide production *in vivo*. The purpose of this study was to address the relative effect(s) of sulphur-containing food additives in addition to Saa intake on faecal sulphide concentrations in healthy volunteers.

Seven healthy male volunteers aged 36–49 years were fed a series of three diets randomized in a metabolic suite, for 15 days each. Study diets were iso-energetic and contained 60 g meat/day without additives (60), 60 g meat with additives (60S) and 420 g meat with additives (420S). Sulphur additives were consumed as sulphited alcohol-free beverages (wine and cider) and dehydrated foodstuffs (dried fruit and potato). These contributed around 10 mmol/d of sulphur additives (8.3 mmol/d sulphate and 1.9 mmol/d sulphite). On days 14 and 15 of each test period, faecal and urine samples were collected for the determination of sulphide (SH⁻) and sulphate (SO₄²⁻) respectively, by ion exchange chromatography (Florin *et al.* 1991). Faecal and urine collections were validated using radio-opaque markers and PABA respectively.

Diet	Inorganic dietary S content (mmol/d)	SD	Urinary SO₄²⁻ excretion (mmol/d) (n 7)	SE	Faecal SH⁻ (mmol/l) (n 7)	SE
60	6.0	0.7	18.3	2.4	0.21	0.05
60S	15.5	1.5	23.2[†]	2.0	0.30[*]	0.04
420S	16.7	0.4	39.8[†‡]	4.8	0.78[**]	0.11

Regression analyses revealed: [†]Significantly different from 60 ($P<0.001$); [‡‡]significantly different from 60 ($P<0.001$) and 60S ($P<0.001$); [*]significantly different from 60 ($P<0.001$); [**]significantly different from 60 ($P<0.001$) and 60S ($P<0.001$).

Mean daily urinary SO₄²⁻ excretion (mmol/d, SE) increased from 18.3 (2.4) on the 60 diet to 39.8 (4.8) on the 420S diet and acted as a useful marker of Saa and S(IV) intake. Mean faecal SH⁻ concentrations (mmol/l) ranged from 0.21 on the 60 diet to 0.78 on the 420S diet. Regression analysis revealed a significant relationship between total faecal SH⁻ and dietary sulphate (r^2 0.43, $P<0.01$).

The present study is the first to examine the contribution of sulphiting agents to total faecal SH⁻ production in healthy volunteers. The findings presented demonstrate a significant relationship between dietary intake of sulphited foods and faecal SH⁻ concentrations. Thus, in attempting to lower faecal SH⁻ levels through diet alone, it is necessary to consider both dietary protein and sulphur-containing food additive intakes.

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Pregnant women in Trinidad have low plasma triacylglycerol and small babies. By R.C. SHERMAN[1], G.C. BURDGE[1], Z. ALI[2], K. LUTCHMAN SINGH[2] and A.A. JACKSON[1]. [1]Institute of Human Nutrition, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, [2]The University of the West Indies, St. Augustine, Port of Spain, Trinidad

In Trinidad, cardiovascular disease and type II diabetes are important causes of morbidity and mortality (Government of Trinidad and Tobago, 1978), and birth weight is significantly less than reference standards (Lenton et al. 1998). Lower birth weight has been associated with an increased risk of both diseases (Forrester et al. 1996). In part, birth weight is dependent upon the effective accumulation of lipid by the fetus in the third trimester. Maternal hyperlipidaemia is usual during late pregnancy (Darmandy & Postle, 1982), and one possible physiological role is to increase the availability of lipid to the fetus during the major growth phase. Low birth weight may therefore reflect reduced delivery of lipid from mother to fetus.

A cross-sectional study of non-pregnant and pregnant African-Trinidadian (AT) and Indian-Trinidadian (IT) women was carried out at Mount Hope Hospital, Trinidad. The women did not differ significantly in terms of age and anthropometry. Cord blood samples were collected at delivery and birth weights recorded. Maternal fasted blood samples were collected the morning after delivery. Plasma triacylglycerol (TAG) concentrations were determined by gas chromatographic analysis at the Institute of Human Nutrition.

On average, infants born to both AT (n 4) and IT (n 5) women were of lower birthweight than reference standards; medians 3.1 (2.7–4.6) kg and 3.2 (2.6–4.0) kg, respectively. Plasma lipid concentrations in non-pregnant and pregnant women, and umbilical cord samples were similar for AT and IT women. Median plasma TAG concentration was significantly ($P<0.05$) greater at term (1.8 (0.6–2.7) mmol/l AT (n 7); 1.9 (1.5–2.7) mmol/l IT (n 5)) compared with non-pregnant women (0.8 (0.5–1.3) mmol/l AT; 1.0 (0.5–1.7mmol/l IT) (n 5/group)). Whilst previous studies have reported a substantial increase in circulating TAG concentrations in late pregnancy, plasma TAG concentrations did not differ significantly between the second (1.5 (0.3–5.1) mmol/l) and third trimester (1.7 (1.2–2.8) mmol/l) of pregnancy and term (1.8 (0.6–2.0) mmol/l) in the Trinidadian women. Term TAG concentrations were markedly lower than reported previously for Caucasian (2.73 mmol/l; Darmandy & Postle, 1995), Indian (2.76 mmol/l; Jagadeesan & Prema, 1980) and Nigerian (2.70 – 2.93mmol/l; Oladunni Taylor et al. 1980) women, while values in non-pregnant and pregnant women at 20–22 weeks and 30-34 weeks were comparable. There was a close relationship between the concentration of TAG in maternal plasma and the concentration in cord blood (r^2 0.88, $P<0.02$).

The results suggest that for women in Trinidad there was an impaired ability to increase plasma TAG concentrations during late gestation. This may result in a limited transfer of lipid to the fetus, thereby reducing fetal lipid accumulation and fetal growth.

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Energy intakes in the Republic of Ireland: analysis of the North/South Ireland Food Consumption Survey. By M.J. McGOWAN[1], K.E. HARRINGTON[1], M.J. GIBNEY[1], M. KIELY[2], A. FLYNN[2], P.J. ROBSON[3], M.B.E. LIVINGSTONE[3] and J.J. STRAIN[3], Irish Universities Nutrition Alliance, [1]Trinity College Dublin, Ireland, [2]University College Cork, Ireland, [3]University of Ulster, Coleraine, Northern Ireland

The North/South Ireland Food Consumption Survey included the collection of food intake data over a 2-year period (1997–1999) on random samples of the population of the Republic of Ireland and Northern Ireland aged 18–64 years. Food intakes were recorded in a food diary for 7 days and quantified using a variety of methods: a food photographic atlas, weighing of foods, standard portion sizes, manufacturer's information and household measures. Energy intakes and the contribution of macronutrients to energy intakes are presented for male and female respondents in three age groups from the Republic of Ireland.

	Energy (MJ)			Percentage energy from						EI/BMR		
				Fat		Carbohydrate		Protein		Alcohol			
	Mean	(SD)	n	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)	n
All	11.3 (3.1)		958	35.2 (5.8)		44.1 (6.4)		15.6 (2.8)		4.8 (6.2)		1.42 (0.41)	892
Males													
All ages	11.3 (3.1)		475	34.7 (5.7)		43.5 (6.5)		15.5 (2.8)		6.0 (7.2)		1.50 (0.43)	428
18-35y	12.0[a] (3.3)		169	35.0[a] (5.4)		42.7[a] (6.3)		14.7[a] (2.8)		7.1[a] (7.8)		1.57[a] (0.43)	156
36-50y	11.4[a] (3.0)		178	35.8[a] (5.5)		42.9[a] (6.3)		15.7[b] (2.5)		5.3[a] (6.2)		1.49[ab] (0.42)	161
51-64y	10.4[b] (2.8)		128	32.9[b] (6.0)		45.2[b] (6.9)		16.2[b] (2.7)		5.3[a] (7.7)		1.40[b] (0.42)	111
Females													
All ages	7.8 (2.1)		483	35.7 (5.9)		44.7 (6.3)		15.6 (2.9)		3.7 (4.8)		1.34 (0.39)	464
18-35y	8.0[a] (2.1)		167	36.1[a] (5.6)		43.9[a] (6.0)		14.6[a] (3.0)		5.3[a] (5.8)		1.38[a] (0.42)	163
36-50y	8.0[a] (2.1)		201	35.9[a] (5.5)		44.3[a] (6.0)		15.8[b] (2.5)		3.6[b] (4.3)		1.36[a] (0.36)	196
51-64y	7.2[b] (2.1)		115	34.8[b] (6.7)		46.4[b] (6.8)		16.8[c] (2.8)		1.6[c] (3.0)		1.24[b] (0.38)	105

[a,b,c] Denotes significance between age groups $P<0.05$ (ANOVA).

Men had significantly higher mean energy intakes ($P<0.01$) than women in all age groups. The percentage contribution to energy intake from fat and carbohydrate was significantly higher ($P<0.05$) in women than in men, whereas the percentage energy from alcohol was higher among men than women ($P<0.01$) for the full sample. These differences were not consistently significant for fat and carbohydrate in all age groups. Men had, however, significantly higher alcohol intakes than women in all age groups.

Mean energy intakes decreased with increasing age in both men and women. The percentage contribution of fat to energy was significantly lower in men aged 51–64 compared with the other two groups. The percentage contribution of carbohydrate and protein to energy increased with age in both sexes. Energy from alcohol, expressed as a percentage of total energy, decreased with increasing age for both men and women. When energy from alcohol was excluded, the mean percentage contribution of fat, carbohydrate and protein to energy intakes for the full sample increased to 37%, 46% and 16% respectively.

Energy intake/estimated basal metabolic rate (EI/BMR$_{est}$) was calculated to further examine energy intakes. Mean EI/BMR$_{est}$ values were significantly higher in men than in women in all age groups. Among both sexes there was a fall in mean EI/BMR$_{est}$ values with increasing age.

When respondents who were considered to be on a diet or who said that they were unwell during the course of the food diary week were excluded (n 128), mean EI/BMR$_{est}$ values increased from 1.42 to 1.46 in the full sample, from 1.49 to 1.54 in men only and from 1.34 to 1.39 in women only.

Maternal fat intake, green leafy vegetable and fruit consumption, and micronutrient status are related to fetal size at birth in rural India: The Pune Maternal Nutrition Study. By S. RAO[1], C.S. YAJNIK[2], A. KANADE[1], B.M. MARGETTS[3], A.A. JACKSON[3], R. SHIER[4], S. JOSHI[1], S. REGE[2], H. LUBREE[2] and C.H.D. FALL[4]. [1]Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD, [2]Agharkar Research Institute, Pune, India, [3]KEM Hospital, Pune, India, [4]Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD, [4]MRC Environmental Epidemiology Unit, Southampton General Hospital, Southampton SO16 6YD

One third of full-term Indian babies weigh less than 2.5 kg at birth. This high prevalence of intra-uterine growth retardation has been attributed to widespread maternal undernutrition. Although reliable data on maternal nutritional status in India and its relationship with fetal growth are scarce, they are crucial if effective interventions to improve birthweight are to be developed. The Pune Maternal Nutrition Study examined maternal nutrition and anthropometry of babies at birth in a large population of rural Indian women. This paper focuses on our findings in relation to maternal macronutrient intakes, dietary quality and micronutrient status, in full-term pregnancies (n 633). At 18 (SD 2) and 28 (SD 2) weeks gestation, macronutrient intakes were assessed by a semi-weighed 24-h recall method; consumption of specific foods was assessed by a food frequency questionnaire, and red cell folate, serum ferritin and vitamin C concentrations were measured.

The mothers were short and underweight. Mean pre-pregnant height was 151.9 (SD 5.1) cm, BMI 18.0 (SD 1.9) kg/m^2 and weight 41.7 (SD 5.1) kg, and the babies were small (birthweight 2665 (SD 358) g). Maternal energy and protein intakes were low (7.4 (SD 2.1) MJ and 45.4 (SD 14.1) g at 18 weeks; 7.0 (SD 2.0) MJ and 43.5 (SD 13.5) g at 28 weeks) and were unrelated to the size of the babies at birth. Intakes of fat were low (34.9 (SD 14.8) g at 18 weeks and 32.4 (SD 14.0) g at 28 weeks) but babies born to mothers with higher intakes at 18 weeks were longer ($P<0.001$) and fatter (triceps skinfold: $P=0.02$; all P values adjusted for sex, parity and gestation). Birthweight and some other measurements were related to the frequency of consumption of milk at 18 weeks (birthweight; $P=0.03$) and of green leafy vegetables (GLV; $P<0.001$) and fruits ($P=0.007$) at 28 weeks, independently of maternal size and social class. Higher GLV consumption from one group to the next higher group was associated with a 45 g higher birthweight (95% CI: 18–73). This effect was stronger (89 g; 95% CI: 48–130) in the lightest mothers (<40 kg). Birth measurements were positively related to erythrocyte folate and serum vitamin C concentrations and inversely related to serum ferritin concentration. Trends associated with GLV intakes and erythrocyte folate concentrations were independent of each other.

	No.	Birth wt (g)	Length (cm)	Head (cm)	Neonatal measurements Mid-arm (cm)	Triceps skinfold (mm)	Subscap skinfold (mm)	Placenta (g)
Maternal intake of GLV at 28 weeks								
Never	60	2571	47.0	32.6	9.6	3.9	3.9	347
<Once/wk	175	2601	47.5	32.9	9.6	4.0	4.1	354
≥Once/wk	225	2675	48.0	33.2	9.7	4.1	4.0	358
≥Alt days	149	2742	47.9	33.3	9.9	4.4	4.3	371
P*		<0.001	0.005	<0.001	0.02	<0.001	0.04	0.05
Maternal erythrocyte folate at 28 weeks (µg/l)								
<360	171	2616	47.5	33.0	9.5	4.1	4.2	347
360–506	171	2637	47.7	32.9	9.7	4.1	4.1	356
≥506	172	2727	48.1	33.3	9.8	4.2	4.1	374
P*		<0.001	0.02	0.03	0.001	0.2	0.6	<0.001

*P derived by linear regression analysis and adjusted for sex, parity and gestational age at delivery.

The lack of association between birthweight and maternal energy and protein intakes, but strong associations with intakes of micronutrient-rich foods and folate and vitamin C status suggest that micronutrients are important limiting factors for fetal growth in undernourished communities. This has implications for the planning of interventions to improve fetal growth in developing countries.

Gastrointestinal handling and metabolic disposal of different fatty acids during rehabilitation from severe childhood malnutrition. By J.L. MURPHY[1], V.A. BADALOO[2], B. CHAMBERS[2], A. HOUNSLOW[1], T.E. FORRESTER[2], S.A. WOOTTON[1] and A.A. JACKSON[1]. [1]Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD, [2]The Tropical Metabolism Research Unit, University of the West Indies, Jamaica

During the treatment of malnourished infants, diets rich in fat are given to increase the energy density of the intake and to promote rapid catch-up growth. However there is no evidence to justify the prescription of any particular choice of dietary fatty acid. We have shown that the gastrointestinal handling of [1,1,1-^{13}C-labelled tripalmitin (TP) varied markedly between subjects (stool ^{13}C excretion ranged from 0 to 31% administered dose) during the rehabilitation of severely malnourished infants with only a small amount of label (<5%) partitioned towards oxidation (Murphy et al. 2000). In the present study we used stable isotope methodologies to examine the gastrointestinal handling and metabolic partitioning of oleic and linoleic acids (given as triacylglycerols, TAG) which are major fatty acids in the feed given during recovery from childhood malnutrition. Two groups of eight children (aged between 6 and 23 months) admitted to the Tropical Metabolism Research Unit (less than 80% weight for age and/or presence of pitting oedema; marasmus, marasmic-kwashiorkor and kwashiorkor) were studied three times: on admission (Phase 1), during rapid catch-up growth (Phase 2) and when weight for height had reached 90% of the reference (Phase 3). The children were given a single oral dose of either [1,1,1-^{13}C]Triolein (TO) or Trilinolein (TLIN) (20 mg/kg body weight) with their usual feed. After label administration, breath samples were obtained at half-hourly intervals for 6 h and at 8, 10 and 24 h and stools were collected for the next 3 days. The enrichment of ^{13}CO$_2$ in breath and ^{13}C in stool samples were analysed by isotope ratio mass spectrometry. The results are shown in the table as means (with SD).

	Phase 1				Phase 2				Phase 3			
	TO	SD	TLIN	SD	TO	SD	TLIN	SD	TO	SD	TLIN	SD
Stool ^{13}C (% admin dose)	9.1	9.2	8.9	8.2	0.5	1.0*	2.3	4.3	1.3*	1.9	1.2*	1.7
Breath ^{13}CO$_2$ (% absorbed dose)	12.5	14.2	16.2	11.3	8.9	7.5	11.9	11.9	6.8	3.9	19.4†	7.7

Significantly different from Phase 1 * $P<0.05$; Significantly different from TO at Phase 3 † $P<0.01$ (ANOVA).

Stool ^{13}C excretion was approximately 10% for both TO and TLIN but varied markedly between subjects. During rehabilitation, stool ^{13}C excretion was significantly reduced for both TAG. Whilst the excretion of ^{13}C on breath (from AUC) tended to decline for TO (NS), there was a significant increase in the amount of label excreted on breath for TLIN. These results suggest that the efficiency with which dietary TAG was handled within the gastrointestinal tract was generally impaired at admission but improved during recovery. The effect is most evident for TO and TLIN when compared to TP (Murphy et al. 2000), which may be related to differences in the physicochemical properties of each TAG. Following absorption, it appears that the postprandial metabolism for labelled linoleic acid during recovery is different to labelled oleic acid.

Murphy JL, Badaloo VA, Robinson EN, Forrester TE, Wootton SA & Jackson AA (2000) Proceedings of the Nutrition Society **59**, 38A.
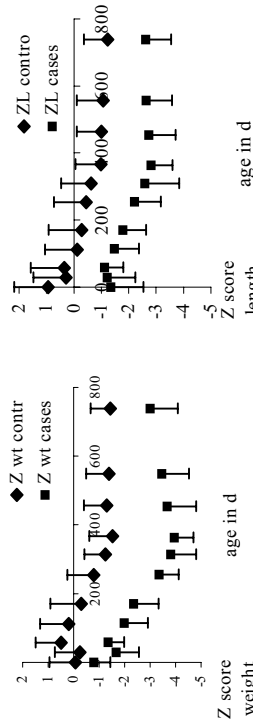
Nutritional stunting: a family continuum? By M.K. DARBOE and E.M.E. POSKITT, *International Nutrition Group, Department of Public Health Nutrition, London School of Hygiene and Tropical Medicine, 49–51 Bedford Square, London WC1B 3DP*

Nutritional stunting (NS; underweight with short stature) is the commonest form of malnutrition in rural Gambia. Catch-up growth is unimpressive and of uncertain duration. We reviewed a group of 3–9 year old children who had attended Keneba (The Gambia) nutritional rehabilitation centre with severe malnutrition (<60% expected weight for age: marasmus). Their present anthropometry, 48-h weighed food intake, past clinical history, social environment, and parental anthropometry were compared with those of control children (two controls/study child) matched for age, sex and village. Information available on health and anthropometry for the children from 0 to 2 years was analysed retrospectively.

Median age for presentation with marasmus was 10 months. At review (mean age 7.1 (SD 1.2) years), these children were still significantly lighter, shorter, and had a smaller head circumference (HC) than control children. MUAC and Z score BMI values were also less in the study children, but not significantly so. Changes in Z scores between 2 and 7 years (17 cases; 33 controls) for W, H, BMI, HC, and MUAC (mm) were +0.7, +0.8, −0.1, +0.8 and 26 mm, respectively, for study children and −0.1, +0.2, −0.4 and 22 mm for control children. Nutrient intakes for the two groups of children were not significantly different at review and, with the exception of calcium intake, achieved DRV for body weight. Parents of the study children were significantly lighter, shorter and had smaller HC (significant only in fathers) than control parents. MUAC and BMI were similar for the two groups of parents.

A retrospective review of growth in the first 2 years of life showed that study children were lighter, shorter, thinner and had a smaller HC at birth (Fig.). Anthropometry of study and control children followed very similar patterns but with different values at birth, so control children paralleled, at a higher level, the growth faltering of the study children.

This study suggests that marasmus in this area is strongly associated with small parents and small size at birth. The marasmic children's growth follows the same pattern as other children but starts off at levels closer to those critical for severe underweight/marasmus. Catching up can continue for years. Efforts to prevent severe malnutrition in this society need to be directed towards improving size at birth and weight gain and growth in very early infancy.

Figure legends: ◆ Z wt contr; ■ Z wt cases; ◆ ZL contro; ■ ZL cases. Axes: Z score weight, Z score length, age in d.

Changes in weight and length Z scores for 1–2 year-old study and control children analysed retrospectively.

Anthropometry of schoolchildren in the Midlands of KwaZulu Natal, South Africa. By E.M.W. MAUNDER, *Dietetics and Human Nutrition Discipline, School of Agricultural Sciences and Agribusiness, University of Natal, Pietermaritzburg, South Africa*

South Africa underwent a major political transformation in 1994, which is expected to result in improved economic and nutritional status of the disadvantaged communities, mainly the African population. Stunting is known to be a significant problem, particularly in African children in South Africa (Vorster *et al.* 1997). This study is a cross-sectional observational study, which sought to determine the effects of race, age and type of school on stunting of schoolchildren in the Midlands of KwaZulu Natal. The data were collected in 1997.

The data on 722 children in Grades 1 and 4 were collected at five primary schools within a 45 km radius of Pietermaritzburg. The schools are classified as private (P), historically white urban (HWU), historically black urban (HBU), historically serving black (African) children living in the white-owned commercial farming sector (HBF) and historically serving black, rural communities living within the former bantustans (HBB). For schools with small classes the whole class was included; for HBU schools a sample of the class was used. The gender composition of the children was fairly evenly divided. The children were mainly African or white. The age range of the study population was 6–15 years of age. Age and height measurements were used to calculate the z scores for height-for-age (HAZ) based on growth reference curves developed by the National Center for Health Statistics.

The mean HAZ differed between races for the total sample, being: −0.885 (African, n 584); −0.286 (Coloured, n 7); 0.093 (Indian, n 26) and 0.102 (White, n 105).

Height-for-age z scores

	African Grade 1*			African Grade 4*			White Grade 1			White Grade 4		
School	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
P	6	0.150	0.737	5	0.580	1.766	22	0.095	1.021	16	0.225	0.919
HWU	9	0.033	0.522	11	−0.473	0.843	27	0.144	0.963	40	0.040	0.840
HBU	141	−0.752	1.349	158	−1.023	1.155	0			0		
HBF	40	−0.793	1.463	27	−0.385	0.912	0			0		
HBB	110	−1.270	1.480	76	−0.992	1.157	0			0		

*Mean results within a grade shown to differ significantly by ANOVA, P<0.01.

Height-for-age z scores

	African Grade 1*			African Grade 4*			White Grade 1			White Grade 4		
Age-group**	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
1	280	−0.845	1.414	1	−0.600	0.000	49	0.122	0.979	0		
2	21	−1.376	1.226	181	−0.609	1.042	0			56	0.093	0.859
3	4	−2.575	1.879	93	−1.459	1.200	0			0		

*Mean results within a grade shown to differ significantly by ANOVA, P<0.05.
**Age-group: 1 = 5–8 years, 2 = 9–11 years, 3 = 12–15 years.

These results show that the mean HAZ was low in the African children but that the small number of African children in the P and HBU schools generally did not have a low mean HAZ. It is interesting to note that the African children who were old for their grade (age-groups 2 and 3 for Grade 1 and age-group 3 for Grade 2) showed lower mean HAZ scores than African children who were within the expected age-group for their grade. In conclusion, the relationship between stunting and race is modified in different schools and also by the child's age in relation to school grade.

Vorster HH, Oosthuizen W, Jerling JC, Veldman J & Burger HM (1997) *The Nutritional Status of South Africans. A Review of the Literature from 1975–1996.* Health Systems Trust.

Food habits in a sample of the immigrant population in Dublin. By K. O'CONNOR[1,2] and E. BALL,[2] [1]*Department of Clinical Nutrition, Trinity College Dublin, Ireland,* [2]*Dublin Institute of Technology, Kevin Street, Dublin, Ireland*

Since 1996 there has been a dramatic increase in the number of people seeking asylum in Ireland, with 7724 applications made in 1999 (Department of Justice, Equality and Law Reform, 2000). Studies have shown that acculturation leads to a change in food habits among immigrants, many of which are adverse, resulting in an increase in chronic disease rates in many such populations (Trostler, 1997). It has also been shown that nutritional services need to be specific for the language and cultural needs of the target population (Stowers, 1992). To date no information is available about the food habits of immigrants to Ireland and no suitable nutritional services are available.

An interviewer-assisted questionnaire was carried out on a non-random sample of twenty-eight immigrants, who were recruited via refugee centres (n 8), English language night classes (n 7), hostels (n 5), native food shops (n 7) and African hair salons (n 1). The data were analysed using SPSS, and descriptive statistics.

Twenty-two males and six females took part in the study, all of whom were aged between 18 and 44 years. All had been living in Ireland for between 3 months and 5 years, and 54% (n 15) had arrived within the last 6 months. 79% (n 22) originated from Africa, including thirteen from Nigeria. 79% (n 22) had tertiary education.

71% (n 20) of the study population were Christian and 29% (n 8) were Muslim. All of the Muslims reported following their religious dietary practices. Muslims were likely (n 5) not to eat out in Ireland, despite frequently eating out in their country of origin. Muslims (4/8) were more likely to think that nutritional advice would not be of benefit to them in Ireland than Christians were (5/20).

57% (n 16) of the sample was on social welfare. Many lacked cooking facilities, for example five subjects did not have a grill. Two subjects shared their cooking facilities with up to sixty other people. The main venue for eating out was fast food outlets (n 11) especially for those on social welfare (n 9). 82% (n 23) of subjects shopped regularly in an ethnic shop, with ten subjects living at least one hour away by foot. 64% (n 18) named price as their number one problem in buying native food in Ireland. The number one choice of intervention to overcome this problem was native foods available in supermarkets (n 15).

46% (n 13) ate their native food every day. Acculturation has resulted in adverse changes such as a decrease in consumption of fruit, vegetables and pulses and an increase in fast food and confectionary, with 43% (n 12), especially Christians (n 10), reporting an increase in weight. Subjects should be encouraged to maintain their native diet as much as possible with some modifications such as a recommendation to reduce frying of food. They should also be advised to reduce frying of food, chosen by 43% (n 12) as their main method of cooking. 79% thought it would be very important (n 16) or somewhat important (n 6) to have native foods available in hospitals. 68% (n 19) believed that nutritional advice would be of benefit to them in Ireland. Their number one choice of intervention was a cooking/nutrition video.

The immigrant population in Dublin is diverse, with varied food habits, cooking practices and religious beliefs. Any intervention should be specific for each group, be in a variety of languages and should ideally be visual. Asylum seekers are suffering financial difficulties, discrimination, and language and cultural barriers in Ireland, all of which will affect their dietary intake. There is also a need for the development of a culturally specific food frequency questionnaire before more specific research can take place.

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Moderate levels of undernutrition have little impact on immune function in rural Gambian children. By S.E. MOORE, A.C. COLLINSON and A.M. PRENTICE, *MRC Keneba, The Gambia and MRC International Nutrition Group, London School of Hygiene and Tropical Medicine, 49–51 Bedford Square, London WC1B 3DP*

The belief has long been held that undernutrition impairs many aspects of both the innate and adaptive immune system, leading to increased levels of morbidity and mortality. More recently, however, it has been suggested that this belief is based only on seminal observations in severely undernourished children and that it may be confounded by the fact that undernourished children frequently carry concomitant infections which may suppress immunity (Morgan, 1997). The current study explored the relationship between nutritional status and immune function in a cohort of 472, 6.5–9.5 year-old moderately undernourished rural Gambian children.

The children recruited into the study were born during the West Kiang maternal supplementation study (Ceesay et al. 1997). The West Kiang region sees a profound seasonality revolving around the annual rains (July–October). The rains coincide with an annual 'hungry season' when weight loss occurs in all adults, including pregnant women, and a seasonal reduction in birth weights is observed. The study ran for a 12-month period from April 1998 covering both seasons. Nutritional status was assessed by anthropometry and micronutrient status (haemoglobin (Hb) and plasma levels of zinc, vitamin C, and vitamin A and related retinoids). Immune function was assessed using a variety of different techniques, chosen to look at different components of the immune system; these included cell-mediated immunity (CMI) using the Merieux 'Multitest' CMI kit, response to T-cell mediated (Human Diploid Cell Rabies vaccine) and B-cell mediated vaccination (Pneumovax® 23 valent pneumococcal capsular polysaccharide vaccine), and mucosal immunity (lactulose mannitol intestinal permeability test and salivary levels of secretory IgA (sIgA)).

Weight-for-age z-scores (WAZ) and micronutrient status indicated moderate levels of undernutrition (mean (range): WAZ –1.6 (–4.7–0.91); Hb 12.66 g/l (6.5–16); zinc 12.55 µmol/l (0.72–63.3); vitamin C 50.0 µmol/l (0.97–185.2); retinol 0.84 µmol/l (0.28–1.6); α-carotene 0.68 µmol/l (0.02–4.8)). A seasonal variation was observed in all measures of nutritional status except plasma zinc and plasma retinol levels, and in all of the measures of immune function.

The table shows the relationship between immune function and aspects of nutritional status. All data were adjusted for season of measurement, age and gender.

Test	WAZ	Hb	Zinc	Vitamin C	Retinol	α-carotene
CMI	NS	NS	0.028	NS	NS	0.034
Pneumococcal vaccine						
Type 1	NS	NS	NS	NS	NS	NS
Type 5	NS	NS	NS	0.015	NS	NS
Type 14	NS	NS	NS	NS	NS	0.038
Type 23	NS	NS	NS	NS	NS	NS
Rabies vaccine						
Dose 1	NS	NS	NS	NS	NS	NS
Dose 2	NS	NS	NS	NS	NS	0.001
Lactulose/mannitol ratio	NS	NS	NS	NS	NS	NS
Salivary sIgA	0.017	NS	NS	NS	NS	NS

Despite a varying degree in both nutritional status and immune response across the children, no consistent associations were found between the two parameters. These results, from a large cohort of Gambian children do not support the concept that nutritional status influences immune function. These findings do not allow us to establish the association between immune function and nutritional status during more severe states of nutritional deficiency.

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Morgan G (1997) *Lancet* **349**, 1693–1695.

The effect of conjugated linoleic acid on the inflammatory response in humans. By A.P. NUGENT[1], E. NOONE[1], A. LONG[2], D.K. KELLEHER[2] M.J. GIBNEY[1] and H.M. ROCHE[1], [1]Unit of Nutrition, , [2]Molecular Immunology, Department of Clinical Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8, Ireland

Conjugated linoleic acid (CLA) is a collective term for a mixture of positional and geometric isomers of conjugated dienoic derivatives of linoleic acid. In vitro models and animal studies show that CLA modulates the inflammatory response. In porcine blood lymphocytes CLA enhanced T-cell mitogen (phytohaemagglutin (PHA) and concanavalin A (Con A)) induced lymphocyte proliferation, inhibited lymphocyte IL-2 production and suppressed the phagocytic activity of macrophages in a dose-dependent fashion (Chew et al. 1997). Turek et al. (1998) showed that a CLA-enriched diet significantly reduced macrophage tumour necrosis factor (TNF) and interleukin (IL)-6 production in male Sprague-Dawley rats. In contrast, Hayek et al. (1999) demonstrated that mice fed a CLA-enriched diet significantly increased splenocyte proliferation and IL-2 production. These studies clearly show that CLA modulates the inflammatory response; however, to date there has been no investigation of the effects of CLA on immune function in humans.

This double-blind placebo-controlled trial investigated the effect of two isomeric mixtures of CLA which contained different proportions of the cis-9 and trans-10 CLA isomers, compared to linoleic acid (control) on ex vivo mitogen stimulated proliferation of human peripheral blood mononuclear cells (PBMC). Fifty-five healthy volunteers (twenty males, thirty-five females, mean age 31.5 years, mean BMI 23.9 kg/m²) were randomly assigned to receive 3 g of (i) 80:20 cis-9, trans-10 isomer blend, (ii) 50:50 cis-9, trans-10 isomer blend, or (iii) a control fatty acid (linoleic acid) daily for 8 weeks. PBMC were isolated from whole blood drawn at weeks 0 and 8 and cultured in triplicate in the presence of 2.5% autologous serum and one of the T-cell mitogens PHA (10µg/ml), Con A (10µg/ml), the CD3 monoclonal antibody, OKT3, (10 µg/ml) the anti-HLA antibody, anti-IE, (30 µg/ml). Lymphocyte proliferation was measured as [³H] thymidine incorporated over the final 18 h of a 72 h culture period and expressed as a simulation index of cpm of stimulated cultures/cpm of unstimulated cultures. Statistical analysis was completed using two-way ANOVA.

Stimulus	50:50 cis-9, trans-10 isomer (n 17)				80:20 cis-9, trans-10 isomer (n 17)				Control: linoleic acid (n 19)			
	Pre		Post		Pre		Post		Pre		Post	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
PHA	203	47	232	53	210	49	319[a]	65	225	38	271	43
Con A	133	30	71[a]	16	155	32	117	25	124	24	118	27
OKT3	98	22	72	17	104	25	106	22	80	15	104	18
Anti-IE	1.4	0.3	1.1	0.3	1.3	0.2	1.7	1.5	1.2	0.1	1.4	0.2

[a] Denotes a significant difference between group mean values pre- and post- supplementation, $P<0.03$.

Supplementation with the 50:50 cis-9, trans-10 isomer blend significantly decreased Con A induced T-cell proliferation of PBMCs, whereas treatment with the 80:20 cis-9, trans-10 isomer blend significantly increased the lymphocyte proliferative in response to PHA. There was no significant effect of CLA on OKT3 or anti-IE (negative control) induced PBMC proliferation.

This is the first study which investigated the effect of CLA on the inflammatory response in humans. It clearly shows that CLA has the ability to modulate the immune function. The difference between isomer blends suggests that the nature of the effect on inflammatory response may be isomer-specific.

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Effects of substituting dietary saturated fatty acids with monounsaturated fatty acids on whole blood platelet aggregation in young healthy volunteers. By C.N.M. KELLY, R.D. SMITH, K.D.R.R. SILVA, M.C. NYDAHL and C.M. WILLIAMS, The Hugh Sinclair Unit of Human Nutrition, University of Reading, Reading RG6 6AP

The substitution of dietary saturated fatty acids (SFA) for monounsaturated fatty acids (MUFA) has been proposed as an alternative approach to reducing risk from cardiovascular disease (CVD). Although disturbances in blood platelets contribute to the development and progression of CVD, surprisingly few well-controlled studies investigating the effects of dietary MUFA on platelet function in humans have been conducted. The aim of this study was to investigate the effects of substituting dietary SFA for MUFA at two levels of MUFA intake on human whole blood platelet aggregation.

Twenty-nine students (thirteen males and sixteen females; age 21 (SD 3) years) residing in a fully catered Hall of Residence at the University of Reading were recruited into the study and consumed a reference (SFA) diet (% energy: 15.6 SFA, 12.4 MUFA, 5.9 PUFA) for 8 weeks. Thereafter the group was split in a randomized manner to receive either a moderate MUFA (MM) diet (% energy: 13.1 SFA, 15.2 MUFA, 5.9 PUFA) or high MUFA (HM) diet (% energy: 10.5 SFA, 17.8 MUFA, 5.9 PUFA) for a further 16 weeks. Substitution of cooking fats in the Hall kitchen, and modified spreads and snacks were used to achieve these dietary targets (Kelly et al. 2000). Fasting blood samples were collected at the beginning (M_0), 8 weeks (M_8) and 16 weeks (M_{16}) of the MUFA diet period. The table shows the mean change from baseline (M_0) in fasting whole blood platelet aggregation levels in response to three agonists (adenosine diphosphate (ADP), collagen and arachidonic acid) for each group, and for the two groups combined (Chrono-Log corp., Labmedics Ltd, UK).

Change in aggregation (ohms)		MM group (n 14)		HM group (n 15)		All (n 29)	
		M_0-M_8	M_0-M_{16}	M_0-M_8	M_0-M_{16}	M_0-M_8	M_0-M_{16}
ADP (10 µM)	Mean	-1.8**	-1.0	-1.5	-1.4**	-1.6***	-1.2*
	SE	0.5	1.0	0.8	0.4	0.4	0.5
Collagen (4 µg/ml)	Mean	-2.8*	+0.9	-2.3	-1.0	-2.5**	-0.1
	SE	1.2	1.0	1.3	1.0	0.8	0.7
Arachidonic acid (1 mM)	Mean	-2.0	-0.3	-1.5	-1.1	-1.7**	-0.7
	SE	1.0	0.7	0.7	0.7	0.6	0.5

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

There was a significant reduction in aggregation for the whole group in response to all three agonists following 8 weeks of MUFA intervention, which was only maintained up to 16 weeks in the case of the ADP response. A dose response effect was not apparent between the MM and HM groups and this was also the case for their blood lipid responses (Smith et al. 2001).

Although the effects observed were not large, they may be important when translated to whole population shifts in SFA and MUFA intakes. Further work is needed to clarify the mechanisms underlying the effects of MUFA on platelet function.

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α-Linolenic acid metabolism in adult men: evidence for synthesis of eicosapentaenoic and docosapentaenoic acids, but not docosahexaenoic acid.

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n-3 Polyunsaturated fatty acids (PUFA), principally eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, are important regulators of cell and tissue function. However, the major n-3 fatty acid in the U.K. diet is their precursor α-linolenic acid (ALNA) (MAFF, 1997). Since maintenance of appropriate cell membrane PUFA balance in UK adults may depend on their metabolic capacity for conversion of ALNA to EPA and DHA, we have investigated n-3 PUFA metabolism in men.

Six healthy men (mean age 36 (27–40) years) consumed their typical diet until one day before the study when they were fed three standardized meals. After an overnight fast, subjects consumed [U-^{13}C]ALNA (700 mg) in an emulsion with a test breakfast designed to reflect UK n-3 fatty acid intakes. Venous blood and breath specimens were collected for up to 21 d. Plasma triacylglycerol (TAG), non-esterified fatty acids (NEFA) and phosphatidylcholine (PC) were isolated by solid phase extraction (Burdge et al. 2000). Fatty acid [13C]enrichments were determined by gas chromatography–combustion–isotope ratio mass spectrometry.

Estimates derived from measurements of $^{13}CO_2$ excretion on breath indicated that 33 (SD 9.1) % of administered [13C]ALNA underwent β-oxidation over the first 24 h after ingestion of label. [13C]ALNA concentration was maximal in plasma TAG at 4–6 h, NEFA at 6 h and PC at 6–10 h. Integrated area-under-the-curve (AUC) for [13C]ALNA in TAG was greater than NEFA (18.1-fold) and PC (9.2-fold) (see Table). AUC for [13C] incorporation into EPA and docosapentaenoic acid (DPA) was greater (4.5- and 3.6-fold, respectively) in plasma PC than TAG. However, no [13C]-labelled n-3 PUFA were detected in plasma NEFA. There was no incorporation of [13C] into DHA in either plasma TAG, NEFA or PC after 21 d.

	Excursions of [13C]-labelled fatty acids in plasma (Integrated AUC; μg/ml over 21d)							
	[13C]ALNA		[13C]EPA		[13C]DPA		[13C]DHA	
Plasma lipid class	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
TAG	41.6	5.6	1.1	0.5	1.2	0.3	ND	
NEFA	2.3*	0.4	ND		ND	0.0	ND	
PC	4.5**	0.6	4.9**	1.5	4.3**	1.3	ND	

Values significantly different (P<0.01) from TAG * or between NEFA and PC **. ND indicates not detected.

The fractional [13C]ALNA oxidation was similar to [13C]palmitic acid (Bennoson et al. 1999) indicating that ALNA was not used preferentially as an energy source in men. Comparison of time courses and relative concentrations of [13C] fatty acids suggested that ALNA was mobilized from the gut primarily as TAG, while the liver was likely to be the major site of EPA and DPA synthesis. The apparent absence of DHA synthesis from [13C]ALNA suggests a disparity between dietary ALNA content and the extent to which this is converted to it's longer chain metabolites. This may have important implications for maintenance of plasma membrane DHA concentrations in these individuals.

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Burdge GC, Wright P, Jones AE & Wootton SA (2000) *British Journal of Nutrition* **84**, 781–787.
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α-Linolenic acid supplementation does not enrich platelet phospholipids with docosahexaenoic acid.

By Y.E. FINNEGAN, E.C. LEIGH-FIRBANK, A.M. MINIHANE and C.M. WILLIAMS, Hugh Sinclair Unit of Human Nutrition, Department of Food Science, University of Reading, Reading RG6 6AP

Isotope tracer studies suggest that α-linolenic acid (ALNA; 18:3n-3), the parent fatty acid of the n-3 family, is converted to its longer chain metabolites eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in human tissue (Emken et al. 1994). However, studies of platelet phospholipid composition have only demonstrated EPA enrichment on administration of ALNA with no increase in DHA. In general, such studies have been short term (average duration of 2–4 weeks) and it is thought that a longer trial period may be needed to detect the formation of DHA from ALNA. The aim of this study was to investigate the enrichment of platelet phospholipids with longer chain n-3 metabolites following supplementation of the diet with ALNA for a period of 6 months.

Twenty-eight men and women were randomly allocated into one of three dietary treatment groups. The medium and high ALNA groups were instructed to maintain a diet higher in ALNA with the use of specially formulated margarines, to be used as replacements for their normal butter or margarine. The control group was provided with a margarine representative of the typical UK fatty acid composition. All margarines provided similar amounts of total fat SFA, PUFA and MUFA as a percentage of total energy. Habitual dietary intakes were assessed using a 180-item food frequency questionnaire. Total dietary ALNA intake, averaged 1.8, 4.8 and 10.2 g/d in the control, medium and high ALNA groups, respectively. Additional ALNA was substituted isocalorically for linoleic acid, the intakes of which were 26.3, 17.4 and 14.6 g/d in the control, medium and high ALNA groups respectively. Blood samples were taken at baseline and after 2 and 6 months on the diet. Platelets were isolated and determination of individual fatty acids was carried out by GLC.

Treatment	Platelet phospholipid EPA				Platelet phospholipid DPA (% total fatty acids)				Platelet phospholipid DHA			
	Baseline		6 months		Baseline		6 months		Baseline		6 months	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	0.71	0.38	0.48	0.35	3.44	0.33	3.28	0.26	2.71	0.71	2.61	0.61
Medium ALNA	0.57	0.17	0.58	0.19	2.94	0.50	3.45[†]	0.55	2.69	0.49	2.40	0.49
High ALNA	0.54	0.23	0.95*	0.33	3.13	0.69	3.78	1.28	2.32	0.53	2.16[†]	0.40

Changes within groups: repeated measures ANOVA with paired t test. Significantly different from baseline *P<0.001, [†]P<0.05.

There was no significant change in platelet phospholipid EPA or DHA in the control or medium ALNA groups. However, docosapentaenoic acid (DPA; 22:5n-3) concentrations were significantly raised by 6 months on the latter. The change in platelet phospholipid EPA concentrations was significant following the high ALNA diet, the effect being evident by 2 months and significantly different from the changes observed in the control group. This was accompanied by a significant decrease in the DHA concentration. However, this change was not significantly different from the control group. Long-term supplementation with high ALNA intakes lead to enrichment of platelet phospholipids with EPA but not DHA, thus limiting its possibilities as a potential alternative to fish oil.

Emken EA, Adolf RO & Gulley RM (1994) *Biochimica et Biophysica Acta* **1213**, 277–288.

Oxidative susceptibility of low-density lipoprotein as measured by a micro-fluorescence assay: effects of dietary polyunsaturated fatty acids. By P.A. WILKINSON, Y.M. JEANES, D.J. MILLWARD, G. FERNS and B.A. GRIFFIN, *Centre for Nutrition and Food Safety, University of Surrey, Guildford, GU2 7XH*

The oxidative modification of LDL is a prerequisite step in atherogenesis (Steinberg *et al.* 1989). The oxidizability of LDL is dependent on many factors, including LDL particle size, lipid and antioxidant composition, all of which can be influenced through diet. The susceptibility of LDL (*ex vivo*) to copper-catalysed oxidation *in vitro* can be determined by measuring the fluorescence spectrum produced by the modification of apoprotein B (Cominacini *et al.* 1991). The pattern of fluorescence develops into inhibitory (I-FDR) and propagatory phases (P-FDR), which correspond to the rates of consumption of endogenous antioxidants (lag phase) and fatty acid oxidation, respectively. In the present study, a standard, cuvette-based, fluorescence assay was adapted to a 96-well micro-titre plate. The aims were to improve the resolution of the oxidation phases by continuously monitoring fluorescence, and to increase sample throughput and thus the sensitivity of detecting diet-induced changes in the oxidizability of LDL. Since dietary polyunsaturated fatty acids (PUFA) have previously been shown to increase the oxidative susceptibility of LDL, fish- and sunflower oil-enriched diets were used to test and validate this new procedure.

The habitual diets of twelve normal, healthy males (mean age 52 (SD 5.2) years) were supplemented with either 30 g/d of sunflower oil (SO) or sunflower oil plus 6 g (3 g/d EPA/DHA) of fish oil (FO) for 12 weeks. Fasting blood samples were collected into EDTA at baseline and after 12 weeks. Plasma was stored at −80° until isolation of LDL by sequential ultracentrifugation. Salt and EDTA were removed by membrane dialysis (4° for 24 h against 3 × 1L of PBS in the dark). 200 μl of LDL standardized to a final protein concentration of 1.0 mg/ml was placed in a microtitre plate and oxidized with 10 μl CuSO₄ (final concentration 5 μM). Fluorescent readings were taken every 6 min for 5 h (excitation 360 nm, emission 430 nm, RT).

Quinine sulphate solution was used as a calibrant between assays. PBS, native LDL and LDL in PBS were run as controls on each plate. A linear regression line was used to determine both I-FDR and P-FDR slopes. Results were expressed as length of I-FDR (min) and P-FDR in relative fluorescence per min (RFmin⁻¹). The table shows the values obtained for I-FDR and P-FDR in four subjects on the diets.

Sample	Diet	I-FDR min (Wk 0)	I-FDR min (Wk 12)	P-FDR RFmin⁻¹ (Wk 0)	P-FDR RFmin⁻¹ (Wk 12)
1	FO	134.4	76.8	71.6×10^{-3}	76.9×10^{-3}
2	FO	128.0	12.0	79.3×10^{-3}	68.3×10^{-3}
3	SF	147.2	115.2	82.5×10^{-3}	92.2×10^{-3}
4	SF	121.6	83.2	79.6×10^{-3}	87.9×10^{-3}

The method successfully detected a dietary effect in I-FDR and P-FDR within and between diets over a 12-week intervention period. Fish oil was associated with a greater reduction in I-FDR than sunflower oil was. This method successfully increased the throughput rate in terms of sample numbers and removed the need for repeated handling of the LDL, during the course of the experiment. This method may benefit intervention studies with a high number of samples, which may undergo subtle changes in their susceptibility of LDL to oxidation.

Cominacini L, Garbin U, Davoli A, Micciolo R, Bosello O, Gaviraghi G Scuro LA & Pastorino AM (1991) *Journal of Lipid Research* **32**, 349–358.
Steinberg D, Parthasarathy S, Carew TE & Witzium JL (1989) *New England Journal of Medicine* **320**, 915–924.

Accounting for variation in baseline diet in fatty acid intervention studies. By C. BOLTON-SMITH[1], I. MURRIE[1], R. BARR[1], I. CURRIE[2], R.J.G. PRICE[1], S.T. FENTON[1], J.F. BELCH[2] and A. HILL[2], [1]*Nutrition Research Group, CVEU,* [2]*Department of Medicine, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY*

Dietary intervention studies are probably confounded by variation in the baseline diet of the participating individuals, even if a standardized lead-in diet phase is included. The monitoring of the participants' compliance is a second major issue. It is necessary to consider these additional study variables if the outcomes of such interventions are to be viewed objectively.

An 8-month fatty acid supplementation study was carried out in 120 men and 56 women. The intervention consisted of 50 ml/d of a peppermint flavoured oil (10 ml) and water (40 ml) suspension. The oil groups were high oleate (C18:1*n*-9) sunflower oil (Oleate SF), γ-linolenate (C18:3*n*-6, GLA)-rich evening primrose oil (EPO), *n*3-PUFA-rich tuna fish oil (Fish), linoleate (C18:2*n*-6)-rich soya oil (Soya), Fish + EPO and a comparison "placebo" group (1:3 soya and medium chain triacylglycerol-rich oat oil), which had previously been established as representative of "the Scottish Diet" (D. Horrobin, personal communication).

Dietary intake of fatty acids was assessed by food frequency questionnaire (Bolton-Smith *et al.* 1991) at the beginning, middle and end of the intervention, and by 24-h recall, using the photographic food atlas (Nelson *et al.* 1996) every 6–8 weeks; and fasted plasma phospholipid fatty acids were analysed at the beginning and end, using routine GLC procedures.

Weight changes were measured, and physical activity ratio (PAR) estimated from questionnaire data at each visit. Based on EI:BMR and PAR the most feasible record of dietary intake was used to calculate the mean daily intake of the different fatty acids from the basal diet (including dietary supplements). The estimated mean consumption of the intervention oil per day was used to calculate the fatty acid intake additional to basal diet, and expressed as a percentage increase over basal diet. Similarly, the percentage change in key plasma phospholipid fatty acids was calculated from baseline to post-intervention phase. The post-intervention dietary (D-) and plasma (P-) values are shown in the table, as a percentage of the baseline values.

	Oleate SF (*n* 30) Mean	Range	Soya (*n* 29) Mean	Range	EPO (*n* 28) Mean	Range	Fish (*n* 29) Mean	Range	Fish+EPO (*n* 31) Mean	Range
D-SFA	103	101–105	116	108–140	114	108–130	119	109–130	104	77–109
D-MUFA	126	113–139	105	102–111	102	101–103	103	101–105	103	101–106
D-*n*-6 PUFA	115	106–133	147	121–209	154	125–181	110	104–120	149	124–219
D-*n*-3 PUFA	100	100–100	134	114–249	106	104–113	266	159–902	204	140–481
P-Oleate	113	72–153	99	65–154	93	69–125	91	72–125	91	85–68
P-DHLA	136	62–153	109	71–174	131	78–150	74	35–105	100	56–143
P-*n*-6 PUFA	107	80–145	107	78–151	108	74–130	93	70–120	98	71–127
P-*n*-3 PUFA	85	50–151	93	41–210	91	36–183	205	74–515	199	91–368

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Oleate, C18:1*n*-9; DHLA, dihomo-gamma-linolenate, C20:3*n*-6.

The partial correlation coefficient (adjusting for sex, age, BMI, weight change, study days) between change in diet and plasma fatty acids for the total study population was *r* 0.16, *P*=0.021 and *r* 0.68, *P*<0.001 respectively for total *n*-6 PUFA and total *n*-3 PUFA. Dietary change in *n*-6 PUFA intake correlated most strongly with change in plasma DHLA (*r* 0.33, *P*<0.001). The range of the change in both dietary and plasma fatty acids, as a result of the intervention, was large. This suggests that the monitoring of the potential influence of basal diet on fatty acid intervention outcomes is important. Either appropriate dietary intake assessments for SFA, MUFA and *n*-6 PUFA or plasma phospholipid fatty acid analyses for n-3 PUFA may be suitable for allowing statistical adjustment in these situations.

Bolton-Smith C, Smith WCS, Woodward M & Tunstall-Pedoe H (1991) *British Journal of Nutrition* **65**, 321–335.
Nelson M, Atkinson M & Meyer J (1996) Ministry of Agriculture, Fisheries and Food, London.

Eicosapentaenoic acid supplementation modulates LPS-stimulated PGE$_2$ release in human blood. By S.A. BONNER[1], D. ROTONDO[2], K.W.J. WAHLE[3] and J. DAVIDSON[2]. [1]Department of Nutrition and Dietetics, The Robert Gordon University, Kepplestone, Aberdeen AB15 4PH, [2]Department of Immunology, University of Strathclyde, Glasgow G4 ONR, [3]The Rowett Research Institute, Greenburn Road, Aberdeen AB21 9SB

A major action of the acute phase response of the immune system to infection is the release of cytokines such as interleukin (IL)-1β and tumour necrosis factor (TNF)-α which in turn stimulate the production of prostaglandin (PG)E$_2$. This eicosanoid produces the symptoms of generalized inflammatory responses. The circulating levels of PGE$_2$ are reported to be increased by almost 10-fold during immunoactivation, indicating a massive up-regulation of biosynthesis of this eicosanoid (Rotondo et al. 1988). One of the main functions of dietary fatty acids is to serve as precursors of eicosanoids. In people eating a standard Western diet, arachidonic acid (ARA) is the major fatty acid substrate in cell membranes for metabolism of eicosanoids and gives rise to PG of the 2-series. However, n-3 and n-6 fatty acids compete for incorporation into the phospholipid structure of the cell membranes. The effect of eicosapentaenoic acid (EPA) on PGE$_2$ release in male volunteers was investigated in this study.

Subjects (18–40 years of age) were given a daily supplement of 94.1% pure EPA (1 g/d, supplied by the National Institute of Health, USA) for 42 d and blood samples were taken pre- and post-supplementation. Blood samples were incubated with various concentrations of lipopolysaccharide (LPS) for 20 h, after which the plasma was collected and the PGE$_2$ level was measured by immunoassay (Bonner et al. unpublished results). All values are the mean of duplicate measurements and are presented as the mean with their SE (n 6).
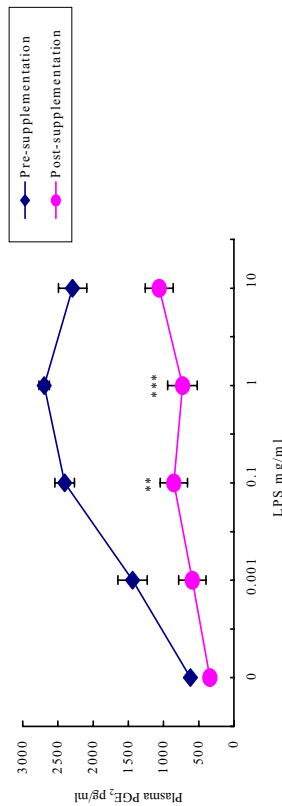
Significant differences from pre-supplementation values are indicated by **($P<0.01$) or ***($P<0.001$).

The levels of PGE$_2$ produced in blood in response to 0.1 and 1 μg LPS/ml blood post-supplementation (day 42) were significantly less than pre-supplementation values.

These results show that EPA suppresses PGE$_2$ production in response to LPS ex vivo, indicating that immune function could be modulated in vivo following EPA supplementation.

Rotondo D, Abul HT, Milton AS & Davidson J (1988) European Journal of Pharmacology **154**, 145–152.

The effect of fish oil supplementation on serum growth factors in healthy humans. By J.M.W. WALLACE[1], A.J. McCABE[1], H.M. ROCHE[2], S. HIGGINS[3], P.J. ROBSON[1], W.S. GILMORE[1] and I.J. STRAIN[1], [1]Northern Ireland Centre for Diet and Health (NICHE), University of Ulster, Coleraine, Northern Ireland, BT52 1SA, [2]Unit of Nutrition and Dietetics, Trinity Centre for Health Sciences, St James Hospital, Dublin 8, Republic of Ireland, [3]Nutritional Sciences, Department of Food Sciences & Technology, University College Cork, Republic of Ireland

Clinical studies report a reduction in all causes of mortality among myocardial infarction patients who consume fish regularly (GISSI-Prevenzione Investigators, 1999). Dietary n-3 polyunsaturated fatty acids (PUFA) have also been reported to reduce lesion formation in animal models (Zhu et al. 1988), an observation which may partly be explained by the effect of fish oil on growth factor levels. The n-3 PUFA, given at moderately high doses, modulate the expression of specific growth factors (Baumann et al. 1999) and alter total serum growth factor activity (Mata et al. 1997). In the present study we investigated whether low-dose fish oil supplementation alters the serum concentrations of specific growth factors and serum growth factor activity.

Sixty-three healthy volunteers, thirty-seven males and twenty-six females (average age 32.7 years, BMI 24.4 kg/m^2) were recruited and randomly assigned to one of four intervention groups receiving 0, 0.3, 0.6, or 0.9 g n-3 PUFA (17% eicosapentaenoic acid / 11.7% docosahexaenoic acid)/d for 8 weeks. Venous blood samples were taken, following an overnight fast (12 h), at baseline and again after the 8-week intervention period and assayed for platelet derived growth factor (PDGF) and transforming growth factor beta (TGFβ) concentration using an ELISA (R&D Systems). The effect of serum obtained from the subjects on arterial smooth muscle cell DNA synthesis (stimulation index, SI) was also measured (Mata et al. 1997). Analysis of covariance was used to analyse for an effect of treatment on each parameter.

	Control (n 15)		0.3 g Fish oil (n 17)		0.6 g Fish oil (n 16)		0.9 g Fish oil (n 15)		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	P=
PDGF concentration (ng/ml)									
Week 0	26.24	3.51	33.41	3.62	36.75	3.66	27.03	3.40	
Week 8	34.83	3.45	38.62	2.45	36.87	3.70	37.69	3.09	0.490
TGFβ concentration (ng/ml)									
Week 0	22.83	2.11	29.10	2.85	28.92	1.70	24.30	2.88	
Week 8	32.82	2.07	34.45	1.47	31.74	2.07	33.07	1.85	0.601
SI (CpmG/cpmG$_0$)									
Week 0	36.28	1.56	36.18	1.61	35.81	1.83	37.17	4.33	
Week 8	38.39	1.68	39.48	1.88	35.40	1.71	35.45	1.56	0.136

The Table shows the week 0 and week 8 values in each of the intervention groups. There was no significant effect of 8 weeks supplementation on the serum levels of PDGF or on the serum levels of TGFβ. Human umbilical arterial smooth muscle cell proliferation following the addition of baseline and week 8 serum samples was not altered significantly by fish-oil supplementation.

The results from the current study suggest that supplementing the diet of apparently healthy volunteers with low doses of fish oil does not alter the serum concentration of PDGF, TGFβ or the total growth factor activity of the serum for arterial smooth muscle cells.

Baumann KH, Hessel F, Larass I, Müller T, Angerer P, Kiefl R & von Schacky C (1999) Arteriosclerosis Thrombosis Vascular Biology **19**, 59–66.
GISSI-Prevenzione Investigators (1999) Lancet **354**, 447–455.
Mata P, Varela O, Alonso R, Lahoz C, de Oya M &Badimon L (1997) Arteriosclerosis Thrombosis Vascular Biology **17**, 2088–2095.
Zhu BQ, Smith DL, Sievers RE, Isenberg WM & Parmley WW (1988) Journal of the American College of Cardiology **12**, 1073–1078.

Effect of using either bovine serum albumin or ethanol as a vehicle for delivery of oleic acid, eicosapentaenoic acid and docosahexaenoic acid to Chinese Hamster V79 cells. By L.C. O'SULLIVAN, J.A. WOODS and N.M. O'Brien, *Nutritional Sciences, Department of Food Science and Technology, University College, Cork, Republic of Ireland*

Previous studies have shown that pre-treatment of mammalian cells with oleic acid (OA), docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA) exacerbates the level of sister chromatid exchanges (SCE) caused by oxidants (Higgins *et al.* 1999). The way in which the fatty acid (FA) is delivered to the cells (i.e. dissolved in solvent or complexed with albumin) may influence the toxicity of these compounds. The objective of the present study was 2-fold. Firstly, to optimize the uptake of OA, DHA or EPA in cultured mammalian cells using either bovine serum albumin (BSA) or ethanol (EtOH) as the delivery vehicle. Secondly, to determine whether the FAs induced toxicity in these cells in the presence or absence of oxidants. Chinese hamster V79 cells were treated for 24 h with FAs. The FAs were prepared either as a stock solution in ethanol, or complexed to BSA, to give a final concentration in the cell growth media of 50 $\geq$mol/l. The final concentration of ethanol in the growth media did not exceed 0.7 $\geq$l/ml.

After 24 h incubation, samples were removed for duplicate determination of FA composition by capillary gas chromatography, membrane integrity using fluorescein diacetate/ethidium bromide (FDA/EtBr) assay and metabolic activity using the neutral red uptake assay (NRUA). Control plates contained equal volumes of either ethanol alone or BSA alone. A second set of samples were treated for 1 h in the presence or absence of hydrogen peroxide (100 $\geq$mol/l). After inactivation of the peroxide, samples were incubated in complete, peroxide-free medium for a further 30 h to mimic the conditions of the earlier SCE experiment. After this time, samples were removed for the NRUA.

Table 1

Treatment	OA		EPA		DHA	
	Mean	SE	Mean	SE	Mean	SE
EtOH	24.8	1.1	0.5	0.1	3.2	0.6
BSA	25.5	0.7	0.5	0.3	3.5	1.4
OA & EtOH	59.0^a	1.1	ND		ND	
OA & BSA	41.8^a	3.3	ND		ND	
EPA & EtOH	ND		12.9^b	1.0	ND	
EPA & BSA	ND		11.1^b	2.5	ND	
DHA & EtOH	ND		ND		21.5^b	2.0
DHA & BSA	ND		ND		18.0^b	3.5

Fatty Acid Uptake (Percent of Total Fatty Acids Measured by Gas Chromatography)

$^a P<0.01$; $^b P<0.05$; significantly different from corresponding control (ANOVA, Dunnett's test). n 3 independent experiments.

Table 2

Treatment	Membrane Integrity (FDA/EtBr) % Viable Cells	
	Mean	SE
EtOH	98	0
BSA	97	1
OA & EtOH	98	0
OA & BSA	99	0
EPA & EtOH	99	1
EPA & BSA	96	0
DHA & EtOH	99	1
DHA & BSA	99	0

Incubation of V79 cells with 50 $\geq$mol/l FA resulted in an enrichment of either OA, EPA or DHA by approximately 2-, 24- and 6-fold, respectively (Table 1). The method of delivery of the FAs to the cell made no difference to the amounts incorporated. The FAs alone had no toxic effects in the cells as measured by FDA/EtBr (Table 2). In the second set of experiments, cells were treated for 24 h with FAs before peroxide treatment, then allowed to recover for 30 h. Again, there were no toxic effects as measured by NRUA (data not shown). Thus FAs were incorporated into V79 cells with no effects on membrane integrity or metabolic activity. Work is ongoing to determine the effect of these FAs on SCE frequency in V79 cells.

Higgins S, Vasconcelos MH & O'Brien NM (1999) *Mutagenesis* **14**, 335–338.

Effect of supplementation with vitamin E and vitamin C on immune response of sows and their litters in hot environments. By A. PINELLI-SAAVEDRA[1,2], J.R. SCAIFE[1], A.M. CALDERON DE LA BARCA[2], J.R. VALENZUELA[2] and H. CELAYA[2], [1]*Department of Agriculture, University of Aberdeen, 581 King Street, Aberdeen AB24 5UA, Scotland*, [2]*Centro de Investigación en Alimentación y Desarrollo, A.C., Carr. a la Victoria Km. 0.6, Hermosillo, Sonora, México*

Heat stress in sows may compromise piglet survival by reducing viability of the new-born and limiting their subsequent growth. There is a little information on the effect of vitamin C on immune response and the interaction between vitamins C and E in hot environments. The aims of this work were to study the effect of vitamin E and C supplementation during pregnancy and lactation on lymphocyte proliferation in gestating sows and their litters exposed to high temperatures, and to determine the IgG concentrations in piglets at 21 (SD 3) days of age. The experiment was carried out in summer on a commercial farm in Sonora, México. Maximum night and day temperatures were 20° and 45°, respectively. At the beginning of gestation, thirty-six multiparous sows were allocated individually (six per treatment) to the following treatments: (1) Control (commercial diet, including vitamin E 30 mg/kg feed), (2) Control + vitamin E 200 mg/kg feed, (3) Control + vitamin E 400 mg/kg feed, (4) Control + vitamin C 1 g/day, (5) Control + vitamin C 10 g/day and (6) Control + vitamin C 1 g/day + vitamin E 200 mg/kg feed. The vitamins were supplemented daily throughout. Sow blood samples for lymphocyte proliferation were collected on day 103 (SD 5) of gestation and day 21 (SD 3) post-farrowing. Piglet blood was collected at weaning (day 21). IgG concentrations were determined by direct ELISA using porcine IgG as control and standard, and the status of cellular immune response was analysed in lymphocyte proliferation assays using concanavalin A (Con A) and phyto-haemagglutinin (PHA) as described by Hernández, *et al.* (1998). Data were analysed by one-way ANOVA using Minitab.

Treatment	Sows Day 103				Sows Day F+21				Piglets Day 21					
	ConA	SE	PHA	SE	ConA	SE	PHA	SE	ConA	SE	PHA	SE	IgG	SE
1	10.0^a	4.9	22.0^a	4.4	60.5	3.4	78.3	3.3	18.9	7.7	47.8^a	6.9	6.22^c	0.6
2	42.7^b	4.1	39.8^a	4.1	59.5	3.2	81.3	3.0	33.4	4.8	65.8^b	5.6	7.15^a	0.6
3	26.8^a	4.9	11.7^c	5.3	59.4	4.4	71.1	4.1	33.0	8.6	40.6^a	7.9	6.98^a	0.5
4	42.3^b	4.7	28.2^a	4.8	58.0	5.9	72.5	5.0	18.5	7.7	70.1^b	7.9	7.81^a	0.5
5	27.6^a	4.5	35.7^a	4.8	55.4	3.8	72.3	3.5	31.4	5.4	59.1^a	6.1	7.02^a	0.6
6	29.1^a	4.9	42.6^b	5.7	54.4	3.8	79.6	3.8	20.7	5.6	39.5^c	6.1	9.20^b	0.6
	***		***		NS		NS		NS		**		*	

Data for lymphocyte proliferation are shown in counts per min $\times 10^3$ (cpm). IgG concentrations are shown in mg/ml. Mean values within a column not sharing a common superscript were significantly different; $*P<0.05$, $**P<0.01$, $***P<0.001$.

On day 103 in sows, Con A induced significantly higher proliferation ($P<0.001$) in the groups supplemented with vitamin E (200 mg/kg fed) and vitamin C (1 g/d) compared with the control and other supplemented groups, but there were no significant effects of treatments post-farrowing and in piglets. PHA-induced proliferation on day 103 was highest in sows fed on the combined supplement and was reduced by the higher level of vitamin E. In contrast, in piglets, PHA-induced proliferation was reduced by the combined vitamin supplement and increased by the low vitamin E or vitamin C supplements. IgG concentrations in piglets were significantly increased by the combined vitamin supplement. There were no consistent effects of vitamin supplementation on lymphocyte proliferative responses. The difference in responses in sows before and after parturition may be associated with factors such as movement to a cooler farrowing environment and the release of immunosuppression due to pregnancy.

Hernández J, Reyes-Leyva J, Zenteno R, Ramírez H, Hernández-Jauregui P & Zenteno E (1998) *Veterinary Immunology and Immunopathology* **63**, 367–381.

Influence of cortisol on uncoupling protein-1 (UCP1) and leptin mRNA expression in perirenal adipose tissue in the late gestation sheep fetus. By A. MOSTYN[1], A. MOSTYN[1], A. FORHEAD[2], D. KEISLER[3], T. STEPHENSON[1], A.L. FOWDEN[2] and M.E. SYMONDS[1], [1]Academic Division of Child Health, School of Human Development, University Hospital, Nottingham NG7 2UH, [2]Physiological Laboratory, Downing Street, Cambridge CB2 3EG, [3]Department of Animal Science, USDA Columbia, USA

Brown adipose tissue contains a unique mitochondrial UCP1 which generates a certain amount of heat rapidly after birth (Symonds et al. 1992). In fetal sheep, mRNA abundance and thermogenic activity of UCP1 remain low throughout gestation and peak immediately after birth (Casteilla et al. 1989). The expression of the16 kDa hormone leptin in perirenal adipose tissue also increases during late gestation (Yuen et al. 1999). Since these changes occur coincidentally with the prepartum surge in fetal plasma cortisol, the aim of this study was to determine whether cortisol influences UCP1 and leptin mRNA expression in perirenal fat from sheep fetuses during late gestation (term $\cong$ 147 d).

Twenty Welsh Mountain sheep fetuses were used. Under halothane anaesthesia (1.5% in O_2/N_2O_2) one of the following procedures was carried out; (1) intravascular catheterization of intact fetuses at 115–118 d coupled with i.v. infusion of saline (3 ml/d, n 5) or cortisol (2–3 mg/kg/d, n 5) for 5 d before delivery at 129–131 d; (2) fetal adrenalectomy at 115–118 d followed by intravascular catheterization at 129–131 d and delivery at 139–142 d (n 5); (3) intravascular catheterization of intact fetuses at 129–132 d and delivery at 139–142 d (n 5). Perirenal adipose tissue was collected from all the animals at delivery after administration of a lethal dose of anaesthetic (Na pentobarbitone 200 mg/kg) and frozen immediately in liquid nitrogen. Total RNA was extracted according to the method of Chomcynski & Sacchi (1987) and expression of UCP1 analysed by Northern Blotting using the oligonucleotide sequence 5'-GGACTTTGGCGGTGTCCAGCGGGAAGGTGA T-3' end-labelled with digoxigenin. Leptin mRNA abundance was measured using a digoxigenin-labelled 192 base pair PCR product encoding base pairs 27–218 of the ovine leptin sequence. Results are expressed as a percentage of 18S rRNA. Statistically significant differences between groups were assessed using a Mann-Whitney test.

Expression of UCP1 mRNA was greater in perirenal adipose tissue of cortisol compared with saline-treated fetuses (cortisol 7.7 (SEM 2.5) %; saline 1.1 (SEM 0.7, P =0.032). Leptin expression was also increased by cortisol infusion (cortisol 18.3 (SEM 3.8) %; saline 27.4 (SEM 1.1, P=0.056)). UCP1 expression was lower in adrenalectomized than in age-matched intact fetuses (adrenalectomized 0.15 (SEM 0.03) %; intact 1.15 (SEM 0.17) % (P=0.008)).

In conclusion, the increase in UCP1 and leptin mRNA expression observed in ovine perirenal adipose tissue during late gestation is mediated, in part, by the prepartum rise in plasma cortisol. These parallel increases in both UCP1 and leptin may be critical in promoting the onset of non-shivering thermogenesis immediately after birth (Symonds et al. 1992).

Casteilla L, Champigny O, Bouillaud F, Robelin J & Ricquier D (1989) Biochemical Journal 257, 665–671.
Chomcynski P & Sacchi N (1987) Analytical Biochemistry 162, 156–159.
Symonds ME, Bryant MJ, Clarke L, Darby CJ & Lomax MA (1992) Journal of Physiology 455, 487–502.
Yuen BSI, McMillen IC, Symonds ME & Owens JA (1999) Journal of Endocrinology 163, R1–R4.

Effect of administration of a recombinant molecular mimic of phosphorylated prolactin (PP-PRL) on thermoregulation in neonatal lambs. By S. PEARCE[1], A. MOSTYN[1], A.M. WALKER[2], T. STEPHENSON[1], R. WEBB[3] and M.E. SYMONDS[1], [1]Academic Division of Child Health, School of Human Development, University Hospital, University of Nottingham, Nottingham NG7 2UH, [2]Division of Biomedical Sciences, University of California, Riverside, CA 92521, USA, [3]Division of Agriculture and Horticulture, School of Biological Sciences, University of Nottingham, Nottingham NG7 2UH

In lambs the rapid increase in heat production after birth is due to the initiation of non-shivering thermogenesis in brown adipose tissue. This occurs in conjunction with an increase in amount and activity of the brown adipose tissue specific uncoupling protein-1 (e.g. Clarke et al. 1997). Both mRNA and protein for the long and short forms of the prolactin receptor are highly abundant in ovine brown adipose tissue up to the time of birth (Symonds et al. 1998). Enhancement of prolactin receptor abundance during late gestation is associated with increased uncoupling protein-1 abundance (Bispham et al. 1999) but it is not known if exogenous stimulation of the prolactin receptor can influence thermoregulation after birth. This was therefore examined using a recombinant molecular mimic of PP-PRL, which acts as an agonist of prolactin (Chen et al. 1998).

Seven pairs of weight-matched, day-old female triplet lambs were entered into the study and randomly assigned to PP-PRL (10 µg) or vehicle treatment. Jugular vein catheters were inserted in each lamb to allow administration of each treatment on day 1. The following day PP-PRL treatment was repeated and colonic temperature measured hourly for a 6 h period. All temperatures are in C and n was 6 per group. Values are means with their standard errors. An asterisk (*) indicates $P<0.05$ (Mann-Whitney U test).
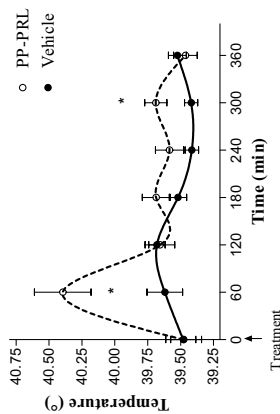
Colonic temperature increased significantly in PP-PRL treated lambs compared with controls 1 h after treatment and then declined to control values 2 h after injection. A further rise in colonic temperature was observed in treated compared with controls 5 h following PP-PRL injection. These responses were observed in the absence of any visible signs of shivering and suggest that agonizing the action of prolactin at the level of the prolactin receptor may stimulate non-shivering thermogenesis in brown adipose tissue.

Bispham JR, Heasman L, Clarke L, Ingleton PM, Stephenson T & Symonds ME (1999) Journal of Neuroendocrinology 11, 849–856.
Chen T-J, Kuo CB, Tsai KF, Liu J-W, Chen D-Y & Walker AM (1998) Endocrinology 139, 609–616.
Clarke L, Heasman L, Firth K & Symonds ME (1997) American Journal of Physiology 272, R131–R1939.
Symonds ME, Phillips ID, Anthony RV, Owens JA & McMillen IC (1998) Journal of Neuroendocrinology 10, 885–890.

Relationship between prolactin receptor (PRLR) and uncoupling protein-1 (UCP1) abundance in perirenal adipose tissue during neonatal development in lambs. By H. BUDGE[1], P. INGLETON[2], T. STEPHENSON[1] and M.E. SYMONDS[1], [1]Academic Division of Child Health, School of Human Development, University Hospital, Nottingham NG7 2UH, [2]Division of Biochemical and Musculoskeletal Medicine, Human Metabolism and Clinical Biochemistry, University of Sheffield Medical School, Sheffield S10 2RX

Uncoupling protein-1 (UCP1) is a unique protein which effects the rapid production of large amounts of heat from brown adipose tissue after birth in non-shivering thermogenesis. Both mRNA and protein for the long and short forms of the prolactin receptor (PRLR) are highly abundant in ovine perirenal adipose tissue up to the time of birth and PRLR mRNA concentrations first appear coincident with the gestational age at which UCP1 is first detected (Symonds et al. 1998). This study was designed to determine whether the peak of UCP1 after birth and its subsequent decline in early postnatal life is accompanied by changes in PRLR.

Twenty lambs were randomly allocated to be sampled at either 145 d gestation (where term is 147 d), within 2 h of birth or at days 4 or 7 of postnatal life (n 5 per sampling age). Perirenal adipose tissue was collected and the abundance of UCP1 and the specific isoforms of the long (PRLR1) and short (PRLR2) forms of PRLR were determined by immunoblotting as described by Schermer et al. (1996) and Bispham et al. (1999). All results are expressed as a percentage of densitometric results obtained from a 4-d-old lamb included on all Western blotting tests. Values are means with their standard errors and significant differences with respect to lamb age were assessed by analysis of variance.
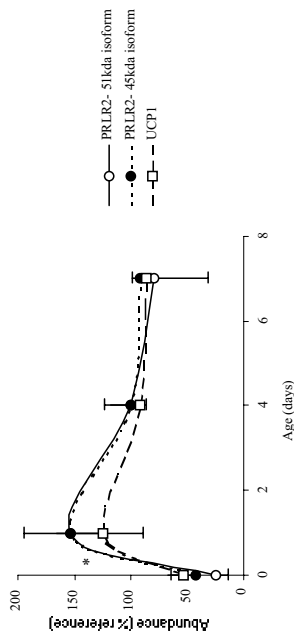
The abundance of the 51 kDa and 45 kDa isoforms of PRLR2 peaked in perirenal adipose tissue sampled within 2 h of birth and were significantly greater ($P<0.05$) than in the late-term fetus. This pattern of change in abundance of PRLR2 paralleled that of UCP1. In contrast, no change in the abundance of PRLR1 was seen in the perinatal period up to day 7 of postnatal life for either the 60 kDa isoform (145-d fetus: 121.4 (SE 16.6) %; 2 h: 64 (SE28.9) %; 4 d: 100 (SE 4) %; 7 d: 86 (SE9) %) or 15kDa isoform (145-d fetus: 77.4 (SE15.8) %; 2 h: 128.4 (SE6.7) %; 4 d: 100 (SE5.8) %; 7 d: 145.8 (SE56) %).

In conclusion, changes in UCP1 abundance during early postnatal life are associated with parallel changes in the short but not the long form of PRLR. The mechanism and extent by which PRLR may regulate postnatal adipose tissue development remains to be elucidated.

Bispham JR, Heasman L, Clarke L, Ingleton PM, Stephenson T & Symonds ME (1999) Journal of Neuroendocrinology **11**, 849–856.

Schermer SJ, Bird JA, Lomax MA, Shepherd DAL & Symonds ME (1996) Reproduction Fertility and Development **8**, 995–1002.

Symonds ME, Phillips ID, Anthony RV, Owens JA & McMillen IC (1998) Journal of Neuroendocrinology **10**, 885–890.

The influence of maternal nutrient restriction between early and mid-gestation on 11β-hydroxysteroid dehydrogenase (11βHSD) mRNA abundance in neonatal sheep tissues. By M.E. SYMONDS[1], K.M. FIRTH[1,2], H. BUDGE[1] and C.B. WHORWOOD[2], [1]Academic Division of Child Health, School of Human Development, University Hospital, Nottingham NG7 2UH, [2]Endocrinology and Metabolism Unit, School of Medicine, University of Southampton, Southampton SO16 6YD

Maternal nutrient restriction (NR) in sheep between early and mid-gestation has been shown to initially restrict individual placentome growth (Clarke et al. 1998) but result in a fetus with a disproportionately larger placenta at term compared with adequately fed controls (Heasman et al. 1998). Glucocorticoids exert significant effects on both fetal growth and glucose metabolism (e.g. Fowden et al. 1998), for which tissue sensitivity is regulated in part by the enzymes 11βHSD (11βHSD1), the other is high affinity and unidirectional (11βHSD2). In rats, feeding 9% vs 18% casein throughout pregnancy results in increased tissue expression of glucocorticoid receptor, decreased expression of renal 11βHSD2, but no change in expression of 11βHSD1 in the fetal and neonatal rat (Bertram et al. 1999). The present study investigates the effects of maternal NR between early and mid-gestation on subsequent expression of 11βHSD in neonatal lambs.

Fourteen singleton-bearing Welsh Mountain ewes of similar body weight and condition score were individually housed from 28 d gestation, with seven animals randomly allocated to each diet group. All ewes were fed a diet comprising concentrate and chopped hay in a ratio of 1:3. NR ewes consumed 2.5–4.1 MJ of metabolizable energy (ME) per day (≡ 50% of ME requirements for maintenance and growth of the conceptus) between 28 and 80 d gestation, with controls consuming 8.6–9.3 MJ/d (i.e. to appetite). After 80 d gestation, until term (147 d) all animals were fed to requirements and consumed 6.8–7.5 MJ/d. At 144–146 d gestation each lamb was delivered by Caesarean section (Clarke et al. 1997), and the lamb sacrificed with an overdose of barbiturate 6 h after birth to enable tissue sampling. Placenta, kidneys, adrenals, lungs and livers were excised, weighed, snap frozen in liquid nitrogen and stored at –80°C for molecular analysis. mRNA encoding 11βHSD1 (1.8 Kb) or 11βHSD2 (1.9 Kb) was analysed by Northern blot using a ^{32}P-labelled antisense cRNA probe and relative levels of expression were quantified in relation to those for 18S rRNA. Statistically significant differences in tissue weights and gene expression between the diet groups were assessed by analysis of variance.

Expression of mRNA for 11βHSD1 was greater in the maternal than the fetal component of the placenta (maternal 2.63 (SEM 0.37, n 4); fetal 0.97 (SEM 0.10, n 4); $P<0.01$), but was unaffected by maternal NR (control 0.32 (SEM 0.09, n 7); NR 0.51 (SEM 0.07, n 7). There was no significant difference in 11βHSD1 between groups in lungs (control 0.94 (SEM 0.2); NR 0.89 (SEM 0.1) or liver (control 0.40 (SEM 0.07); NR 0.30 (SEM 0.05). Expression of mRNA for 11βHSD2 was not detected in the placenta. 11βHSD2 expression was significantly lower in RNA extracted from the kidney cortex of the NR group compared to controls (control 0.90 (SEM 0.03; NR 0.59 (SEM 0.11, $P<0.05$). There was no difference in adrenal expression of 11βHSD2 between groups (control 0.065 (SEM 0.014); NR 0.048 (SEM 0.0194). Organ weights were similar between groups, with the exception of kidneys, which were larger in the NR group (control 16.9 (SEM 2.3) g; NR 20.8 (SEM 1.4) g, $P<0.05$).

In conclusion, maternal nutrient restriction followed by adequate feeding up to term markedly decreases 11βHSD2, but not 11βHSD1 mRNA expression in a tissue-specific manner in the resulting offspring. These changes may result in altered kidney function in later life, thereby predisposing these individuals to hypertension.

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Metabolic responses to glucose and essential amino acid infusions in calves. By F. SADIQ, M.C. WALLACE, J. STRUTHERS, J.R. SCAIFE, L.M. BIRNIE and M.A. LOMAX, *Department of Agriculture, Aberdeen University, 581 King Street, Aberdeen AB24 5UA, Scotland*

Vascular infusions of branched chain amino acids (BCAA) have been shown to increase the sensitivity of rat muscle protein synthesis to insulin (Garlick & Grant, 1988). In fed and underfed sheep, insulin has been shown to stimulate muscle protein accretion, but this response was not altered by BCAA infusion (Wester *et al.* 2000). The aim of the present study was to examine the effects of essential amino acids (EAA) alone or in combination with high and low doses of glucose (to stimulate plasma insulin concentrations) on muscle protein degradation estimated from urinary 3-methylhistidine excretion in cattle.

Six Holstein-Friesian calves (approximately 4 months of age and mean body weight 90 kg) were housed separately in metabolism units. Calves were kept on a restricted diet to achieve 0.3 kg daily body weight gain. All animals were randomized to six treatments in a 3×2 factorial design: saline (control); high-dose glucose (HDG); low-dose glucose (LDG); mixture of essential amino acids (EAA); HDG + EAA and LDG + EAA. Dose rates for HDG, LDG and EAA were 20 µmol/kg body weight (BW)/min, 9.6 µmol/kg BW/min and 0.8 mg/kg BW/min, respectively. Treatments were continuously infused for 5 d via jugular vein catheters and blood samples were collected on day 5 of the infusion period. Urine samples were also collected during the last four days of the infusion period. Plasma was analysed for glucose, insulin and blood urea nitrogen (BUN), while urine was analysed for creatinine and 3-methylhistidine (3MH) concentrations. Results were statistically analysed by one-way ANOVA.

Compared with control, both doses of glucose (HDG and LDG) caused an increase in plasma glucose and insulin concentrations in calves ($P<0.05$). Addition of EAA to glucose doses did not significantly increase glucose or insulin levels above those obtained without EAA. Plasma urea nitrogen levels were significantly ($P<0.05$) decreased after LDG and HDG treatments and were increased ($P>0.05$) following EAA infusions. Glucose infusion reduced BUN concentrations in a dose-dependent manner both in the absence or presence of infused EAA. Urinary 3MH excretion, corrected for muscle mass (3MH:creatinine), was significantly decreased after HDG infusion with or without EAA ($P<0.05$).

	Control		LDG		HDG		EAA		LDG+EAA		HDG+EAA	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Glucose mmol/l	4.06[a]	0.198	5.19[bc]	0.222	5.74[c]	0.208	4.43[ad]	0.146	5.03[cd]	0.349	5.49[bc]	0.174
Insulin ng/ml	0.57[a]	0.031	0.95[bd]	0.066	1.31[c]	0.170	0.63[ab]	0.056	0.80[abd]	0.113	1.01[cd]	0.168
BUN mg/dl	8.78[a]	0.932	6.62[bc]	0.444	5.09[b]	0.416	11.00[d]	0.804	8.14[ac]	0.652	5.66[b]	0.522
3MH µmol/kgBW/d	1.81[a]	0.094	1.52[bc]	0.308	1.82[a]	0.113	1.67[ac]	0.283	1.86[a]	0.215	1.62[ac]	0.209
Creatinine g/d	2.31[abc]	0.164	2.01[b]	0.190	2.57[c]	0.163	2.22[abc]	0.215	2.48[abc]	0.189	2.20[b]	0.146
3MH:Creatini ne mg/g	13.33[a]	0.523	12.43[ab]	0.461	11.87[b]	0.494	12.5[ab]	0.245	12.52[ab]	0.290	12.00[b]	0.314

[a,b,c,d] Values within the same row with different superscripts are significant ($P<0.05$)

The significant alterations in plasma urea levels suggest that amino acid deamination was decreased under conditions of increased plasma insulin; however, this response was not affected by co-infusion of EAA. There was some evidence that muscle protein degradation, as indicated by urinary 3MH:creatinine ratios, contributed to this response. EAA alone or in combination with glucose did not significantly increase the sensitivity of muscle proteolytic response to increased plasma insulin levels. The significant response in sensitivity due to the HDG + EAA treatment was similar to that seen for HDG alone, suggesting that the response was due to glucose and not to EAA.

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Purine derivative excretion by one-week-old crossbred (*Bos indicus* × *B. taurus*) calves. By F. HERRERA-GOMEZ, F.D.DeB. HOVELL and C.A. SANDOVAL-CASTRO, *Faculty of Veterinary Medicine and Animal Science, University of Yucatan, Apdo. 4-116, Mérida, Yucatán, 97100, México*

The use of purine derivative (PD) excretion as an estimator of microbial protein supply (MPS) to ruminants is a technique which offers considerable potential, particularly in the tropics where protein is an important nutritional constraint. Knowledge of the endogenous excretion of PD (PD_E) is fundamental to the models relating MPS to PD excretion. The accepted value for PD_E of cattle was estimated with *B. taurus* steers nourished by intragastric infusion (Verbic *et al.* 1990). It is unclear whether this value holds for *B. indicus* and its crosses. An alternative approach to the estimation of endogenous excretion is the use of young suckling ruminants. This study measured the PD_E of seven newborn crossbred (*B. indicus* × *B. taurus*) calves weighing 33.7 (SD 3.52) kg. Calves were allowed to stay with their mother for 2 d after birth. From days 3–8 they were kept in individual pens to allow daily total urine collection. Calves were allowed to suckle their mother for 20 min after morning milking (about 09.00 hours). Urine was collected into 200 ml H_2SO_4 25% (v/v) to ensure a final urine pH <3, diluted five times with tap water, and subsamples were stored at -10^0 before analysis for allantoin and uric acid.

The mean PD_E excretion of the calves of 0.818 (SD 0.067) mmol/kg $LW^{0.75}$ was higher than that of 0.486 mmol/kg $LW^{0.75}$ reported for growing steers nourished intragastrically (Fujihara *et al.* 1987; Verbic *et al.* 1990), but agrees with that of 0.705 (SD 0.079) mmol/kg $LW^{0.75}$ from five *Bos taurus* calves at about 3 weeks of age given milk replacer and reported by Funaba *et al.* (1997). It is more than the 0.513 mmol/kg $LW^{0.75}$ per d of the 3-week-old calves studied by Chen *et al.* (1990). The allantoin content in the urine was consistently around 81% (mmol/mmol) of the total PD, slightly less than the 90% reported by other workers. The high PD_E found by ourselves and others may have been caused by lack of ruminal epithelium, a reduction in possible recycling to the rumen (even intragastrically nourished animals have a microbial population on the rumen wall), and hence higher excretion of PD. However, this effect is difficult to quantify.

Daily purine derivative excretion by week-old crossbred calves (n 7)

Animal no	$LW^{0.75}$	Total PD (mmol)	(mmol/kg $LW^{0.75}$)	Allantoin (mmol/kg $LW^{0.75}$)	Uric acid (mmol/kg $LW^{0.75}$)
181	14.2	12.21	0.858	0.755	0.103
502	14.1	14.86	1.055	0.905	0.150
4	14.7	12.36	0.841	0.704	0.136
12	15.0	11.17	0.744	0.620	0.124
226	14.8	7.08	0.479	0.344	0.135
284	13.2	10.84	0.821	0.718	0.103
400	11.8	10.99	0.928	0.637	0.290
Mean	14.0	11.36	0.818	0.669	0.149
SD	1.11	2.336	0.178	0.171	0.065

There may be an age effect as suggested by the data of Funaba *et al.* (1997) and collectively reported in the literature. An alternative explanation could be that a higher purine turnover in both young and early weaned calves may be due to the stress caused by birth and weaning, leading to greater PD excretion. The coefficient of variation at 22%, similar to the 25% of Funaba *et al.* (1997), may be indicative of variable metabolic activity. In conclusion, this work suggested that PD_E of *B. taurus* and *B. indicus* is similar.

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Rumen degradation of stover of ten maize varieties cultivated in Yucatan, Mexico. By A.J. AYALA-BURGOS, G. ROMERO-MORALEDA, G. RIOS-ARJONA and F.D.DEB. HOVELL, *Faculty of Veterinary Medicine and Animal Science, University of Yucatán, Apdo. 4–116 Itzimna, Mérida, Yucatán, 97100, México*

Maize has been cultivated in the Americas for thousands of years and still forms a major part of the diet. Much of the production in rural areas is with traditional varieties which take a wide range of times to grow to maturity, thus enabling a spread of the risk associated with annual variations in the timing and amount of rain. Ten maize varieties collected in Yucatán had biomass yields which ranged from 5 to 13 t ha⁻¹, of which 2.6–10.6 t was stover (maize straw), and which took from 12 to 20 weeks from sowing to harvest (Ayala-Burgos et al., unpublished observations). We have estimated that in Yucatan between 1 and 1.5 million t of stover is produced annually. This resource is mostly unused but is a potential ruminant feed. Given the wide variations between varieties, it seems likely that the stover would also be variable. This work measured the rumen degradability of stover from ten local maize varieties.

Three zubu heifers of 329–340 kg live weight, fitted with permanent rumen cannulae, were used to incubate the samples. They were given freshly cut, chopped Napier grass (Pennisetum purpureum) ad libitum plus 1.5 kg of a sorghum/soya concentrate. 3–4 g samples of stover were incubated for 6, 12, 24, 36, 48, 72, 96 and 120 h in nylon cloth bags (pore size 45μ, 1.8 g sample per 100 cm² bag). Dry matter losses were fitted to a negative exponential when degradation (P) after t hours $= a + b(1-\exp^{-ct})$ and a, b and c are constants. The asymptote (potential degradability) is given by $a+b$. Material washed from bags without incubation is W_0, and the insoluble degradable material (B) is described as $(a+b)-W_0$. The truly soluble material that washed through filter paper is S_0. The local Mayan names of the ten maize varieties are given in Table 1. These are the names given by the local farmers. Two varieties were given the same name (Nal Xoy, or maize from (the village of) Xoy), but appeared to be different.

Table 1. Local (Mayan) names of the maize varieties tested.

No.	Maize variety		No.	Maize variety	
1	Sac Nal	(White maize)	6	Sac Tux	(White turkey hen)
2	Nal Tel Sac	(White cockerel maize)	7	Xnuc Nal Sac	(Large white maize)
3	Nal Xoy	(Maize from Xoy)	8	Xmejen Nal Kan	(Little yellow maize)
4	Nal Tel Kan	(Yellow cockerel maize)	9	Nal Xoy	(Maize from Xoy)
5	Xnuc Nal Kan	(Large yellow maize)	10	Ek-Hub	(Purple maize)

There were clear and significant differences between varieties (Table 2). The potential degradabilities ($a+b$, the asymptote of the degradation curve) ranged from 60 to 94%. These values represent considerable extrapolation of the data, and are greatly in excess of 120 h degradability, and therefore are thought to be unlikely. A better idea of the potential nutritive value is given by the effective degradability (D) at a 3% outflow rate from the rumen, also given in Table 2. Again considerable between-variety differences were found, maize-1 being 25% more degradable than maize-7. However, part of these differences may have been due to environmental or other factors.

Table 2. Rumen degradation characteristics of ten local maize varieties from Yucatan, Mexico when percentage degradability after t hours is described as $a + b(1-\exp^{-ct})$ (n 3).

	Maize variety										SED
	1	2	3	4	5	6	7	8	9	10	
a	25.2	24.5	23.3	21.1	16.1	23.1	19.5	22.2	21.7	22.7	0.85
b	61.0	57.6	70.2	44.4	43.9	71.1	64.1	54.5	64.9	49.2	7.51
c (×10²)	0.87	1.00	0.67	1.07	2.17	0.57	0.73	0.73	0.57	1.13	0.20
$a+b$	86.2	79.1	93.5	65.5	60.0	94.2	83.7	76.7	86.7	71.9	7.81
B	69.8	65.8	81.0	51.7	48.9	80.6	73.9	62.2	73.3	59.1	7.81
W_0	16.4	13.3	12.5	13.8	11.1	13.6	9.8	14.5	13.4	12.8	0.89
S_0	12.3	12.3	8.6	11.6	7.7	8.9	5.3	8.4	11.3	10.5	1.24
D when											
$k=0.03$ h⁻¹	37.8	34.4	34.8	32.6	34.5	34.2	30.3	32.2	31.0	35.3	0.38
120 h	60.7	56.9	60.5	51.3	51.4	57.7	53.0	52.1	50.6	57.4	0.76

Short-term intake of six local forage trees in Yucatan, Mexico to local zebu cattle (Bos indicus). By C. NIETO-MARIN, G. MONFORTE-BRAGA, A. AYALA-BURGOS, G. RIOS-ARJONA, C.A. SANDOVAL-CASTRO, L. RAMÍREZ-AVILÉS and F.D.DEB. HOVELL, *Faculty of Veterinary Medicine and Animal Science, University of Yucatán, Apdo. 4–116 Itzimna, Mérida, Yucatán, 97100, México*

Yucatán is rich in tree species, many of which have been used as forage by generations of farmers. There is increasing interest in the use of Silvo-Pastoral systems in Yucatán; however, the amount of recorded and systematic information is sparse. Farmers describe some trees as being very palatable to cattle, others less so. As part of a programme investigating the value of forage trees, we made measurements of the palatability of a range of forage trees. The objective of this experiment was to measure the palatability to local cattle of the foliage of six commonly used forage trees.

Six individually penned zebu cattle (mean live weight 328 (SD 16) kg) were offered cut and carried foliage from each of six forage trees. The experimental design was a 6 × 6 Latin square with six periods Each period was of 3 d duration, as follows: on day 1 animals were offered 15 kg fresh chopped Napier grass (Pennisetum purpureum, variety Taiwan). This was below their voluntary intake, and was done to ensure that the animals were hungry and had rumen volumes below capacity on the following day. On day 2 fresh tree foliage was offered ad libitum for 6 h (12.00–18.00 hours), followed by 15 kg chopped grass. On day 3 animals were offered 30 kg fresh chopped grass (i.e. more than their voluntary intake).

There were clear and significant differences between intakes of different forages (Table 1). The intake of Ramón was the greatest, and was significantly (P<0.05) greater than that of Tzlam or Jabin. Ramón is the most widely used and most highly rated of the tree forages. The intake of Ramón was 260% greater than that of Tzlam, but virtually identical to (i.e. only 7% greater than) that of Huaxim, the local name for Leucaena leucocephala, a tree forage widely used around the world. Differences in intake were almost entirely due to the time spent eating; rates of eating being almost identical for all six foliages. There were also large differences between individual animals as shown by Table 2. Again the differences were due to time spent eating.

Table 1. Voluntary intake of the foliage of six forages by zebu cattle during a 6-h period (n 6).

Tree species		Consumption of tree foliage		
Local name	Scientific name	(kg DM/d)	Eating time (min)	Rate (g/min)
Huaxim	(Leucaena leucocephela)	4.51	205	21
Pixoi	(Guazuma ulmifolia)	3.63	180	20
Jabin	(Piscidia pscipula)	3.18	141	21
Ramón	(Brosimum alicastrum)	4.84	222	23
Chaca	(Bursera simaruba)	3.65	178	22
Tzalam	(Lysiloma bahamense)	1.86	95	23
SEM		0.51	18	3.2

Table 2. Differences between animals in eating behaviour when offered tree foliage (n 6) (see Table 1).

Animal No	Weight (kg)	Consumption of tree foliage		
		(kg DM/d)	Time spent eating (min)	Rate (g/min)
1	190	3.22	165	23
2	393	4.69	231	20
3	286	2.02	118	18
4	334	1.94	117	17
5	328	5.23	202	25
6	337	4.56	186	25
SEM		0.51	18	3.2

This experiment demonstrated clear differences in the short-term intakes of local forage trees by cattle. The procedure was able to distinguish between the different forage trees tested, and provides a useful method for the initial and rapid screening of a large number of materials of potential value. Short-term trials should be considered as an addition to, not a substitute for, longer trials. However, even in this short-term trial, the subjective assessment of the local farmers was clearly vindicated, with the addition of information as to the relative value of the trees examined.

Effect of the amount of maize stover offered to goats and sheep on intake and digestibility. By N.E. MARTÍNEZ-VIERA, G. RIOS-ARJONA and F.D.DeB. HOVELL, *Faculty of Veterinary Medicine and Animal Science, University of Yucatán, Apdo. 4–116 Itzimna, Mérida, Yucatán, 97100, México*

Goats and sheep could become important sources of animal protein in Yucatán. We have estimated that in Yucatán some 1.5 million t year⁻¹ maize stover is produced, little of which is used. In the tropics, a major constraint to ruminant production is voluntary intake. Maize stover consists (essentially) of stem with a very low rumen degradability and leaf of better degradability. Ruminants given the opportunity will select the better material (Wahed *et al.* 1990). The objective of this work was to compare the voluntary intake and digestibility of maize stover by goats and sheep, and whether the amount offered affected intake and digestibility.

Six male goats (17 (SD 2) kg) (local Creole), and six male Peliguey sheep (16 (SD 2) kg) kept in metabolism cages were offered chopped maize stover (length 50–200 mm, stems 10–35 mm diameter) to give refusals of 30, 50 or 70% (R-30, R-50 and R-70). The stover was supplemented with minerals and urea (30 g kg⁻¹ stover) and Na₂SO₄, plus 115 g d⁻¹ (goats) or 109 g d⁻¹ (sheep) of ground maize. The design was a change-over with three periods of 17 d (10 d adaptation, 7 d measurement). All animals received all treatments. Fresh food was offered daily. Samples of stover offered and refused were bulked and stored at –3°. Degradation characteristics of the stover were measured *in sacco* (incubated for 6, 12, 24, 48 and 72 h.) in two bulls fed a similar diet. Zero-time washing loss (W₀) was measured. Intake and digestibility values for stover were corrected for their maize grain contribution. Degradation data were fitted to the model P = a+b(1–exp⁻ᶜᵗ), when P is degradation after *t* hours, and *a*, *b* and *c* are constants. Insoluble degradable material (*B*) was potential degradability (a+b) – W₀.

Although 15% more stover was eaten by both goats and sheep when more was offered, this was not significant statistically. The sheep ate 22% more stover (P<0.01) than the goats (65.5 *vs* 53.9 g DM/kg W⁰·⁷⁵, SED 3.4). OM digestibility was greater by goats (0.552 *vs* 0.510, SED 0.0117; P<0.01). The net effect was that ME intake (per kg W⁰·⁷⁵) was greater by goats, but with animals of essentially the same size, rumen retention time was greater with goats. Although intakes did not differ significantly, the data suggest that when given more opportunity to select, selection did occur. Thus ADF of the refusal declined (P<0.01) as the amount of stover offered was increased. The 72-h degradation values, together with the trends in fractional rates of degradation, *c*, are consistent with selection. The tendency for potential degradability (a+b) to decrease as stover offered was increased (opposite to that expected, since the quality of the refusal should have improved), with values greater than the 72-h degradation values, arising from the *c* values and providing a warning about extrapolation of data.

Table 1. Intake, digestibility and composition of maize stover offered to, and refused by, goats and sheep.

Parameter	Stover offered	Goats (*n* 6)				Sheep (*n* 6)			
		R-30	R-50	R-70	SEM	R-30	R-50	R-70	SEM
Offered (g DM d⁻¹) (g DM d⁻¹/kg W⁰·⁷⁵)		717 85.3	999 118.3	1526 178.4		717 89.2	999 123.7	1526 189.9	
Refused (%)		42	54	64		29	45	63	
Eaten (g DM d⁻¹)		419	457	556	4.8	506	457	558	3.5
g DM (d⁻¹ kg W⁰·⁷⁵)		49.9	54.6	57.2		59.5	68.0	69.0	
ME (kJ D⁻¹ kg W⁰·⁷⁵)		530	570	570	20	520	560	560	20
Digestibility OM		0.560	0.559	0.537	0.017	0.519	0.509	0.501	0.011
Stover ADF (g kg DM⁻¹)	535	619	597	583	8.1	626	605	587	8.8
Degradation characteristics (*n* 2)									
a	18.3	16.7	14.8	18.7		17.3	16.7	17.2	
b	49.4	50.3	47.2	44.6		53.5	50.6	41.2	
c (%h⁻¹)	2.30	1.15	1.99	1.96		1.15	1.72	2.39	
a+b	67.7	67.0	62.0	63.3		70.8	67.3	58.4	
B	53.2	59.2	54.8	47.3		65.2	60.5	48.0	
72-h. degradation	58.2	44.9	48.3	50.2		47.4	49.2	50.6	

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Disorders of iron homeostasis in the pregnancy disease pre-eclampsia. By M.P. RAYMAN[1], J. BARLIS[1], R.W. EVANS[2], C.W.G. REDMAN[3] and L.J.KING[1] *[1]School of Biological Sciences, University of Surrey, Guildford GU2 7XH, [2]Division of Biomolecular Sciences, King's College London, Guy's Campus, London SE1 9RT, [3]Nuffield Department of Obstetrics and Gynaecology, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU*

Pre-eclampsia is a disorder of pregnancy that is believed to affect one in ten of all pregnancies to some degree. Risk factors include: first pregnancy, change of partner or use of barrier contraception (suggesting a protective role for previous antigen exposure); a family history of the condition; a large placenta e.g. multiple pregnancy; hydatidiform mole; a fetus with hydropic placenta and pre-existing conditions such as hypertension, diabetes, obesity and collagen vascular diseases (Redman, 1995; Robillard *et al.* 1993). In this condition, damage to the vascular endothelium, resulting in the symptoms of hypertension, proteinuria, and sudden oedema, is believed to be caused by high levels of circulating lipid hydroperoxides (Wickens *et al.* 1981). Disturbances in iron homeostasis have been observed in pre-eclampsia and may contribute to lipid peroxidation and endothelial-cell injury through the ability of this transition element to catalyse lipid peroxidation reactions (Entman *et al.* 1987; Hubel *et al.* 1996).

Pre-delivery samples of blood serum were collected from pre-eclamptic women and pregnant controls, matched for age, gestation and parity. Total serum iron concentration, serum ferritin and unsaturated iron-binding capacity (UIBC) were measured in these sera by a colorimetric method using ferrozine. Total iron-binding capacity (TIBC) was calculated as the sum of the serum iron and UIBC. The extent to which transferrin was saturated with iron was determined both directly, by gel electrophoresis, and indirectly (as serum iron × 100/TIBC) by the colorimetric method. A good correlation was found between the two methods.

Parameter	Patient group	No. of pairs	Median	Range	P-value*
Serum iron (mmol/l)	Pre-eclampsia Control	39	21.7 12.9	6.3–79.3 4.3–39.0	<0.001
Serum ferritin (mg/l)	Pre-eclampsia Control	19	53.1 9.4	8.7–1259 5.3–43.5	<0.001
UIBC (mmol/l)	Pre-eclampsia Control	39	47.4 68.7	0.3–94.3 46.7–117.1	<0.0001
TIBC (mmol/l)	Pre-eclampsia Control	39	75.5 85.7	39.3–106.8 68.7–121.4	<0.0001
% Saturation of iron-binding capacity (colorimetric method)	Pre-eclampsia Control	39	32.2 15.9	7.3–99.6 3.5–42.1	<0.0001
%Transferrin saturation (gel electrophoresis method)	Pre-eclampsia Control	20	27.6 11.6	10.8–73.7 3.2–37.3	<0.0001
Apotransferrin (arbitrary units)	Pre-eclampsia Control	20	289 530	27–639 241–809	<0.0001

*Matched pairs analysed by Wilcoxon's signed rank test.

Total serum iron concentration, serum ferritin and percentage saturation of transferrin or iron-binding capacity were significantly higher in the pre-eclamptic patients than in the controls, whereas UIBC, TIBC and apotransferrin levels were significantly lower.

Results show reduced serum-iron buffering in pre-eclampsia, indicating a decrease in the antioxidant capacity of serum that may exacerbate lipid peroxidation and endothelial-cell injury. Haemolysis is the likely source of the excess iron which is a hazardous feature of this condition. Our findings suggest that raised serum iron and ferritin might be used diagnostically to warn of incipient pre-eclampsia. As eighteen percent of pre-eclamptic subjects had levels of transferrin saturation within the range normally associated with iron overload, the use of iron supplements in women at high risk of this condition may warrant further research.

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Serial studies of the gastrointestinal handling of ^{13}C-labelled tripalmitin in preterm infants. By D.J. MITCHELL[1], T.R.J. TUBMAN[1], B.G. McCLURE[1], L. GENKEER[2], J.L. MURPHY[2] and S.A. WOOTTON[2], [1]*Department of Child Health, The Queens University of Belfast, Belfast BT12 6BJ, [2]Institute of Human Nutrition, University of Southampton SO16 6YD*

The maturation of the gastrointestinal tract that takes place during uterine development continues after parturition in response to exposure to food intake. These developmental changes are reflected in the functional capacity of the gastrointestinal tract, both in terms of digestion and absorption of dietary nutrients. Lipid is the major energy source in human milk and most infant formulas and plays a critical role in the growth and development of the newborn infant. Whilst the digestion and absorption of [1,1,1-^{13}C]tripalmitin was relatively complete in older children (Murphy *et al.* 1998), preterm infants exhibited an obvious impairment in lipid handling (Craig *et al.* 2000). The extent to which the digestion and absorption of dietary lipid may improve during early life growth and development in preterm infants remains unclear. The aim of the present study was to examine the gastrointestinal handling of [1,1,1-^{13}C]tripalmitin on two occasions during hospitalization in preterm infants.

Eight well preterm infants were studied on the first occasion at 4 (2–23) d (median and range) after full enteral feeds had been established, equivalent to 32 (30–33) weeks post-conceptional age. All the infants were studied again 2 weeks later, apart from one infant who was studied 4 weeks later. The infants received their usual feed by orogastric tube which was either breast milk (*n* 4) or formula milk (*n* 4; Nutriprem®). All were given a single oral dose of emulsified [1,1,1-^{13}C]tripalmitin (20 mg/kg body weight) in one milk feed. A baseline stool sample was collected before the labelled feed and all stools were collected for at least 72 h. The total excretion of ^{13}C-label in stool was analysed by isotope ratio mass spectrometry. The figure shows the individual values for ^{13}C-label in stool (% administered

dose) observed during each study. As a group, the proportion of ^{13}C recovered in stool was 23.3% (2.1–77.5% administered dose) for the first study. On the second occasion, stool ^{13}C excretion declined to 16.0% (6.6–29.6%; NS). There was a reduction in stool ^{13}C excretion for five subjects, whilst two subjects exhibited similar stool losses on both occasions. However an increase in label excretion was observed in one subject. These results suggest that in some preterm infants there may be an improvement in the gastrointestinal handling of labelled tripalmitin during this period of enteral feeding in hospital. In these infants the excretion of ^{13}C label in stool was comparable to that observed for healthy children (Murphy *et al.* 1998). Further studies are needed to examine the factors underlying these observations, such as the effect of feeding strategy on the digestion and absorption of dietary lipids during maturation of the gastrointestinal tract.
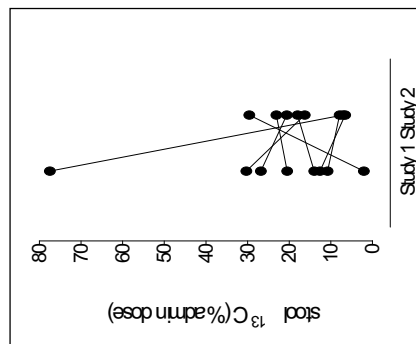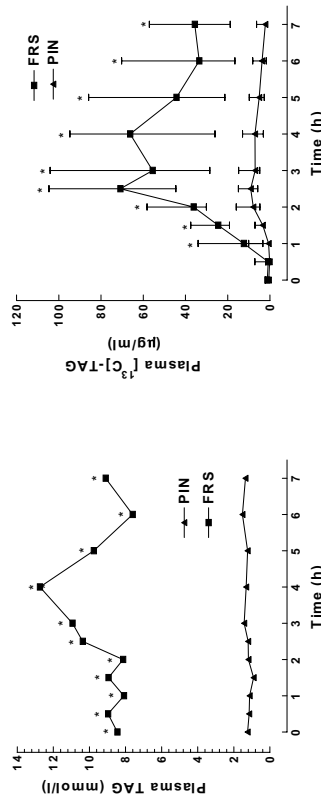
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Highly Active Antiretroviral Therapy (HAART) in Human Immunodeficiency Virus 1-positive men increases the retention time of dietary lipid within the circulation. By L.J. WARE[1], J. MORLESE[2], B. GAZZARD[2], G.C. BURDGE[1], A.A. JACKSON[1] and S.A. WOOTTON[1], [1]*Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD and [2]St. Stephen's Centre, Chelsea and Westminster Hospital, London W6 8RF*

Highly Active Antiretroviral Therapy (HAART) consists of a combination of drugs, usually including a viral protease inhibitor (PI), and is widely used to reduce viral load and increase T-lymphocyte number in HIV-positive patients. While extremely effective at reducing the viral burden (Markowitz *et al.* 1995), PI-containing HAART has been associated with the development of a "Fat Redistribution Syndrome" (FRS), the symptoms of which are a wasting of subcutaneous adipose tissue, with a central accumulation of visceral adipose tissue, marked hypertriacylglyceridaemia, hyperglycaemia and insulin resistance (Carr *et al.* 1998). The aim of the present study was to examine differences in the handling of dietary lipid during the postprandial period in HIV patients on PI-containing HAART with FRS and HIV patients not yet exposed to PI's, using stable isotope tracer methodologies.

Following an overnight fast, six HIV-positive men on PI-containing HAART with fasting plasma triacylglycerol (TAG) of >4 mmol/l (FRS) and five HIV-positive, PI-naive men with normal fasting plasma TAG (PIN) ingested [1-^{13}C]palmitic acid within a lipid-casein-glucose-sucrose emulsion as part of a test meal (3.7 MJ; 45 g lipid; 93 g carbohydrate). Plasma triacylglycerol (TAG) and ^{13}C-TAG concentrations were determined by GC-IRMS following solid phase extraction of plasma lipid (Burdge *et al.* 2000) before and hourly for 7 h after the meal.

Both plasma TAG and [^{13}C]-TAG concentration were significantly elevated in the FRS group compared with the PI-naive HIV+ patients in the postprandial period, suggesting an accumulation of dietary lipid within the circulation. This may be due to a reduced clearance of TAG from lipoproteins, either as a direct result of PI-containing HAART, or metabolic changes that may occur during the resulting immune reconstitution. The extent to which this alteration in the capacity to clear lipid from the circulation influences the other symptoms of FRS remains unclear.

* Significantly different from PIN group (*P*<0.05 using Kruskal-Wallis test), data presented as medians and 95% CI.

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Effects of dietary *n*-3 and *n*-6 PUFA intake on LDL oxidizability and fatty acid composition in patients with advanced atherosclerosis. By J.M. GARRY[1], F. THIES[1], P. YAQOOB[1], A. CHULAKADABBA[2], J. WILLIAMS[2], P.J. GALLAGHER[2], C.P. SHEARMAN[2], P.C. CALDER[1] and R.F. GRIMBLE[1], [1]*Institute of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO16 7PX,* [2]*Department of Surgery, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD*

Cardiovascular diseases are the main cause of mortality in Western countries. The development of atheromatous plaques plays a key role in the progression of such diseases. The extent of lipid accumulation within the plaque is a major determinant of its stability and is a potent risk factor for coronary heart disease and stroke. Recent studies have indicated that the unsaturated fatty acid composition of lipid within the plaque may have an impact on its stability (Felton *et al.*, 1997). The relationship between the fatty acid composition of dietary fat and of plaque lipid is unknown, although dietary intake influences the fatty acid composition of the lipoprotein pool from which plaque lipid is derived.

The aims of this study are to determine the effects of moderate supplementation with *n*-3 and *n*-6 polyunsaturated fatty acids (PUFA) on the fatty acid profile and oxidizability of plasma LDL from patients undergoing carotid endarterectomy.

Subjects (*n* 54/group) were given placebo, sunflower oil (SO) or fish oil (FO) in capsules (6 g oil/d) for at least 2 weeks (mean 8 weeks) before undergoing surgery. The FO capsules provided 0.86 g eicosapentaenoic acid (EPA) and 0.50 g docosahexaenoic acid (DHA) per day. Blood samples were taken before and after supplementation. Copper-induced oxidation of isolated LDL (conjugated diene formation) and the fatty acid composition of lipid classes from LDL were determined, as well as LDL peroxide concentrations and plasma triacylglycerol (TAG), and total cholesterol (TC) concentrations.

FO supplementation increased the amount of EPA and DHA in phospholipid (PL), TAG and cholesteryl ester (CE) fractions of LDL (see Table). No significant effect was observed in the placebo or SO groups (data not shown). Plasma TAG decreased by 23% and plasma TC concentration decreased by 14% after supplementation with FO (see Table). FO supplementation significantly decreased the lag time (−27%) of copper-induced-LDL oxidation (see Table); no changes were observed in the other groups (data not shown). Peroxide levels in LDL were not affected by any of the supplements (data not shown).

	Before FO		After FO	
	Mean	SEM	Mean	SEM
Plasma TC (mmol/l)	4.78	0.14	4.13*	0.14
Plasma TAG (mmol/l)	1.70	0.18	1.32	0.12
LDL lag time (min)	63.8	3.3	47.1*	2.4
LDL PL EPA (g/100 g fatty acid)	1.39	0.19	3.69*	0.22
LDL PL DHA (g/100 g fatty acid)	3.58	0.27	6.16*	0.28
LDL TAG EPA (g/100 g fatty acid)	0.53	0.06	1.69*	0.16
LDL TAG DHA (g/100 g fatty acid)	0.84	0.11	2.18*	0.21
LDL CE EPA (g/100 g fatty acid)	1.28	0.12	4.19*	0.30
LDL CE DHA (g/100 g fatty acid)	1.06	0.11	2.17*	0.19

* Indicates significantly different from before FO (Student's *t* test; *P*< 0.05).

This study demonstrates that modest changes in dietary *n*-3 PUFA intake significantly influence LDL fatty acid composition and oxidizability, which may affect plaque progression and stability.

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Children on home enteral tube feeding: feeding practices. By E. McNAMARA[1], M. BRESLIN[2], P. FLOOD[3] and N.P. KENNEDY[1], [1]*Department of Clinical Medicine, Trinity Centre for Health Sciences, St James's Hospital, Dublin 8, Republic of Ireland,* [2]*Department of Clinical Nutrition, The Children's Hospital, Temple Street, Dublin 1, Republic of Ireland,* [3]*Department of Clinical Nutrition, St James's Hospital, Dublin 8, Republic of Ireland*

Forty-eight parents were invited by letter to take part in an interviewer-assisted questionnaire survey of children on home enteral tube feeding (HETF). There was a poor response rate using this approach, as only twelve written replies were received (25%). There were three boys and nine girls in the sample, with an average age of 7 years and 3 months (SD 4 years 2 months). The principal indication for HETF was cerebral palsy (*n* 5). The remaining seven children had a variety of indications for HETF (including spina bifida, polycystic kidney disease, and Retts syndrome). Their collective experience on HETF was 28 years.

Feeding practices	*n*
Feeding tube	
Mic-Key® gastrostomy buttons	8
Foley catheters	2
Percutaneous endoscopic gastrostomy tube	1
Surgical gastrostomy tube	1
Mother replaced feeding tubes at home	7
Method of feed delivery	
Bolus feeds*	9
Continuous feeding using pump	3
Full nutritional support (i.e. no oral intake)	8
Supplementary feeding (i.e. some oral intake)	4
Feeds and equipment	
Varieties of enteral feed utilized	11
Mothers who experienced problems with the feeding pump	5
Mothers who collected enteral feeds from local chemist shop†	12
Mothers for whom collecting feeds posed a problem	4

*Eight mothers used a pump to administer the bolus feed, but one mother preferred gravity feeding to administer the bolus.
†Two mothers had the feed delivered occasionally.

The number of tube changes since discharge varied, one child had had the same tube *in situ* for 12 months, another had used 15 tubes within the same time period. Seven mothers had replaced the feeding tubes themselves at home, three never had, and two intended to change the tube themselves the next time it became necessary. There were approximately 91 tube changes in the 28 years on HETF. One mother had continual problems endeavouring to keep her child's tube *in situ* (the reason for the problems was unknown). Three mothers fed their children continuously, for an average of 7.5 h daily.

For children living at home, it was the mother who normally administered the feed to the child, although fathers, sisters and aunts were also involved in some families. Four children were fed whilst in bed at night and one of these children experienced sleep disturbance occasionally. No child was fed for longer than 12 h per day. All mothers who had problems with feeding pumps reported that commercial companies were prompt in replacing the pump if malfunctioning. No parent found the pump too noisy, although one mother complained that flashing lights on the pump were too bright in her child's bedroom at night, another mother noticed that her pump continually under-delivered feed (even after servicing by the manufacturer).

One mother had to make three visits to a chemist shop and carry crates of feed over a reasonably long distance before carrying them up a flight of stairs to her apartment. Feeding bags and giving sets were collected by some families (*n* 6) and delivered to others (*n* 6). In conclusion, children are fed by variety of methods that generally suit their lifestyles, families experienced few major problems, but when a problem is consistently experienced this can be stressful for the patient and/or parent. For this minority of families, a clearly defined support pathway should be established.

Fish consumption and self-reported physical and mental health status. By K.M. SILVERS[1] and K.M. SCOTT[2], [1]New Zealand Institute for Crop and Food Research, Private Bag 11600, Palmerston North, [2]Ministry of Health, New Zealand

Long chain *n*-3 polyunsaturated fatty acids (PUFA) may be important in the aetiology of mood disorders (Edwards *et al.* 1998; Peet *et al.* 1998; Stoll *et al.* 1999). New Zealand has been shown to have one of the highest rates of major depression and lowest consumption of fish per capita in the world (Hibbeln, 1998). As fish is a particularly rich dietary source of *n*-3 PUFA, the aim of this study was to assess whether self-reported mental health status, measured using the SF-36 questionnaire, in a sample of 4644 New Zealand adults aged 15 years and over, was associated with fish consumption assessed using a food frequency questionnaire.

The data were collected in the 1996/97 New Zealand Health and 1997 Nutrition Surveys, which were conducted using the same sampling frame (Ministry of Health, 1997; Quigley & Watts, 1997). Fish consumption was categorized into those who consumed no fish of any kind, and those who consumed some kind of fish, at any frequency (ranging from less than once per month to two or more times per day). "Fish" could include canned tuna, salmon, sardines, eel, fish battered, fried, steamed, baked, grilled or raw, shellfish, or other seafood (dietary supplements were not included). Data were analysed using the SUDAAN® (Research Triangle Institute) statistical package and adjusted for age, household income, alcohol use and smoking. Other demographic variables (e.g. sex, ethnicity and education) and potential confounding nutrients (e.g Fe, Se, niacin, vitamin B12, cholesterol, total fat and energy intake) were not included in the final model, as they were not associated with fish consumption.

Fish consumption was significantly associated with higher self-reported mental health status, even after adjustment for age and household income. By contrast, there was no association between fish consumption and self-reported physical health status, which supports the main interpretation that low self-reported mental health is a function of fish consumption itself. After additional adjustment for smoking and alcohol use, the difference in self-reported mental health scores between fish consumption groups was reduced slightly, but remained significant. Non-fish consumers then had significantly higher Physical Functioning Scale (PFS) scores.

Fish consumption	Mental health scale	PFS	Mental component scale	Physical component scale
		Adjusted for age and household income		
Yes	78.8	86.5	50.5	50.0
No	69.9***	89.7	42.9***	50.5

Mean scores on SF-36 scales and summary measures, significantly different from the fish consuming groups; ***$P < 0.001$.

This study is the first cross-sectional national survey to demonstrate a significant relationship between fish intake and self-reported mental health status. These findings are consistent with the hypothesis that *n*-3 PUFA may act as mood stabilizers in human health, and are of clinical and public health significance. Experimental evidence is now required to determine whether the link is causal and whether the mediating factors are indeed *n*-3 PUFA.

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An energy deficit approach to weight management during nutritional counselling post myocardial infarction. By C.R. HANKEY, W.S. LESLIE and M.E.J. LEAN, *University of Glasgow Department of Human Nutrition, Glasgow Royal Infirmary, Glasgow G31 2ER*

There is good evidence for the value of specific dietary changes (increasing fruit, vegetable and oily fish consumption, lowering saturated fat intake) and weight reduction in those with a BMI >25 kg/m^{2} in reducing CHD risk (Burr *et al.* 1989; Singh *et al.* 1992; De Logeril *et al.* 1994; Williamson *et al.* 1995). This study examined the effectiveness of an energy deficit approach to weight loss within a comprehensive nutritional counselling package in overweight post myocardial infarction patients, within their cardiac rehabilitation. This approach was based on estimated basal metabolic rate, multiplied by an activity factor of 1.3. Energy prescription was calculated by subtracting 2510 KJ (600 kcal) from the total.

Of the eighty-five patients attending routine post-infarction cardiac rehabilitation, fifty-four were recruited to a randomized controlled study; twenty-eight intervention and twenty-six control subjects had a BMI >25 kg/m^{2}. Of these, one control and three intervention subjects failed to complete the study (two deaths and one refusal). All intervention subjects (*n* 28, 24 men, mean age 57, range 41–72 years) received individual, comprehensive nutritional counselling which focused on the achievement of quantitative Scottish dietary guidelines (Scottish Office, 1996) with weight loss advice. This advice included an eating plan, without advice to increase physical activity. A further three consultations over 12 weeks were given. Control subjects (*n* 26, 20 men, mean age 57, range 40–75 years) attended only a baseline screening appointment, although all patients attended follow-up appointments at 12 and 52 weeks.

At 12 and 52 weeks there were no differences in weight. Scottish dietary targets, which were achieved by all subjects at baseline, were maintained only by intervention subjects. In contrast, control subjects showed deterioration in diet composition and body weight at 12 (+2.0 kg, SD 3.7, *P*<0.01) and 52 (+2.4 kg, SD 5.0, *P*<0.05) weeks.

Measurement	Control subjects BMI >25 kg/m^{2}						Intervention subjects BMI >25 kg/m^{2}					
	Baseline (*n* 26)	SD	12 weeks (*n* 26)	SD	1 year (*n* 25)	SD	Baseline (*n* 28)	SD	12 weeks (*n* 28)	SD	1 year (*n* 25)	SD
BMI (kg/m^{2})	30.4	3.9	30.5	4.4	30.9	4.6*	28.6	2.8	28.4	2.9	28.4	3.4
Body wt (kg)	87.9	12.8	88.2	14.1	89.6	15.1*	81.3	8.4	80.6	8.3	80.7	8.4
% body fat	38.4	8.4	37.5	8.5*	38.5	8.7	34.4	5.4	33.3	6.8	33.0	8.5
Waist (cm)	103.8	12.2	102.9	12.4	104.7	12.4**	97.5	7.8	95.9	8.4*	96.2	10.2

Significantly different from baseline *P>0.05, **P<0.01.

Weight management advice as part of a comprehensive nutritional counselling programme failed to induce weight loss but was successful in preventing the weight gain observed in control subjects. The programme was effective in maintaining diet composition in line with dietary targets.

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Bioavailability of cancer-protective isothiocyanates following ingestion of cooked cabbage, coleslaw and mustard by healthy human subjects. By G. ROUZAUD[1], A.J. DUNCAN[1], S. YOUNG[1] and B. RATCLIFFE[2], [1]*Macaulay Land Use Research Institute, Craigiebuckler, Aberdeen AB15 8QH,* [2]*The Robert Gordon University, School of Health Sciences, Aberdeen AB15 4PH*

Isothiocyanates (ITC) are hydrolysis products of glucosinolates (GLS), a prominent group of thioglucosides found in brassica vegetables. ITC can modulate the metabolism of carcinogens (Zhang & Talalay, 1994) but little is known about the bioavailability of ITC in the human digestive tract following brassica vegetable consumption. White cabbage contains the allyl ITC precursor, sinigrin. Cabbage, however, can be eaten either cooked or raw and the preparation of food may modify the bioavailability of allyl ITC by denaturing thioglucosidase, the enzyme responsible for GLS hydrolysis. Allyl ITC is found at high concentrations in household mustard. Hydrolysis of watercress-derived phenethyl GLS provides a good source of phenethyl ITC which can control for systematic variation in the post-absorptive recovery of ITC. An experiment was therefore conducted where excretion of the urinary end-products of allyl ITC and phenethyl ITC was measured following cabbage consumption.

Twelve non-smoking, healthy, male volunteers were offered a standard meal including either 10 g of ready-made mustard (Mustard treatment), 150 g of chopped raw white cabbage (Coleslaw treatment) or 150 g of white cabbage which had been microwaved for 4 min (Cooked treatment). Each meal also included a watercress juice drink with known concentrations of phenethyl ITC. Each treatment was offered to each volunteer at 48 h following each meal and analysed for urinary markers of isothiocyanate metabolism, the mercapturic acids, by HPLC (Mennicke *et al.* 1984).
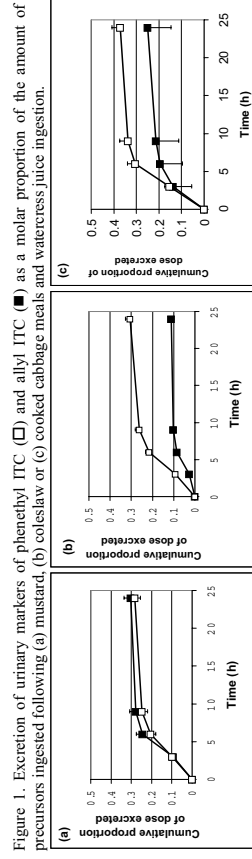

Figure 1. Excretion of urinary markers of phenethyl ITC (□) and allyl ITC (■) as a molar proportion of the amount of precursors ingested following (a) mustard, (b) coleslaw or (c) cooked cabbage meals and watercress juice ingestion.

Excretion of allyl and phenethyl ITC, when administered in their pre-formed state (Mustard treatment), was substantially complete after 24 h (Fig. 1a) and the recovery of phenethyl ITC did not vary systematically with treatment (*P*>0.05). Cumulative excretion after 24 h was close to 0.3 of the amount administered for both ITCs. Microwaved cabbage had lower sinigrin concentrations than raw cabbage (4.8 μmol g⁻¹DM in the Cooked treatment *vs* 11.2 μmol g⁻¹DM in the Coleslaw treatment). Conversion of sinigrin to allyl ITC was not influenced (*P*>0.05) by the method of preparation (cumulative amount of allyl ITC marker excreted over 24 h as a molar proportion of sinigrin ingested = 0.112 (SEM 0.0113) for the Coleslaw treatment (Fig. 1b) and 0.249 (SEM 0.1020) for the Cooked treatment (Fig. 1c). Excretion of the urinary allyl ITC marker was highly variable on the Cooked treatment and this may reflect variation in the capacity of the intestinal microflora to hydrolyse sinigrin when plant thioglucosidase is inactive.

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***In vivo* antioxidant status and levels of lipid peroxidation in subjects with Type I diabetes.** By M.P.A. HANNON-FLETCHER[1], M.J. O'KANE[2], K.W. MOLES[2], C. WEATHERUP[1], C.R. BARNETT[1] and Y.A. BARNETT[1], [1]*Cancer and Ageing Research Group, University of Ulster, Coleraine, County Londonderry, BT52 1SA, Northern Ireland* [2]*Altnagelvin Area Hospital, Glenshane Road, Londonderry, BT47 1SB, Northern Ireland*

Type I diabetes is a chronic metabolic disorder characterized by a raised blood glucose and, while insulin is used to control the life-threatening hyperglycaemia, numerous long-term complications are common (Oberley, 1988). There is strong evidence for oxidative stress in the aetiology and pathogenesis of diabetes and its complications (Baynes & Thorpe, 1999). In addition, lipid peroxidation has been shown to be involved in the pathogenesis of diabetes (Parthiban *et al.* 1995).

The aim of this study was to determine *in vivo* antioxidant status and plasma levels of the lipid peroxidation product, malondialdehyde (MDA), in well-controlled type I diabetic subjects. *In vivo* antioxidant status was assessed in non-fasting peripheral blood samples by determining; erythrocyte superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activities, serum uric acid (UA), and plasma vitamin C and E concentration. Glycated haemoglobin (%HbA₁c) and cholesterol levels were also determined.

Parameters	Control (n 50)		Type I diabetes whole group (n 50)		Type I diabetes without complications (n 38)		Type I diabetes with complications (n 12)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
% HbA1c	4.28	0.06	7.71***	0.21	7.79***	0.24	8.12***	0.35
Cholesterol (mmol/l)	4.74	0.12	4.90	0.15	4.62	0.16	5.66†	0.34
Plasma MDA (μmol/l)	0.40	0.02	0.47†	0.03	0.45	0.03	0.55*	0.07
SOD (U/gHb)	1339	10.0	1158***	40.0	1164***	43.9	1138**	95.3
GPx (U/gHb)	44.2	1.54	39.3†	1.64	39.8	1.87	37.9	3.50
CAT (k/gHb)	40.4	2.03	58.6***	2.67	56.9***	3.31	63.6***	4.71
Vitamin E (μmol/l)	32.1	1.53	36.0	1.63	35.5	1.85	37.3	3.59
Vitamin C (μmol/l)	23.2	1.81	17.3†	1.63	18.84	1.80	13.9†	3.65
UA (μmol/l)	276	11.6	235†	9.40	235*	9.92	227	21.9

Statistical analysis was performed using a Students *t*-test (unpaired) and least squares linear regression analysis. Mean value were significantly different: *** *P*<0.0001; ** *P*<0.001; * *P*<0.01; and † *P*<0.05, when compared to controls.

Levels of HbA₁c were significantly higher in the type I diabetic subjects compared to controls (*P*<0.0001). However, HbA₁c levels in the type I diabetic subjects were indicative of acceptable glycaemic control. In the type I diabetic subjects with complications, cholesterol concentration was significantly higher compared to controls (*P*<0.05). There were significantly lower activities of SOD (*P*<0.0001) and GPx (*P*<0.05) and levels of vitamin C (*P*<0.05) and UA (*P*<0.05) and a significantly higher activity of CAT (*P*<0.0001) in the type I diabetic subjects, compared to controls. Plasma MDA was significantly higher in the type I diabetic subjects, compared to controls (*P*<0.05). When the type I diabetic subjects, who presented with complications, were excluded from the statistical analysis, there was no significant difference in plasma MDA between type I diabetic subjects without complications and controls.

In conclusion, the well-controlled type I diabetic subjects showed alterations in *in vivo* antioxidant status while additionally, in those subjects who presented with diabetic complications, plasma levels of MDA were significantly elevated compared to controls.

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Antioxidant potential of green and black tea determined using the ferric reducing power (FRAP) assay. By S.C. LANGLEY-EVANS, *Division of Health and Life Sciences, University College Northampton, Boughton Green Road, Northampton NN2 7AL*

Tea is one of the most commonly consumed beverages in the world and is a rich source of dietary flavonoids (Hertog *et al.* 1993*a*). The flavonoids of tea have been demonstrated, using a variety of methodologies, to possess antioxidant properties. Epidemiological studies have provided a suggestion that these properties might, *in vivo*, contribute to a reduction of coronary heart disease risk by inhibiting the oxidation of low density lipoproteins (Hertog *et al.* 1993*b*). To date there has been little interest in the possible effects of tea preparation methods on the antioxidant properties of the drink.

A series of *in vitro* studies were performed to assess the antioxidant potentials of green and black teas prepared under varying conditions. The FRAP assay of Benzie & Strain (1996) was used to determine antioxidant capacity of all tea preparations. Experiments were performed to determine the optimum temperature for the preparation of tea infusates. Green leaf tea, black leaf tea and black tea bags were infused in water for 5 min at temperatures ranging from 20° to 90°. Leaf teas liberated antioxidants into the water even at low temperatures and at all temperatures green tea preparations had significantly greater (4- to 5-fold, $P<0.005$) antioxidant capacity than black tea preparations. Between 20° and 70°, tea infusates prepared from tea bags had lower antioxidant potential than from black leaf tea. The optimum infusion time for black teas was between 1 and 2 min at 90°. This was sufficient to release 90% of the maximal antioxidant potential. Green tea antioxidants were released at 90° in a biphasic manner over 15 min, and only 61% of the maximal FRAP was liberated by 2 min of infusion. To determine the effect of adding milk to tea upon antioxidant potential, black tea infusates were prepared from tea bags at 90° and mixed with soya milk, whole cows milk, semi-skimmed cows milk or skimmed cows milk, in a ratio of 1.5 g milk to 20 ml tea. FRAP was determined in the tea preparations and compared to the expected FRAP, which was calculated from the measured antioxidant potential of the tea without milk plus the measured antioxidant potential of the milk. The addition of milk produced measured FRAP values that were 12–28% lower than expected. This effect was greatest with whole cows milk and appeared to be related to the fat content of the milk.

Tea preparation	Observed FRAP (µmol/g tea/L)			Expected FRAP (µmol/g tea/L)	% reduction
	Mean	n	SE		
No milk	683	3	11	683	0
Whole cows milk	693	3	44	962	28
Skimmed cows milk	696	3	72	788	12
Semi-skimmed cows milk	724	3	68	930	22
Soya milk	745	3	55	910	18

The percentage reduction refers to the difference between observed and expected FRAP values.

It has been estimated that tea flavonoids may account for 35–45% of total dietary antioxidant consumption. The data indicate that the method of tea preparation may lead to considerable variation in the antioxidant potential of tea infusates. Tea is at present being widely promoted within the United Kingdom as a healthy drink. The full potential of tea as an antioxidant source may in fact not be exploited by the methods of tea preparation currently practiced by most consumers in this country.

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Consumption of black tea elicits an increase in plasma antioxidant potential in humans. By S.C. LANGLEY-EVANS, *Division of Health and Life Sciences, University College Northampton, Boughton Green Road, Northampton NN2 7AL*

Tea provides a rich dietary source of polyphenolic compounds, which are known to possess potent antioxidant properties *in vitro*. Although catechins, the major flavonoids of green and black teas, are known to be absorbed across the human digestive tract (Van het Hof *et al.* 1999), few studies have demonstrated that they retain their antioxidant properties in the circulation (Benzie *et al.* 1999). This is an important issue, as tea flavonoids have been proposed to reduce risk of coronary heart disease, stroke and cancer related deaths by virtue of their free radical scavenging capacity.

Nine healthy volunteers (one male, eight female), aged between 26 and 59 years, gave informed consent to take part in three study days. These days, conducted with each subject in random order, required the volunteers to drink either black tea with milk, black tea without milk, or no tea. For 12 h before commencing and during each study day subjects refrained from eating or drinking sources of dietary flavonoids. On the no tea drinking day, free access was given to water, whilst on the tea drinking days the subjects consumed a standard measure of tea at hourly intervals between 09.00 and 14.00 hours (six measures in all). Blood samples were obtained at 09.00 (baseline sample), 12.00 and 15.00 hours. The antioxidant potential of plasma was determined using the FRAP assay of Benzie & Strain (1996).

Subjects consuming no tea exhibited no significant change in FRAP across the 6 h of the study. The mean baseline FRAP value obtained was 670 (SE 58) µmol/l. Consumption of three measures of milky tea between 09.00 and 12.00 hours had no significant effect on plasma antioxidant potential. A further three measures of tea produced a 50% increase in antioxidant potential, but this failed to achieve statistical significance. Consumption of tea without milk significantly elevated FRAP by 12.00 hours ($P=0.02$, relative to the 09.00 hours baseline) and this effect persisted until 15.00 hours ($P=0.002$, relative to the 09.00 hours baseline).

Tea protocol	Increase in FRAP relative to the 09.00 hours baseline (µmol/L)					
	12.00 hours			15.00 hours		
	Mean	n	SE	Mean	n	SE
No tea	46	9	65	68	9	80
Tea with milk	59	9	129	357	9	219
Tea without milk	333*	9	121	389*	9	84

Antioxidant potential (FRAP) differed from the baseline value, *$P<0.05$.

The data are consistent with the findings of Benzie *et al.* (1999) in that consumption of tea significantly increased circulating antioxidant capacity. The consumption of moderate to heavy amounts of tea may thus afford some protection against free radical mediated disease. These findings do, however, suggest that the consumption of tea with milk may negate the potential health benefits, presumably through the complexing of tea flavonoids with milk fats or proteins.

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The effects of phenolic components of tea on the production of inflammatory cytokines in human whole blood cultures. By B. POWELL, S. CROUVEZIER, D. KEIR and P. YAQOOB, *The Hugh Sinclair Unit of Human Nutrition, Department of Food Science and Technology, University of Reading, Whiteknights, Reading RG6 6AP*

Epidemiological studies have suggested a protective effect of dietary flavonoids on mortality from coronary heart disease. Tea is the major dietary source of flavonoids in many countries, although consumption varies both between countries and within any given population. The main components of (unfermented) green tea are epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). These phenolic compounds are also found at significant levels in (fermented) black tea, which additionally contains theaflavins and thearubigens. Many phenolic compounds, including the catechins described above, exhibit antioxidant properties *in vitro*. However, several studies have failed to show any effect of tea-derived phenolic compounds on the oxidation of low-density lipoprotein, suggesting that their potential protective effects in atherogenesis may be exerted through other mechanisms.

Atherosclerosis is increasingly being recognised as a disease involving chronic inflammation in the vasculature. There is some evidence that flavonoids such as quercetin have suppressive effects on immune function, but to date there has been no systematic study of the effects of tea-derived phenolic compounds on the inflammatory response in humans. The current study investigated the effects of EC, ECG, EGC and EGCG on the production of the inflammatory cytokines, interleukin (IL)-1β, IL-6 and tumour necrosis factor (TNF)-α by human whole blood cultures.

Blood samples were obtained from twelve healthy, fasted volunteers, diluted 1:10 with culture medium containing 2 mM glutamine and antibiotics and cultured for 24 h in the presence of EC, ECG, EGC or EGCG (1–20 μM; dissolved in a final concentration of 0.1% aqueous dimethyl sulphoxide (DMSO)) and lipopolysaccharide (10 μg/ml). Medium was collected and cytokine production measured by ELISA. Cytokine production was expressed as a percentage of the control samples (no tea polyphenol, 0.1% DMSO).

Conc (μM)	Cytokine production (% of control)											
	IL-1β				IL-6				TNFα			
	EGC		EGCG		EGC		EGCG		EGC		EGCG	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	100		100		100		100		100		100	
0.63	94.3	5.2	92.9	3.8	102.1	6.7	107.9	9.9	107.5	8.3	100.1	4.8
1.25	95.2	4.4	94.8	6.2	108.3	9.8	115.2	13.3	99.7	7.4	98.6	5.1
2.50	96.4	4.3	94.8	5.7	111.8	8.7	97.3	5.4	102.1	7.9	112.6	8.4
10.00	88.0[a]	3.5	88.8	6.8	109.0	6.7	108.8	9.5	104.6	7.4	102.9	8.1
20.00	82.1[b]	2.5	84.7	5.8	95.5	5.8	98.8	9.9	100.5	5.8	98.2	9.7

Data are mean values with their standard errors for twelve subjects. Statistically significant differences (paired *t*-test with Bonferroni correction) compared with controls (no tea compounds) are shown as [a]*P*<0.01 and [b]*P*<0.001.

There was no effect of EC or ECG on the production of inflammatory cytokines by human whole blood cultures (data not shown). However, there was a progressive decrease in the production of IL-1β in the presence of increasing concentrations of EGC and EGCG, which became significant at 10 μM EGC (*P*<0.01), but was not significant for EGCG. In contrast, there was no effect of either EGC or EGCG on the production of IL-6 or TNFα. This suggests that some, but not all, of the major phenolic components of tea are able to suppress the production of IL-1β (albeit at slightly higher than physiological concentrations) and that similar effects are not observed on the production of other inflammatory cytokines. This raises interesting questions about the mechanism of action of EGC and EGCG on the production of IL-1β, but not IL-6 or TNFα, which is the subject of ongoing studies.

The effects of phenolic components of tea on phagocytosis and respiratory burst by human neutrophils and monocytes. By S. CROUVEZIER and P. YAQOOB, *The Hugh Sinclair Unit of Human Nutrition, Department of Food Science and Technology, University of Reading, Whiteknights, Reading RG6 6AP*

Dietary flavonoids such as quercetin have been demonstrated to inhibit immune functions, including antigen presentation by mononuclear cells, production of interleukin-2, lymphocyte proliferation, adhesion of mononuclear cells, natural killer cell activity and antibody production by B-lymphocytes (Middleton, 1998). While some of these effects may be due to the antioxidant properties of flavonoids, others are suggested to be mediated through antioxidant-independent mechanisms.

Tea is the major dietary source of flavonoids in many countries, although consumption varies both between countries and within any given population. The main components of (unfermented) green tea are epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). These phenolic compounds are also found at significant levels in (fermented) black tea, which additionally contains theaflavins and thearubigens. Animal studies have suggested that there may be effects of tea-derived polyphenols on immune and inflammatory responses, although these have often used excessively high concentrations of catechins in their experiments (e.g. Yang *et al.* 1998). However, to date, there have been no systematic studies examining the effects of tea-derived phenolic compounds on human immune function. This is important, since suppression of immune function could result in impairment of host defence against invading pathogens. The current study investigated the effects of EC, ECG, EGC and EGCG on phagocytosis of *E. coli* and on respiratory burst by human neutrophils and monocytes.

Blood samples were obtained from ten healthy, fasted volunteers. Samples of whole blood were incubated with EC, ECG, EGC or EGCG (0-5 μg/ml; dissolved in a final concentration of 0.25% aqueous dimethyl sulphoxide (DMSO) or control (DMSO only) for 10 min at 37° (or on ice for control samples) and phagocytosis or respiratory burst was determined using commercial kits (Becton Dickinson), based on flow cytometric methods. Fluorescence data for both experiments were collected on 2 × 10⁴ viable cells and analysed using a CellQuest software package. Sample data for neutrophil respiratory burst only are shown below.

Conc (μg/ml)	Neutrophil respiratory burst							
	Cells undergoing respiratory burst (% control)				Burst index (mean fluorescence intensity; % control)			
	EC		EGCG		EC		EGCG	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	100		100		100		100	
0.1	99.0	0.8	99.5	1.2	102.3	2.9	100.7	3.8
0.3	98.0	0.7	98.0	1.1	99.5	4.2	98.6	3.7
0.5	97.7	1.7	99.2	1.2	106.5	3.8	101.8	4.2
1.0	97.9	0.9	98.4	1.0	103.4	6.1	95.5	3.1
2.0	96.8	0.9	99.5	1.6	100.6	3.0	102.9	6.7
4.0	96.9	1.4	99.3	1.1	100.6	3.7	104.9	4.2
5.0	97.4	1.2	99.4	1.2	106.4	3.7	101.4	4.0

Data are mean values with their standard errors for ten subjects.

There was no effect of any of the tea-derived phenolic compounds tested on either phagocytosis or respiratory burst by neutrophils or monocytes over the range of concentrations tested. It is therefore unlikely that tea consumption affects the function of these cells *in vivo*.

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The potential of the flavonoids, quercetin and rutin, butylated hydroxytoluene and iron chelators to protect against DNA single strand breaks in Caco-2 cells. By S.A. AHERNE and N.M. O'BRIEN, *Nutritional Sciences, Department of Food Science and Technology, University College Cork, Republic of Ireland*

Flavonoids are naturally occurring polyphenolic compounds found in many fruits, vegetables and beverages and are an integral part of the human diet. They are reported to exhibit a wide variety of biological effects and may protect tissues against reactive oxygen species which are thought to be involved in the pathogenicity of numerous human diseases such as cardiovascular disease and certain cancers. In the present study the potential protective effects of the flavonoids, quercetin and rutin, against DNA strand break formation was investigated in human colonic carcinoma Caco-2 cells. The action of the flavonoids was compared with the synthetic antioxidant, butylated hydroxytoluene (BHT), and the iron chelators 1,10-phenanthroline (o-phen) and deferoxamine mesylate (DFO).

Caco-2 cells were cultured in Dulbecco's modified Eagle's medium and maintained in a humidified atmosphere of 37°. Caco-2 cells were pre-incubated with either quercetin (50 µM), rutin (50 µM) or BHT (50 µM) for 24 h, or o-Phen (100 µM) or DFO (15 mM) for 30 min. After the pre-treatments, cells were exposed to the genotoxins $tert$-butylhydroperoxide ($tert$-BOOH, 200 µM) or menadione (10 µM) for 30 min at 37°. After oxidant treatment, DNA single strand break formation was assessed using the alkaline single cell gel electrophoresis (alkaline comet) assay (Tice *et al.* 1990). One hundred nuclei were scored for each condition and given a value between 0 (undamaged nuclei) and 400 (maximally damaged nuclei).

	Genotoxin-induced DNA single strand breaks (arbitrary units)			
	$tert$-BOOH (200 µM)		Menadione (10 µM)	
	Mean	SE	Mean	SE
Control	39*	2	41*	5
Genotoxin alone	342	3	351	8
Genotoxin + Quercetin (50 µM)	158*	8	270*	27
Genotoxin + Rutin (50 µM)	281*	15	325	8
Genotoxin + BHT (50 µM)	329	6	312*	15
Genotoxin + o-Phen (100 µM)	178*	17	320	11
Genotoxin + DFO (15 mM)	97*	5	303*	21

* Statistical analysis by one-way ANOVA, followed by least significant difference (LSD; $P<0.05$); n 4 for treatments.

Both $tert$-BOOH and menadione induced DNA single strand breaks in Caco-2 cells. Menadione at 10 µM induced a similar level of DNA damage as $tert$-BOOH at 200 µM. Pre-incubation with either quercetin or rutin for 24 h significantly decreased the formation of DNA single strand breaks evoked by $tert$-BOOH ($P<0.05$). Iron chelators, o-Phen and DFO, also protected against $tert$-BOOH-induced DNA damage whereas BHT had no effect. Only quercetin, DFO and BHT protected against menadione-induced DNA single strand break formation ($P<0.05$). We demonstrated that the flavonoids, quercetin and rutin, protected against $tert$-BOOH-induced DNA strand breaks in a similar way to the metal ion chelators, whereas quercetin protected against menadione-induced DNA damage by behaving like both a metal chelator and radical scavenger.

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Modulation of rat tissue xenobiotic-metabolizing enzymes by nomilin, a citrus phytochemical. By C. KELLY, C. JEWELL and N.M. O'BRIEN, *Nutritional Sciences, Department of Food Science and Technology, University College Cork, Republic of Ireland*

Citrus fruits contain a class of biologically active compounds called limonoids. These compounds have highly oxygenated triterpenoid structures and contain a furan ring. Limonoids impart the bitter taste characteristic of citrus fruit. One of these compounds, nomilin, has been shown to possess chemopreventative activity (Lam & Hasegawa, 1989). The aim of this study was to assess the effect of nomilin on glutathione S-transferase (GST) and a cytochrome P450 isoform (CYP1A1) activity in the liver, lungs and small intestine (SI) of the rat.

Male Wistar rats were divided into six groups (mean weight 47.9g). A control group received an AIN 76 (AIN, 1977) diet alone. Four further groups were fed the AIN 76 diet containing increasing doses of nomilin. The positive control group was fed the AIN-76 diet containing 3-methylcholanthrene (3-MC; 15 mg/kg), a known inducer of GST and CYP1A1. All animals received 15 g of diet/d for 10 d. The animals were killed by cervical dislocation and the organs were excised and used to prepare subcellular fractions. GST activity was measured in the cytosolic fraction using dinitrochlorobenzene (DNCB) as a substrate (Habig *et al.* 1974). Cytochrome P450 activity was assessed in the microsomal fraction by measuring 1A1-linked ethoxyresorufin-O-dealkylation (Burke *et al.* 1985).

Treatment	GST activity[†]						P450 1A1 activity[‡]			
	SI		Lung		Liver		Lung			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	187	12	20	2	38	2	1810		ND	–
3-MC	338*	20	18	3	6419*	3	554		ND	64
Nomilin 1 mg/d	269*	20	36	3	41		ND	6	ND	–
Nomilin 2 mg/d	314*	19	21	3	32		ND	5	ND	–
Nomilin 5 mg/d	365*	14	20	1	35		ND	4	ND	–
Nomilin 10 mg/d	452*	25	20	3	27		ND	3	ND	–

Results expressed as mean (and SE), n 6–10. Values marked * differ from control (ANOVA, Dunnetts test, $P<0.05$).
[†] nmol/min/mg cytosolic protein, [‡] pmol/min/mg microsomal protein. ND = not detected.

The results demonstrate that treatment with nomilin caused a dose-dependant increase in GST activity in the rat SI. The positive control (3-MC) also showed a substantial increase in GST activity above the control value. GST activity in rat lung was low, not induced by 3-MC and unaffected by nomilin treatments. We have reported previously that nomilin treatment resulted in the induction of GST activity in rat liver (Kelly *et al.* 2000).

In the hepatic microsomal fraction, 3-MC induced a significant increase in CYP1A1 activity compared with control. Treatment with nomilin at higher doses caused a slight reduction in CYP1A1. In the lung, CYP1A1 was undetectable in all nomilin treatment groups. 3-MC did induce CYP1A1 activity in the lung.

Nomilin induced GST activity in the SI and liver (previous report) but did not appear to have any effect on GST activity in the lung. Metabolism of nomilin may have occurred before it reached the lung. GSTs play a significant role in carcinogen detoxification. Therefore, induction of the GST enzyme system by this limonoid could potentially increase the capacity of the body to protect against toxicants. On the other hand, nomilin had little effect on liver CYP1A1 activity.

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Generation of oxidative stress during oxysterol-induced apoptosis in U937 cells. By Y.C. OCALLAGHAN, J.A. WOODS and N.M. OBRIEN, *Nutritional Sciences, Department of Food Science and Technology, University College Cork, Republic of Ireland*

Oxysterols have been shown to induce apoptosis in a number of cell lines by a mechanism that is as yet unclear. A number of studies indicate that the central pathway in the induction of apoptosis by certain agents may involve the generation of oxidative stress (Lizard *et al.* 1998). The endogenous antioxidant, glutathione, is decreased in the early stages of apoptosis in certain cell lines and this has been implicated in the regulation of events leading to cell death such as the release of cytochrome *c* from the mitochondria. Levels of the cellular antioxidant enzymes, catalase and superoxide dismutase, have also been shown to be altered during apoptosis. We have shown previously that the oxysterol 7β-hydroxycholesterol (7β-OH) induces apoptosis in the human monocytic cell line, U937 cells, while 25-hydroxycholesterol (25-OH) does not (OCallaghan *et al.* 1999). The aim of the present study was to determine whether glutathione levels or the activities of catalase or superoxide dismutase are altered in U937 cells exposed to these oxysterols.

U937 cells were adjusted to a density of 1×10^5 cells/ml in RPMI 1640 medium supplemented with 25ml/l fetal calf serum. Cells were treated with 30 μM 25-OH or 7β-OH and incubated at 37°, air:CO$_2$ (95:5). Control cells were treated with an equal volume of the carrier, ethanol. The glutathione levels and activities of catalase and superoxide dismutase were determined following both a 3 h and 12 h incubation with oxysterol. Proteins were determined by the BCA method.

| | 3 h treatment | | | | | | | | 12 h treatment | | | | | | | |
| | Control | | 25-OH | | 7β-OH | | | | Control | | 25-OH | | 7β-OH | | |
	Mean	SE	Mean	SE	Mean	SE			Mean	SE	Mean	SE	Mean	SE
Glutathione nmol GSH/mg protein	59.4	3.8	61.6	6.4	56.6	3.2			52.9	3.7	67.4	7.1	13.4*	2.5
Catalase units of activity/mg protein	87.9	8.2	83.5	16.3	76.0	7.2			65.3	3.1	65.1	4.6	75.9	9.6
Superoxide Dismutase units of activity/mg protein	9.3	1.3	12.8	1.8	6.6	1.7			6.7	0.4	7.2	0.8	10.3*	0.6

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

25-OH did not alter the antioxidant status of the cells at either of the time points examined under these experimental conditions. Also, 25-OH does not induce apoptosis in this cell line (OCallaghan *et al.* 1999). Incubation with 7β-OH, which we have shown previously to induce apoptosis after 24 h, resulted in a significant decrease in glutathione levels following a 12 h incubation period and also increased activity of superoxide dismutase, while catalase activity remained unchanged. Therefore, it appears that one of the early events in 7β-OH-induced apoptosis in U937 cells may involve the generation of oxidative stress within the cell, possibly as a result of the decrease in glutathione and the increase in superoxide dismutase activity.

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The effects of an extract of cocoa powder on induced DNA damage measured by the Comet assay. By R.J. HAMBLY[1], E.A. OFFORD-CAVIN[2], A.M. PFEIFER[2], I.R. ROWLAND[1] and P.C. RUMSBY[1]. [1]*TNO BIBRA, Woodmansterne Road, Carshalton, Surrey SM5 4DS, UK,* [2]*Nestlé Research Center, PO Box 44, Vers-chez-les Blanc, 1000 Lausanne 26, Switzerland*

There has been a great deal of interest in the components of plants that may have a role in protection against cancer. There are a large number of polyphenolic compounds found in plants that have been shown to be biologically active (Rice-Evans *et al.* 1997). Studies with green tea have shown that it the polyphenols present may have properties associated with protection against cancer such as anti-proliferative and anti-oxidant effects (Droesti, 1996). Cocoa is a rich source of polyphenols, which make a major contribution to both colour and flavour. The polyphenolic content of the cocoa bean includes anthocyanins, anthocyanidins, leucoanthocyanidins, phenolic acids and flavonols. The main flavonol in cocoa is (–)-epicatechin (Williams, 1971).

In this study the effects of an ethanol (80:20 w/v) or water-soluble extract of defatted cocoa powder on induced DNA damage in the human colonic adenocarcinoma cell line, CACO-2 was assessed by the single cell gel electrophoresis (Comet) assay (Singh *et al.* 1988). The Comet assay provides a simple indicator of DNA damage in single cells by visualizing single strand breaks. Briefly the cells are lysed and the DNA allowed to unwind in alkali and then electrophoresed through agarose gel. The relaxed DNA migrates under the current to form a tail that is stained with ethidium bromide and measured as a tail moment. The greater the tail moment, the greater the DNA damage. The DNA damage was induced *in vitro* by incubation with 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) or hydrogen peroxide to assess effects on mutagenic and oxidative damage respectively. The CACO-2 cells (2×10^5) were incubated for 24 h with the cocoa extract containing 250 μM gallate equivalents or a solvent control and then exposed to MNNG (1 μM) or hydrogen peroxide (50 μM) for 30 min at 37°.

| | Saline | | Hydrogen peroxide | | MNNG | |
		SEM		SEM		SEM
Cocoa extract						
–	2.7	1.4	21.4	0.5	9.5	0.9
+	1.4	0.0	9.6	2.7*	11.4	2.3
Water extract						
–	1.25	0.0	28.1	0.6	15.4	2.5
+	1.25	0.0	28.1	2.5	7.1	0.9*

Values shown are mean 75th percentile of tail moment (and SEM) (n 3). One hundred cells were assessed for each replicate.

There was a dose-dependent increase in DNA damage in CACO-2 cells exposed to MNNG or hydrogen peroxide. Incubation with the ethanol extract was significantly reduced (*$P<0.05$) the DNA damage induced by hydrogen peroxide by 55% (see Table), while the water-soluble extract reduced MNNG-induced damage (*$P<0.05$) by 56%.

This preliminary study suggests that there is a protective effect of this fraction of cocoa powder on DNA damage as measured by the Comet assay. Offord *et al.* (1999) have previously shown in *in vitro* experiments that the same cocoa extract decreased the induction of aflatoxin-induced DNA adduct formation and induced the expression of glutathione-S-transferase and the anti-oxidant responsive element (ARE). The active components of the cocoa extract remain to be elucidated. However, it is clear that there are biologically active compounds present in cocoa which are potentially beneficial.

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Offord EA, Huynh-Ba T, Avanti O & Pfeifer AM (1999) In: *Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease* [JT Kumpulainen and JT Salonen, editors], Cambridge: RSC.
Rice-Evans CA, Miller NJ & Paganga G (1997) *Trends in Plant Science* **2**, 152–159.
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Williams JA (1971) *Journal of Biology and Applied Chemistry* **14**, 10–19.

α-Tocopherol, but not α-tocopherol, prevents oxysterol-induced apoptosis in a human monocytic cell line. By N.M. LYONS, J.A. WOODS and N.M. O'BRIEN, *Nutritional Sciences, Department of Food Science and Technology, University College Cork, Republic of Ireland*

A considerable body of evidence implicates oxysterols (which are readily incorporated into oxidized LDL) as a critical atherogenic component. Oxysterols, particularly those oxidized at position 7 (C-7) on the sterol nucleus, have been shown to induce apoptosis in a wide variety of cell lines including vascular endothelial cells, smooth muscle cells and monocytes. Additionally, recent investigations have implied a role for reactive oxygen species (ROS) in the apoptotic process initiated by such oxysterols (Lizard *et al.* (1999). The antioxidant compounds α- and γ-tocopherol (TOC) are isomeric forms of vitamin E which differ in structure only by a single methyl group (Tran & Chan, 1992). While γ-TOC is the more biologically active. Also, despite the large dietary intake of γ-TOC in the typical western diet, α-TOC is the most abundant form of vitamin E in the typical western diet, α-TOC is the more biologically active. Also, despite the large dietary intake of γ-TOC with respect to α-TOC (often 2:1), plasma concentrations of the latter are consistently higher.

The objective of the present study was to determine if either α-TOC or γ-TOC (10 μM) could modulate apoptotic cell death induced by 7β-hydroxycholesterol (7β-OHC) in a human monocytic cell line. U937 cells were grown in a humidified atmosphere of air:CO_2 (95:5 v:v) at 37°, and were treated with 7β-OHC (30 μM) in the presence or absence of either 10 μM α-TOC or γ-TOC for 48 h. The carrier vehicle for all test compounds was ethanol.

Cell viability was assessed after 48 h using the fluorescein diacetate/ethidium bromide (FDA/EtBr) assay and the percentage of apoptotic cells was determined by staining with Hoechst 33342. Uptake of the tocopherols was determined by high performance liquid chromatography (HPLC). HPLC analysis showed that U937 cells contained almost 3.5 times more γ-TOC (409 (SE 40) picomol/10^6 cells) than α-TOC (115 (SE 8) picomol/10^6 cells).

Treatment	Cell viability (percentage)		Apoptotic cells (percentage)	
	Mean	SE	Mean	SE
Control	95.4	1.1	8.8	1.4
7β-OHC	55.5**	5.1	29.0**	3.8
7β-OHC & α-TOC (3:1 v:v)	86.1*	3.6	11.7	1.7
7β-OHC & γ-TOC (3:1 v:v)	50.1**	3.5	32.4**	3.4

** <0.01, *P<0.05 (ANOVA followed by Dunnett's test). *n* 3 independent experiments.

The results presented show that treatment of U937 cells with 30 μM 7β-OHC significantly decreased cell viability and increased the number of apoptotic cells over the 48 h treatment period. Although γ-TOC was present in higher amounts in U937 cells, apoptosis was inhibited by 10 μM α-TOC, and not by γ-TOC, suggesting that there is indeed a role for ROS in oxysterol-induced apoptosis.

Lizard G, Monier S, Cordelet C, Gesquiere L, Deckert V, Gueldry S, Lagrost L & Gambert P (1999) *Atherosclerosis, Thrombosis and Vascular Biology* **19**, 1190–1200.
Tran K & Chan A (1992) *Lipids* **27**, 38–41.

International evaluations of cancer preventive agents: results from first volumes of the IARC Handbooks of Cancer Prevention. By H. VAINIO, J.A. WOODS and F. BIANCHINI, *Unit of Chemoprevention, International Agency for Research on Cancer, 150 cours Albert Thomas, F-69372 Lyon, France*

With the aim of evaluating the efficacy of cancer chemopreventive agents, the International Agency for Research on Cancer (IARC) initiated in 1997 a new series of '*IARC Handbooks of Cancer Prevention.*' The volumes so far published deal with several non-steroidal anti-inflammatory drugs (vol. 1), carotenoids (vol. 2), vitamin A (vol. 3) and retinoids (vol. 4). The fifth volume, on the use of sunscreens in skin cancer prevention, is under preparation. In the IARC *Handbooks*, the available published data on cancer preventive efficacy are critically reviewed by an International Working Group of Experts convened by IARC; the mechanisms involved and other beneficial and adverse effects of the agent are also discussed. In evaluating the evidence for cancer preventive efficacy, either human or experimental animal models are considered, using the index terms '*sufficient*' or '*limited*' or '*inadequate evidence of cancer-preventive activity*' or '*evidence suggesting lack of cancer-preventive activity*'. The degree of evidence for agents evaluated in the first *Handbooks* are given in the table below.

DEGREE OF EVIDENCE EVALUATIONS FROM THE IARC HANDBOOKS

Agent	Evidence in humans	Evidence in animal models
Non-Steroidal Anti-inflammatory Drugs		
Aspirin	Limited	Sufficient
Sulindac	Limited	Sufficient
Piroxicam	Inadequate	Sufficient
Indomethacin	Inadequate	Sufficient
Carotenoids		
=Carotene (high dose supplements)	Lack of activity	Sufficient
=Carotene (usual dietary levels)	Inadequate	Sufficient
Canthaxanthin	Inadequate	Sufficient
-Carotene	Inadequate	Limited
Lycopene	Inadequate	Limited
Lutein	Inadequate	Limited
Fucoxanthin	Inadequate	Limited
Retinoids		
all-*trans*-Retinoic acid	Inadequate	Inadequate
13-*cis*-Retinoic acid	Limited	Limited
9-*cis*-Retinoic acid	Inadequate	Limited
Fenretinide (4-HPR)	Inadequate	Sufficient
Etretinate	Inadequate	Limited
Acitretin	Inadequate	Inadequate
N-Ethylretinamide	Inadequate	Lack of activity
Targretin	Inadequate	Inadequate
LGD 1550	Inadequate	Inadequate
Preformed Vitamin A	Lack of activity	Limited

In addition to the evaluations given above, the Expert Group also makes an 'Overall Evaluation'. This is done in a narrative text, without specific terminology. Instead, the Expert Group is invited to offer, on the basis of all available data, a perspective in relation to the use of the agent in question for cancer preventive purposes. The following extract from vol. 3 on vitamin A illustrates the scope of such evaluation: 'The benefits to health of correcting vitamin A deficiency are clear …… There is little evidence to support the idea that, within the wide range of doses bordered by deficiency and toxicity, modulating preformed vitamin A intake will have any substantial cancer-preventive effect.'.

The *IARC Handbooks of Cancer Prevention* are available from the IARC electronic bookstore (Press@iarc.fr).

Volume 1. *Non-Steroidal Anti-inflammatory Drugs*, 1997. Volume 2 *Carotenoids*, 1998. Volume 3. *Vitamin A*, 1998. Volume 4. *Retinoids*, 1999. Volume 5. *Use of Sunscreens* (in preparation).

Phylloquinone (vitamin K_1) status in British elderly people: findings from a recent National Diet and Nutrition Survey. By C.W. THANE[1], C.J. BATES[1], A.A. PAUL[1] and M.J. SHEARER[2], [1]MRC Human Nutrition Research, Downhams Lane, Milton Road, Cambridge CB4 1XJ, [2]Vitamin K Research Unit, Haemophilia Centre, St Thomas' Hospital, London SE1 7EH

Vitamin K circulates in blood mainly as phylloquinone (vitamin K_1), which is also the major dietary source (Shearer, 1997). Sub-optimal vitamin K status has been linked to both osteoporosis (Jie et al. 1995). This may be due to impaired actions of several vitamin K-dependent proteins (e.g. osteocalcin and matrix Gla protein (MGP)) which regulate bone turnover, while MGP inhibits arterial wall calcification. Deteriorating bone and cardiovascular health are both likely to become manifest in older people. It is therefore desirable to profile plasma K_1 (as a measure of status) in the elderly, in order to gain a better understanding of the relationship between plasma K_1 and intake, and with other nutrients and socio-demographic factors.

This analysis used fasting plasma K_1 values from 859 people aged 65 years and over living in private households in mainland Britain (Finch et al. 1998). Associations were examined between plasma K_1 and intake, and with consumption of main food groups and socio-demographic factors (age, sex, region of habitation, season, social class, household income and composition, smoking habit), including the relationship of the latter with low plasma K_1 (defined as values in the lowest fifth of the distribution). Dietary K_1 intake was estimated from 4-d weighed dietary records using K_1 content values for a comprehensive range of foods (Bolton-Smith et al. 2000; plus unpublished data). Plasma K_1 levels were determined by HPLC with fluorescence detection, with a lower limit of quantification of 0.11 nmol/l (0.05 ng/ml). Subjects with values below this limit (87/859) were arbitrarily assigned a value of 0.055 nmol/l (0.025 ng/ml). Since plasma K_1 was correlated with plasma triacylglycerol (TAG), associations were adjusted for TAG levels in all statistical analyses, with $P<0.05$ deemed significant.

Plasma K_1 values showed a positively-skewed distribution (geometric mean 0.34, 95% interval of distribution 0.06–1.83 nmol/l), with no significant sex difference ($P=0.64$, ANCOVA). Of the other socio-demographic factors, plasma K_1 values were significantly lower in those aged 85 years and over, and in samples taken during autumn (Oct–Dec) and winter (Jan–Mar) (ANCOVA). Consequently, higher proportions from these groups had low plasma K_1 (multiple logistic regression). Significant associations were also noted between plasma K_1 and intake values, and with total vegetable consumption and selected blood analytes. Associations were not altered significantly after further adjustment for energy and protein intakes.

Association* between plasma K_1[†] and:	Men (n 420)		Women (n 395–398)		All (n 815–818)	
	Partial r	P	Partial r	P	Partial r	P
Dietary K_1 intake[†]	0.17	<0.001	0.33	<0.001	0.25	<0.001
Total vegetable consumption[†]	0.10	0.04	0.31	<0.001	0.19	<0.001
Plasma lutein[†]	0.31	<0.001	0.31	<0.001	0.31	<0.001

*Adjusted for plasma TAG levels. [†]Log₁-transformed values.

Significant partial correlations were also noted between plasma K_1 and µ- and -tocopherols in men and women, and with serum ferritin, percentage iron saturation, and 25-hydroxyvitamin D, in women only. The significance of these findings, especially with respect to the iron status indices, is presently unclear. The association between plasma K_1 and lutein is explained by vegetables, especially green leafy vegetables, being the primary source of both micronutrients.

These plasma K_1 results provide a definitive reference range for plasma K_1 in the elderly population and enable a better understanding of its variability according to dietary and socio-demographic factors.

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Finch S, Doyle W, Lowe C, Bates CJ, Prentice A, Smithers G & Clarke PC (1998) National Diet and Nutrition Survey: People aged 65 years and over. Volume 1: Report of the Diet and Nutrition Survey. London: The Stationery Office.
Jie K-SG, Bots ML, Vermeer C, Witteman CM & Grobbee DE (1995) Atherosclerosis **116**, 117–123.
Shearer MJ (1997) Proceedings of the Nutrition Society **56**, 915–937.

Vitamin E intake of national samples of 4-year-old children in 1950 and 1992/93. By K.M. COLLINS[1], C.J. PRYNNE[1], A.A. PAUL[1], N.S. MALIK[1], C.W. THANE[1] and M.E.J. WADSWORTH[2], [1]MRC Human Nutrition Research, Milton Road, Cambridge CB4 1XJ, [2]MRC National Survey of Health and Development, University College and Royal Free Medical School, 1–19 Torrington Place, London WC1E 6BT

Epidemiological studies have suggested that individuals with high dietary intakes of antioxidants, such as vitamin E, have a 20–40% lower risk of coronary heart disease (CHD) (Buring & Hennekens, 1997), suggesting that vitamin E has a protective role. Factors predisposing to the development of adult CHD may start in childhood. To investigate this hypothesis, the vitamin E intake of 4599 children in the MRC National Survey of Health and Development (NSHD), who were aged 4 years in 1950, was examined using one-day food records which have recently been coded (Prynne et al. 1999). In the 1950s, foods were fried in bacon fat or dripping and cooked with margarines made from oils such as coconut and whale. Estimations of the vitamin E content of these foods were calculated using analyses of contemporary margarines (Ward, 1958). Dietary vitamin E intakes are compared below with similar data from the 493 children aged 4 years in the 1992/93 National Diet and Nutrition Survey (Gregory et al. 1995), obtained from the Data Archive.

Food groups	Daily intake of vitamin E (∝-tocopherol equivalents) (mg)			
	1950 NSHD (n 4599)		1992/93 NDNS (n 493)	
	Mean	SD	Mean	SD
Cereals and cereal products	0.68	0.35	0.86[†]	0.57
Milk and milk products	0.32	0.17	0.24[†]	0.43
Eggs and egg dishes	0.23	0.30	0.15[†]	0.28
Fat spreads	0.46	0.28	1.14[†]	1.39
Meat and meat products	0.14	0.14	0.37[†]	0.39
Fish and fish dishes	0.03	0.11	0.14[†]	0.30
Vegetables, potatoes and savoury snacks	0.48	0.52	1.28[†]	0.84
Fruit and nuts	0.09	0.17	0.21[†]	0.26
Sugars, preserves and confectionery	0.00	0.04	0.21[†]	0.26
Beverages and miscellaneous	0.06	0.12	0.17[†]	0.31
Total vitamin E	**2.50**	**0.84**	**4.75[†]**	**2.01**

[†]Significantly different from 1950, $P<0.001$, Mann-Whitney U test.

Overall, the children in 1950 consumed significantly less vitamin E than those in 1992. Th lower intake in 1950 was due to lower provision of vitamin E by most food groups. However, almos 50% of the vitamin E intake from the vegetable group in 1992 was from savoury snacks such as potat crisps which are prepared in vegetable fats which have a high vitamin E content. Consumption o vegetables, potatoes and savoury snacks contributed 27% to the average daily intake of vitamin E i 1992 compared with 19% in 1950 where savoury snacks existed but were not widely consumed. Ther were only two food groups from which the children in 1950 had a significantly higher intake of vitami E, milk/milk products and eggs/egg dishes. At that time, milk was provided free or subsidized t children of nursery/school age and breakfasts often included eggs, whereas in 1992 breakfast cereal were more popular.

The NSHD cohort members are now 53 years of age and their health continues to be monitorec Hence the association of cardiovascular outcomes and risk factors resulting from their childhood die can be investigated as the study progresses.

Buring JE & Hennekens CH (1997) Nutrition Reviews **55**, S53–S57.
Gregory JR, Collins DL, Davies PSW, Hughes JM & Clarke PC (1995) National Diet and Nutrition Survey: Children aged 1.5 to 4.5 years. Volume 1. Report of the Diet and Nutrition Survey. London: HMSO.
Prynne CJ, Paul AA, Price GM, Day KC, Hilder WS & Wadsworth MEJ (1999) Public Health Nutrition **2**, 537–547.
Ward RJ (1958) British Journal of Nutrition **12**, 231–236.

Micronutrient intake of free-living elderly people. By N. SAINI[1], S.M. MAXWELL[2], L.DUGDILL[2] and A.M. MILLER[2], [1]The Hugh Sinclair Unit of Human Nutrition, The University of Reading, Reading RG6 6AP, [2]Liverpool John Moores University; Barkhill Road, Liverpool L17 6BD

Vitamin and mineral status of elderly people can be compromised by reduced food intake, impaired state of repletion of vitamins, the presence of diseases and increased intakes of drugs. Combined supplementation could be the best way of preventing accelerated ageing and of reducing the risk of age-related diseases (Richard & Roussel, 1999). A survey to assess the nutritional status of elderly people was carried out in the four boroughs of Merseyside. Eighty elderly subjects; forty-one women (mean age 74.6 (SD 6.98) years) and thirty-nine men (mean age 73.9 (SD 6.12) years), living freely in the community were selected at random from the Family Practice Register. Three-day diet diaries quantified by a food photographic atlas (Mullan & Luke, 1994) were used to estimate nutrient intake. Information on type and frequency of dietary supplements taken was also recorded. Dietary diaries were analysed using Microdiet (Salford University) and micronutrient intakes were compared to those recommended by The Department of Health (1991).

Micronutrient	Men (n 39)					Women (n 41)				
	Mean (/d)	SD	%below RNI	%below EAR	%below LRNI	Mean (/d)	SD	%below RNI	%below EAR	%below LRNI
Ret Eq† (µg)	567.2	204.15	72	41	15	537.2	194.28	66	24	5
Vitamin D (µg)	2.6	2.20	97	–	–	2.9	2.46	98	–	–
Thiamin (mg)	1.2	0.37	3	0	0	1.2	0.48	0	0	0
Riboflavin (mg)	1.6	0.42	23	5	3	1.5	0.49	12	5	2
Vitamin B_{12} (µg)	3.5	1.80	8	3	0	3.5	2.40	15	12	12
Vitamin B_6 (mg)	9.6*	2.70	61	20	5	7.8*	2.34	44	15	0
Folate (µg)	43.6	16.37	95	–	36	44.8	16.52	83	–	44
Vitamin C (mg)	104.9	38.30	82	–	36	94.8	45.60	88	–	34
Calcium (mg)	1.6	0.48	10	5	3	1.5	0.47	12	2	0
Iron (mg)	199.5	60.90	59	20	5	198.4	61.30	54	29	0
Zinc (mg)	49.8*	32.60	46	28	5	67.9*	44.4	32	15	2
Selenium (µg)	749.6	236.57	46	15	5	687.5	199.50	51	22	7
Iodine (µg)	10.1	2.80	38	10	3	9.0	3.35	58	19	2

†Retinol equivalents.
*Significantly different for men and women $P<0.05$.

Although 33% of the men and 41% of the women took dietary supplements, their contribution towards the micronutrient status of the elderly was disregarded due to irregularity of intake. Men had significantly higher intakes of zinc and lower intakes of vitamin C than women. Compared with dietary reference values, major shortfalls from reference nutrient intake were observed for retinol equivalents, vitamin D, iodine, selenium and folate. Women also had low intakes of calcium.

These findings highlight the possibility that elderly people living in the community and eating self-selected diets may be vulnerable to vitamin and mineral deficiencies. Although non-prescribed dietary supplements may make a sizeable contribution towards the micronutrient intake of these elderly people, irregularity of intake and incomplete food composition data makes it difficult to ascertain their impact on the overall micronutrient status of elderly people.

Department of Health (1991) Report on Health and Social Subjects 41: Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. London: HMSO.
Mullan A & Luke V (1994) Photographic Atlas of Average Portions. Liverpool: Liverpool University Department of Medicine.
Richard MJ & Roussel AM (1999) Proceedings of the Nutrition Society 58, 573–578.

The North/South Ireland Food Consumption Survey 2000: Mineral intakes in 18–64 year old adults. By E.M. HANNON[1], M. KIELY[1], A. FLYNN[1], M.J. GIBNEY[2], K.E. HARRINGTON[2], P.J. ROBSON[3] and J.J. STRAIN[3], [1]University College Cork, [2]Trinity College Dublin, Republic of Ireland, [3]University of Ulster, Northern Ireland

The North/South Ireland Food Consumption Survey 2000 has established a database of habitual food and drink consumption in a representative sample of Irish adults aged 18–64 years. A 7-d estimated food record was used to collect food intake data from 1379 adults who were selected randomly from the electoral register. Analysis of dietary intake data was carried out using Wisp© (Tinuviel Software, Warrington) which contained McCance and Widdowson's The Composition of Foods, 5th Edn (Holland et al. 1995). Mineral intake data (including intake from supplements) from the Republic of Ireland for n 958 subjects are presented here.

	Ca		Mg		P		Fe		Zn		Cu	
	Mean (mg/d)	SD	Mean (mg/d)	SD	Mean (mg/d)	SD	Mean (mg/d)	SD	Mean (mg/d)	SD	Mean (mg/d)	SD
Males												
18–35y	1018	372	364	110	1722	498	15.1	6.4	11.4	4.2	1.6	0.7
36–50y	984	371	370	113	1704	486	15.0	5.6	12.4	4.7	1.6	0.9
51–64y	836	268	361	138	1574	381	14.5	4.9	12.0	4.0	1.6	0.9
All ages	956	354	366	119	1675	468	14.9	5.7	11.9	4.4	1.6	0.9
Females												
18–35y	755	352	253	76	1136	306	13.5	13.8	7.9	3.7	1.2	0.6
36–50y	768	289	270	90	1225	318	14.9	18.5	9.1	6.5	1.3	0.7
51–64y	741	296	261	84	1181	321	11.3	5.0	9.1	4.3	1.2	0.7
All ages	757	313	262	84	1184	317	13.6	14.7	8.7	5.2	1.3	0.7

The percentage of the population with mineral intakes below the Estimated Average Requirement (EAR) is presented for selected minerals as an estimation of the percentage of the population with inadequate intakes (Food and Nutrition Board, 1997).

Mineral	Males EAR*	18–35y	36–50y	51–64y	Females EAR	18–35y	36–50y	51–64y
Calcium	615	14.2	15.7	20.3	615	33.5	30.3	41.7
Iron	7.7	4.7	3.9	4.7	10.8**	52.7	52.2	13.0
Zinc	7.5	12.4	11.2	8.6	5.5	19.2	6.0	11.3
Copper	0.8	7.7	6.7	7.0	0.8	21.6	14.9	27.0

*Food Safety Authority of Ireland, 1999. ** The EAR for females in the 51–64 year old age category is 6.9.

These are preliminary estimates of the occurrence of inadequate mineral intakes. Further analysis will be carried out to evaluate the impact of misreporting on these values.

Food and Nutrition Board, Institute of Medicine (1997) In Dietary Reference Intakes: Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride, pp. 314–374. Washington DC: National Academy Press.
Food Safety Authority of Ireland (1999) Recommended Dietary Allowances for Ireland 1999.
Holland B, Welch AA, Unwin ID, Buss DH, Paul AA & Southgate DAT (1995). McCance & Widdowson's The Composition of Foods, Fifth Edn. The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food.

Individual differences in the P ratio during pregnancy. By S.L. DUGGLEBY and A.A. JACKSON, *Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD*

It has been suggested that the P ratio is an innate characteristic of an individual which identifies their tendency to mobilize or deposit energy as protein (lean tissue) or as fat. This implies that for each individual the P ratio is a constant (Payne & Dugdale, 1977; Henry, 1992; Dulloo, 1997). Fatter people tend to mobilize or deposit less energy as protein than thinner individuals. They have a lower urinary nitrogen loss during starvation as they derive less energy from the oxidation of amino acids. The P ratio has been estimated as fasting urinary nitrogen loss (FUNL), a measure of amino acid oxidation, divided by the resting metabolic rate (Henry, 1992). However, urea production provides a direct measure of amino acid oxidation (Jackson, 1998). This approach was used to determine the P ratio twice during pregnancy and to investigate the intra-individual constancy of the P ratio.

Twenty-five pregnant women took part in studies at 16–18 and 26–28 weeks' gestation. Resting metabolic rate was measured by indirect calorimetry for 30 min after the subjects had fasted overnight, using a ventilated hood system and a gas exchange measurer. Urea production was calculated from the excretion in urine of $[^{15}N^{15}N]$urea over 48 h following a single oral dose of $[^{15}N^{15}N]$urea (Jackson *et al.* 1993). In converting urea production to its energy equivalent, it was assumed that body protein is 16% nitrogen and the metabolizable energy of 1 g protein is 16.7 kJ.

The P ratio was 0.206 (SD 0.044) and 0.189 (SD 0.048) in mid- and late pregnancy respectively. There was almost a three-fold difference in the P ratio between individuals, which ranged from 0.114 to 0.340. Although as a group the change between mid- and late pregnancy was statistically non-significant (mean difference 0.016, *P*=0.10), individuals showed a variety of responses as gestation advanced.

P ratio in mid- and late pregnancy
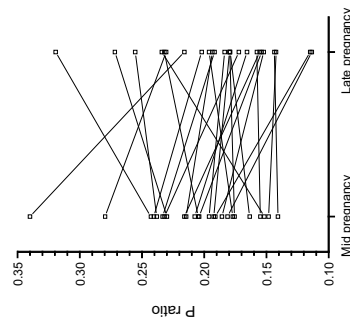
It would be valuable to establish a method of measuring the P ratio in normal people which does not require extended periods of either fasting or consuming a zero-protein diet. The approach used here needs to be validated against other methods to determine the extent to which the P ratio may vary between and within individuals and whether the changes observed in pregnancy are real. It would then be possible to know with confidence whether pregnancy leads to a change in the P ratio in some individuals.

Ethical permission was granted by the local research ethics committee.

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Henry CJK (1992) *Protein-Energy Interactions.* Lausanne: I/DE/C/G.
Jackson AA (1998) *Biochemical Society Transactions* **26**, 231–236.
Jackson AA, Danielsen MS & Boyes S (1993) *Journal of Nutrition* **123**, 2129–2136.
Payne PR & Dugdale AE (1977) *Annals of Human Biology* **4**, 525–535.

Estimation of energy expenditure in adolescents using Caltrac™ and its use to validate reported energy intake as estimated from 3-day food diaries. By C. FROBISHER and S.M. MAXWELL, *Liverpool John Moores University, School of Education, Community and Social Science, IM Marsh Campus, Barkhill Road, Liverpool L17 6BD*

A comparison between energy expenditure (EE) and energy intake (EI) can help validate dietary surveys. EE has been measured in adolescents using doubly-labelled water (Davies *et al.* 1991), but this is an expensive procedure. Caltrac™ is a cheap and simple device which also estimates EE.

Ten adolescents were recruited, but one failed to provide an adequate dietary record. EI was calculated from 3-d food diaries and a food atlas to quantify portion sizes. EE was recorded for the same 3 d using a Caltrac™ worn on the waistband. This gives an estimation of the energy expended over a specific time, based on computed BMR and continuous measurements by an internal accelerometer, which detects the subject's movements.

Sex	Age (years)	Weight (kg)	BMI (kg/m²)	BMR* (kcal/d)	Mean EI (kcal/d)	Mean EE (kcal/d)	FIL EI:BMR	PAL EE:BMR
F	11.10	34.20	15	1150	2300	1539	2.00	1.34
F	11.04	31.60	17	1115	1332	1572	1.19	1.41
M	15.04	41.25	17	1387	2315	1943	1.67	1.40
F	11.10	36.00	18	1174	1410	1768	1.20	1.51
M	13.10	45.90	18	1469	2354	2115	1.60	1.44
F	13.08	52.30	20	1583	2714	2196	1.72	1.39
F	11.10	58.00	22	1469	2737	2422	1.86	1.65
M	15.02	63.90	23	1788	1976	2946	1.11	1.65
M	14.05	84.05	29	2145	1742	2762	0.81	1.29
Mean	12.74	49.69	19.94	1476	2098	2140	1.46	1.45
STD	1.71	16.95	4.46	333.7	517.6	497.49	0.40	0.13

*Calculated using Schofield equations (Department of Health, 1991).

Davies *et al.* (1991) found a mean physical activity level (PAL) value of 1.76 for 9, 12 and 15 year olds, which is higher than the values reported here but their subjects were thought to have moderately to very active lifestyles. Assuming that Caltrac™ gave a valid estimate of EE and the subjects were in energy balance, food intake level (FIL) should be equal to PAL, and in fact FIL values were not significantly different (Wilcoxon) from the PAL values (*P*=0.953, *r* 0.0). Four subjects had a FIL value below their PAL value. It is suggested that Caltrac™ is a simple way of estimating EE, making due allowance for physical activity, and is an improvement on using BMR alone. It could be a useful tool in dietary surveys.

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Inter- and intra-individual variability in the glycaemic response to white bread. By A.M. SMITH[1,2] and M. MOLONEY[2], [1]University of Dublin, Trinity College, Dublin, Ireland, [2]Dublin Institute of Technology, Kevin Street, Dublin 8, Ireland

Carbohydrate foods vary considerably in their effects on hormonal and glucose responses after a meal (Jenkins *et al.* 1988). In an attempt to classify carbohydrate foods in terms of quality (i.e. on their acute glycaemic impact), the concept of the glycaemic index was introduced. The glycaemic index (GI), as defined by the FAO/WHO (1998), is the incremental area under the blood glucose response curve of a 50 g carbohydrate portion of a test food expressed as a percentage of the response to the same amount of carbohydrate from a standard food (typically white bread or glucose) taken by the same subject. However, despite the continuing level of interest in the GI, the degree of inter- and intra-individual variability involved in calculating the eventual GI for a food has remained largely unstudied.

Inter-individual variability (variation between subjects) was investigated in this study in twelve normal subjects on three different days, after the ingestion of 50 g of available carbohydrate from white bread. Intra-individual variation (day-to-day variation within a subject) was evaluated using identical methodology in one of the twelve subjects on ten different days. The subject pool consisted of six males and six females of normal glucose tolerance with a mean age of 23 years and a mean BMI of 24 kg/m² .

	Inter-individual variation CV (%)	Intra-individual variation CV (%)
Fasting blood glucose (FBG)	5.8	5.9
Peak blood glucose (PBG)	8.8	10.4
Peak as a percentage of FBG (PPFBG)	44.4	28.0
Peaking time (PT)	35.7	35.4
Blood glucose at 120 min (BG120)	6.6	5.9
BG120 as a percentage of FBG (BG120PFBG)	74.7	55.0
Area under the curve (AUC)	43.3	22.3
Incremental area under the curve (IAUC)	6.3	2.7

Values for the inter-individual variation were taken as the mean of three trials in twelve subjects (six male, six female).
Values for the intra-individual variation were taken as the mean of three trials in subject 1 (female)

Inter-individual variation was greater for the peak as a percentage of fasting blood glucose (PPFBG), and the blood glucose value at 120 min (BG120PFBG), while the values for the area under the blood glucose response curve (AUC) and the incremental area under the curve (IAUC), for the inter-individual group were almost twice those of subject 1, representing the intra-individual variation. The results of this study thus indicate that inter-individual variability was generally greater than intra-individual variability with respect to the glycaemic response to white bread.

A recent study by Caballero-Plascencia *et al.* (1999) found that the gastric emptying of digestible solids and liquids was significantly slower in women than in men. The effect of gender on the estimation of GI values, however, has not been published to date. Differences between males and females were found in the glycaemic response profiles recorded in this study. For the most part, the males generally had a more elevated response than the females, as measured by the PPFBG (35 *v.* 21%, *P*<0.05), with a shorter time to peak (40 *v.* 65 min, *P*<0.05) and a more rapid and more complete return to baseline, as measured by the BG120PFBG (5 *v.* 12%, *P*>0.05). Furthermore, the males had both a larger AUC (672 *v.* 607 mmol/l/min, *P*<0.01) and IAUC (99 *v.* 68 mmol/l/min, *P*>0.05) than the females in this study. The effect of gender on inter- and intra-individual variability of the glycaemic response to reference foods such as white bread is an area that may merit further exploration to elucidate its relative contribution to the GI.

Caballero-Plascencia AM, Valenzuela-Barranco M, Martin-Ruiz JL, Herrerias-Gutierez JM & Esteban-Carretero JM (1999) *Scandinavian Journal of Gastroenterology* **34**, 772–776.
Food & Agricultural Organisation/World Health Organisation (1998) *FAO Food and Nutrition Paper* **66**, 1–140.
Jenkins DJA, Wolever TMS, Buckley G, Lam KY, Giudici S, Kalmusky J, Jenkins AL, Patten RL, Bird J, Wong GS & Josse RG (1988) *American Journal of Clinical Nutrition* **48**, 248–254.

The effect of aspartame and aspartame constituents on gastric emptying, plasma cholecystokinin and glucagon-like peptide-1 response and self-rated appetite scores. By W.L. HALL[1], A. GIOUVANOUDI[2], P.J. ROGERS[3], N. SPYROU[2] and L.M. MORGAN[1], [1]Centre for Nutrition and Food Safety, University of Surrey, Guildford GU2 7XH, [2]Department of Physics, University of Surrey, Guildford GU2 7XH, [3]Department of Experimental Psychology, University of Bristol, Bristol BS8 1TN

Aspartame has previously been found to increase satiety in human subjects (Rogers *et al.* 1995). It may exert this effect via phenylalanine, a component of aspartame that appears to affect satiety via cholecystokinin-mediated mechanisms (Ballinger & Clark, 1994). The main objective of this study was to determine possible mechanisms, such as delayed gastric emptying or altering plasma hormonal profiles, whereby aspartame could exert its effect on satiety. The amino acid constituents of aspartame, phenylalanine and aspartic acid were also administered in equivalent amounts.

Six lean healthy volunteers (four females and two males; 24–31 years; BMI<25kg/m²) were recruited for a within-subjects, repeated measures experimental design. They were studied on three occasions and instructed to avoid alcohol, caffeine and products containing aspartame or paracetamol for 12 h before each study day. After an overnight fast, subjects were given, in random order, either one gelatine capsule containing 400mg aspartame, 176mg aspartic acid +224mg phenylalanine, or 400 mg cornflour (control treatment) together with 450ml water +1.5g crushed paracetamol. One hour after the treatment, a 1983kJ liquid meal of fixed size containing 22g fat and 67g carbohydrate was consumed by the subjects. Venous blood samples were taken at intervals before and after the capsule for 120min for the measurement of plasma cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) concentrations. Gastric emptying was measured both by the paracetamol absorption test and electric impedance epigastrography (EIE) from 0 to 60min, and by EIE from 60 to 120min (McClelland & Sutton, 1985). Hunger, desire to eat and fullness ratings were assessed with 100mm visual analogue scales before the capsules and at intervals over the following 120min.

	CCK (pmol/l per min)		GLP-1 (pmol/l per min)		T50 (min)	
	Mean	SEM	Mean	SEM	Mean	SEM
Control	132.2	42.4	2096.9	709.7	25.2	2.2
Aspartame	130.1	26.5	536.6	161.9	23.1	1.2
Amino acids	125.4	30.6	861.8	309.3	18.4	1.3

Mean values for CCK and GLP-1 expressed as incremental area under the curve following the liquid test meal (pmol/l by time (60 – 120 min)). Gastric half-emptying times, measured by EIE, are expressed as T50s. Repeated measures ANOVA, significant treatment effect: GLP-1, *P*<0.05, T50s, *P*<0.005.

There were no differences in gastric emptying rates measured by either method following the capsules for the first 60min. However, following the liquid meal, a repeated measures ANOVA revealed that percentage meal retained in the stomach overall (measured by EIE) was significantly less following the aspartame and constituent amino acids (*P*<0.0005). Plasma hormone concentrations were analysed using repeated measures two-way analysis of variance, with treatment and time as within-subjects factors. There was no effect of treatment on plasma CCK concentrations either before or after the liquid test meal. There was a treatment effect on plasma GLP-1 concentrations after the liquid meal (*P*<0.05), with the *post-hoc* Duncan test showing this to be the product of lower GLP-1 concentrations following the aspartame and amino acids at +75 and +90min. There was an effect of treatment on desire to eat following the liquid test meal (*P*<0.05); the *post-hoc* Duncan test revealed this to be due to subjects having significantly less desire to eat after the aspartic acid + phenylalanine treatment between +65 and +120min compared with the control and aspartame treatments. There were no significant differences in hunger or fullness ratings.

The results showed that: (1) aspartame administered in amounts previously shown to increase satiety does not have any significant effect on cholecystokinin concentrations nor gastric emptying; (2) the gastric emptying rate of the liquid meal was faster following the constituent amino acids and aspartame, the faster delivery of nutrients to the intestine may have prompted the reduction in desire to eat; (3) GLP-1 secretion was suppressed following the aspartame and amino acids, which was consistent with the gastric emptying effects; (4) the satiety effect of aspartame could not be reproduced in these circumstances, however, subjective ratings of desire to eat were reduced following the amino acids. In conclusion, the study failed to provide evidence that aspartame increases satiety via CCK-mediated mechanisms, but both the aspartame and constituent amino acids did produce some unanticipated results. This illustrates the complexity of nutrient–gut interactions and the difficulties involved in extricating cause and effect.

Ballinger AB & Clark ML (1994) *Metabolism* **43**, 735–738.
McClelland GR & Sutton JA (1985) *Gut* **26**, 607–614.
Rogers PJ, Burley VJ, Alikhanizadeh LA & Blundell JE (1995) *Physiology and Behavior* **57**, 489–493.

Different postprandial responses of larger and smaller chylomicrons and remnant particles. By K.D.R.R. SILVA[1], A.E. JONES[2], R.D. SMITH[1], C.N.M. KELLY[1], J.A. LOVEGROVE[1], S.A. WOOTTON[2] and C.M. WILLIAMS[1], [1]*Hugh Sinclair Unit of Human Nutrition, Department of Food Science and Technology, University of Reading, PO Box 226, Whiteknights, Reading RG6 6AP*, [2]*Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD*.

Chylomicrons (CM) are synthesized exclusively by the enterocytes to transport dietary triacylglycerols (TG). Elevated postprandial plasma TG is an independent risk factor for cardiovascular disease and the atherogenic potential of CM and their remnants have been identified. Studying the dynamics of the postprandial CM responses may lead to a greater understanding of the cellular mechanisms of heterogeneously-sized CM synthesis and secretion.

The postprandial responses of CM in plasma and lipoprotein fractions (Svedberg flotation rate (Sf >400 (large CM), 60–400 (small CM and large remnants) and Sf 20–60 (small remnants)) to a standard breakfast (45 g fat) given after an overnight fast were studied using apolipoprotein (apo) B48 as a marker of the intestinally-derived lipoproteins (Lovegrove *et al.* 1996) in nine healthy, normolipidaemic male and female subjects (age 21 (SD 2) years, BMI 23.3 (SD 4.0) kg/m²).

a Different from Sf >400 (Estimates; P < 0.05)
b Different from Sf 60–400 (Estimates; P < 0.05)
c Different from baseline (Duncan multiple range test; P ≤ 0.05)
d Different from baseline (Duncan multiple range test; P 0.1)

The concentrations of different particle sizes of CM changed with time ($P=0.08$) and showed significantly different temporal patterns of response ($P=0.001$) (see Figure). The concentration of CM remnant particles (Sf 20–60) significantly ($P=0.05$) decreased compared with baseline values by 120 min, increasing again around 150 min. In contrast, smaller CM and larger remnants (Sf 60–400) showed an early increase followed by a decrease up to 300 min ($P=0.05$) and a second rise up to 480 min. Large CM (Sf >400) were released into the circulation at a slower rate than these smaller particles so that the concentrations were lower than that of smaller CM and large remnants during the early postprandial stage, rising to reach a broad peak between 150 and 360 min.

The plasma concentrations of apo B48 corresponded well to the sum of the apo B48 in three lipoprotein fractions (Sf 20–60, 60–400 and >400) (data not shown). It is suggested that the marked decline in apo B48 in the Sf 20–60 fraction represents a decline in secretion of small remnant-like particles due to diversion of apo B48 into formation of the small (Sf 60–400) and large (Sf >400) CM particles in response to fat ingestion. These observations are in line with the model proposed by Hussain (2000) that small immature particles of CM are secreted during the fasted state and that after feeding, larger CM are produced in a process involving fusion of 'immature' CM with variously sized TG-rich lipid droplets formed during the postprandial state.

Hussain MM (2000) *Atherosclerosis* **148**, 1–15.
Lovegrove JA, Isherwood SG, Jackson KG, Williams CM & Gould BJ (1996) *Biochimica et Biophysica Acta* **1301**, 221–229.

Relationship between measures of adiposity, insulin sensitivity and lipaemia in post-menopausal women. By J.A. LOVEGROVE, J.W. WRIGHT and C.M. WILLIAMS, *Hugh Sinclair Unit of Human Nutrition, University of Reading, Whiteknights, Reading RG6 6AP*

Obesity, and more specifically central fat distribution, is recognised as major preventable risk factor for a range of debilitating diseases such as coronary heart disease (CHD). There are few studies investigating fat distribution in relation to CHD risk factors in the postprandial state in post-menopausal women, a group which have an increased risk of CHD development (van der Schouw *et al.* 1996). The aim of this study was to investigate any associations between total body fat and central fat accumulation with measures of insulin sensitivity and lipaemia in the fasted and postprandial state in post-menopausal women.

Twenty-eight healthy, post-menopausal women were recruited with a mean age 62 (52–76) years, BMI 27.2 (20.5–38.8) kg/m² and waist circumference 86 (64–124) cm. Volunteers were asked to come to the investigation unit after fasting for a 12 h period where they were cannulated and two fasting blood samples were collected. The subjects were given a breakfast (0 h) providing 2469 kJ, 30 g fat, 11 g protein and 76 g carbohydrate, followed by a lunch (4.5 h) providing 3138 kJ, 44 g fat, 11 g protein and 81 g carbohydrate. No other food or drink, except water and sugar-free drinks, was consumed during the study period. Blood samples were taken every 30 min for the first 90 min after a meal, and hourly thereafter for 10 h. Plasma was prepared and stored at –20° for future analysis. Plasma glucose and triacylglycerol (TAG) were measured using an enzymatic method on an automated analyser (Monarch) and plasma insulin (ins) was measured using a specific insulin ELISA (Mohamed-Ali & Yudkin, 1992). After normality testing, bivariate and partial Pearson correlations and, where appropriate, Spearman correlations were calculated. Stepwise multiple regression analysis was performed to establish independent associations between metabolic variables and measures of adiposity.

Variable		Insulin fast	Insulin IAUC	Glucins fast	Gluc:ins AUC	TAG fast	TAG IAUC	TAG:ins fast	TAG:ins AUC
BMI	a	0.600***	0.821***	−0.491**		0.416*	0.203	−0.329	−0.745***
	b	−0.165	0.535**	0.351		0.160	0.127	0.258	−0.447*
	d				−0.797***				
Waist	a	0.740***	0.738***	−0.634***		0.393*	0.163	−0.493**	−0.667**
	c	0.559*	0.238	−0.161		0.062	−0.034	−0.456*	−0.031
	d				−0.730***				

[a]Bivariate Pearson, [b]partial Pearson (controlling for waist), [c]partial Pearson (controlling for BMI), [d]Spearman correlation. AUC = area under time response curve. IAUC = incremental area under time response curve.
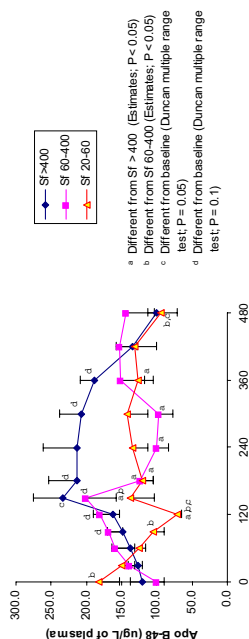*$P<0.05$, **$P<0.01$, ***$P<0.001$.

Measures of adiposity (BMI) and central fat distribution (waist) were found to be significantly associated with measures of insulin, insulin sensitivity in relation to glucose (Gluc:ins fast and AUC) and TAG (TAG:ins fast and AUC). After controlling for confounding factors, waist remained significantly correlated with fasting insulin concentrations and insulin sensitivity in relation to TAG in the fasted state (TAG:ins fast). BMI remained significantly correlated with measures of insulin sensitivity in the postprandial state (TAG:ins AUC). This was supported by multiple regression analysis where waist contributed 53% of the variability in fasting insulin levels and BMI remained an independent predictor for postprandial insulin response (contributing 66% of the variability). These data are consistent with previous studies investigating relationships between body fat and central fat distribution with parameters of insulin resistance in pre-menopausal women and middle-aged men. However they are in contrast with our findings in a group of similarly aged men with the atherogenic lipoprotein phenotype, who showed a paradoxical negative relationship between measures of lipaemia and central adiposity (Minihane *et al.* 2000).

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Mohamed-Ali V & Yudkin JS (1992) *Clinical Science* **82**, Suppl. **27**, 4p.
van der Schouw YT, van der Graaf Y, Steyerberg EW, Eijkemans MJK & Banga JD (1996) *Lancet* **347**, 714–718.

Effects of substituting dietary saturated fatty acids with monounsaturated fatty acids on blood lipids in young healthy volunteers. By R.D. SMITH, C.N.M. KELLY, K.D.R.R. SILVA, M.C. NYDAHL and C.M. WILLIAMS, *Hugh Sinclair Unit of Human Nutrition, University of Reading, Reading RG6 6AP*

The substitution of dietary saturated fatty acids (SFA) with monounsaturated fatty acids (MUFA) has been recognised as a potential strategy for reducing cardiovascular disease (CVD). MUFA-enriched diets have been shown to reduce plasma total and LDL cholesterol in middle-aged men, and young men with a family history of CVD (Williams et al. 1999). However, more information is needed about the longer term effects of such diets in young healthy individuals. The aim of this study was to investigate the effects of substituting dietary SFA with MUFA at two levels of MUFA intake on blood lipids, in students living in a fully catered Hall of Residence.

Fifty-one students (twenty-six males and twenty-five females; age 18–28 years) consumed a reference (SFA) diet (% energy: 15.6 SFA, 12.4 MUFA, 5.9 PUFA) for 8 weeks. They were then randomized to receive either a moderate MUFA (MM) diet (% energy: 13.1 SFA, 15.2 MUFA, 5.9 PUFA) or high MUFA (HM) diet (% energy: 10.5 SFA, 17.8 MUFA, 5.9 PUFA) for a further 16 weeks. The dietary targets were to be achieved by the substitution of cooking fats in the Hall kitchen and the provision of specially manufactured spreads and snack foods to the volunteers (Kelly et al. 2000). Fasting blood samples were collected at the beginning (M_0), 8 weeks (M_8) and 16 weeks (M_{16}) of the MUFA diet period. The mean fasting plasma total, LDL and HDL cholesterol concentrations for each group, and the two groups combined, are shown in the Table.

	MM group (n 25)			HM group (n 26)			All (n 51)		
	M_0	M_8	M_{16}	M_0	M_8	M_{16}	M_0	M_8	M_{16}
TC (mmol/l) Mean	4.59	4.35*	4.13**†	4.60	4.48	4.41	4.59	4.42*	4.28**†
SD	0.90	0.74	0.76	0.80	0.72	0.65	0.84	0.72	0.72
LDL-C (mmol/l) Mean	2.67	2.47*	2.33**	2.50	2.40	2.43	2.58	2.43*	2.38*
SD	0.77	0.66	0.68	0.64	0.64	0.58	0.71	0.64	0.63
HDL-C (mmol/l) Mean	1.49	1.45	1.38*	1.57	1.55	1.45*†	1.53	1.50	1.42**†
SD	0.33	0.32	0.32	0.38	0.33	0.29	0.35	0.33	0.30

Significantly different from M_0, *$P<0.05$, **$P<0.001$; Significantly different from M_8, †$P<0.05$.

There was a significant reduction in both total and LDL cholesterol for the whole group following 16 weeks of MUFA intervention. The mean reduction in LDL-C (0.18 mmol/l) was similar to that predicted (Yu et al. 1995). Surprisingly, the MM group showed a greater LDL-C response than the HM group (P=0.019). Both groups also showed a significant reduction in HDL-C, which was greater than predicted and was more marked during the second half of the MUFA diet period. There was no effect of either diet on fasting TAG concentrations (data not shown).

Substitution of SFA with MUFA, even at a moderate level, had a modest beneficial effect on LDL-C in this young healthy group, although this may be offset by the potentially adverse reduction in HDL-C. Unexpected disparity in the magnitude of response of the two groups may be due in part to genetic differences, or issues relating to dietary compliance, and these are being investigated.

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Yu S, Derr J, Etherton TD & Kris-Etherton PM (1995) *American Journal of Clinical Nutrition* **61**, 1129–1139.

The acute effects of ethanol on postprandial lipid metabolism: a follow-up study. By A.J. HAWDON, N. MOHD ESA, E. MALOUTAS, J.W. WRIGHT, B.J. GOULD and B.A. GRIFFIN, *Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 7XH*

In a previous study by Hawdon et al. (1999), the consumption of ethanol with food was shown to augment postprandial lipaemia, decrease lipoprotein lipase (LPL) activity and elevate markers of intestinally derived lipoproteins (retinyl palmitate and apo B-48) in the late postprandial phase (>6 h). Whilst these effects are consistent with a delayed clearance of chylomicron remnants (CMR), very low density lipoproteins (VLDL) are likely to contribute to this ethanol-induced lipaemia, since ethanol promotes the synthesis of triacylglycerol (TAG) and VLDL in the liver. The present study was designed to confirm these previous findings in a larger cohort of subjects and to establish the relative contributions of CMRs and VLDL to this phenomenon. In a crossover design, eight normal, healthy males aged 22–56 years were given a test meal on two separate occasions with and without ethanol (0.6 g/kg body weight ~5–6 units). The meal contained 80 g of mixed fat, and retinyl palmitate (RP) (170 000 IU). Blood samples were taken over 8 h for the determination of total plasma TAG, RP and apo B-48 by ELISA. Post-heparin (7500 U) LPL (*EC* 3.1.1.34) activity was measured at 8 h by a new fluorometric method (Progen). After the initial separation of CMs, the remaining TAG-rich lipoproteins (TRL) were separated on a self-generating iodixanol gradient and collected into pools representative of CM remnants (P_1 & P_2) and two VLDL pools (P_3 & P_4) as previously described (Hawdon et al. 2000).

All subjects showed an increase in postprandial lipaemia in response to ethanol (incremental area under the TAG curve 295 (SD 90) v. 933 mmol/l (SD 316.24), RP (1047 (SD 73) v. 1553 (SD 1047) mmol/l, TRL (225 (SD 39) v. 295 (SD 157) mmol/l). Ethanol was associated with a decrease in the activity of LPL in seven out of eight subjects and a significant increase in postprandial TAG from 6 h onwards (P<0.05). At 6 h (time at which the control and ethanol postprandial TAG curves diverged), the VLDL pools (P_3 & P_4) made a significant contribution to the increased lipaemia (see Table). The increase in plasma TAG at 6 h was strongly associated with the decrease in LPL activity (r –0.89, P<0.03). As shown in the Table, at 6 h the latter correlated significantly with TRL pools containing CM remnants and larger, less dense TRL (CM, P_1–P_3).

Treatment	CM	P_1	P_2	P_3	P_4
Control	1.04	0.34	0.49	0.57	0.36
Ethanol	1.00	0.28	0.57	0.66	0.63
P value	n.s.	n.s.	n.s.	0.04	0.01
ΔLPL	ΔCM	ΔP_1	ΔP_2	ΔP_3	ΔP_4
r	0.71	0.86	0.74	0.83	0.29
P value	0.05	0.01	0.04	0.01	n.s.

Data show medians (mmol/l plasma) and changes in TAG values for TRL pools (r = Spearman correlation coefficient; n.s. = non-significance P>0.05).

These results indicate that VLDL contributes to ethanol-induced lipaemia in the late postprandial phase. The reciprocal relationship between LPL activity and TRLs may be explained by competition between TRLs for this common lipolytic pathway and LPL showing greater substrate specificity for larger TRLs. The potentially unfavourable effects of ethanol-induced lipaemia on cardiovascular health warrants further study.

Hawdon AJ, Mohd Esa N, Wright JW, Gould BJ & Griffin BA (1999) *Proceedings of the Nutrition Society* **58**, 32A.
Hawdon AJ, Gould BJ & Griffin BA (2000) *Proceedings of the Nutrition Society* **59**, 18A.

Conjugated linoleic acid: the effect of supplementation on plasma lipid metabolism. By E.J. NOONE, A.P. NUGENT, H.M. ROCHE and M.J. GIBNEY, Unit of Nutrition, Department of Clinical Medicine, Trinity Centre for Health Sciences, St. James' Hospital, James' Street, Dublin 8, Ireland

Conjugated linoleic acid (CLA) is the term given to a group of positional and geometric isomers of conjugated dienoic derivatives of linoleic acid. CLA is found in foods such as ruminant meats and dairy products. Recent animal studies have shown that CLA has positive nutritional effects on cardiovascular risk factors. Nicolosi et al. (1997) demonstrated in hamsters that CLA significantly reduced plasma and VLDL TAG and cholesterol concentrations by 26-28%, resulting in a significant reduction of aortic streak formation (26%). The present double-blind placebo-controlled study was the first to investigate the effects of CLA on lipid metabolism in humans.

Fifty-one healthy volunteers (eighteen males, thirty-three females, mean age 31.4 years, mean BMI 23.41 kg/m²) were randomized into three groups receiving 3 g/d of either (1) 80:20 cis-9, trans-10 CLA isomer blend, (2) 50:50 cis-9, trans-10 CLA isomer blend, or (3) a control fatty acid (linoleic acid) for 8 weeks. Fasting blood samples were drawn at weeks 0 and 8 and analysed for total plasma cholesterol, triacylglycerol, glucose, LDL and HDL cholesterol concentrations. Statistical analysis was completed using a two-way ANOVA.

	50:50 cis-9, trans-10 isomer				Control: linoleic acid				80:20 cis-9, trans-10 isomer			
	Week 0		Week 8		Week 0		Week 8		Week 0		Week 8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cholesterol (mmol/l)	4.93	1.36	4.84	0.95	5.25	1.17	5.28	1.02	4.96	0.49	4.92	0.70
Triacylglycerol (mmol/l)	1.20	0.39	0.95*	0.31	1.03	0.43	0.94	0.33	1.08	0.32	1.00	0.40
Glucose (mmol/l)	4.94	0.31	4.85	0.40	5.03	0.37	5.21	0.5	5.01	0.49	4.884	0.91
HDL cholesterol (mmol/l)	1.56	0.57	1.54	0.42	1.31	0.27	1.42	0.36	1.44	0.43	1.46	0.37
LDL cholesterol (mmol/l)	1.65	0.83	1.53	0.64	1.77	1.04	1.71	0.71	1.69	0.70	1.54	0.59

Mean values were significantly different between week 0 and week 8: *$P=0.005$.

Supplementation with the 50:50 cis-9, trans-10 isomer blend significantly ($P=0.005$) decreased plasma triacylglycerol concentrations. There were no significant changes in plasma cholesterol, glucose, HDL or LDL cholesterol concentrations.

This study clearly shows that CLA reduces plasma TAG concentrations, an effect which may be exclusive to the trans-10 isomer. In vitro studies have shown that cis-9 and trans-10CLA are peroxisome proliferator-activated receptor (PPAR) γ ligands. Since PPARγ is the transcription factor for the genes involved in adipose tissue TAG metabolism, this may explain the molecular basis of the hypotriacylglycerolaemic effect of CLA. This finding has important clinical implications because the Physicians' Heart Health Study showed that plasma TAG concentrations were a significant predictor of future myocardial infarction (Stampfer et al. 1996).

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Platelet docosahexaenoic acid but not eicosapentaenoic acid levels following fish oil supplementation are related to changes in low-density lipoprotein cholesterol (LDL-C). By A.M. MINIHANE[1], E.C. LEIGH-FIRBANK[1], S. KHAN[2], M.C. MURPHY[2], B.A. GRIFFIN[2] and C.M. WILLIAMS[1], [1]Hugh Sinclair Unit of Human Nutrition, University of Reading, Reading RG6 6AP, [2]School of Biological Sciences, University of Surrey, Guildford GU2 5XH

The hypercholesterolaemic effects of fish oil supplementation are well documented, with intakes of 1–7 g of the long-chain n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) shown to increase low density lipoprotein cholesterol (LDL-C) (Harris, 1997). Moreover, the greatest increases have been observed in hypertriacylglycerolidaemic individuals. Although fish oil supplementation has been shown to have an impact on the composition of very low-density lipoprotein (VLDL) produced by the liver, with a shift towards smaller VLDL particles, which act as the precursors of LDL, a full understanding of the hypercholesterolaemic effects of fish oils is currently lacking. Raised fasting and postprandial triacylglycerol (TG) levels have been implicated as a major metabolic component of a combined dyslipidaemia referred to as the 'atherogenic lipoprotein phenotype' (ALP). The ALP profile occurs in up to 25% of middle-aged males and is associated with a three-fold increased risk of CHD. Despite the recognised benefits of fish oil intervention with respect to TG metabolism, no study has yet investigated the efficacy of this dietary intervention in counteracting the lipid abnormalities of the ALP. However, concern exists regarding the impact of fish oil supplementation on cholesterol metabolism in this moderately hypertriacylglycerolaemic group.

Fifty ALP males (age 56 years, BMI 28.0 kg/m²) completed a double-blind, placebo-controlled crossover study in which participants consumed either fish oil (6 g of oil providing 3.0 g EPA+DHA per day) or olive oil (6 g per day) for 6 weeks with a 12-week washout in between. A fasted blood sample was taken at 0, 3 and 6 weeks on each arm of the study for the determination of plasma lipids and platelet membrane fatty acid composition in a randomly chosen subgroup (n 21).

	Baseline		% change			Correlation between FA % change & cholesterol % change					
						EPA		DHA		Total n-3	
(nmol/l)	Mean	SE	Mean	SE	P†	r‡	P	r‡	P	r‡	P
TC	6.56	0.12	-1.5	2.1	0.627	-0.25	0.326	0.31	0.170	-0.10	0.666
HDL-C	0.97	0.02	-0.8	3.3	0.446	-0.27	0.310	-0.16	0.494	-0.21	0.382
LDL-C	4.48	0.12	7.1	3.2	0.000	0.27	0.322	0.77	0.000	0.39	0.086

TC = total cholesterol; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; FA = fatty acids; % change = [(Fish oil wk6–Olive oil wk6)/Olive oil wk 6]× 100; †The statistical significance of overall group changes determined using paired t-tests; ‡The statistical significance of the associations were determined using Spearman's rho correlations.

In the group as a whole, fish oil intervention was effective in counteracting the proatherogenic lipid profile of the ALP (Minihane et al. 2000). With respect to circulating cholesterol levels, little changes in TC or HDLC were evident. However, a significant 7% increase in LDLC levels was observed ($P=0.000$). The supplementation regime resulted in a highly significant increase in platelet membrane EPA and DHA concentration from 0.53 to 3.16% ($P=0.000$) and 2.50 to 3.61% ($P=0.000$) respectively (data not shown). No significant association between EPA membrane enrichment and cholesterol responsiveness was observed. However, a highly significant correlation between DHA membrane enrichment and LDL-C changes were seen (r 0.77, $P=0.000$), suggesting that the hypercholesterolaemic effects of fish oil supplementation are attributable to its DHA content.

In conclusion, DHA rather than the EPA appears to be the component of fish oil which is responsible for the hypercholesterolaemic effects reported here and elsewhere. Further work is merited in order to establish the underlying mechanisms involved.

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The effect of maternal iron deficiency on fetal growth in hooded Lister rats. By H.J. McARDLE, S. GAIR, C. ANTIPATIS and L. GAMBLING, Nutrition and Development Division, Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB

During pregnancy as many as 30% of women are diagnosed as suffering from anaemia. A significant proportion of anaemia cases during pregnancy are due to iron (Fe) deficiency. The effect of maternal iron deficiency is serious, with babies being born prematurely and/or smaller, having an altered placental:fetal ratio and an increased risk of cardiovascular disease in adult life (Godfrey et al. 1991; Scholl & Hediger, 1994). This study examines the effect of maternal Fe deficiency in rats on the growth and development of the fetus.

Female hooded Lister rats were fed a control synthetic diet (containing Fe at 50 mg/kg) for 2 weeks after weaning and then diets of varying Fe content (50, 37.5, 12.5 and 7.5 mg/kg) for 4 weeks before mating and throughout pregnancy. Pregnant rats were killed by stunning and cervical dislocation on day 21 of pregnancy. The fetuses were killed by decapitation, counted and weighed. Samples were taken and stored for analysis. All results are presented as means $\pm$ SEM. For clarity, only the results for the 50 mg/kg (control diet) and 7.5 mg/kg (Fe-deficient diet) groups (n 35) are shown. Linear regression analysis was used to determine statistical significance, where significance was assumed at $P \leq 0.05$.

The effect of the diet on the mother was studied by the measurement of indicators of Fe status in maternal blood. There was a significant diet-dependent decrease in packed cell volume ($P<0.0001$), haemoglobin ($P=0.001$) and transferrin saturation ($P<0.0001$) values, whilst total Fe binding capacity remained unaltered.

Maternal Fe-deficient diets had no affect on the number or viability of fetuses. However, maternal Fe deficiency did affect fetal growth, as evidenced by a significant decrease in fetal weight ($P=0.003$), a significant increase in the placental:fetal ratio ($P=0.003$) and a significant decrease in both fetal liver weight ($P=0.01$) and fetal packed cell volume values ($P=0.01$).

There was a significant ($P<0.05$) diet-dependent decrease in the Fe content of all tissues examined (see Table). The maternal liver showed the greatest susceptibility to Fe deficiency, supporting the contention that maternal liver Fe content is the best indicator of Fe status. There was a positive correlation ($P=0.01$) between maternal liver Fe content and fetal weight, indicating that fetal growth is dependent on maternal Fe status (see Figure).

Fe content	Control diet	Fe-deficient diet
(μg/g dry wt.)	(Fe 50 ppm)	(Fe 7.5 ppm)
Maternal Liver	1013 ± 59	310 ± 64
Placenta	1362 ± 64	1097 ± 45
Fetal Liver	1470 ± 79	792 ± 63
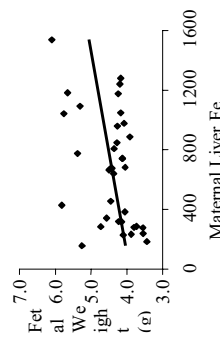
In conclusion, mild maternal anaemia in the rat has a significant effect on the growth and development of the fetus and may lead to problems in neonatal health and well being.

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Scholl TO & Hediger ML (1994) American Journal of Clinical Nutrition **59**, 492S–500S.

Iron deficiency during pregnancy affects maternal and fetal vitamin A metabolism in the rat. By L. GAMBLING, C. ANTIPATIS, C. ANTIPATIS and H.J. McARDLE, Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB

Iron deficiency is one of the most common nutritional problems in pregnant women and is associated with premature birth and low birth weight (Scholl & Reilly, 2000). Fe supplementation is therefore widely used during pregnancy. In pregnant women suffering from Fe-deficient anaemia, supplementation with both Fe and vitamin A has an increased beneficial effect on Fe status compared with Fe supplementation alone (Suharno et al. 1993). This suggests a relationship between vitamin A and Fe metabolism and status. We have shown with the use of rat models that maternal status of both Fe and vitamin A is important for fetal development and neonatal survival (Antipatis et al. 2000; Gambling et al. 2000). The aim of this study was to assess the interactions on maternal and fetal liver Fe and vitamin A metabolism using the rat model of maternal Fe deficiency.

Rowett hooded Lister rats (~100 g) received either a control diet (C; n 8) containing 50 mg/kg Fe or an Fe-deficient (ID; n 7) diet containing 12 mg/kg Fe for 4 weeks prior to mating and during pregnancy. Pregnant rats were killed by stunning and cervical dislocation on day 21 of pregnancy. The fetuses were killed by decapitation, counted and weighed. Maternal and fetal livers were dissected, weighed and stored for further analysis.

Maternal and fetal liver Fe and retinol levels were measured. Although maternal liver weight (C: 13.3 (SE 0.6) v. ID: 13.1 (SE 0.5) g, $P>0.05$) was not affected by dietary Fe deficiency, Fe (C: 289 (SE 5) v. ID: 85 (SE 22) μg/g, $P<0.001$) and retinol (C: 18.7 (SE 1.3) v. ID: 15.2 (SE 0.9) μg/g, $P<0.05$) contents were reduced by 70% and 20% respectively. Consequently, maternal liver Fe stores were positively correlated with liver retinol stores ($y=13.656+0.017*x$, $P<0.05$; Figure 1A).

Under these experimental conditions fetal weight was not significantly affected (C: 4.4 (SE 0.2) v. ID: 4.0 (SE 0.1) g, $P=0.14$) by Fe deficiency. However, relative fetal liver weight (C: 0.073 (SE 0.002) v. ID: 0.065 (SE 0.002), $P<0.05$) was reduced. Dietary maternal Fe deficiency reduced fetal liver Fe levels (C: 6611 (SE 122) v. ID: 866 (SE 84) μg/g, $P<0.001$) but fetal liver retinol (C: 4.8 (SE 0.2) v. ID: 5.6 (SE 0.4) μg/g, $P=0.06$) levels were not significantly affected. Consequently, there was an inverse relationship between fetal liver Fe and retinol levels ($y=6.739-0.001*x$, $P<0.05$; Figure 1B).

Figure 1. Relationship between liver iron and retinol levels, (A) maternal and (B) fetal liver.
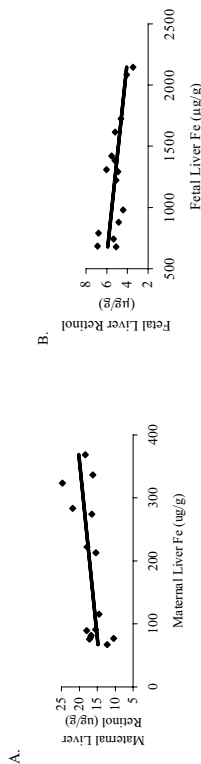
These data suggest that in pregnant rats subjected to dietary Fe deficiency maternal liver vitamin A metabolism is affected, resulting in mobilization of retinol stores, which is then transported to the fetus. It remains to be established whether this is specific to the fetus or if it also results in increased retinol levels in the peripheral maternal organs. It is possible that changes in the expression of vitamin A transport proteins may mediate the effects of Fe deficiency on maternal hepatic retinol metabolism.

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Comparison of faecal short-chain fatty acids in breast-fed, formula-fed and mixed-fed neonates. By A.M. PARRETT, K. FARLEY, A. FLETCHER and C.A. EDWARDS, *Department of Human Nutrition, Glasgow University, Yorkhill Hospitals, Glasgow G3 8SJ*

Previous work has shown marked differences between the faecal flora of breast-fed and formula-fed infants (Balmer & Wharton, 1989). Breast-fed infants have predominantly bifidobacteria and lactobacilli whereas formula-fed infants have more *enterobacteriaceae* and anaerobic bacteria. This difference is reflected in the profile of faecal short-chain fatty acids (SCFA; Edwards *et al.* 1994). Whilst the differences between these two feeding groups are well documented, very little is known about infants who are fed a mixture of breast milk and formula milk. Fresh faeces were obtained from ten exclusively breast-fed (BF) infants, nine exclusively formula-fed (FF) infants and thirteen mixed-fed (MF) infants (aged 1–2 months) and from similar infants during early weaning (4 weeks after receiving solid food; BF 13, FF 8, MF 11 infants at 4–6 months of age). The MF infants were receiving a range in quantity of formula milk from one bottle in combination with breast milk to complete transfer to formula after a period of breast-feeding. Samples were processed within 1 h of passage, made up to >pH 9 with NaOH and freeze-dried before analysis. SCFA were measured in acidified ether extracts of the sample by gas liquid chromatography (Spiller *et al.* 1980) and lactic acid after methylation (Holdeman & Moore, 1973). Results were compared by Kruskal-Wallis and Mann-Whitney U tests.

	SCFA (μmol/ml)					
	Breast-fed (n 10)		Formula-fed (n 9)		Mixed-fed (n 13)	
	Median	Range	Median	Range	Median	Range
Pre-weaning						
Total	342.2	174.8–592.6	489.8	264.9–608.5	249.5	132.9–626.3
Acetate	265.0	76.0–363.9	390.6†	156.2–497.1	168.9	82.7–451.3
Propionate	9.2	0.0–97.9	56.6**	24.6–129.9	56.8‡‡	16.9–180.5
n-Butyrate	1.1	0.0–58.7	8.9	0.0–23.4	2.7	0.0–17.9
Lactate	21.5	3.4–331.5	4.8†	0.0–71.1	0.0‡	0.0–66.8
	Breast-fed (n 13)		Formula-fed (n 8)		Mixed-fed (n 11)	
Early weaning						
Total	436.0	124.9–678.1	476.6	276.8–644.8	441.4	298.0–962.3
Acetate	352.4	83.2–589.2	388.3	208.9–534.3	282.3	193.7–487.8
Propionate	34.5	7.8–67.5	77.9***	41.1–117.0	77.0†‡	22.6–411.8
n-Butyrate	1.0	0.0–57.7	14.7	4.7–39.7	15.0	0.0–34.7
Lactate	4.1	0.0–221.8	0.0†	0.0–0.0	1.8	0.0–87.2

*$P<0.05$, **$P<0.01$, ***$P<0.001$ breast-fed compared with formula-fed; †$P<0.05$ formula-fed compared with mixed-fed; ‡‡$P<0.01$ breast-fed compared with mixed-fed;

Mixed-fed infants had significantly more propionate and less lactate at pre-weaning than BF infants, although by early weaning differences in faecal lactate had disappeared. In contrast, MF infants had significantly less faecal acetate and lactate than FF infants. By early weaning these differences had disappeared but MF had significantly more faecal lactate. This indicated that the mixed fed infants are a distinct group and not the same as exclusively FF infants. As many infants are mixed fed rather than exclusively breast-fed until weaning, it is important to understand the activity of their colonic microflora. Larger studies with more characterized feeding practices are needed.

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New continuous culture model of the colon of the breast-fed infant. By A.M. PARRETT[1], C.A. EDWARDS[1], S.A.S SAVAGE[1], A. FLETCHER[1], F MARTIN[2], C.A. GRAMMET[2] and J. DORE[2], and colleagues in project FAIR CT97 3181, [1]*Department of Human Nutrition, Glasgow University, Yorkhill Hospitals, Glasgow G3 8SJ*, [2]*INRA 78350, Jouy en Josas, France*

The colon of the newborn infant is rapidly colonized by a simple microflora that is dependent on feeding practice. Breast-fed infants have a flora dominated by lactic acid bacteria which produce mainly acetic and lactic acid from carbohydrate fermentation. Modern formula-fed infants have more bacteroides and enterobacteria in their flora, which produce an acetic/propionic acid-dominated short-chain fatty acid (SCFA) profile (Edwards *et al.* 1994). There are many ethical and practical problems when studying factors that affect the colonization of the infant gut, so a continuous culture *in vitro* model has been developed. This model should allow well controlled and detailed study of the dietary factors affecting bacterial growth and metabolism.

A single 1-litre culture vessel was used for the model with a working volume of 300 ml (Bioflo 1000 New Brunswick Scientific, Hatfield; Biolab CP Braun Biotech UK, Reading). The medium was based on all available data of the digestive capability of the newborn infant and in general assumed a 10% malabsorption of nutrients. Medium was pumped at a continuous rate of 1 volume/d. Cultures were inoculated with faeces from 1–2 breast infants. Faeces were used within 1 h of passage. The inoculum ranged from 1.4–4 g. pH was maintained at 5.5–5.7 and anaerobic conditions were achieved by continuous gassing with 95% N_2/5% CO_2. The results of five cultures were validated against the measured characteristics of faeces from human infants. SCFA concentration of the culture supernatant fraction was measured each day by capillary GLC of ether extracts (Spiller *et al.* 1980; Trace 2000, Thermoquest, Italy). Bacterial populations were assessed by semi-quantitative plating on selective media and by ribosomal RNA analysis using six probes for *Bacteroides, Lactobacilli*, Enterics, *C. leptum* and *C. coccoides* (Martin *et al.* 2000).

Steady state was reached by day 5 in each culture as determined by short-chain fatty acid production. The culture flora was dominated by lactic acid bacteria, lactobacilli and bifidobacteria. The SCFA profile was characteristic of the faeces of a breast-fed infant with a predominance of acetic acid and lactic acid. The variability also reflected that of the faecal SCFA in breast-fed infants.

	Short-chain fatty acids				Total SCFA
	Acetic acid	Propionic acid	n-Butyric acid	Lactic acid	μmol/ml
	Molar proportions				
Culture fluid (days 5–10) (n 4)					
Median	259	32	3	575	91.3
Range	125–580	7–248	0–16	408–841	10.4–342.8
Faeces of breast-fed babies 1–2 months (n 28)					
Median	766	26	0	129	75.8
Range	159–1000	0–199	0–51	0–830	24.7–257.9

This validated model of the colon of the human breast-fed infant should prove useful in the study of dietary components in infant foods.

Edwards CA, Parrett AM, Balmer SE & Wharton BA (1994) *Acta Paediatrica* **83**, 459–462.
Martin F, Savage SAS, Parrett AM, Gramet G, Dore J & Edwards CA (2000) *Proceedings of the Nutrition Society* **59**, 64A
Spiller GA, Chernoff MC, Hill RA, Gates JE, Nasser JJ & Shipley EA (1980) *American Journal of Clinical Nutrition* **33**, 754–759.

Caecal short-chain fatty acids and colonic microflora in a human flora-associated rat model of the breast-fed infant. By C.A. EDWARDS[1], C.J. RUMNEY[2], F. MARTIN[3], S.A.H. SAVAGE[1], C.A. GRAMMET[3], J. DORE[3] A. FLETCHER[3], D. SMITH[2], I.R. ROWLAND[4], I.R. ROWLAND[4], J. SCHMITT[5] and colleagues in project FAIR CT97 3181, [1]*Department of Human Nutrition, Glasgow University Yorkhill Hospitals, Glasgow G3 8SJ*, [2]*Department of Microbiology and Nutrition, TNO BIBRA International, Woodmansterne Road, Carshalton, Surrey SM5 4DS*, [3]*INRA 78350, Jouy en Josas, France*, [4]*Northern Ireland Centre for Diet and Health, Biomedical Sciences, University of Ulster, Coleraine BT52 1SA*, [5]*Milupa Research, Bahnstrasse 14–30, D-61381 Friedrichdorf, Germany*

The first months of life may be critical for the development of the colonic microflora. It is very difficult to study the effects of diet in human infants. A human flora-associated (HFA) rat model has been developed which successfully mimics the flora of the human infant.

Twelve germ-free Fischer F344 rats (3–3.5 weeks) were inoculated with pooled fresh infant faeces from up to three breast-fed infants. The rats were maintained in isolators on an *ad libitum* diet prepared from Aptamil formula milk with protein, vitamin and mineral supplements. At 13 weeks the rats were killed and caecal samples collected. Samples from infants and rats were collected and processed within 1 h of passage, frozen and subsequently analysed. SCFA concentration in faeces was measured by capillary GLC of ether extracts (Spiller *et al.* 1980; Trace 2000, Thermoquest, Italy). Bacterial populations were assessed by semi-quantitative plating on selective media and by ribosomal RNA analysis using six probes for *Bacteroides, Lactobacilli, Enterics, C. leptum* and *C. coccoides* (Martin *et al.* 2000).

	Short-chain fatty acids (µmol/ml)				
	Acetic acid	Propionic acid	*n*-Butyric acid	Lactic acid	Total SCFA
Rat caecum (*n* 12)					
Median	176.1	4.6	1.9	386.1	645.9
Range	60–236	0–37	0–15.7	0–1071	138–1274
Faeces of breast-fed babies 1–2 months (*n* 28)					
Median	197	8.0	0	30.4	284
Range	46–485	0–96	0–10	0–462	46–955

The caecal flora of the HFA rats was dominated by lactic acid bacteria in a similar manner to the faeces of the breast-fed infant. The SCFA also reflected those of breast-fed infants rather than that of formula-fed infants (Edwards *et al.* 1994). The differences may be due to the different site of sampling. This validated model of the colon of the human breast-fed infant should prove useful in the study of dietary components in infant foods.

Edwards CA, Parrett AM, Balmer SE & Wharton BA (1994) *Acta Paediatrica* **83**, 459–462.
Martin F, Savage SAS, Parrett AM, Gramet G, Dore J & Edwards CA (2000) *Proceedings of the Nutrition Society* **59**, 64A.
Spiller GA, Chernoff MC, Hill RA, Gates JE, Nasser JJ & Shipley EA (1980) *American Journal of Clinical Nutrition* **33**, 754–759.

The effect of breast milk, infant formula and soya-milk formula on faecal ammonia concentration and bacterial enzyme activity in infants. By L. HOEY[1], I.R. ROWLAND[1], P.M. HEAVEY[1] and I.S. BROWN[2], [1]*Northern Ireland Centre for Diet and Health (NICHE), University of Ulster at Coleraine, Cromore Road, Coleraine BT52 1SA*, [2]*Mountsandel Surgery, Mountsandel Road, Coleraine BT52 1JB*

Marked changes in gut microflora composition and gut bacterial metabolism occur during early stages of infant development. Studies have shown differences between breast-fed and formula-fed infants. To date there are no data on the influence of soya-milk formula feeding on infant gut microflora. This study compares the concentration of faecal ammonia (product of bacterial breakdown of proteins) and bacterial enzyme activity in infants fed infant formula, soya-milk formula and breast milk during the first year of life.

Subjects were ten healthy infants (six males, four females) aged 5–13 months. All infants were weaned. In addition to solid foods, the infants consumed a soya-milk formula (six received SMA Wysoy, three consumed C&G Infasoy and one consumed Farley's soya-milk). Two of the infants also consumed infant formula (C&G Step-up) in conjunction with Infasoy. Faecal samples were collected and transported to the laboratory within 1 h of being passed. The samples were homogenized in 0.9% saline to form a 20% suspension and pH was determined. Ammonia concentration and enzyme activity were measured using a spectrophotometer. All measurements were made in duplicate. Results were compared with data from ten infants fed on breast milk and ten infants fed on infant formula (Aptamil, Milupa). The infants were matched for age and sex. The table shows the mean and the standard deviation for pH, ammonia concentration, β-glucuronidase and β-glycosidase in faecal samples collected from infants aged 5–13 months, who were fed on the three types of infant milks.

	Study Group		
	Breast-milk (*n* 10)	Infant formula (*n* 10)	Soya-milk formula (*n* 10)
Ammonia Concentration (µmol/g)			
Mean	7.17	11.78	18.99**†
SD	3.04	5.51	9.77
β-glucuronidase activity (µmol/h/g)			
Mean	8.18	18.68	21.24*
SD	5.58	9.88	17.46
β-glycosidase activity (µmol/h/g)			
Mean	17.50	19.82	27.94
SD	10.31	14.76	17.95
pH			
Mean	6.48	7.02	7.09*
SD	0.59	0.61	0.47

Significantly different from breast-fed infants *$P<0.05$, **$p<0.001$ (one-way ANOVA).
Significantly different from formula-fed infants †$P<0.05$ (one-way ANOVA).

Mean values for all faecal measurements were higher in infants fed on formula milks compared with breast-milk, with soya-fed infants having approximately twice the mean ammonia concentration of the other two groups. Values in all groups were lower than those reported for adults. The difference between the means for ammonia for soya-fed infants compared with both infant formula and breast-fed infants was highly significant ($P<0.05$, $P<0.001$ respectively). The means for β-glucuronidase activity and pH for soya-fed infants were significantly different from breast-fed infants ($P<0.05$) but not formula-fed infants. No significant differences in β-glycosidase activity were noted. These results suggest that the type of infant feeding does influence ammonia concentration and some enzyme activities. In our current studies we are attempting to relate the metabolic changes to diet-induced alterations in gut microflora composition.

Childhood microalbuminuria may be a marker of reno-vascular programming. By M.C. MARCHAND[1], S.C. LANGLEY-EVANS[1], A. AIHIE SAYER[2,4], R.C. SHERMAN[2], M.O. NWAGWU[2] and T. WHEELER[3], [1]Centre for Healthcare Education, University College Northampton, Broughton Green Road, Northampton NN2 7AL, [2]Institute of Human Nutrition, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, [3]Department of Gynaecology and Obstetrics, Princess Anne Hospital, Coxford Road, Southampton SO16 6YD

Epidemiological evidence suggests that babies who exhibit characteristics of growth retardation at full term have an increased risk of developing hypertension and coronary heart disease in adult life. Maternal nutrition is considered to be an important determinant of fetal growth and development. These findings have been supported by observations in a variety of animal models (Langley-Evans, 1999). Studies on offspring of rats fed a low protein diet have demonstrated that the kidney, an important regulator of blood pressure, is particularly sensitive to the effects of fetal undernutrition during nephrogenesis, with both morphology and function compromised (Langley-Evans, 1999; Nwagwu et al. 2000). The number of nephrons in the kidney of the growth-retarded human are also reduced (Konje et al. 1996). In order to maintain renal function in these kidneys, it has been proposed that glomerular vascular perfusion is increased by adaptive responses that raise systemic blood pressure, promoting an accelerated progression toward glomerulosclerosis and subsequent renal failure in a self-perpetuating cycle (MacKenzie & Brenner, 1995). Furthermore, offspring of rats exposed to a maternal low-protein diet become hypertensive and develop increasing albuminuria (Nwagwu et al. 2000). The aim of the present study was to examine parallels between rats subjected to undernutrition in utero and human subjects. The study was conducted on a selected group of 64 children whose weight and ponderal index at birth encompass that of the general population. The children provided samples of urine at ages 10 and 12 years, which were frozen at −20° prior to biochemical analysis. Albumin was assayed using the bromocresol green method corrected for creatinine concentration using the alkaline picrate assay.

Ponderal index (kg/m³)	n	Albumin:creatinine ratio (mg/mg)			
		Age 10 years		Age 12 years	
		Mean	SE	Mean	SE
<25.12	12	0.40	0.26	0.53	0.31
−26.56	12	0.35	0.35	0.46	0.16
−28.37	13	0.24	0.15	0.44	0.11
−29.97	14	0.27	0.22	0.45	0.12
>29.97	13	0.17	0.10	0.46	0.17

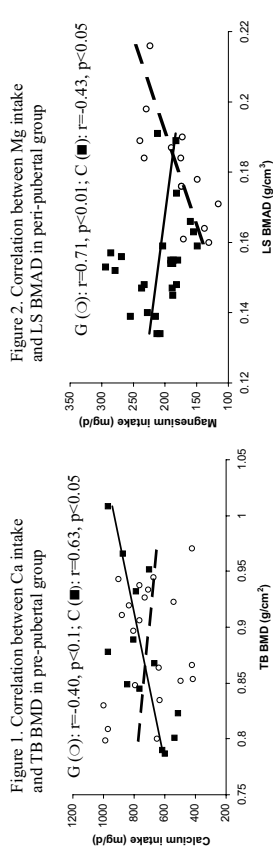Data grouped by quintiles of ponderal index. P for trend = 0.023 at age 10 years.

At age 10, significant relationships were found between the albumin:creatinine ratio and ponderal index at birth (see Table) and birth weight (r −0.325, P=0.017). The data are similar to observations made in the rat (Nwagwu et al. 2000), and are consistent with the hypothesis that intrauterine programming of renal function may provide a link between fetal undernutrition and later hypertension. No associations were found with albumin excretion at age 12. It is possible that these were masked by the additional effects of puberty.

Konje JC, Bell SC, Morton JJ, DeChazel R & Taylor DJ (1996) Clinical Science **91**, 169–175.
Langley-Evans SC (1999) In Development of the Hypertensive Phenotype: Basic and Clinical Studies. Handbook of Hypertension 19, pp. 539–574 [R McCarty, DA Blizard and RA Chevalier, editors]. Amsterdam: Elsevier.
MacKenzie HS & Brenner BM (1995) American Journal of Kidney Disease **26**(1), 91–98.
Nwagwu MO, Cook A & Langley-Evans SC (2000) British Journal of Nutrition **83**, 79–85.

Intrauterine exposure to a maternal low-protein diet shortens lifespan in rats. By S.C. LANGLEY-EVANS[1], A. AIHIE SAYER[2,4], R. DUNN[3] and C. COOPER[4] [1]Division of Health and Life Sciences, University College Northampton, Broughton Green Road, Northampton NN2 7AL, Departments of [2]Geriatric Medicine, [3]Human Nutrition, MRC Environmental Epidemiology Unit, University of Southampton, Southampton General Hospital, Tremona Road, Southampton SO16 6YD

Restricting the diets of rodents in the postweaning period is associated with prolongation of lifespan, reduction of disease risk and attenuation of age-related changes in physiological function (Merry, 1991). Nutritional manipulations in fetal life are known to permanently alter organ structures, activities of hormone axes and promote chronic conditions including hypertension and diabetes (Langley-Evans, 1999). The effects of fetal undernutrition on lifespan have, however, not been extensively studied.

Ten virgin female Wistar rats (200–225 g) were mated and on the day of conception were allocated to either a 180 g casein/kg (control) or a 90 g casein/kg (low-protein) diet. Feeding of these diets was maintained throughout pregnancy (five rats per group). At littering all the rat dams were transferred to a non-purified laboratory rat diet (189 g protein/kg) and this same diet was used to wean the offspring generated, at 4 weeks of age. At the time of weaning the offspring had their blood pressures determined using a tail-cuff method. The rats were weighed at birth and at monthly intervals until 68 weeks of age and other than this they received minimal handling. To determine the effects of prenatal undernutrition on lifespan, the rats were allowed to die without intervention unless they exhibited evidence of pain, distress or discomfort. To prevent unnecessary suffering, animals rapidly losing weight or otherwise exhibiting distress were culled. The age (weeks from birth) of each animal was noted at death and Kaplan-Meier analysis was performed on the survival curves all male and female rats from each maternal dietary group.

At birth the offspring of the rats fed the low-protein diet were of similar weight to the pups in the control group. Rats exposed to the low-protein diet in utero exhibited altered patterns of postnatal growth and by maturity both male and female animals weighed less than control rats. At 4 weeks of age, the low-protein exposed group had significantly elevated blood pressures.

Maternal diet (g casein/kg)	Sex	Systolic blood pressure (mm Hg)			Age at death (weeks)		
		Mean	n	SE	Mean	n	SE
180	Male	105	15	3	76	15	3
90	Male	127*	16	4	73	16	3
180	Female	105	11	2	97	15	5
90	Female	113*	10	5	86*	14	4

Mean values were significantly different from the 180 g casein/kg control group. *P<0.05.

Within this colony of rats the average lifespan of male rats was 74 weeks (SE 2) and for females the mean age at death was 92 weeks (SE 4). Lifespan was significantly reduced in the female rats exposed to a low-protein diet in utero (Kaplan-Meier test, P=0.044). Although the mean age at death among male rats tended to be lower in the low-protein exposed than in the control group, there was no statistically significant effect of prenatal dietary experience on lifespan in males (Kaplan-Meier test, P=0.481).

The findings of this study support for the concept that undernutrition in the fetal period is associated with reduced lifespan. This is in contrast to the extension of longevity noted following postweaning diet restriction.

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Merry BJ (1991) Reviews in Clinical Gerontology **1**, 203–213.

Associations between dietary factors and bone health indices in pre- and peri-pubertal elite female gymnasts and sedentary controls: baseline findings from a longitudinal study. By J.A. NURMI[1], J.A. BISHOP[2], P. TAYLOR[3], C. COOPER[3] and S.A. NEW[1], [1]Department of Clinical Nutrition, University of Kuopio, Finland, [2]Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH, [3]Osteoporosis Centre, University of Southampton SO16 6YD

Peak bone mass (PBM) is predominantly achieved during longitudinal growth. Although PBM is dependent on genetic factors, there is still sufficient scope for exogenous factors such as nutrition, physical activity and hormones to play an important role. The influence of diet on PBM development, especially in physically active children, is still unclear due to the focus of research studies on Ca nutrition only.

We are currently investigating the relationship between nutrients that have plausible biological mechanisms associated with bone health (such as protein, Ca, Mg, P, K, Zn, vitamin C, fibre) and bone health indices (total body bone mineral density (TB BMD), lumbar spine bone mineral apparent density (LS BMAD), broadband ultrasound attenuation (BUA), velocity of sound (VOS)) in physically active and non-active adolescents, and we report here the baseline analysis. Elite British female gymnasts (G n 21, C n 13) and peri-pubertal (G n 14, C n 26) groups have been investigated. Dietary intakes were assessed by 7 d estimated food diaries, and bone density by dual x-ray absorptiometry (DXA) and a contact ultrasound bone analyser (CUBA). Preliminary dietary and bone growth indices have been reported previously (New et al. 2000; Nurmi et al. 2000).

Figure 1. Correlation between Ca intake and TB BMD in pre-pubertal group

G (O): r=-0.40, p<0.1; C (■): r=0.63, p<0.05

Figure 2. Correlation between Mg intake and LS BMAD in peri-pubertal group

G (O): r=0.71, p<0.01; C (■): r=-0.43, p<0.05

The associations between nutrients (e.g. Ca, Mg and K) and bone mass showed a trend, where correlations (adjusted for weight and height) were negative in pre-pubertal gymnasts but positive in peri-pubertal gymnasts. In the control group, these findings were reversed (see Figures). The baseline analyses may suggest that high intensity exercise in childhood suppresses the influence of nutrition on bone health before puberty, but becomes detectable at a later stage. There is some evidence in the literature to support this: Johnston et al. (1992) reported a greater effect of Ca supplementation on bone mass in pre-pubertal than in peri/post-pubertal girls. Further analysis of the longitudinal bone growth data is, however, required.

Johnston CC, Miller JZ, Slemenda CW, Reister TK, Hui S, Christian JC & Peacock M (1992) New England Journal of Medicine 327, 82–87.
New SA, Nurmi JA, Bishop JA, Carr-Bains S, Taylor P & Cooper C (2000) Proceedings of the Nutrition Society 59, 28A.
Nurmi JA, Bishop JA, Carr-Bains S, Gardner J, Taylor FS, New SA (2000) Proceedings of the Nutrition Society 59, 31A.

Hypertension in the mouse following intrauterine exposure to a maternal low-protein diet. By R.L. DUNN[1], S.C. LANGLEY-EVANS[2], A.A. JACKSON[1] and C.B. WHORWOOD[3], [1]Institute of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO16 7PX, [2]Centre for Healthcare Education, University College Northampton, Boughton Green Road, Northampton NN2 7AL, [3]Endocrinology and Metabolism Unit, School of Medicine, Southampton General Hospital, Southampton SO16 6YD

Studies of the pregnant rat have demonstrated that intrauterine exposure to a maternal low-protein diet results in low body weight at birth and programmes hypertension in the resulting offspring (Langley-Evans, 1999). Mild protein restriction in this species thus provides a useful experimental system to study the fetal origins of adult disease hypothesis (Barker, 1998). Work using the rat model of maternal protein restriction has resulted in the elucidation of several possible glucocorticoid-dependent mechanisms of programming, which include the modification of renin–angiotensin system activity (Langley-Evans, 1999, Trowern et al. 2000).

Whilst the rat model has allowed considerable progress to be made in studying the molecular basis of fetal programming, there are major limitations to future activities. Through the wealth of knowledge concerning the genetic make-up of the mouse it is possible to use transgenic mouse systems to investigate the molecular basis of disease. A murine model of intrauterine programming effects would allow investigations of the interaction between genotype and diet. The aim of this study was thus to determine whether the programming effects of a low-protein diet in pregnancy, observed in the rat, could be also generated in a mouse model.

Thirty virgin female MF1 mice (6 weeks of age) were randomly allocated to receive either a control diet (180 g casein/kg) or a low-protein diet (90 g casein/kg). Conception was determined by the presence of a vaginal plug and the mice were fed the diet from the day of conception throughout gestation (19 days). All litters were weighed within 12 h of delivery and the mother put onto a standard laboratory chow. The litters were weaned at between 3 and 4 weeks and fed standard laboratory chow. Systolic blood pressures were determined in a random selection of pups at 8 weeks of age using a tail-cuff method. The results for the two groups were compared using Student's t-test.

Maternal diet g casein/kg diet	Birth weight (g)						Systolic blood pressure at 8w (mmHg)					
	Males			Females			Males			Females		
	Mean	n	SE	Mean	n	SE	Mean	n	SE	Mean	n	SE
180	1.71	64	0.03	1.64	71	0.03	109.7	16	2.7	113.0	16	3.7
90	1.55*	30	0.04	1.44*	38	0.04	123.4*	18	1.7	124.5*	15	5.1

*Denotes significantly different from 180 g casein/kg group (P<0.001).

In preliminary studies it became clear that great care was needed in handling the animals and in measuring blood pressure if reproducible results were to be obtained. Male and female pups of mothers fed a low-protein diet during pregnancy had significantly lower birth weights than the controls (P<0.001). There were no significant differences observed between the size of the litters. Systolic blood pressures at 8 weeks of age were significantly higher than those of control animals (P<0.001). These results show that in the mouse, as in the rat, it is possible to induce high blood pressure in the offspring of mothers consuming a diet low in protein during pregnancy. This model will be useful in future work exploring the interactions between genotype and diet in the development of the cardiovascular system.

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Langley-Evans SC (1999) In Handbook of Hypertension 19, 539–574.
Trowern AR, Sherman RC, Bertram C, Dunn R, Gardner DG, Langley-Evans SC & Whorwood CB (2000) In Proceedings of the 3rd International Symposium on Angiotensin II Antagonism, London, Abstract 13.3.

Attitudes to food and handling of nutrition concepts among school-aged children in Guildford. By K.H. HART[1], J.A. BISHOP[1], E. BuCHANAN-BARROW[2] and H. ANTHONY[1], [1]School of Biological Sciences, [2]School of Human Sciences, University of Surrey, Guildford GU2 7XH

There is evidence that behaviours in childhood continue throughout life (Kelder et al. 1994) and the reported link between childhood diet and adult coronary heart disease risk (Willett, 1990) illustrates a need to maximize the quality of nutritional intake in this population. The disparity between nutritional knowledge and behaviour change is likely to be pronounced in this group due to the limitations of cognitive development (Lytle et al. 1997) and in this heterogeneous population effective education must be appropriate in context and content. The aim of this study was to determine the attitudes and approaches used by children of different ages, gender and socio-economic status (SES) when dealing with nutrition issues, using qualitative methodology.

114 children from four local schools participated in focus groups investigating parental food rules, and knowledge of diet–disease links and grouping of foods. Two schools were classified as lower and two as higher SES on the basis of their free school meal ratio. Groups were separated by age (7 and 11 years) and gender and systematically sampled from alphabetical class lists. Transcripts from twenty-three focus groups were coded and qualitatively analysed to establish areas of between-group consensus and variance.

An effect of gender was apparent. As well as being more likely to report food restriction by parents, girls in both age groups were more likely than boys to correctly identify a diet–disease link, particularly in relation to fattening foods, and were less likely to use taste or preference as an indicator of a food's health value. Differences were also noted between the low and high SES schools, particularly in relation to rules, with those attending a high SES school more likely to be prescribed or restricted foods and less likely to be given free choice than those attending a low SES school. Overall nutritional knowledge and handling of the nutritional concepts were good, although recognition of more advanced ideas such as "moderation" and "variety" was restricted to a small sub-section of the group, primarily older children and those attending a high SES school. However, the older children did not show a consistently better grasp of the nutritional concepts, with 11-year-olds more likely to mention single foods when asked about food groupings than 7-year-olds. Overall, foods were most frequently grouped by nutrients or Balance of Good Health categories (Health Education Authority, 1994), although knowledge in this area appeared to vary greatly between individuals, independent of age or SES. Direct questions provoked greater accuracy than asking children to develop their own food groups and deviations from standard categories were logical and based on biological food sources, meal combinations or primary ingredients.

This investigation demonstrated that the interpretation of nutritional topics differs among children, varying with gender and SES. These findings may affect the way in which any new healthy eating guidelines are accepted and acted upon. Further development of this research will elucidate the extent to which these differences are mirrored in actual consumption, enabling a feed-forward approach to new, effective healthy eating guidelines for school-aged children.

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Kelder SH, Perry CL, Klepp K-I & Lytle LL (1994) American Journal of Public Health **84**, 1121–1126.
Lytle LA, Eldridge AL, Kotz K, Piper J, Williams S & Kalina B (1997) Journal of Nutrition Education **29**(3), 128–136.
Willett WC (1990) Nutritional Epidemiology. New York: Oxford University Press.

Nutrient intake in elite female gymnasts and healthy age-matched controls: analysis of longitudinal data. By J.A. NURMI[1], J.A. BISHOP[2], S. CARR-BAINS[3], V. GREEN[2], B. HELBY[2], M. IERITI[2], E. SHEPPARD[2] and S.A. NEW[2], [1]Department of Clinical Nutrition, University of Kuopio, Finland, [2]Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH, [3]Guildown Medical Practice, Student Health Centre, University of Surrey, Guildford GU2 5XH

Studies published to date examining the relationship between nutritional influences and bone health in elite female athletes are limited due to several reasons: low subject numbers, weak assessment of dietary intake, severe under-reporting and lack of longitudinal study designs. Athletes, especially children and females involved in low body weight activities, are a challenging group to work with due to their commitment to the sports.

In order to address these issues, we are investigating the modifiable factors (e.g. diet and physical activity) affecting bone health in elite British female gymnasts and healthy age-matched sedentary controls and have collected information on anthropometry, pubertal development, diet and indices of bone health at baseline (New et al. 2000; Nurmi et al. 2000), 6 months and 12 months. Dietary intake was assessed using repeated estimated dietary records (7 d at baseline and 3 d each at 6, 9 and 12 months: making 16 d in total) and analysed using the Diet5 for Windows-program. This abstract reports the follow-up dietary information on younger (8–10 year old) gymnasts (G n 19) and controls (C n 25). Results are shown in the Table.

	Baseline				6 months				12 months			
	G (n 16)		C (n 23)		G (n 12)		C (n 24)		G (n 9)		C (n 21)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Height and height increase from baseline (cm)	126	0.05	136	0.07	+2.3*	1.0	+2.7*	0.9	+5.2*†	0.9	+5.8*†	1.2
Weight and weight increase from baseline (kg)	24.3	2.1	32.9	6.1	+1.2[a]*	0.5	+2.2[a]*	1.6	+2.8[a]*†	1.3	+4.4[b]*†	2.1
Energy (MJ)	7.2	1.6	7.6	1.0	6.1[a]	1.2	7.2[b]	1.5	6.9	1.0	7.0	1.3
Carbohydrate (g)	229	50	242	52	198[a]	35	232[b]	55	215	32	223	53
Protein (g)	55	11	56	10	48	12	53	12	58	9	52*	11
Fat (g)	70	20	74	10	56[a]	16	70[b]	16	67	15	67	17
Ca (mg)	700	197	717	141	595	197	635	238	592	201	666	204
Fe (mg)	9.6	3.7	9.9	3.3	7.5	2.4	8.3*	3.5	8.8	3.1	8.5*	3.1
EI:BMR	1.64	0.4	1.56	0.2	1.36	0.6	1.45*	0.3	1.51	0.3	1.37*	0.3

[a,b]Values with unlike superscripts were significantly different (Independent Student's t test). *Significantly different from baseline, $P<0.05$; †Significantly different from 6 months, $P<0.05$.

Changes in weight, but not in height, were significantly smaller in the gymnasts. There were few significant changes in dietary intakes over the 12 months, with only energy, carbohydrate and fat intakes being significantly different at 6 months between the groups. The mean EI:BMR for the 12 month period (16 d) was 1.48 for gymnasts and 1.50 for controls, although it should be noted that some subjects did not always return their diaries. Further analysis of the older age group (11–14 years) is required and the energy expenditure of the gymnast group needs further quantification.

New SA, Nurmi JA, Bishop JA, Carr-Bains S, Taylor P & Cooper C (2000) Proceedings of the Nutrition Society **59**, 28A.
Nurmi JA, Bishop JA, Carr-Bains S, Gardner J, Taylor FS & New SA (2000) Proceedings of the Nutrition Society **59**, 31A.

Moderate vitamin A deficiency during pregnancy affects fetal organ growth in the pig. By C.J. ASHWORTH[1,2] and C. ANTIPATIS[1], [1]Rowett Research Institute, Bucksburn, Aberdeen, AB21 9SB, [2]Applied Physiology Department, SAC, Bucksburn, Aberdeen, AB21 9YA

In humans maternal vitamin A deficiency is believed to affect fetal development in the absence of clinical symptoms in the mother (Underwood, 1994). We have shown that maternal vitamin A deficiency also retards fetal development and reduces neonatal survival in the rat (Antipatis et al. 2000a). The effects of moderate maternal vitamin A deficiency in a large animal species have not been reported. In the present study, we investigated the effects of dietary maternal vitamin A deficiency on pregnancy outcome, and fetal and neonatal organ growth in a single-stomached species, often regarded as the preferred model for studying neonatal organ (e.g. lung) maturation.

Large White × Landrace gilts received 2.3 kg/day of either a vitamin A-sufficient (control (C); n 10) or a vitamin A-free (VAF; n 9) diet for one oestrous cycle prior to mating and throughout pregnancy. Five gilts from the C group and four gilts from the VAF group were killed on day 100 of pregnancy; the remaining gilts were slaughtered 48 h after birth. The effects of vitamin A restriction on maternal retinol status were assessed at these times. Values obtained 48 h after birth are reported elsewhere (Antipatis et al. 2000b). On day 100 (fetal) and 12 h after birth (neonatal), organ growth, survival and retinol status were examined. Neonatal survival and retinol status are reported elsewhere (Antipatis et al. 2000b).

On day 100, maternal plasma (C: 0.178 (SE 0.009) v. VAF: 0.117 (SE 0.009) µg/ml, P<0.05) and liver (C: 171.3 (SE 4.3) v. VAF: 105.1 (SE 11.1) µg/g, P<0.05) retinol levels and fetal plasma (C: 0.068 (SE 0.002) v. VAF: 0.053 (SE 0.003) µg/ ml, P<0.05) and liver (C: 9.53 (SE 0.96) v. VAF: 5.88 (SE 0.46) µg/g, P<0.05) retinol levels were reduced due to dietary vitamin A deficiency. Fetal survival (C: 0.95 (SE 0.05) v. VAF 0.92 (SE 0.05), P 0.73), fetal weight (C: 0.89 (SE 0.02) v. VAF: 0.89 (SE 0.012) kg P 0.92) and placental weight (C: 0.20 (SE 0.01) v. VAF: 0.24 (SE 0.02) kg, P 0.11) were not affected. However, placental:fetal ratio (C: 0.224 (SE 0.005) v. VAF: 0.272 (SE 0.021), P<0.05) was increased.

Relative organ weights (as percentage (%) of total fetal and neonatal body weight) in fetuses (day 100) and neonates (day of birth) from gilts fed a control or a vitamin A-free (VAF) diet.

	Control				VAF				Probability*		
	Fetus (day 100)		Neonate (day of birth)		Fetus (day 100)		Neonate (day of birth)				
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Diet	Day	Interaction
Liver	2.55	0.02	2.93	0.10	2.32	0.03	2.55	0.12	0.030	0.009	NS
Lungs	3.59	0.11	1.68	0.06	3.40	0.09	1.48	0.06	0.002	0.001	NS
Heart	0.75	0.01	0.74	0.03	0.75	0.01	0.71	0.02	NS	NS	NS
Kidneys	0.87	0.03	0.80	0.01	0.83	0.04	0.70	0.05	0.030	0.009	NS
Spleen	0.15	0.01	0.11	0.01	0.14	0.01	0.08	0.01	0.040	0.001	NS

*Data were analysed by two-way analysis of variance.

Vitamin A deficiency reduced relative fetal and neonatal liver, lung, kidney and spleen weights. Relative liver weight increased from day 100 to day of birth whereas relative lung, kidney and spleen weights decreased. Vitamin A deficiency and day did not affect relative heart weight.

Given that neonatal survival was not affected in this experiment and retinol levels were higher than those observed in our rat model (Antipatis et al. 2000b), these data suggest that the degree of vitamin A deficiency obtained in the pig was less severe than that described in the rat. Nevertheless, in both studies, relative liver, lung and kidney weights were reduced. The absence of changes in the relative heart weight, one of the first organs to develop and function in both species, suggests that the severity and timing of the deficiency during pregnancy can lead to selective effects on organ development.

Antipatis C, Grant G &Ashworth CJ (2000a) British Journal of Nutrition **84**, pp 125-132.
Antipatis C, Rooke JA, Ewen M &Ashworth CJ (2000b) Proceedings of the Nutrition Society **60** (In the Press).
Underwood B (1994) American Journal of Clinical Nutrition **59**, 517S–524S.

Impact of milk intake on bone health of peri- and early post-menopausal Scottish women: confounding effects of menopausal status and HRT use. By H.M. MACDONALD[1,3], S.A. NEW[2], M.H.N. GOLDEN[3], D.A. GRUBB[4] and D.M. REID[1,3], [1]Osteoporosis Research Unit, University of Aberdeen, Woolmanhill Hospital, Aberdeen AB25 1LD, [2]Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH, [3]Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen AB25 2ZD, [4]Computing Department, Rowett Research Institute, Aberdeen AB21 9SB

A longitudinal study of diet and bone health was carried out on 1064 Scottish women as they moved through the menopause, to investigate what dietary factors influence bone density at this time. At their initial assessment in 1990–3, 93% of the women (aged 45–54 years) were menstruating normally. At that time, energy-adjusted intakes of K, Mg, Zn, fibre and vitamin C were positively associated with BMD at the hip and the spine (New et al. 1997, 2000). Bone mineral density (BMD) was measured at hip and spine sites by dual x-ray absorptiometry (DXA) (using a Norland XR26/36). Dietary intake was assessed by a validated food frequency questionnaire (FFQ) (New & Bolton-Smith, 1993; Bodner et al. 1998). Five to seven years later the women, now aged 50–59 years, were reassessed. 85% of the women had a repeat DXA scan and 99% of these again completed the FFQ. At their follow-up visit, 17% of the women were still menstruating, 38% were post-menopausal and 45% had taken hormone replacement therapy (HRT) at some time (13% past and 32% current users). Bone loss, which occurred at hip and spine, was predominantly influenced by menopausal status and HRT use when included as four dummy variables to account for the five categories (Macdonald et al. 1998).

We recently showed that nutrient intake had not changed significantly for the majority of the women since their last visit (Macdonald et al. 1999). At their follow-up visit, however, there was no longer a relationship between nutrient intake and BMD at 5 years for the whole group. This loss of association could be explained by the strong confounding effects of different menopausal status and HRT use. To examine the data further, the FFQ was analysed according to food groups. Milk intake was associated with higher BMD at the femoral neck (Spearmans coefficient r 0.102; P=0.002). Milk intake (follow-up and baseline) accounted for 1.0% and 0.3% respectively of the variation in FN BMD by forwards stepwise multiple regression.

Dependent variable:	log FN BMD at follow-up	
Independent variables:	Weight	10.5
	Height	1.6
	Age	0.5
	Smoking	NS
	Physical activity level	NS
	menopause/HRT (as dummy variables)	2.3
	Milk intake (follow-up)	1.0 (or milk intake at baseline 0.3)

This suggests that there may be particular food groups associated with higher BMD. Analysis of FFQ by food groups may give clearer insights into the effects of dietary quality on health, than analysis by individual nutrients.

Bodner CH, Soutar A, New SA, Scaife AR, Byres M & Henderson GD (1998) Journal of Human Nutrition and Dietetics **11**, 373–380.
Macdonald HM, New SA, Golden MHN & Reid DM (1998) Bone **23**, S304.
Macdonald HM, New SA, Golden MHN, Grubb DA & Reid DM (1999) Proceedings of the Nutrition Society **59**, 27A.
New (née Lanham) SA & Bolton-Smith C (1993) Proceedings of the Nutrition Society **52**, 330A.
New SA, Bolton-Smith C, Grubb DA & Reid DM (1997) American Journal of Clinical Nutrition **65**, 1831-1839.
New SA, Robins SP, Garton MJ, Bolton-Smith C, Lee SM, Grubb DA & Reid DM (2000) American Journal of Clinical Nutrition **71**, 142–51.

Comparison of nutritional status in elderly patients with a fractured neck of femur with age-matched controls. By M.C. MURPHY[1], S.A. NEW[2] and M. LUMBERS[3], [1]European Institute of Health and Medical Sciences, [2]Centre for Nutrition and Food Safety, School of Biological Sciences, [3]Centre for Food and Health Care Management, School for Management in the Service Sector, University of Surrey, Guildford GU2 5XH

Fractured neck of femur (FNF) occurs mostly in the older female population and is generally caused by falls. Malnutrition has been postulated as a factor that increases the tendency to sustain falls (Bastow et al. 1983). Older female hospital patients admitted for emergency surgery for FNF were recruited (n 75) and compared with an age-matched control group of older females attending a local Day Centre (n 50). An anthropometric and dietary assessment was undertaken using three consecutive 24-h dietary recalls and, in the hospital group, using completed menu cards as memory prompts.

Age and anthropometry variables	Cases (n 73) Mean	SD	Controls (n 50) Mean	SD	50% centile (Pen Group, 1998)
Age (years)	80.5	8.2	79.8	7.5	63.0
Weight (kg)	59.6***	11.9	67.5	13.1	
Demispan (mm)	727.2*	38.7	711.2	35.7	736.0
Mid upper arm circumference (MUAC; cm)	27.1***	4.3	31.3	4.7	29.9
Triceps skinfold thickness (TSF; mm)	17.0***	2.7	18.9	2.8	23

Nutrient intake	Cases (n 73) Mean	SD	Controls (n 50) Mean	SD	EAR/RNI (DoH, 1991)
Energy (MJ)	4.27*	1.25	5.40	1.88	7.61
Fat (g)	44	25	50	22	110
Carbohydrate (g)	133	56	164	63	172
Protein (g)	44*	53	53	21	47
Thiamine (mg)	0.86*	0.33	1.39	1.44	0.76
Pyridoxine (mg)	1.0*	0.4	1.4	0.6	1.2
Folate (µg)	154	94	196	165	200
Vitamin C (mg)	60.3	32.7	55.2	38.8	40.0
Vitamin A (µg)	800	199	613	361	700
Vitamin D (µg)	1.7	1.1	2.2	1.6	10.0
Calcium (mg)	543	183	654	265	700
Phosphorous (mg)	759***	240	929	263	550
Magnesium (mg)	153***	52	191	61	270
Potassium (mg)	1629***	483	2052	675	3500
Selenium (µg)	30.3**	14.1	39.2	17.7	60
Zinc (mg)	5.4	1.9	6.0	1.9	7.0
Non-starch polysaccharides (g)	7.9	3.8	12.5	5.2	12-18

Mean values were significantly different from the control group *P<0.05, **P<0.01, ***P<0.005.

Mean intakes of both groups were below the EAR for energy and below the RNI for folate, Ca, Mg, Se and Zn. The mean dietary intakes of pyridoxine in the hospital patients were also below the RNI and vitamin D, potassium, and selenium intakes were below the LRNI. The mean anthropometric values in the FNF group were below the 25th centile, indicating possible poor nutritional status prior to admission, which may have been exacerbated by the sub-optimal dietary intakes observed. Further research is required to assess whether these findings significantly affect clinical outcomes, such as length of hospital stay and risk of complications.

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Department of Health (1991) Dietary Reference Values for Food Energy and Nutrients for the United Kingdom: Report on Health and Social Sciences 41. London: HM Stationery Office.
Pen Group (1998) A Pocket Guide to Nutrition, 2nd Edn. [VE Todorovis and A Micklewright, editors]. Birmingham: British Dietetic Association.

Diet and systemic lupus erythematosus. By E.M. DUFFY[1], J.J. STRAIN[1], B.M. HANNIGAN[1] and A.L. BELL[2], [1]Northern Ireland Centre for Diet and Health, University of Ulster at Coleraine, Northern Ireland, BT52 1SA, [2]Lupus Research Group, Musculoskeletal Education and Research Unit, Musgrave Park Hospital, Belfast BT9 7JB

Systemic lupus erythematosus (SLE) is an autoimmune disease with the potential to involve multiple organ systems directly. It displays a broad spectrum of clinical and immunological manifestations including vascular inflammation. Empirical approaches to treatment with corticosteroids and immunosuppressants remain central to therapy but there is scope for exploration of less potentially toxic treatments.

Questionnaires were circulated to 400 individuals with all three types of lupus throughout the island of Ireland via Lupus Support groups. There was a 58.9% (n 235) response rate. Of those who responded, 81.4% had SLE, 16.7% had discoid lupus and 1.8% had drug-induced lupus. The mean age of the patients was 48.1 (SD 4.38) years, with a ratio of 9:1 female:male. The average BMI was 23.6 (SD 4.38) and the average age of diagnosis was 37.3 (SD 11.99) years. All individuals were white Caucasians.

A total of 12.8% of respondents had a family history of lupus and 41.9% had a family member with another autoimmune disease. Of those who reported specific times when symptoms were most active (n 167), flares in lupus activity were associated with the summer season (18.1%), pre-menstrual cycle (15.1%) and during times of stress (45.2%). Some 47.1% had never smoked, another 24.5% had given up smoking since diagnosis while 62.4% drank alcoholic beverages of which 65.9% only drank once per month or less.

A total of 48.8% had consciously changed their diet since diagnosis with lupus, but only 52.8% of these patients had received the dietary advice from their doctor, consultant or a dietitian. Tomatoes and dairy products were seen by 6.3% and 8.5% of respondents, respectively, as foods that aggravate symptoms. Some 20.8% of the respondents reported taking dietary supplements. Fish oils, evening primrose oils, garlic and vitamin C supplements were most often perceived by respondents as being beneficial to their condition. A total of 44.5% had tried a form of alternative medicine with benefits perceived from acupuncture and spiritual/faith healing.

The use of complementary medicine, especially the use of dietary supplementation, for the treatment of rheumatic diseases has increased enormously. Few clinical research studies, however, have been carried out to establish any links between diet and lupus activity. Poor patient disclosure to the physician may complicate identification of any benefits as well as any long-term adverse side effects of supplements or dietary modifications. There is an obvious need for rigorous nutritional studies involving patients with lupus.

Effects of radiotherapy treatment on dietary intake in patients with cancer of the head and neck. By E.E. BROADHEAD[1], P.M. HUGHES[1], C.G. KELLY[2] and C.J. SEAL[1], [1]*Human Nutrition Research Centre, Department of Biological and Nutritional Sciences, University of Newcastle upon Tyne NE1 7RU,* [2]*The Northern Centre for Cancer Treatment, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE*

Cancer treatments often have a profound effect on quality of life and in some cases can affect taste and appetite. These are important factors in the enjoyment of food and may lead to patients changing their diet. Lowering intake and avoiding specific foods are contributory factors in reducing nutritional status and this may affect outcome from treatment. Prolonged poor diet and associated weight loss may also have longer term consequences and lead to the development of anorexia-cachexia. The aim of this study was to investigate the effects of radiotherapy treatment on dietary intake and nutritional status in patients with oral and oro-pharyngeal cancer.

Seventeen patients (fourteen male) average age 63 years (range 38–89) completed a 4-d retrospective food frequency questionnaire (FFQ) with a food atlas to estimate portion sizes (Nelson *et al.* 1997) before commencement of a 6-week course of radiotherapy, at mid-treatment and at the end of the treatment period. Basic anthropometric measurements were made at each time point. Nutrient intakes were calculated from the FFQ and portion size estimates using Microdiet (University of Salford). Data were analysed by ANOVA with *post hoc* analysis of the difference between pre-treatment values and data for each treatment stage using Dunnett's two-sided test.

	Treatment stage				Significance of effects	
Dietary intake	Before	Mid	End	SEM[1]	Before v. Mid	Before v. End
Energy (kJ/d)	7984	5665	6030	521.1	0.129	0.228
Protein (g/d)	75.7	50.4	60.3	5.52	0.115	0.423
Total fat (g/d)	73.6	45.1	49.8	4.80	0.028	0.076
SFA (g/d)	27.3	17.5	21.8	1.62	0.025	0.271
PUFA (g/d)	7.4	4.5	3.1	0.76	0.191	0.038
MUFA (g/d)	21.2	10.8	11.5	1.27	0.001	0.002
Carbohydrate (g/d)	232.4	183.2	182.2	17.40	0.418	0.414
Sugars (g/d)	102.9	101.7	94.5	10.00	0.998	0.923
Starch (g/d)	107.3	47.3	30.6	8.26	0.002	0.000
Fibre (g/d)	13.4	7.5	6.5	1.38	0.145	0.085
Body weight (kg)	72.6	70.6	67.5	2.05	0.893	0.516
% body fat (determined from triceps skinfold)	22.0	23.3	22.2	1.71	0.941	0.997
Mid upper arm circumference (cm)	24.8	24.5	24.4	0.47	0.962	0.921

[1]SEM, pooled standard error.

Energy intakes were below recommended levels, and there was a further reduction in energy intake during radiotherapy treatment, despite three patients at mid-treatment and nine patients at the end of treatment consuming dietary supplements in addition to their normal diet. There were some changes observed in the pattern of macronutrients consumed, especially fat intake, which fell significantly during treatment. There was a fall in total carbohydrate intake which appeared to be due to a significant reduction in intake of starch but not simple sugars. The variety of foods consumed fell as treatment progressed from an average of eighteen different food items reported over the 4-d period before treatment to thirteen items at mid-treatment and ten items at the end of treatment. These changes reflect the extensive use of supplements and increased consumption of liquid foods such as soups. Most patients experienced some weight loss, but otherwise there were no major changes in body composition. Fluid intake remained constant throughout treatment and changes in fluid retention may mask loss of body tissue. These results show that dietary intake and nutritional status are adversely affected by radiotherapy, although the consequences of these changes on outcome from treatment have yet to be evaluated.

Nelson M, Atkinson M, Meyer J (1997) *An Atlas of Food Portion Sizes.* London: MAFF Publications.

Changes in taste, appetite and quality of life in patients with cancer of the head and neck during radiotherapy treatment. By E.E. BROADHEAD[1], P.M. HUGHES[1], C.G. KELLY[2] and C.J. SEAL[1], [1]*Human Nutrition Research Centre, Department of Biological and Nutritional Sciences, University of Newcastle, Newcastle upon Tyne NE1 7RU,* [2]*The Northern Centre for Cancer Treatment, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE*

Taste and appetite are important features in the enjoyment of food and have a major impact on quality of life. Loss of taste and development of food aversions are recognised side effects of many forms of cancer treatment which may result in patients changing their diet. Removing the pleasure of eating and the development of anxiety-related eating problems are key determinants of quality of life. The aim of this study was to investigate the effects of radiotherapy treatment on quality of life in patients with particular emphasis on eating habits, taste and appetite in patients with oral and oro-pharyngeal cancer. The results of dietary evaluations from this study are reported elsewhere (Broadhead *et al.* 2000).

Seventeen patients (fourteen male) average age 63 years (range 38–89) took part in the study. Quality of life (QoL) was assessed using the EORTC QoL questionnaire (Aaronson *et al.* 1993) before commencement of a 6-week course of radiotherapy, at mid-treatment and at the end of the treatment period. Changes in taste perception and eating habits were assessed by questionnaire at mid- and end-treatment only. Data were analysed by ANOVA with *post hoc* analysis of the difference between pre-treatment values and data for each treatment stage using Dunnett's two-sided test.

	Treatment Stage				Significance of effects	
During the past week….	Before	Mid	End	SEM[1]	Before v Mid	Before v. End
Have you lacked appetite?	1.8	2.6	3.2	0.19	0.117	0.004
Have you had problems with your sense of taste?	1.8	2.8	3.5	0.19	0.054	0.000
Have you had problems swallowing liquids?	1.6	2.6	2.7	0.32	0.057	0.042
Have you had problems swallowing pureed foods?	1.5	2.9	2.9	0.20	0.007	0.007
Have you had problems swallowing solid foods?	2.0	3.4	3.4	0.18	0.002	0.002
Have you had trouble eating?	1.6	3.5	3.4	0.19	0.000	0.000
Have you had trouble eating in front of your family?	1.2	2.1	2.6	0.19	0.087	0.006
Have you felt ill?	1.4	2.2	2.4	0.15	0.081	0.027
Have you been constipated?	1.4	1.8	2.3	0.15	0.318	0.021

Score index; 1 = not at all, 2 = A little, 3 = Quite a bit, 4 = Very much.
[1]SEM, pooled standard error.

Results of the QoL questionnaire showed that there was a significant deterioration in the sense of taste and appetite during treatment. Patients reported a significant increase in trouble with eating, which extended to problems eating in front of family members. These observations were associated with an increase in reported symptoms of dry mouth, sticky saliva, painful throat, problems with opening the mouth and overall soreness within the mouth area. By the mid-treatment stage, all of the patients reported that their food tasted worse and 93% of patients reported that their appetite or desire for food had become worse. 71% of patients had changed their diet because their appetite had changed and 65% changed their diet because their food tasted different. Changes in the pattern of nutrients consumed, and in particular reduced fibre and complex carbohydrate consumption during treatment (Broadhead *et al.* 2001) may be responsible for the reported increase in constipation.

The results demonstrate the progressive decline in patients' quality of life during the course of radiotherapy treatment. Changes in eating habits and problems in eating should be carefully monitored during treatment in order to provide adequate advice and support for the patients. Recovery of appetite and quality of life after treatment have yet to be evaluated.

Aaronson NK, Ahmedzai S, Bergman B *et al.* (1993) *Journal of the National Cancer Institute* **85**, 365–376.
Broadhead EE, Hughes PM, Kelly CG, Seal CJ (2001) *Proceedings of the Nutrition Society* **60** (In the Press).

Vitamin E metabolism: tocotrienols are metabolized to their carboxyethyl-hydroxychromanol derivatives and excreted in human urine. By J.K LODGE[1,2] and M.G TRABER[1], [1]Linus Pauling Institute, 571 Weniger Hall, Oregon State University, Corvallis, Oregon, OR 97331, USA, [2]School of Biological Sciences, University of Surrey, Guildford GU2 7XH

Vitamin E encompasses eight structurally different compounds: four tocopherols (α, β, γ, δ), which have a saturated phytyl chain, and four tocotrienols, in which the chain is unsaturated. All compounds have similar antioxidant activity but differ in their biological activity. Although the properties of vitamin E are well researched and understood, relatively little is known regarding its metabolism. The carboxyethyl-hydroxychromanol (CEHC) derivatives of α- and γ-tocopherol (α-, γ-CEHC) have both been detected in human urine. No metabolites of the tocotrienols have yet been reported. We have developed a new extraction and analytical method for the determination of CEHCs (Lodge et al. 2000a) and have used this method to investigate whether the tocotrienols are metabolized and excreted as urinary CEHC derivatives (JK Lodge and MG Traber, unpublished results).

Typically, 24-h urine collections were taken 2 d before (baseline) and for 4 d after ingestion of a 75 or 300 mg dose of either α- or γ-tocotrienyl acetate. Large inter-individual differences were observed in baseline values, which ranged from 80–300 µg/d and 150–600 µg/d for total α- and γ-CEHC excretion, respectively. Analysis of unconjugated α- and γ-CEHC showed that these forms represent 5–25% of the total CEHC in human urine. After a 75 mg dose of γ-tocotrienyl acetate, total γ-CEHC concentration increased 5-fold in the urine on the day of intake and decreased back to baseline levels in 2 d. No increase in α-CEHC levels was observed after a similar dose of α-tocotrienyl acetate. This indicates that the efficiency of metabolism for this form is lower. However, a 300 mg dose of α-tocotrienyl acetate increased urinary α-CEHC excretion up to 30-fold. Similarly, γ-CEHC excretion increased up to 40-fold after a similar dose of γ-tocotrienyl acetate. Urinary excretion of γ-CEHC was also followed during a 24-h period after a dose of γ-tocotrienyl acetate. Urinary γ-CEHC was detected 6 h after ingestion and peaked at 9 h, indicating that metabolism is rapid.

Typically between 2 and 7 % of the ingested dose of the tocotrienyl acetates were detected as the urinary CEHC metabolites. Based on literature values, the metabolism of tocotrienols to their CEHC derivatives appears to be approximately 10-fold less than the corresponding tocopherols. This leaves the question as to the fate of absorbed tocotrienols.

Lodge JK, Traber MG, Elsner A & Brigelius-Flohé R (2000a) Journal of Lipid Research **41**, 148–154.

The North/South Ireland Food Consumption Survey 2000: Vitamin intakes in 18–64 year-old adults. By M.M. O'BRIEN[1], M. KIELY[1], A. FLYNN[1], M.J. GIBNEY[2], K. HARRINGTON[2], P. ROBSON[3], M.B.E. LIVINGSTONE[3] and J.J. STRAIN[3], Irish Universities Nutrition Alliance: [1]University College Cork, [2]Trinity College Dublin, Republic of Ireland, [3]University of Ulster, Northern Ireland

The North/South Ireland Food Consumption Survey 2000 was conducted to establish a database of habitual food and drink consumption of Irish adults aged 18–64 years. A 7-d food diary method was used to collect food intake data from 1379 adults who were selected randomly from the electoral register. Analysis of the dietary intake data was carried out using Wisp© (Tinuviel Software, Warrington) which contained McCance and Widdowson (5th Edn) The Composition of Foods (Holland et al. 1995). The mean daily intakes of vitamins from all sources including supplements for men (n 475) and women (n 483) from the Republic of Ireland are presented here.

Vitamin/Units	RDA*	Men Mean	SD	Median	RDA*	Women Mean	SD	Median
Vitamin A (µg RE)	700	1080	955	887	600	980	817	750
Vitamin D (µg)	0–10	3.6	3	2.4	0–10	4.0	10.4	2.1
Vitamin E (mg)	None	11.2	40	6.1	None	11.0	27.3	6.1
Thiamin (mg)	[100µg/MJ]	2.4	1.6	2.4	[100µg/MJ]	2.3	4.9	1.5
Riboflavin (mg)	1.6	2.2	1.6	2.0	1.3	2.2	4.0	2.2
Niacin (mg NE/MJ)	1.6	49.7	14.2	48.1	1.6	35.2	12.2	33.8
Vitamin B$_6$ (mg)	[15µg/g protein]	3.6	2.1	3.2	[15µg/g protein]	3.9	8.1	2.1
Vitamin B$_{12}$ (µg)	1.4	5.4	3.7	4.6	1.4	4.3	3.8	3.4
Folate (µg)	300	342	130	316	300	264	126	231
Pantothenic acid (mg)	None	6.7	3	6.2	None	5.4	5.1	4.4
Biotin (µg)	None	44.3	23.9	40.9	None	34.7	22.2	30.5
Vitamin C (mg)	60	119	240	75	60	109	200	67

*Food Safety Authority of Ireland, 1999.

The percentage of the population with vitamin intakes below the Estimated Average Requirement (EAR) is presented for selected vitamins as an estimation of the percentage of the population with inadequate intakes (Food and Nutrition Board, 1997).

Percentage of the population with vitamin intakes below the Estimated Average Requirement.

Vitamin	EAR*	Men 18–25y (n 169)	36–50y (n 178)	51–64y (n 128)	EAR*	Women 18–35y (n 167)	36–50y (n 201)	51–64y (n 115)
Vitamin A	500 µg	24	14	20	400 µg	14	13	17
Riboflavin	1.3 mg	12	11	9	1.1 mg	19	16	17
Folate	230 µg	17	15	16	230 µg	45	49	53
Vitamin C	46 mg	25	22	15	46 mg	24	27	26

*Food Safety Authority of Ireland, 1999.

These are preliminary estimates of the occurrence of inadequate vitamin intakes. Further analysis will be carried out to evaluate the impact of misreporting on these values.

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Ascorbic acid intake and biochemical status in the National Diet and Nutritional Survey of people aged 65 years and older. By R.L. THOMPSON, B.M. MARGETTS and A.A. JACKSON, *Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD*

The report of the National Diet and Nutrition Survey (NDNS) of people aged 65 years and over (Finch *et al.* 1998) stated that 14% of free-living elderly people had a low biochemical status for ascorbic acid (<11 µmol/l for plasma ascorbate). However, mean dietary ascorbic acid intakes were 150% of the reference nutrient intake (RNI) for men and 127% for women (Department of Health, 1991).

We sought to determine possible predictors of plasma ascorbate status in a group of older people not considered to be at increased risk of ascorbate deficiency, using the NDNS for people aged 65 years and over. The analysis included 479 people who were free-living, completed a 4-d weighed dietary record, were non-smokers and provided a blood sample. People with a dietary intake below the lower reference nutrient intake were excluded on the assumption that their intake was not adequate. The median ascorbic acid intake was 59 mg/d (148% of RNI) and 8% had a plasma ascorbate value of less than 11 µmol/l. Of those with low plasma ascorbate, 37% had dietary intakes above the RNI for ascorbic acid. A stepwise logistic regression analysis with biochemical status (low; <11 µmol/l *v.* adequate; ≥11 µmol/l) as the dependent variable was carried out. The analysis included demographic (including age and sex) and socio-economic factors, dietary food patterns (derived from a principal components analysis), nutrient intakes and biochemical measures. Continuous variables were divided into thirds of the distribution.

Variable	Comparison (thirds unless indicated otherwise)	Crude		Adjusted	
		Odds ratio	95% confidence interval	Odds ratio	95% confidence interval
Dietary ascorbic acid	Low versus high	35.6†	4.8–204.8	23.8*	2.8–205.8
Dietary vitamin E	Moderate versus low	1.5	0.7–3.2	4.5*	1.5–13.6
Intrinsic & milk sugars	Low versus high	7.5†	2.5–22.3	16.8†	3.3–87.1
More milk, sugary foods, tea & coffee; less potatoes	High versus low	2.5	1.0–6.1	14.3†	3.6–57.5
Plasma copper	Moderate versus low	10.1*	2.3–44.6	8.5*	1.7–42.5
	High versus low	11.8†	2.7–51.7	20.9†	4.0–109.4
Dietary supplements	No versus yes	5.3*	1.9–15.3	7.3*	1.9–27.3
Region	North versus south	5.3†	2.6–10.6	6.2†	2.5–15.2
Height	High versus low	0.9	0.4–2.1	5.1*	1.5–17.0

Significantly different: *$P < 0.01$, †$P < 0.001$.

Low ascorbic acid status was associated with lower dietary intakes of ascorbic acid and intrinsic and milk sugars, and a dietary pattern with more milk and sugary foods. Low ascorbate status was also associated with being in the top two-thirds for plasma copper, not taking dietary supplements, living in the North of Great Britain and being taller.

The reference nutrient intake is set to cover the requirements of the majority of the population, being set at a level to maintain health in an otherwise healthy population on the assumption that the requirements for energy and all other nutrients are already satisfied. The analysis showed that although dietary intake of ascorbic acid is predictive of ascorbate status, other dietary and non-dietary factors are also associated with ascorbate status.

Department of Health (1991) *Dietary Reference Values For Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects no. 41.* London: HM Stationery Office.
Finch S, Doyle W, Lowe C, Bates CJ, Prentice A, Smithers G & Clarke PC (1998). *National Diet and Nutrition Survey: People aged 65 years and over. Volume 1: Report of the Diet and Nutrition Survey.* London: The Stationery Office.

Dietary intake and sources of phylloquinone (vitamin K1): regional differences in British elderly people. By C.W. THANE[1], A.A. PAUL[1], C.J. PRYNNE[1] and C. BOLTON-SMITH[2], [1]*MRC Human Nutrition Research, Downhams Lane, Milton Road, Cambridge CB4 1XJ,* [2]*Nutrition Research Group, Cardiovascular Epidemiology Unit, University of Dundee, Ninewells Hospital, Dundee DD1 9SY*

In addition to its role in blood clotting, vitamin K, present mainly as phylloquinone (K1), is also essential for conferring functional calcium-binding to several bone proteins (Shearer, 1997). Low K1 intake may lead to poor vitamin K status and contribute to bone conditions, such as osteoporosis, and increase the risk of fracture (Szulc *et al.* 1993). Sub-optimal vitamin K status has also been associated with atherogenesis and vascular calcification (Jie *et al.* 1995). In order to translate the relationships between biochemical markers of vitamin K status and health outcomes into public health and dietary recommendations, it is first necessary to obtain accurate estimates of K1 intake.

Dietary intake of K1 and the relative contributions of different food groups were estimated in a nationally representative sample of free-living British people aged 65 years and over who participated in the 1994–5 National Diet and Nutrition Survey (Finch *et al.* 1998). After excluding those who were unwell with eating affected, complete 4-d weighed dietary records were analysed for 582 men and 570 women. Dietary K1 intake was estimated using the K1 composition of a comprehensive range of foods (Bolton-Smith *et al.* 2000; plus unpublished data). Average content values were used for eighty-seven food groups, with a further fifteen groups (main contributors of K1 or of variable content) analysed using content values for their individual constituents, to obtain a more accurate estimate of intake. Dietary K1 intake was examined according to several socio-demographic factors (age, sex, season, social class, educational attainment, household income and composition, state benefits, smoking, drinking habits, and region). The regional differences are reported here, with adjustment for these other factors.

K1 intakes were positively-skewed, with overall geometric means (95% CI) for men and women of 66 (62–70) and 57 (53–60) µg/d respectively. Whether expressed in terms of µg/d, µg/4.18 MJ or as µg/kg body weight/d, K1 intakes were significantly lower in the North than in the South, for both men and women, even after further adjustment for possible under-reporting (energy intake:calculated BMR <1.2). Regional variation also existed in the sources of K1. Vegetables and vegetable dishes contributed most to K1 intake (57% overall), with green leafy vegetables alone providing around 26% (cabbage>Brussels sprouts>broccoli). The broad food groups of cereals, meat and milk provided 12–15, 6–9 and 3% respectively, while fat spreads contributed only 5% to K1 intake.

	Scotland & North (n 325–399)	Central, S West & Wales (n 402–459)	London & S East (n 266–294)	P*
K1 intake (µg/d)†	50^a (47–54)	64^b (61–69)	73^b (67–79)	<0.001
K1 intake (µg/4.18 MJ)†	33^a (31–35)	41^b (38–43)	47^c (44–51)	<0.001
K1 intake from vegetables (%)‡	49^a (47–52)	60^c (58–62)	63^b (60–66)	<0.001
of which: leafy green vegetables (%)‡	20^a (17–23)	28^c (25–31)	32^b (29–36)	<0.001
K1 intake /kg body weight/d†	0.76^a (0.71–0.81)	0.96^b (0.89–1.02)	1.10^c (1.00–1.21)	<0.001
K1 intake <1 µg/kg body weight/d (%)	68^a	55^ab	50^b	0.01

†Geometric means (95% CI) obtained from antilog of log₁₀-transformed data; ‡arithmetic means (95% CI). *Adjusted for other socio-demographic factors. Different superscript letters indicate significant regional differences ($P<0.05$; Scheffé test for continuous variables, following ANCOVA; ‡ test for discontinuous proportions, following multiple logistic regression).

Notwithstanding the caveats inherent with all estimates of nutrient content and intake (Bolton-Smith *et al.* 2000), these data suggest regional differences in K1 intake, which may be reflected in vitamin K status and be pertinent to the prevalence of some chronic diseases in the elderly.

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Szulc P, Chapuy M-C, Meunier PJ & Delmas PD (1993) *Journal of Clinical Investigation* **91**, 1769–1774.

Implications of fortification of white flour with folic acid on total folate intake by adolescents. By A.J. ADAMSON, A.J. RUGG-GUNN, T.J. BUTLER and P.J. MOYNIHAN, *Human Nutrition Research Centre, University of Newcastle, Newcastle upon Tyne NE1 4LP*

The role of folic acid in the prevention of neural tube defects is well documented. However, compliance with the Department of Health recommendation (Department of Health, 1992), to consume a supplementary intake of folic acid of 400 μg/d peri-conceptually is low. A more effective means of achieving a supplementary intake of folic acid is through food fortification. The Committee on Medical Aspects of Food Policy recently recommended that all flour be fortified with folic acid at 240 μg/100g (Department of Health, 2000). This study determined the consequences of this on additional folic acid intake and total folate intake of adolescents. The level of fortification of white flour required to achieve 400 μg/d from this source and the consequences of this for high white-flour consumers were also determined.

Existing dietary data on 379 adolescents (aged 11–12 years) collected in 1990 (Adamson *et al.* 1992) were analysed for mean daily intake of total dietary folate and white flour from all sources. White flour intake (g/d) was multiplied by 2.4 to give the theoretical intake of folic acid from flour fortification at 240 μg/100g. Based on the distribution of values for flour intake, calculations were carried out to determine the level of fortification required to ensure that 97.5% of girls would receive 400 μg/d from this source. Analyses were carried out by gender and social class using the Mann-Whitney U test and the statistical significance of social class trends tested using parametric and non-parametric ANOVA.

			Flour intake		Dietary total folate (without fortification)		Folic acid from fortification (240 μg/100g)	
	Social class	n	Mean (g/d)	SE	Mean (μg/d)	SE	Mean (μg/100g)	SE
Girls	All	195	80	2.2	153	3.4	191	6.0
Social class	I&II	67	79	4.3	160	6.0	189	10
	III	60	81	3.9	147	5.8	193	9
	IV&V	38	80	4.4	148	7.4	192	10
Boys	All	184	83	2.6	166	4.4	199	5.4
Social class	I&II	70	84	5.0	171	7.5	202	12
	III	56	78	3.9	173	8.6	187	10
	IV&V	32	90	5.9	161	10.4	216	14

No statistically significant differences were found between gender and social class for dietary intakes of flour or folate. The percentage of girls and boys who failed to achieve the LRNI (100 μg/d) for total folate were 6.9 and 9.7, respectively. The average intake of total folate that would be achieved (from diet and white flour fortification) if white flour were fortified at 240 μg/100g would be 365 (SEM 8) and 343 (SEM 6) μg/d for boys and girls respectively. To ensure that 97.5% of girls received 400 μg/d from flour, fortification at 1043 μg/100g would be necessary – resulting in an additional intake of 1422 μg/d in the highest 2.5th percentile of flour consumers.

Fortification of white flour with folic acid at 240 μg/100g would deliver, on average, approximately 200μg/d to adolescent girls, which is half the desirable dose. This would have the potential of reducing risk of NTD births by 35% (Department of Health, 2000).

Adamson AJ, Rugg-Gunn AJ, Butler TJ, Appleton DR & Hackett AF (1992) *British Journal of Nutrition* **68**, 543–563.
Department of Health (1992) *Folic Acid in the Prevention of Neural Tube Defect.* London: HMSO.
Department of Health (2000) *Folate and the Prevention of Disease.* London: HMSO.

Effect of vitamin B₁₂ status on homocysteine levels in healthy male subjects. By N.J. MANN, A.J. SINCLAIR, N. LI D, N.P.B. DUDMAN, X.W. GUO and K. KELLY, *Department of Food Science, RMIT University, Melbourne, Australia*

Moderate to extreme cases of hyperhomocysteinaemia have for some time been associated with genetically determined enzymatic faults of methionine metabolism. More recently, interest has been directed at the dietary factors involved in homocysteine metabolism, which are methionine, the *in vivo* precursor of homocysteine and the three B group vitamins: folate and vitamin B₁₂ (remethylation) and vitamin B₆ (transulfuration), involved in homocysteine removal.

The aim of this study was to determine if habitual diet (omnivorous and vegetarian) resulted in differing plasma levels of homocysteine and whether these levels were related to dietary intake of methionine or serum concentrations of folate and vitamin B₁₂. Healthy, non-smoking male subjects (n 139) aged 20–55 years were recruited from the Melbourne area. They were divided into four groups based on habitual dietary intake: Vegan (n 18), Ovo-lacto vegetarian (n 43), Moderate meat-eaters (n 60, <260 g/day) and High meat-eaters (n 18, >280 g/day). Meat consumption was plotted on a scattergram and subjects self selected into >280 and <260 g meat per day, with no subjects in between. Plasma total homocysteine concentration was determined by a standard HPLC method (Dudman *et al.* 1996) and serum folate and vitamin B₁₂ by a competitive immunochemiluminometric assay. Dietary methionine intake was estimated from extensive dietary information collected from six randomly chosen subjects from each of the four dietary groups.

Plasma homocysteine levels exhibited a clear and significant stepwise increase from the high meat diets through to vegan diets, with the inverse pattern being observed for serum vitamin B₁₂ levels (see Table). A highly significant (*P*<0.01) inverse correlation was established between these two parameters. A small but significant variation in serum folate levels was detected between the vegan and moderate meat-eating groups. Methionine intake was significantly higher in the two omnivorous groups compared with the vegetarian groups (*P*>0.001), indicating no direct association with the elevated homocysteine levels shown by vegetarians. These data suggests that low dietary vitamin B₁₂ can elevate plasma homocysteine levels, whereas methionine intake is independent of homocysteine status (Mann *et al.* 1999).

	Habitual dietary group							
	High-meat		Moderate-meat		Ovo-lacto		Vegan	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Plasma homocysteine (μmol/l)	11.0[a]	2.5	11.6[a]	2.7	15.8[b]	9.3	19.2[b]	10.7
Methionine intake (mg/day)	4145[a]	931	3017[a]	515	1300[b]	567	1606[b]	647
Serum vitamin B₁₂ (pg/ml)	544[a]	228	452[a]	134	285[c]	132	196[d]	92
Serum vitamin B₁₂ (pg/ml)	6.7[ab]	2.3	5.6[b]	1.7	6.3[ab]	2.4	7.8[a]	3.4

All figures on the same line with differing superscripts are significantly different (*P*<0.001)

Dudman NPB, Guo XW, Crooks R, Xie L & Silberberg JS (1996) *Clinical Chemistry* **42**, 2028–2032.
Mann NJ, Li D, Sinclair AJ, Dudman NPB, Guo XW, Elsworth GR, Wilson AK & Kelly FD (1999) *European Journal of Clinical Nutrition* **53**, 895–899.

How does dietary non-starch polysaccharide intake affect plasma micronutrient levels? Results from the UK Women's Cohort Study. By J. CADE[1], D. GREENWOOD[2] T. NEENAN[1], C. CALVERT[1], K. WHITE[3] and C. SCHORAH[3] [1]*Nutrition Epidemiology Group,* [2]*Sub-unit for Medical Statistics, Division of Public Health Medicine, Nuffield Institute for Health, 71–75 Clarendon Road, Leeds LS2 9PL,* [3]*Chemical Pathology, University of Leeds, Leeds LS2 9PL*

Current emphasis on increasing intake of complex carbohydrates might lead to changes in intakes of other nutrients. Bioavailability of micronutrients and trace metals may be affected due to a concomitant increase in phytic acid and dietary fibre. This project investigated the effect of long-term consumption of dietary fibre on micronutrient levels in a free-living population.

The women in this study are responders to the UK Women's Cohort study and include a high percentage of vegetarians. All subjects had completed the baseline data collection including a previously validated food frequency questionnaire (FFQ) which included 217 food/drink items, and details of lifestyle habits including exercise, supplement use, current illness, smoking and drug use. Subjects included were those living within a 30 mile radius of Leeds (or 1 h drive: postcodes in Huddersfield/Halifax/York/Leeds/Wakefield/Bradford) to facilitate the collection of blood samples. A nurse/field-worker collected a fasting blood sample and 4-d food diary and questionnaire. Samples were collected into lithium heparin (8 ml) for carotenoid (α-, β-carotene, lycopene, cryptoxanthin, lutein), ascorbic acid, total vitamin C (ascorbic acid + dehydroascorbic acid), α-tocopherol and trace metal analysis (iron, copper, zinc) and into EDTA (5 ml) for plasma and red cell folate.

634 women were contacted and 283 women participated, of whom 274 had blood samples taken. These women tend to be above average social class and have an interest in their diet and health. Nevertheless, women in the lowest fibre quartile had mean intakes of non-starch polysaccharide (NSP) of 9.5 g, which is about half of what might be expected for a high social class group (Gregory *et al.* 1990). They also had a mean BMI which was in the overweight category (25.4 kg/m^2). Mean NSP intake in the top fibre quartile was 25.2 g. The FFQ and the food diary showed similar correlations between dietary nutrient intake and blood levels, with the food diary having higher correlations for some nutrients. As expected, vegetarians had higher NSP intakes compared with the fish- or meat-eating groups. Although we had small numbers of new vegetarians, we did not find any impact of length of time as a vegetarian on nutrient intakes or blood nutrient levels.

Nutrient	% increase in nutrient for 10% increase in NSP*	95% CI	R^2	*P*-value
Beta-carotene	4	1 to 7	0.31	0.011
Lutein	2	0 to 4	0.35	0.037
Cryptoxanthin	3	1 to 6	0.20	0.017
Lycopene	1	–1 to 4	0.31	0.323
Vitamin E	0	0 to 1	0.45	0.273
Folate – red cell	1	–1 to 3	0.16	0.250
Ascorbic acid	2	0 to 4	0.30	0.020

*Adjusting for lifestyle factors, energy intake and supplement use.

Exploring the sample according the quartiles of NSP intake showed increasing blood measures of β-carotene, folate and vitamin C across the NSP groups. These persisted in linear regression analysis and also following a multiple regression analysis adjusting for other lifestyle factors. In this analysis including β-carotene, lutein, cryptoxanthin and ascorbic acid showed positive increases in the plasma with increasing NSP intake. We did not find any evidence of NSP negatively influencing plasma micronutrient levels including trace metals. In fact, there was evidence in the multiple regression model that NSP had an independent positive effect on plasma levels of carotenoids, ascorbic acid and perhaps plasma folate.

Gregory J, Foster K, Tyler H & Wiseman M (1990) *The Dietary and Nutritional Survey of British Adults.* London: OPCS, HMSO.

Iron status, diet and cognitive function in British adolescent girls. By M. NELSON[1], R. ASH[2], C. MULVIHLL[1], T.J. PETERS[1] and P. ROGERS[3] [1]*Department of Nutrition and Dietetics, King's College London, 150 Stamford Street, London SE1 8WA,* [2]*Nutrition Group, School of Health and Sport Science, University of North London, 166–220 Holloway Road, London N7 8DB,* [3]*Department of Experimental Psychology, 8 Woodland Road, University of Bristol, Bristol BS8 1TN*

Previous studies in the UK suggest that between 10 and 30% of adolescent girls have poor iron reserves or mild iron deficiency anaemia (Nelson 1996). There is growing evidence that borderline iron status in this age group has adverse effects on cognitive function (Bruner *et al.* 1996).

595 girls aged 11–18 attending three comprehensive schools in North London provided finger-prick capillary blood samples which were assessed for haemoglobin (Hb) and packed cell volume (PCV). Mean (SD) age was 13.4 (1.6) years. Mean Hb was 12.9 (1.2) g/dl and mean PCV was 36.7 (3.2)%. 21.8% of girls had Hb <12 g/dl (ranging from 17.2% in White girls to 29.2% in girls of Indian, Pakistani or Bangladeshi origin) and 23.2% of girls had PCV <35% (ranging from 17.2% in White girls to 27.0% in Black girls).

152 of these girls provided venous blood samples and measures of cognitive function and diet. Venous blood was measured for Hb and serum transferrin receptor (TfR). Girls in the bottom third of the Hb distribution (<12.3 g/dl) and the top third of the TfR distribution (>3.11 mg/l) were classified as iron-deficient anaemic (IDA, *n* 25). Those in the top two-thirds of the Hb distribution *and* in the bottom two-thirds of the TfR distribution were classified as iron-deplete (IR, *n* 71). All other girls were classified as iron-deficient (ID, *n* 56). Cognitive function was measured using the short version of the British Ability Scales (BAS) test for IQ. Diet was measured using a 4-d food checklist with portion sizes expressed in household measures. The nutrient content of the diet was calculated using IDA (Integrated Dietary Analysis).

There was a highly statistically significant difference in IQ between IDA girls and ID and IR girls. Even after the effects of social class, ethnic origin, the onset of menstruation, taking vitamin and mineral supplements, attempting to lose weight, and being vegetarian were taken into account in a multivariate analysis of variance, the effect of iron status remained statistically significant (*P* 0.025).

Iron status at baseline	*n*	Mean IQ*	SE
Iron-deficient anaemic	25	97.9	3.1
Iron-deficient	56	107.7	2.0
Iron-replete	71	108.5	1.7

*IDA significantly different from ID and IR, *P* 0.006 (ANOVA)

Dietary iron intake was significantly correlated with Hb levels (*r* 0.204, *P* 0.024). Dietary intakes of energy, protein, carbohydrate, calcium, iron and vitamin C were positively and significantly correlated with IQ. In a stepwise multiple regression analysis including Hb, TfR, energy, protein, carbohydrate, calcium, iron and vitamin C, only energy, vitamin C and TfR were significant predictors of IQ.

We conclude that poor iron status is common amongst British adolescent girls, and that diet and iron status play an important role in determining cognitive function independent of factors such as menstrual status or social class. Indeed, part of the apparent difference in cognitive function between social class groups is likely to be explained by differences in diet and iron status. These differences in cognitive function are likely to have important consequences in relation to learning ability and academic achievement.

Bruner AB, Joffe A, Duggan AK, Casella JF & Brandt J (1996) *Lancet* **348**, 992–996.
Nelson M (1996) *Proceedings of the Nutrition Society* **55**, 359–367.

Intake and sources of selenium in British elderly people. By C.W. THANE and C.J. BATES, *MRC Human Nutrition Research, Downhams Lane, Milton Road, Cambridge CB4 1XJ*

Concern has recently been expressed over low selenium (Se) intakes in the UK population (Rayman, 1997). Declining intakes in the last three decades have been attributed mainly to a change in the source of wheat for the manufacture of bread and other cereal products, from predominantly North American to European origin (high to low Se content). The decline in Se intake may have deleterious health consequences since, through its incorporation in a number of selenoproteins, it exhibits antioxidant activity which may help to limit free radical damage and protect against some chronic diseases, such as cardiovascular disease and some forms of cancer (e.g. lung, colorectal and prostate). Its involvement in thyroid hormone function and immunity are also of particular relevance in the elderly.

Se intake, and the relative contribution of different food groups and supplements, were estimated in a nationally-representative, cross-sectional sample of free-living British people aged 65 years and over who participated in the 1994–5 National Diet and Nutrition Survey (Finch *et al.* 1998). After excluding those who were unwell with eating affected, complete 4-d weighed dietary records were analysed for 582 men and 570 women. Se intake was estimated using content data for a comprehensive range of foods. Average Se content values were used for ninety-seven food groups, with a further five groups (main contributors of Se or of variable content) analysed using values for their individual constituents, to obtain a more accurate estimate. Contributions from four broad types of dietary supplement were also obtained using data from MAFF, and from manufacturers. Se intake was examined according to several socio-demographic factors (age, sex, region, season, social class, educational attainment, household income and composition, state benefits, smoking and drinking habits, and consumption of Se-containing dietary supplements).

Se intakes for men and women were 47 (18) and 37 (16) μg/d respectively (mean (SD), $P<0.001$, ANOVA). Se intake (whether expressed in terms of μg/d, μg/4.18 MJ or μg/kg body weight/d) declined significantly with age, in both men and women. Omission of possible under-reporters (energy intake:calculated BMR <1.2) had little impact on results.

	Men				Women			
	65–74 y (n 254)	75–84 y (n 245)	85+ y (n 83)	$P*$	65–74 y (n 224)	75–84 y (n 197)	85+ y (n 149)	$P*$
Se intake (μg/d)	51[a] (20)	45[b] (15)	40[b] (15)	<0.001	41[a] (21)	36[a] (12)	33[b] (13)	<0.001
Se intake <LRNI[§] (%)	30[a]	40[b]	64[c]	<0.001	57[a]	67[ab]	74[b]	0.02
Se intake (μg/4.18 MJ)	26[a] (10)	25[ab] (9)	24[b] (7)	<0.001	28[a] (14)	26[b] (9)	24[b] (9)	0.01

Values are arithmetic means (SD), except for %. *Adjusted for other socio-demographic factors. Different superscript letters indicate significant differences between age groups, in men and women respectively ($P<0.05$; Scheffé test for continuous variables, following ANOVA; χ^2 test for discontinuous proportions, following multiple logistic regression). §40 μg/d (Department of Health, 1991).

Se intake was not significantly related to region or season, in men or women, but was significantly lower in those of manual social class or with no educational qualifications, after adjustment for other socio-demographic factors. Male smokers tended to have lower Se intakes than non-smokers, as did men living alone compared to those living with others. Cereals, meat and fish contributed most to Se intake (26, 22 and 21% respectively) with no sex differences. Of cereals and cereal products, breads alone contributed around 13%, while milk and vegetables provided 11 and 8% respectively. Overall, dietary supplements contributed <0.5% to Se intake, although significant differences in intake were noticed between groups consuming and not consuming Se-containing supplements.

These data show significant differences in Se intake according to a number of socio-demographic factors and a high proportion of older British people with daily intakes below the Government's recommended levels (95% <RNI, 52% <LRNI). If these findings are reflected in sub-optimal status, then higher Se intakes may be desirable in the elderly.

Department of Health (1991) *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects No. 41.* London: HMSO.
Finch S, Doyle W, Lowe C, Bates CJ, Prentice A, Smithers G & Clarke PC (1998) *National Diet and Nutrition Survey: People aged 65 years and over. Volume 1: Report of the Diet and Nutrition Survey.* London: The Stationery Office.
Rayman MP (1997) *British Medical Journal* **314**, 387–388.

Implications of reduced red and processed meat consumption for iron intakes among British women. By S. GIBSON[1] and M. ASHWELL[2], [1] *Woodway, Merrow, Guildford GU1 2TF*, [2] *Ashwell Associates, Ashwell St, Ashwell, North Hertfordshire, SG7 5PZ*

Red meat (beef, lamb and pork) is a rich source of highly bioavailable haem Fe, but its consumption appears to be in long-term decline. The COMA Report on Cancer (Department of Health, 1998) concluded that a reduction in red and processed meat (RPM) consumption may reduce the risk of colorectal cancer. Although the report suggested that only high consumers need reduce their consumption of RPM, this is likely to be misinterpreted as general advice to eat less red and processed meat.

We estimated the likely impact on Fe intakes of reductions in RPM of up to 50% compared with the baseline diet in 1986–7 (Gregory *et al.* 1990). We assumed two scenarios: (1) no replacement by other foods, or (2) isocaloric replacement of RPM by poultry and poultry products. Mean RPM was 90 g/d (71 g/d among women, 110g/d among men), calculated from estimates of the meat content of the ten meat groups in the Dietary and Nutritional Survey of British Adults (Gregory *et al.* 1990).

In 1986–7, the prevalence of low Fe intakes among women, defined as below the LRNI (8.0 mg/d under 50 years, 4.7 mg/d over 50 years), was 20% (Table). This ranged from 25%, among non-consumers of RPM, to 8% among women consuming >140 g/d, a difference mirrored in the Fe status indices (ferritin and haemoglobin). These showed a high prevalence of low Fe stores (24%) and anaemia (33%) among non-consumers of RPM.

A 50% reduction in RPM consumption would theoretically reduce mean Fe intake by about 9%, from 10.5 mg/d to 9.6 mg/d. The prevalence of low Fe intakes would rise to 29%, with a greater relative impact in high consumers of RPM. More realistically, if the missing RPM were substituted isocalorically by poultry and poultry products, this would replace about half the deficit in Fe (mean intake 9.9 mg/d) and result in 25% of all women having low Fe intakes. In relative terms these changes are modest, but represent a significant reduction in haem Fe. Given the marginal Fe status of many women, any reduction may have relevance for nutrition policy and advice. The intake of RPM is already estimated to have declined by 25% since 1986 (MLC data).

Women aged 16–64 years	Non-consumers (n 32)	<90 g/d (n 766)	90–140 g/d (n 233)	>140 g/d (n 79)	Total (n 1110)
Percentage of women with:					
Fe<LRNI @ baseline RPM (1986–7)	**25**	**23**	**12**	**8**	**20**
Fe <LRNI @ RPM less 25%	25	27	19	14	24
Fe <LRNI @ RPM less 25% + poultry	25	26	15	11	22
Fe <LRNI @ RPM less 50%	25	30	27	24	29
Fe<LRNI @ RPM less 50% + poultry	25	28	20	14	25
Iron status (1986–7)					
% with ferritin <12 μg/l	24	13	17	5	14
% with haemoglobin <120 g/l	33	12	14	6	13

Department of Health (1998) *Nutritional Aspects of the Development of Cancer. RHSS 48.* London: The Stationery Office.
Gregory J, Foster K, Tyler H & Wiseman M (1990) *The Dietary and Nutritional Survey of British Adults.* London: HMSO.

The Mediterranean diet in Australia: sustainability issues. By S. SOMERSET[1] and D. COLQUHOUN[2] for the OLIVE study investigators, [1]School of Health Science, Gold Coast Campus, Griffith University, PMB 50, Gold Coast Mail Centre, Qld 9726, Australia, [2]University of Queensland, Brisbane, Australia

The Mediterranean diet (MD) describes traditional dietary patterns bordering the Mediterranean Sea. Pasta, coarse breads, beans, nuts, seeds, olive oil, wine and seasonal fruits and vegetables predominate. Importantly, the MD is sustained by cultural and geographic equilibrium developed over centuries, making the diet readily accessible (Gussow, 1995). The MD may be useful for coronary heart disease prevention in non-Mediterranean countries such as Australia. We are currently comparing the effects of the MD versus a low-fat diet on the progression of atherosclerosis using coronary angiography in 180 patients (Colquhoun et al. 1998). The results of this and other studies may increase the popularity of the MD in Australia.

Prescriptive interpretation of MD simply transfers lists of foods to other regions, without reference to climatic, agricultural or social factors. Currently, it would be difficult for the Australian food system to meet widespread MD adoption. For example, requirements (t per annum) of some foods for a Cretan 1960s MD in Australia are:

	1° prevention (13.6 million people)	2° prevention (500 000 people)	Current production (t per annum)*
Fats/oils	175 000 t	6 400 t	Olive oil 842, rapeseed oil 557 000
Fish	136 000 t	5 000 t	Fish 127 000, beef 1 700 000
Fruit	2 000 000 t	73 000 t	Orange 442 000, apple 280 000, banana 220 000

*From Agriculture, Australia: 1997–8.

The above figures treat Australia as a single geographic region. Despite its size, Australia's food distribution is centralized, functioning as a single food system. Consuming local foods locally becomes problematic under these circumstances. However, some foods (e.g. wine, cheese, fish, fruit) are grown only in certain parts of Australia, and public recognition of regionality of food is emerging. Local edible oil production presents a particular challenge.

In Australia, an extensive programme of olive tree plantings is underway. There is a 5-year lag between planting and first harvest, so that in 5-10 years time far more olive oil will be available locally. However, this position will depend on how widespread the adoption of a MD becomes. We have based our extreme scenario on the total adult population of 13 million people adopting this diet, and in this case even current planting rates would not meet such a local demand. Even for secondary prevention of CHD (500 000 individuals), current olive oil production is insufficient.

If, however, canola oil is deemed equivalent to olive oil as a suitable oil source for the prescriptive Mediterranean diet, then current production levels are far in excess of even the primary prevention requirements above. This raises other issues. These oils are more than just mixtures of fatty acids. While oleic acid predominates in both, there is a range of non-nutrient components which induce physiological effects (Visioli & Galli, 1998). These oils may differ in terms of health impact in general, and in terms of effects on CHD risk in particular.

Social, cultural and environmental contexts of the MD are important. Although some parts of Australia enjoy a typical Mediterranean climate, agricultural profiles are diverse. Climatic conditions range from tropical in the north, desert in the centre to temperate in the south, with many other variations interspersed. Australia probably needs a series of regional diets to address these contextual aspects. Widespread adoption of MDs, defined by local culture, environment and society, is feasible in Australia. The specific details of population dietary recommendations needs further development.

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Colquhoun DM, Somerset SM, et al. (1998) Australian Journal of Nutrition and Dietetics 55 (Suppl.), S24-29.
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Visioli F & Galli C (1998) Nutrition Reviews 56, 142-147.

The evidence for high meat intake during the evolution of hominids. By N.J. MANN, Department of Food Science, RMIT University, Melbourne, 3001, Australia

For a number of years our research group have conducted dietary intervention studies examining the health aspects of meat in the diet. The interest in this area resulted from our studies in the mid 1980s dealing with Australian aborigines returning to their natural diets. These studies revealed striking health improvements in these subjects, including dramatic reductions in plasma cholesterol levels when consuming large quantities of wild game meats (O'Dea, 1991). These studies have prompted a review of the knowledge in the area of evolution and food consumption patterns. A clear role for lean red meat in a healthy balanced diet becomes evident as the diet history of our species is uncovered.

In recent times anthropological investigation has revealed many new insights into the evolution of human diet and specifically the role of meat consumption. In this review, the aim was to establish the extent of meat consumption during the evolution of the human diet, by reference to a number of fields of scientific evidence.

Findings show clear cranio-dental changes including, a decrease in molar teeth size, jaws/skull becoming more gracile and front teeth becoming well-buttressed, all indicative of less emphasis on grinding and more on biting and tearing (Eaton et al. 1997). Carbon isotope studies indicate the incorporation of C4 grasses, undoubtedly in the form of meat from herbivorous animals, at a level which increased substantially during the progression of our genus from A. aferensis to H. sapiens. Even as far back as 3.5 million years ago, the Sr:Ca ratio falls in between those typical for herbivores and carnivores, possibly indicative of scavenging (Sponheimer & Lee-Thorp, 1999). Gut morphology studies indicate a closer structural analogy with carnivores than the folivorous or frugivorous mammals (Martin, 1992) and even considerable difference to other primates (see Table).

Relative gut volume proportions for some primate species (percentage of total volume).

Species	Stomach	Small Intestine	Caecum	Colon
Gorilla	25	14	7	53
Orangutan	17	28	3	54
Chimpanzee	20	23	5	52
Gibbon	24	29	2	45
Human	17	67	na	17

(Adapted from Milton, 1986)

Energetic requirements of a relatively enlarged brain have been balanced by reduction in size and energy requirement of the digestive system, a phenomena requiring a high quality diet typical of meat consumption (Aiello & Wheeler, 1995). Investigation of food procurement habits of hunter-gatherer societies indicates the advantage of hunting of game animals compared with plant foraging in terms of energy gain versus expenditure (Hawkes et al. 1982). Investigation of the dietary habits of unacculturated hunter–gatherer societies in modern time, as an approximation of paleolithic practices, has shown a high reliance on animal foods compared with plant foods for basic energy requirements. An approximate subsistence ratio of 65:35 (animal:plant) has been recently determined (Cordain et al. 2000). Study of macronutrient proportions in the diet show a clear relationship between high protein content and the evolution of insulin resistance as a survival advantage, compared with the current incidence of NIDDM on diets higher in carbohydrate and lower in protein (Brand-Miller & Colagiuri, 1994).

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Validity of assessment of fruit and vegetable consumption using a simple cross-check question. By A. GREENHALGH and J. CADE, *Nutritional Epidemiology Group, Division of Public Health, Nuffield Institute for Health, 71–75 Clarendon Road, University of Leeds, Leeds LS2 9PL*

Large epidemiological studies often use food frequency questionnaires in the estimation of fruit and vegetable consumption. There is evidence that food frequency questionnaires can over-estimate total fruit and vegetable intake (Feskanich *et al.* 1993), with the inclusion of more items in the questionnaire leading to a greater tendency to over-estimate intakes. A simple cross-check question such as "what is your weekly number of servings of fruit" is often used as a weighting factor to improve estimation of total weekly fruit and vegetable intakes (Calvert *et al.* 1997). However there is little data reporting what fruit and vegetable items subjects consider when using such a question.

A sub-sample of 208 women from the UK Women's Cohort Study (Woodhouse *et al.* 1997) were contacted in a brief telephone survey. Subjects were first asked how many portions of fruit and vegetables were consumed in a typical week. Definition of a portion was self-assessed by the subjects. This was checked against a list of possible fruits (nine items) and vegetables (twenty-one items) to determine whether these items were, firstly, consumed on a regular basis and, secondly, whether they were included in the count. Finally subjects were asked to recalculate weekly intakes taking into account food items that they may have omitted.

Mean intakes of fruit and vegetables were found to be 14 (SD 9) and 16 (SD 12) portions per week respectively. From the twenty-one vegetable items listed, 35% of subjects had missed key items consumed on a regular basis (range 1–15), and for the fruit 33% (range 1–9). Of these, fruit juice was not included by 36% of subjects, canned fruit by 33%, or dried fruit by 35% of the subjects in the count. Vegetables reflected a similar pattern. The most common vegetable items omitted were salad in sandwiches or in a side salad, and vegetables in soup, or vegetables in bakes or casseroles and pasta dishes.

There was no change in the mean weekly vegetable intake following recalculation. However there was an increase in mean weekly fruit intake from 14 to 17 (SD 10) portions per week following recalculation, although this was not statistically significant. Furthermore 69% of subjects claimed to take into account seasonal variation when estimating their fruit and vegetable intakes and by doing so would try and make the estimation representative of the whole year's intake.

Results from this study highlight the fact that many fruit and vegetable items are not included in a simple cross-check question despite being consumed on a regular basis. However when subjects were asked to recalculate weekly consumption of fruit and vegetables including key items previously omitted, the total weekly intakes of both fruits and vegetables varied only with the fruit, and the difference between the original and recalculated estimates of fruit and vegetable intakes were not found to be statistically significant. Thus estimates of fruit and vegetable intakes using this simple method may be used with reasonable confidence, especially when used to identify potential mis-reporting of specific food groups on food frequency questionnaires.

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Feskanich D, Rimm EB, Giovannucci EL, Colditz GA, Stampfer MJ, Litin LB & Willett WC (1993) *Journal of the American Dietetic Association* **7**, 790–796.

Woodhouse A, Calvert C & Cade J (1997) *Proceedings of the Nutrition Society* **56**, 64A.

Measuring the fat and energy content of the family diet: how do supermarket receipts compare with the four-day weighed food intake method and a food frequency questionnaire? By J.K. RANSLEY[1], J.K. DONNELLY[1], H. BOTHAM[1], T.N. KHARA[1], H. ARNOT[1], J.E. CADE[2] and D.C. GREENWOOD[2], [1]*The Public Health Nutrition Unit, Trinity and All Saints, University of Leeds, Brownberrie Lane, Horsforth, Leeds LS18 5HD,* [2]*Division of Public Health, Nuffield Institute for Health, The University of Leeds, 71–74 Clarendon Road, Leeds LS2 9PL*

Food frequency questionnaires and permutations of the food diary method are well-established ways of recording the food intake of individuals. Each method has distinct advantages and disadvantages, which have been well documented elsewhere (Margetts & Nelson, 1997). When it comes to recording the food consumption of households, family budget surveys such as the National Food Survey (NFS) use a diary method which involves the diary keeper in the manual recording of foods purchased. Household budget surveys use food purchases as a proxy measure of population intake of nutrients. Approximately 90% of the population now purchase most of their foods from supermarkets, which issue itemized receipts at the point of sale. These receipts provide a prospective list of food and drink purchased, together with their price. Using this information, together with databases provided by Tesco Stores Ltd, a novel procedure was developed to establish the fat and energy composition of foods purchased from supermarkets.

214 households were recruited from a random sample of Tesco Clubcard members in Leeds. Itemized supermarket receipts were collected for a period of 28 d to estimate household food purchases. Members of each household completed a 4-d weighed intake (WI) and a food frequency questionnaire (FFQ) to estimate household dietary intake of fat and energy (Tinuviel Software, Warrington, UK).

	Itemized receipts				WI				FFQ			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
% energy from fat	11.81	53.26	35.89	7.02	9.82	46.75	34.02	5.71	23.12	49.01	36.06	4.51
Daily purchase or intake fat (g)	22	496	185	94	18	511	190	102	39	978	248	148
Daily purchase or intake (MJ)	4.18	49.19	19.16	8.75	4.84	51.18	20.65	10.19	5.04	96.05	25.79	14.78

Mean household size = 2.4 people

The Table compares the two measures of household energy and fat intake i.e. 4-d weighed food intake (WI) and food frequency questionnaire (FFQ) with the estimate of household fat and energy composition of food purchased, using supermarket receipts. The figures represent mean values and SD for each of the three measures of percentage energy from fat, daily intake of fat (g) and energy (MJ). There is better agreement between the measurement of these dietary variables by WI and till receipts than with the FFQ. In this case the comparison of means shows the FFQ method to over-report fat and energy consumption of individual households. These data are slightly lower than recent figures from the NFS which show 38.8% of energy in the diet is derived from fat (Ministry of Agriculture, Fisheries and Food, 1999).

Margetts B & Nelson M (1997) *Design Concepts in Nutritional Epidemiology.* Oxford: Oxford University Press.

Ministry of Agriculture, Fisheries and Food (1999) *National Food Survey, 1998. Annual Report on Household Food Consumption and Expenditure.* London: HMSO.

Systematic review of the validation of food frequency questionnaires. By D.L. WARM[1], V.J. BURLEY[2], R.L. THOMPSON[1], B.M. MARGETTS[1] and J.E. CADE[2], [1]Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD, [2]Nuffield Institute for Health, University of Leeds, Leeds LS2 9JT

Food frequency questionnaires (FFQ) have been widely used in epidemiological research. Although they offer considerable advantages in terms of ease of administration and analysis, they may be limited in their usefulness and, if not designed and used appropriately, may not yield the required information. The results presented are part of a systematic review on the design, validation and utilization of FFQs.

Studies were identified by computerized searches of databases including MEDLINE, EMBASE, CANCERLIT, CAB Abstracts and Dissertation Abstracts, and were English-language based. Searching also included hand searches of conference proceedings, key journals and reference lists of retrieved articles. Search terms used were based on 'food frequency questionnaires', 'reproducibility', 'validity/validation' and 'calibration' and covered the period 1980–99.

The searches yielded 1982 references of which 779 papers were obtained. The references included review articles and a number of publications from the same studies. Finally 227 studies were identified (196 single studies and thirty-one groups of studies) and data were extracted.

The 227 studies originated from thirty different countries, with the most from the USA (n 102) and 183 of the studies had samples drawn from the general population. The review also showed that 54% of the validation studies used a modified version of a previously developed questionnaire. Of the 104 questionnaires adapted from previous FFQs, twenty-six were adapted from the Block questionnaire (Block *et al.* 1986) and twenty-eight were adapted from the Willett questionnaire (Willett *et al.* 1985).

Results showed that 171 (75%) of FFQs were validated against another dietary assessment method, 43 (19%) against a biomarker(s) and 27 (12%) against another method (e.g. doubly-labelled water or energy expenditure studies).

Method of validation	Number (%)
Dietary assessment only	141 (62)
Biomarker(s) only	17 (7)
Other method only	10 (4)
Dietary assessment and biomarker(s)	16 (7)
Dietary assessment and other method	7 (3)
Biomarker(s) and other method	3 (1)
All (dietary assessment, biomarker(s) and other method)	7 (3)
Method not stated	26 (11)

The review showed that a variety of dietary assessment tools were used as a reference measure. The weighed record was used by 56 (25%) studies; a food record/diary (not including weighed diaries) by 59 (26%); a 24-h recall by 50 (22%) studies; a diet history questionnaire by 14 (6%) and another food frequency questionnaire by 27 (12%) studies. One hundred and forty-four validation studies used only one dietary assessment methodology as the reference method (14 another FFQ, 43 weighed record, 29 24-h recall, and 58 either a food record or diet history questionnaire). Seven validation studies used both the weighed record and 24-h recall as reference methods.

The impact that the use of different (and multiple) reference measures has on the interpretation of the relative validity of the FFQ needs to be carefully considered, bearing in mind the purpose and timeframe for which the FFQ has been designed.

Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J & Gardner L (1986) *American Journal of Epidemiology* **124**, 453–469.

Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH & Speizer FE (1985) *American Journal of Epidemiology* **122**, 51–65.

The 24-h recall: a combined qualitative and quantitative nutrition research tool. By P.M. KENYON[1], P. NEWTON[2] and M.E. BARKER[1] [1]University of Sheffield, Centre for Human Nutrition, Northern General Hospital, Sheffield S5 7AU, [2]University of Sheffield, Institute of General Practice and Primary Care, Northern General Hospital, Sheffield S5 7AU

There is a need to understand food and nutrition as a phenomenon within the context of peoples' lives. In the naturalistic paradigm this is termed the emic or insider's perspective. Specific qualitative methods for use in nutrition and diet-related research and assessment are needed. The 24-h recall method is an established research tool that is conventionally used to obtain quantifiable dietary intake information. We report here on the development of a qualitative nutrition research tool using the 24-h recall method as a framework.

This study was a component of a community nutrition intervention involving adult, type II diabetes and/or cardiovascular disease patients living in a deprived inner city area of Sheffield, South Yorkshire, England. The 24-h recall method was used to produce quantitative dietary information and as the introductory component of semi-structured, depth interviews using descriptive questioning techniques of the type advocated by Spradley (1979). A total of eight patients participated in the first instance, and provided a total of fifteen tape-recorded, transcribed depth-interviews, twelve of which contained transcribed 24-h recalls. Transcripts were analysed using the first step of the interpretative phenomenological analysis (IPA) method espoused by Smith *et al.* (1999).

Typical influences on dietary behaviour emerged, often almost by default, as the previous day's intake was described. Therefore, it was possible to simultaneously generate quantitative and qualitative data. The following are examples of qualitative data contributed during description of the previous day's dietary intake:

After that, cup o' tea, roughly about four o'clock, and then I start to prepare 't tea for when my missus comes home from work, she gets home about half past six.

No, home made, missus made it, home made pancake.

Milk, yeah. Plenty o' milk, 'cause I love milk.

Like Eric'll say, oh, this one's buy one get one free and I'll say; we're not 'avin' any, you know, I'll say we don't want two lots like.

I usually go to me friend's on a Thursday……she did lunch about one o'clock and we had Salmon Mornay; weight watcher's, the packet, that you just have to slip int't oven.

Round about half past twelve time, depends what I'm watching on the telly. Has to fit in with me television programmes and I think right, I'll get it all made ready and I'll sit there and watch me programmes. Everything runs round that. Right, so this week I've been havin' fish sandwiches.

From a full analysis of the interview transcripts, the themes of *the family* and *eating familiar things* were the two predominant issues determining food choices. The same two issues were evident from qualitative analysis of the 24-h recalls. These data provide an easily obtainable insight into factors that are likely to prevent or promote dietary change. This method, facilitating the simultaneous generation of qualitative and quantitative data, would be valuable to workers in both clinical and research contexts.

Smith JA, Osborn M & Jarman M (1999) In *Qualitative Health Psychology: Theories and Methods.* London: Sage Publications.

Spradley JP (1979) *The Ethnographic Interview.* London: Harcourt Brace Jovanovich.

Dietary restraint and the mis-reporting of anthropometric measurements by middle-aged adults. By A. CULLUM, D. GUNNELL, G. DAVEY SMITH and Y. BEN-SHLOMO, *Department of Social Medicine, University of Bristol, Whiteladies Road, Bristol, BS8 2PR*

The mis-reporting of anthropometric measures is a common problem in epidemiological research, and has been shown to vary with body size, age, sex and education (Nieto-Garcia et al. 1990; Rimm et al. 1990). Dietary restraint has previously been associated with mis-reporting of dietary intakes in women (Bingham et al. 1995) but, to our knowledge, its association with mis-reporting of anthropometric measures by middle-aged adults has not been assessed.

As part of a cohort study investigating the development of adiposity in young adults, the ability of cohort member's parents to accurately report body mass index (BMI), waist and waist:hip ratio (WHR) was assessed. All parents (n 631) were mailed a questionnaire and asked to record their height, weight, waist and hip circumference. A paper tape measure with instructions for use was attached. Parents also completed the restraint section of the Dutch Eating Behaviour Questionnaire (van Strien et al. 1986), and provided information on smoking and employment. 435 mothers (69%) and 332 fathers (55%) completed the questionnaire. A sample of parents was then invited to attend a clinic where detailed anthropometric measures were taken; of those invited, 182 (85%) mothers (aged 51.9 (SD 4.29) years) and 102 (61%) fathers (age 54.7 (SD 5.6) years) attended. Mean self-reported and measured values (SD) are shown in the table below.

		$BMI (kg/m^2)$	Waist (cm)	WHR	Dietary restraint score
Females	Self-report	26.5 (4.63)	81.1 (12.49)	0.79 (0.074)	2.7 (0.95)
	Measured	27.5 (5.09)	86.2 (13.12)	0.84 (0.079)	
Males	Self-report	26.6 (3.43)	93.3 (9.05)	0.91 (0.062)	2.1 (0.88)
	Measured	27.5 (3.86)	97.0 (10.71)	0.97 (0.065)	

Pearson's correlation coefficients between measured and self-reported values were high for BMI (0.94 for both parents), moderate for waist (mothers 0.81, fathers 0.84) and low for WHR, particularly for fathers (0.68 and 0.49, respectively). As expected, height tended to be over-reported whereas weight, waist and hip circumference were under-reported. With the exception of height, mis-reporting was positively associated ($P<0.05$) with the measured value, suggesting that self-reported values were systematically biased. Mean maternal under-reporting of BMI increased across tertiles of restraint score from -0.9 to -1.5 kg/m^2 (P 0.02). This trend was not found in fathers. Paternal under-reporting of waist, hip and WHR was lowest in the lowest tertile of restraint score, but this was not significant.

Multivariable regression analyses showed that height, social class, smoking and the time difference between self-report and clinic measurement were not associated with mis-reporting. Dietary restraint score was independently associated with mis-reporting of BMI by mothers (P 0.01) but not by fathers. However, a formal test of interaction between sex and restraint on the mis-reporting of BMI was not significant. Restraint was associated with maternal mis-reporting of waist and paternal mis-reporting of WHR in univariable analyses but these associations were attenuated with the addition of BMI to the models. The body size or restraint score of one parent was not associated with mis-reporting by the other.

In conclusion, dietary restraint score may be a useful tool for identifying individuals more likely to mis-report anthropometric measures, although associations may vary by gender.

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Nieto-Garcia FJ, Bush T & Keyl P (1990) *Epidemiology* **1**, 146–152.
Rimm EB, Stampfer MJ, Colditz GA, Chute C, Litin L & Willett WC (1990) *Epidemiology* **1**, 466–473.
van Strien T, Frijters JER, Bergers GPA & Defares PB (1986) *International Journal of Eating Disorders* **5**, 295–315.

Characteristics of under- and over-reporters from the UK Women's Cohort Study (UKWCS). By L. BUCKLEY, D.C. GREENWOOD and J.E. CADE, *Nuffield Institute for Health, University of Leeds, 71–75 Clarendon Road, Leeds LS2 9PL*

In recent years the issue of under-reporting has been highlighted as a major source of bias in studies, where self-reported dietary intakes are relied upon for their accuracy (Smith et al. 1994). Comparatively few studies have identified over-reporters of dietary intake and Goldberg's work on cut-off limits made no measure of an upper range of cut-off limits (Goldberg et al. 1991). This study aimed to explore the issue of potential under- and over-reporting in the first 17 500 female respondents aged 35–69 in the UKWCS, using a baseline Food Frequency Questionnaire (FFQ). The women were divided into ten equal groups according to their EI:BMR (ratio of energy intake to basal metabolic rate) using a previous FFQ collected at baseline. Thirty subjects were selected from each of the following deciles: the bottom decile (under-reporters), the fifth decile (normal reporters) and the top decile (over-reporters). A second, repeat FFQ, Health and Lifestyle Questionnaire (H&LQ) and a Dutch Eating Behaviour Questionnaire (DEBQ) were mailed to all subjects.

Mean (95% CI) values for various characteristics.

Character variable	Under-reporters (n 22)		Normal reporters (n 20)		Over-reporters (n 17)		P value
Age (years)	54	(51–58)	54	(50–58)	53	(48–59)	0.94
BMI (kg/m^2)	26	(24–29)	27	(24–30)	23	(22–25)	0.10
BMR (MJ)	6.00	(5.7–6.2)	6.10	(5.8–6.4)	5.70	(5.5–5.9)	0.05*
EI:BMR	1.16	(1–1.3)	1.52	(1.3–1.7)	2.31	(2.0–2.6)	0.001*
Weight (kg)	71	(63–79)	71	(63–79)	60	(63–71)	0.05*
Height (m)	1.64	(1.6–1.67)	1.64	(1.6–1.67)	1.60	(1.56–1.64)	0.13
Vegetarian (%)	5	(23%)	8	(40%)	9	(53%)	0.15
Dietary supplement use (%)	10	(50%)	8	(42%)	7	(50%)	0.86
Alcohol consumers (%)	18	(82%)	14	(70%)	12	(71%)	0.62
Daily smoking habit (%)	2	(10%)	3	(15%)	2	(12%)	0.89

Where numbers do not add up to the total, this is because of missing data.

The response rate was 66% (n 59) and the mean age of all subjects was 54 years. Mean weights for under- and over-reporters were 71 kg and 60 kg, whilst mean BMIs were 26 kg/m^2 and 23 kg/m^2 respectively. In contrast, the mean BMI (27 kg/m^2) for normal reporters was greater than under- and over-reporters. Restraint scores calculated from the DEBQ for under- and over-reporters were 3.1 and 2.2, whilst the mean score for normal reporters was 2.9. In comparison, the mean restraint score for combined Dutch females was 2.2 (n 1169), the scores increased to 2.66 (n 114), when non-obese subjects were excluded from analysis (van Strien et al. 1986). The under-reporters consumed bread, milk and potatoes as major stated energy sources, whilst the over-reporters reported chocolates and sweets as high contributors of energy. Under-reporters were less likely to be vegetarian and more likely to consume alcohol than the normal and over-reporters.

Results from this study showed that over-reporters have significantly different characteristics from normal and under-reporters. Under-reporters are similar to normal reporters in terms of lifestyle characteristics. This study identified under-, normal and over-reporters, using an original approach which avoids using an arbitrary cut-off value.

Goldberg GR, Black AE, Jebb SA, Cole TJ, Murgatroyd PR, Coward WA & Prentice AM (1991) *European Journal of Clinical Nutrition* **45**, 569–581.
Smith WT, Webb KL & Heywood PF (1994) *Australian Journal of Public Health* **18**, 311–314.
van Strien T, Frijters IER, Bergers GPA & Defares PB (1986) *International Journal of Eating Disorders* **5**, 295–315.

The consumption of fruits and vegetables by Scottish children of primary school age. By E. FOSTER[1], A.J. ADAMSON[1], L.E.G. PORTEOUS[2], C. HIGGINS[3], M.M. HETHERINGTON[3] and A.S. ANDERSON[2], [1]Human Nutrition Research Centre, University of Newcastle, Newcastle upon Tyne NE1 7RU, [2]Centre for Applied Nutrition Research, [3]Psychology Department, University of Dundee, Dundee DD1 4HN

There is increasing evidence that the quality of food intake in childhood is important for health in adult life (Berenson et al. 1998; Gaziano, 1998). Intake of fruit and vegetables is particularly important as they are rich sources of vitamins and a range of components with antioxidant properties and have been associated with a reduced risk of developing heart disease and cancer (Department of Health, 1998). Current guidelines are for both adults and children to consume at least five portions of fruit and vegetables (excluding potatoes) daily, equivalent to 400 g (Department of Heath, 1998).

This study aims to develop a whole-school-based intervention to increase fruit and vegetable intake. As part of baseline measurements 3-d food diaries were collected from 175 children aged 5–11 years. Each child attended a detailed interview during which food weights were estimated using food photographs (MAFF, 1997) and by gathering information on brands and packaging of foods consumed. From these food diaries, frequency of consumption and estimated weights of fruits and vegetables eaten were determined. The 'top five' vegetables and fruits by frequency of consumption during the 525 days recorded are presented in the Table along with average estimated portion size.

Vegetable	Frequency	Portion (g)	Fruit	Frequency	Portion (g)
Baked beans	100	114	Apples	178	142
Tomatoes	67	37	Orange juice	139	223
Carrots	53	41	Bananas	88	129
Lettuce	51	19	Oranges	45	155
Peas	50	57	Apple juice	37	214

Baked beans, which were included as vegetables as they are often eaten as such by children, were the most popular vegetable both in terms of frequency and amount eaten. Apples, bananas and oranges were the most popular fruits, with orange and apple juices also featuring in the top five. The children recorded eating fruit or vegetables on 1240 occasions. Of these, 635 were fruit and fruit juices. On average, fruit or vegetables were consumed 2.4 times per day. The approximate mean daily intake was 256 g, 64% of the recommended 400 g, 70% of this intake was from fruit or fruit juices. This high fruit compared with vegetable intake may be because a large proportion of children's intakes is derived from snacks (Hurren & Stockley, 1987) and while fruits are easy snack items, vegetables are usually only consumed as part of a meal and are less accessible as snack items. Previous work with adults (Cox et al. 1998) showed that, as the result of an intervention, increased consumption of fruit and vegetables was primarily due to an increase in fruit intake. The current intervention will explore opportunities to promote vegetables and fruits both as snacks and as a part of main meals to primary school children.

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MAFF (1997) A Photographic Atlas of Food Portion Sizes. London: MAFF Publications.

The North/South Ireland Food Consumption Survey 2000: Dietary fibre intakes of Irish adults. By M. GALVIN[1], M. KIELY[1], A. FLYNN[1], K.E. HARRINGTON[2], M.J. GIBNEY[2], P.J. ROBSON[3], M.B.E. LIVINGSTONE[3] and J.J. STRAIN[3], Irish Universities Nutrition Alliance: [1]University College Cork, [2]Trinity College Dublin, Republic of Ireland, [3]University of Ulster, Northern Ireland

The North/South Ireland Food Consumption Survey 2000 has established a database of habitual food and drink consumption in a representative sample of Irish adults aged 18–64 years. Food intake data were collected using 7-d estimated food records and processed using Wisp[β] Tinuviel Software (Warrington, UK), which contains McCance and Widdowson's The Composition of Foods, 5th ed. (Holland et al. 1995). The dietary fibre intakes (dietary fibre (DF) measured by the Southgate method and non-starch polysaccharide (NSP) measured by the Englyst method) of men (n 475) and women (n 483) from the Republic of Ireland are presented.

Sex	Age category	n	Mean DF intake (g/d)	(SD)	Mean NSP intake (g/d)	(SD)	Mean DF intake (g/MJ/d)	(SD)	Mean NSP intake (g/MJ/d)	(SD)
Males	18–35 y	169	22.7	(8.7)	16.0	(6.8)	1.93	(0.60)	1.35	(0.47)
	36–50 y	178	23.8	(7.9)	17.6	(6.8)	2.15	(0.68)	1.59	(0.63)
	51–64 y	128	24.4	(9.2)	18.0	(7.4)	2.37	(0.67)	1.76	(0.58)
All	18–64 y	475	23.6	(8.5)	17.1	(7.1)	2.13	(0.67)	1.55	(0.59)
Females	18–35 y	167	16.4	(5.2)	12.0	(4.2)	2.11	(0.62)	1.55	(0.53)
	36–50 y	201	18.4	(6.0)	13.8	(5.2)	2.35	(0.66)	1.77	(0.62)
	51–64 y	115	17.6	(5.7)	13.8	(5.2)	2.50	(0.69)	1.96	(0.65)
All	18–64 y	483	17.5	(5.7)	13.2	(4.9)	2.30	(0.67)	1.74	(0.62)
Total	18–64 y	958	20.5	(7.9)	15.1	(6.4)	2.22	(0.70)	1.64	(0.60)

Overall, men consumed more fibre than women; however, when examined in relation to energy intake (i.e. g/MJ/d), no difference was observed. This indicates that fibre intake was related to the food intake.

The mean daily NSP intake of 24% of men and 46% of women was below the minimum individual intake of 12 g recommended by the UK Department of Health (1991). Eighty-nine percent of men and 88% of women had mean daily dietary fibre intakes below the recommended 3 g/MJ (Netherlands Food and Nutrition Council, 1986; Sandstrom et al. 1996).

The food groups that contributed most to the mean daily intakes of DF (NSP) in the total sample were: bread and rolls 31% (24%), potato and potato products 20% (23%), vegetable and vegetable dishes 16% (18%), fruit, juice, nuts, seeds, herbs and spices 8% (8%), breakfast cereals 7% (8%), biscuits, cakes and pastries 7% (5%).

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Holland B, Welch AA, Unwin ID, Buss DH, Paul AA & Southgate DAT (1995) McCance & Widdowson's The Composition of Foods, 5th edn. London: Royal Society of Chemistry / Ministry of Agriculture, Fisheries and Food.
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Gender differences in dietary habits in a population of primary school children. By P.M.L. SKIDMORE[1], J.W.G. YARNELL[1], G.E. McGEOUGH[1], J. McMAHON[2], M. SHIELDS[3], A. SCOTT[1] and A. EVANS[1], Departments of [1]Epidemiology and Public Health, [2]Respiratory Medicine, [3]Child Health, The Queen's University of Belfast, Belfast BT12 6BJ

In most countries in the Western world obesity is a serious emerging problem, not only in adults but also in children (Freedman et al. 1999). In 1996 we found the prevalence of obesity (BMI μ30 kg/m²) to be 1.5% in boys and girls aged 13 and 14 years (n 2305) across Northern Ireland (Yarnell et al. 1997). In this present study we measured the height and weight of 1468 boys and 1271 girls aged 6–8 years in a stratified random sample of primary schools in Northern Ireland. We also assessed whether there were any gender differences in dietary intake, and the relationship of BMI and parents' socio-economic status with food intake. Qualitative food intake questionnaires were completed by the children's parents. We find that in comparison with data from the UK population (Cole et al. 1995) that the children were of similar height but higher in weight.

Age	Height as a percentage of UK median value		Weight as a percentage of UK median value	
	Boys	Girls	Boys	Girls
6	100.6	100.3	104.1	104.2
7	100.9	100.9	107.0	106.2
8	100.3	100.1	105.3	105.9

Diets were generally poor. Almost half of the children ate ≤1 portion of fruit per day and 44% ate crisps or savoury snacks six or seven times a week Consumption of high-fat and sugary foods was high. Girls tended to consume cakes, biscuits, pastries and savoury snacks less frequently (55% ate these more than six days per week) than boys (63%, P<0.05) and consumed more fruit and vegetables (P>0.02).

	Days per week	0	1	2	3	4	5	6	7
Green vegetables/salad	% of Girls	15.6	8.3	12.8	15.5	10.6	14.5	7.0	15.8
	% of Boys	22.8	7.7	13.6	13.7	12.2	12.2	6.6	11.2
Other vegetables	% of Girls	18.1	13.2	21.8	16.1	9.3	6.7	3.8	11.0
	% of Boys	23.1	13.9	21.5	14.7	8.1	7.0	3.8	7.8

In conclusion, the children studied ate foods that were generally high in sugar and fats and low in fruit and vegetables, especially boys. Parents' awareness of healthy food choices should be increased and children should be encouraged to follow a more healthy diet.

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A city-wide survey of the eating habits of 9 and 11 year old children in Liverpool. By A.F. HACKETT[1], M. GIBBON[2], G. STRATTON[1] and E. HAMILL[2], [1]Liverpool John Moores University, School of Education, Community and Social Science, IM Marsh Campus, Barkhill Road, Liverpool L17 6BD, [2]Leisure Services Directorate, Liverpool City Council, Millennium House, Victoria Street, Liverpool L1 6JH

Regular information about the eating habits of children within a health authority would be an invaluable tool in promoting healthy eating and evaluating the success of attempts to do so. This is a comparison of the eating habits of two year groups of children collected as part of the city wide SportsLinx project, the main aims of which are to increase levels of physical activity in children, and to provide an annual supply of dietary and nutritional information on Liverpool children.

The data were collected using a questionnaire (FIQ) which records the respondent's age, sex and school, followed by a stem question ('Did you at any time yesterday, eat any amount of ...') and a validated list of key foods in children's diets (Johnson et al. 1999). The FIQ has been used extensively (Hackett et al. 1990, 1997). In 1996–7, as a pilot study, five secondary schools took part (649 11–12 year olds completed the FIQ). In 1998–9 77 (of 122) Liverpool primary schools took part (3556 children aged 9–10 years completed the FIQ).

About 90% of the younger children reported having breakfast compared with only 70% of the older children, about 25% of whom ate on the way to school, compared with only 12% of the 9–10 year olds. The table summarizes a few more of the findings.

Foods eaten on the previous day:	Primary children:		Secondary children:	
	Boys (%) n 1803	Girls (%) n 1746	Boys (%) n 375	Girls (%) n 273
Fruit	74	77	70	69 **[2]
Sugary soft drinks	67	61 **[1]	60	55 **[2]
Chocolate biscuits	61	60	52	53 **[2]
Crisps	66	69	50	52 **[2]
'Sweets'	58	61	58	55
Chips	52	51	52	47
Sausages / Burgers	34	25 **[1]	45	29 **[1]
'Brown' breads	23	20 *[1]	30	28 **[2]
Vegetables	40	39	26	26 **[2]

*[1] Boys / girls P<0.05; **[1] Boys / girls P<0.01. **[2] Primary / secondary P<0.01.

These data suggest that key changes in eating habits occur with the transition to secondary school; some for better, some for worse. The fall in the number of children reporting eating fruit and vegetables is of particular concern. The findings also confirm the need for substantial changes in (and lack of progress in achieving better) eating habits of children and indicate some aspects of diet which could be targeted.

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The influence of parental adiposity and behaviours in middle age on offspring adiposity. By A. CULLUM, D. GUNNELL, G. DAVEY SMITH, A. McCARTHY and Y. BEN-SHLOMO, *Department of Social Medicine, University of Bristol, Canynge Hall, Whiteladies Road, Bristol BS8 2PR*

Environment in early life is thought to influence the development of adiposity in adults. Parental factors are likely to be important in any observed associations. Maternal exposures during pregnancy and childhood social class have been associated with adult adiposity, and parental adiposity may influence the growth trajectory of offspring (Parsons et al. 1999). Whether parental adiposity, social class and behaviours in middle age continue to influence offspring adiposity is unclear.

Participants in the Barry Caerphilly Growth Study (1972–9, n 951) (Elwood & Sweetnam, 1987) were followed up at age 25–26; 678 (71%) attended a clinic where anthropometric measurements were made and details collected on current lifestyle and parents' address. Parents (n 631) were mailed a questionnaire and asked to record their height, past and present weight, waist and hip circumference, complete the restraint section of the Dutch Eating Behaviour Questionnaire (van Strien et al. 1986), and to provide information on current lifestyle. 435 (69%) mothers and 332 (55%) fathers responded. A sample of parents were then invited to attend a clinic where anthropometric measures were taken; of those invited, 182 (85%) mothers and 102 (61%) fathers attended. Differences between clinic and self-reported values were used to adjust the latter for measurement error.

Parent and offspring variables were assessed separately in the first instance. They were then combined in multivariate models to determine which factors independently predicted ($P < 0.05$) body mass index (BMI) at age 25–26. Offspring variables included childhood adiposity, current behaviours and current social class; parental variables included maternal measures during pregnancy, current behaviours, and adiposity and social class through adulthood.

Parental adiposity throughout life continued to predict BMI of offspring at age 25-26. For females, maternal BMI during pregnancy, and for males, current maternal BMI and change in BMI since pregnancy were independently, positively, associated with offspring BMI at age 25–26. Father's highest BMI in adulthood was positively associated with BMI of female offspring and father's current BMI was positively associated with BMI in male offspring. Childhood social class was inversely associated with BMI in both sexes; change in maternal social class since the original study was only associated with male BMI. The following were also assessed, but were not significant in multivariate analyses: parental age, maternal or female parity, offspring age at menarche or shaving, offspring or parental birthweight.

Few current parental or offspring behaviours were associated with BMI of offspring at age 25–26. Television viewing in parents, and exercise frequency, smoking and alcohol intake in parents or offspring were all non-significant. Dietary restraint score was not available for young adults, but maternal ($P < 0.01$) restraint scores were inversely associated with BMI of female offspring in multivariate analyses. This was not the case for male offspring and a test for interaction between parental restraint score and offspring gender was statistically significant.

These analyses show that adiposity in young adulthood is influenced by parental adiposity and social circumstances measured at different stages of life. Few current parental behaviours were associated with BMI of offspring. An exception was parental dietary restraint score which may indicate shared family attitudes to weight control.

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Food intakes of Scottish schoolchildren aged 5½ to 8½ years: are mothers' eating habits related? By P. J. LONGBOTTOM[1], W.L. WRIEDEN[1], C.M. PINE[2] and J.A. DAVIES[2], [1]*Centre for Applied Nutrition Research, University of Dundee, Dundee DD1 4HT*[1], [2]*Dental Health Services Research Unit, Dental School, University of Dundee, Dundee DD1 4HR*

In an earlier study (Wrieden & Longbottom, 1997) a positive correlation was found between mothers and their 8–9-year-old children for intakes of fruit and vegetables, NSP and vitamin C. Other work (Wardle, 1995) has shown some correlation in nutrient intake within families but there is little information on specific foods. A follow-up survey of the diet and dental health of the Scottish children who participated in the NDNS survey of pre-school children (Gregory et al. 1995) collected 4-d dietary records from thirty-six children (twelve boys and twenty-four girls aged 5½ to 8½ years) and their mothers. A questionnaire on family eating habits was also obtained from the child–parent pairs. Daily intakes of foods were calculated after appropriate weighting to 7 d of the 2 weekdays and 2 weekend days. Although mean energy intakes of children and mothers were similar (6.92MJ and 6.80MJ respectively), food intakes were expressed as densities (g per 4.18MJ) to take into account any energy intake differences between individual child–mother pairs. Median densities of bread, meat and meat products, vegetables and potatoes were significantly greater and confectionery significantly lower in mothers compared with children.

Type of foodstuffs	Median densities (g/4.18MJ)		Correlation	
	Children	Mothers	r_s	P value
Total breakfast cereals	20.3	15.0	0.107	0.536
Total bread	31.5	58.8***	0.360	0.031+
Wholemeal bread	0.0a	5.0***	0.700	<0.001+++
Milk and milk products	125.5	132.2	0.344	0.040+
Meat and meat products	33.4	75.6***	0.209	0.222
Fruit	26.1	27.8	0.735	<0.001+++
Vegetables	20.8	33.3**	0.213	0.212
Chips	15.5	18.3	0.651	<0.001+++
Potatoes and potato products	35.0	62.5***	0.572	<0.001+++
Total confectionery	9.0	0.0***a	0.466	0.004+
Carbonated drinks	28.8	17.2	0.526	<0.001+++

a Eaten by less than 50% of subjects, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ using Wilcoxon Matched-Pairs Signed-Rank test, +++$P < 0.001$, ++$P < 0.01$, +$P < 0.05$ using Spearman's Rank Correlation Coefficient test.

Significant positive correlations between children and mothers were seen for a range of foods using a non-parametric correlation coefficient (r_s). In the main these were for foods that can be eaten independently rather than as part of a family meal, and included the foods that are usually targeted for change to improve the overall diet. Thus the data suggest that children's intakes of bread (particularly wholemeal), fruit, chips, confectionery and carbonated drinks reflected that of their mothers. Nearly 80% of families claimed that they ate together once a day or more but it would appear that the influence of parents on children's eating habits may be through the availability of certain foods in the home rather than through a traditional family meal.

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The relationship between dental status and intake of fruits and vegetables by older adults. By P.J. MOYNIHAN[1], R. GREAVES[1], A.W.G. WALLS[1], N. STEEN[2], A. SHEIHAM[3], W. MARCENES[3] and J. STEELE[1], [1]The Dental School, University of Newcastle, Framlington Place, Newcastle upon Tyne NE2 4BW, [2]Centre for Health Services Research, 21 Claremont Place, Newcastle upon Tyne NE2 4AA, [3]Department of Epidemiology and Public Health, University College London Medical School, London WC1E 6BT

An oral health survey was included as part of the National Diet and Nutrition Survey of people aged 65 years and over, which studied 955 free-living persons aged 65 years and over (Steele et al. 1998). This study presents further analysis of these data with respect to fruit and vegetable consumption. The aims were to compare the intake of fruits and vegetables between edentate (no natural teeth) and dentate subjects and to investigate whether fruit and vegetable intake is related to dental function (the number of posterior opposing pairs of teeth (POPS)).

Total fruit and vegetable intake, total vegetable intake, raw vegetable intake, fresh fruit intake (collected using a 4-d food diary) and dental indices (edentate, dentate, number of POPS) were entered into SPSS. The relationships between dietary intake of fruits and vegetables and dental indices were investigated using analysis of variance and linear regression. Oral risk factors for a low consumption of fruit and vegetables (<400 g/d) were determined by logistic regression.

	Edentate n 301		All n 332			Dentate 0 POPS n 60		1–4 POPS n 153		>4 POPS n 110		
	Mean (g/d)	SD	Mean (g/d)	SD	P*	Mean (g/d)	SD	Mean (g/d)	SD	Mean (g/d)	SD	P**
Total fruit and vegetables	181	130	267	141	0.000	235	148	247	127	312	146	0.000
Total vegetables	101	73	139	75	0.000	125	76	128	65	160	83	0.001
Raw vegetables	24	38	38	38	0.001	27	29	32	33	53	43	0.000
Total fruit	79	85	128	98	0.000	110	111	118	92	141	95	0.000
Fresh fruit	52	66	82	79	0.000	60	64	80	54	97	77	0.014

*Analysis of variance comparing edentate with dentate accounting for age, sex and social class.
**Analysis of variance comparing POPS groups (0 POPS, 1–4 POPS and >4 POPS) accounting for age, sex, and social class.

Regression analysis showed significant relationships between POPS group and the intake of total fruits and vegetables (r 0.33, P 0.000), total vegetables (r 0.31, P 0.001), raw vegetables (r 0.29, P 0.000), all fruit (r 0.26, P 0.000) and fresh fruit (r 0.24, P 0.007). A relationship existed between social class and all categories of fruit and vegetable with the exception of raw vegetables. Logistic regression showed that edentate subjects were twice as likely to consume insufficient vegetables (<400 g/d) and that with each additional POP, the likelihood of consuming sufficient vegetables increased by 15% (Odds Ratio 1.15).

Edentulism is associated with a low intake of vegetables and fruits. However, dentate elderly adults with few pairs of natural opposing teeth are also at risk of consuming a diet low in vegetables and fruits. Retention of teeth into older age is therefore important to provide the functional means to consume a healthy diet.

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The effect of the oral contraceptive pill on food intake, dietary restraint and iron status. By M. BROWN, O. COTTER and S.L. REEVES, Department of Sports, Health and Exercise Science, St Mary's, Strawberry Hill, Twickenham TW1 4SX

In Britain, the oral contraceptive pill (OCP) has been available for 35 years and currently more than 50% of women aged 18–35 years use it as their main method of contraception (Drife, 1998). Even so, information regarding the effect of the OCP on nutritional status, mood and athletic performance is conflicting. The objective of this study was to investigate possible differences in food intake, dietary restraint, Fe status and VO2max in women who take the OCP with those who do not.

Forty females who habitually exercised aged 24 years (SD 2.3) were recruited into two equal groups, those who took the oral contraceptive pill (OCP) and those who had never taken it (non-OCP). Food intake (by estimated 7-d diarys), body weight, and dietary restraint were continually monitored over an 8 week period and VO2max and Fe status were recorded at the end of the study. Comparisons between the two groups were made and variations over the study period were examined using standard techniques. Both groups included women on weight-reducing diets.

	OCP Mean	SD	Non-OCP Mean	SD
Body weight (kg)	59.5	6.1	65.0	7.0
Energy (kJ/day)	8502*	1629	7707	2312
Carbohydrate (g)	241	56	218.4	57
Fat (g)	77.5	31	63.7	20
Protein (g)	66.3*	28	55.9	21
Alcohol (g)	30.4*	21	24.3	22
Iron (mg)	8.3	0.3	9.1	0.5
Haemoglobin (g/dl)	13.7	0.8	13.2	0.7
Serum ferritin (µg/l)	34.7*	16.7	22.2	13.8
VO2max (ml/kg/min)	43.3	7.1	44.3	7.9

*P<0.05. Student's t test.

Reported energy intake was significantly greater in the OCP group, as was the consumption of both protein and alcohol (Table). Fat intake, however, was non-significantly greater in the OCP group. In addition, the variation in food intake was observed to be greater in the non-OCP group, who had on average coefficients of variation of 30% over the 8 weeks of the study compared to 19% in the OCP group. These differences in energy intake could be attributed to the differences and variations in basal metabolic rate between women who take the OCP and as a result of the menstrual cycle as suggested by Curtis et al. (1996) and Diffey et al. (1997). There were no significant differences between the groups in dietary restraint; however, there was a tendency for dietary restraint to be higher during the luteal phase in the non-OCP group. There were no significant differences in iron intake or blood haemoglobin, although there were significant differences in serum ferritin levels with 20% of the non-OCP group having depleted iron stores (serum ferritin <10 µg/l). No significant differences were identified in the VO2max tests between the two groups.

In conclusion, differences were observed between the OCP and non-OCP groups in macronutrient intake and iron stores. Further investigations are warranted to study the variations in dietary intake and energy expenditure that occur as a result of both oral contraceptive use and the menstrual cycle.

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Diffey B, Piers LS, Soares MJ & O'Dea K (1997) British Journal of Nutrition 77, 853–862.
Drife JO (1998) The Benefits and Risks of the Oral Contraceptive Today. Lancashire: Parthenon Publishing.

Left column

The nutrient intakes of vegetarian and omnivorous adolescents in North-West England. By L. BURGESS, A.F. HACKETT, S. MAXWELL and M. ROUNCEFIELD, Liverpool John Moores University, School of Education, Community and Social Science, IM Marsh Campus, Barkhill Road, Liverpool L17 6BD

Traditional vegetarian diets have been associated with higher intakes of fruit, vegetables and fibre and lower intakes of fat than omnivorous diets. However, a study by Nathan et al. (1996) found that vegetarian children consumed significant quantities of vegetarian (and other) convenience foods and growth in retail sales in this area have increased dramatically (Mintel, 1998). Adolescence is a crucial period during which adequate nutrient intake is essential to support growth and development. However, very little is known about the dietary intake of vegetarian adolescents. Vegetarians consumed no meat or poultry but some consumed fish. This study assessed the nutrient intakes of thirty vegetarians (males n 8, females n 22) aged 13–17 years and compared them with those of thirty omnivores (males n 9, females n 21) of the same age.

Subjects kept a 3-d dietary diary on three separate occasions over a period of 1 year. A follow-up visit was made on each occasion to clarify the amount and exact type of foods eaten. The results are the mean of the 9-d recorded intake. Nutrient intake was determined using Microdiet (1999) and data were analysed with SPSS (1998). The Table below summarizes findings in respect of selected nutrients.

	Vegetarians (n 30)	Omnivores (n 30)	SE	P-value	CI
Energy (kJ)	8360	8942	494	0.244	−1570,406
Total fat (g)	79	91	5.9	**0.049**	−24,−0.079
Saturated fat (g)	12	13	2.6	0.208	−8.4,1.86
Polyunsat. fat (g)	17	18	1.5	0.301	−4.6,1.45
% Energy fat	35	37	1.1	0.051	−4.5,0.01
Iron (mg)	11	11	0.8	0.397	−0.87,2.2
Zinc (mg)	7	8	0.5	0.721	−1.2,0.83
Calcium (mg)	914	756	68	**0.024**	22,294
Vitamin C (mg)	105	72	12	**0.009**	8.8,58
EI:BMR	1.26	1.44	0.23	0.24	−0.32,−0.024
BMI	22	20	0.75	0.047	0.018,3.06

Energy intakes and % of energy from fat were lower in the vegetarians, although the differences were not statistically significant. Fat intakes were significantly lower in the vegetarian group; however, some differences may be due to under-reporting by the vegetarians. Iron intakes in the two groups were the same although that consumed by the vegetarians was non-haem iron, which is less easily absorbed. Calcium intakes were significantly higher in the vegetarians. Both groups consumed adequate amounts of vitamin C although the vegetarians consumed significantly more.

This project was sponsored by the Meat and Livestock Commission.

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Right column

Dietary intake and nutritional status of young vegans and omnivores in Sweden. By C.L. LARSSON and G. JOHANSSON, Department of Food and Nutrition, Umeå University, SE-90187 Umeå, Sweden

Five percent of Swedish students (16–20 years) eat a vegetarian school lunch (Larsson & Johansson, 1997). Vegetarians may have a well-balanced diet with plenty of food variety, reflecting the recommendations of dietary guidelines. However, some young vegetarians may exclude meat without replacing it with nutritionally equivalent vegetarian food. Vegans are more likely to have an inadequate dietary intake than other types of vegetarians who are not equally strict. Dietary intake and nutritional status of thirty young Swedish vegans (50% female, 17.5 years) and thirty sex/age/height-matched omnivores, was assessed. The diet history method was validated against doubly-labelled water, nitrogen, sodium and potassium excretion in urine. Nutritional status regarding iron, vitamin B₁₂, folate and vitamin D was measured in blood samples.

Reported nutrient intake divided by measured biomarker or lowest recommended intake.	Females		Males	
	Vegan (n 15) % (SD)	Omnivore (n 15) % (SD)	Vegan (n 15) % (SD)	Omnivore (n 15) % (SD)
Energy[1]	84 (25)	92 (19)	87 (19)	85 (16)
Protein (nitrogen)[1]	100 (32)	98 (13)	114 (27)	114 (14)
Vitamin B₁₂[2]	1700* (4100)	540 (270)	2200 (3500)	640 (130)
Vitamin D[2]	140* (110)	240 (110)	200*** (77)	340 (100)
Iron[2]	260 (130)	210 (140)	310 (96)	290 (110)
Calcium[2]	190* (180)	330 (93)	150*** (47)	420 (110)
Selenium[2]	120* (110)	180 (180)	110*** (68)	230 (86)

[1] Reported intake divided by biomarker. (n 32 for energy and n 59 for protein.)
[2] Reported intake divided by lowest recommended intake.
* P 0.05, ** P 0.01, *** P 0.001, using a Mann–Whitney U test.

The diet history had a bias towards underestimating energy and potassium intakes but showed good agreement for protein (nitrogen) and sodium intake (P<0.05). Both dietary groups had mean nutrient intakes (including those obtained from supplements) above the lowest recommended intake (LRI) according to The Nordic Nutrition Recommendations (Sandström et al. 1996) (Table). To prevent an inadequate intake of vitamin B₁₂, vitamin D and selenium, the vegans were dependent on supplements. Furthermore, some individuals had nutrient intakes (including supplements) that failed to reach the LRI for vitamin B₁₂ (30% females and 6.7% males), vitamin D (23% females) iron (3.3% females), calcium (17% females and 6.7% males) and selenium (37% females and 20% males).

The nutritional status assessment showed no significant differences in either iron or vitamin D status between the dietary groups, but the prevalence of inadequate iron balance was 25% among the female adolescents. Vegans had significantly lower (P<0.01) vitamin B₁₂ concentrations and higher folate concentrations in blood compared with the omnivores, but values were within the reference range except for the vitamin B₁₂ concentrations of two vegans.

The results of the study are consistent with those of other studies which showed that vegans had significantly lower intakes of protein (Huang et al. 1999), vitamin D, calcium and selenium but no difference in energy (Wilson & Ball, 1999) and iron intakes (Ball & Bartlett, 1999) compared with the omnivores. Planning nutritionally adequate vegetarian meals involves the same principles as those followed in planning omnivorous meals. These are eating a variety of foods, meeting energy needs and limiting the intake of nutrient-poor foods, saturated fat and cholesterol. However, the present study demonstrates that different considerations about nutrient intakes need to be given for vegan and omnivorous adolescents.

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Wilson A & Ball M (1999) European Journal of Clinical Nutrition **53**, 189–194.

The relationship between starch and fat consumption in a UK population. By P.J. CURTIS, A.J. ADAMSON and J.C. MATHERS, *Human Nutrition Research Centre, University of Newcastle, Newcastle upon Tyne NE1 7RU*

Current UK intake of fat, at 39% food energy (%FE) (MAFF, 1999), is undesirably high. An intake of less than 35%FE from fat is targeted for the year 2005 (Department of Health, 1992). To achieve this, it is recommended that carbohydrate (CHO) intake, predominantly in the form of starchy foods, should increase to 50%FE. Macdiarmid *et al.* (1998) have reported an inverse relationship between %FE fat and %FE sucrose but there is little evidence of a similar relationship between %FE fat and %FE starch.

Prior to entry into a dietary intervention, participants completed a 3-d estimated food diary, followed by interview, and quantified using a photographic atlas of food portion sizes (MAFF, 1997). Dietary data were coded and nutrient composition determined. Results are presented for 187 subjects (30 boys and 30 girls <16 years, and 50 men and 77 women >16 years) for whom mean energy intake was between 1.1 and 2.0 times the estimated BMR (Goldberg *et al.* 1991). The mean %FE from fat (y), CHO, sucrose (x_1), and starch (x_2) were 36.6, 48.7, 10. 2 and 26.9 %, respectively.

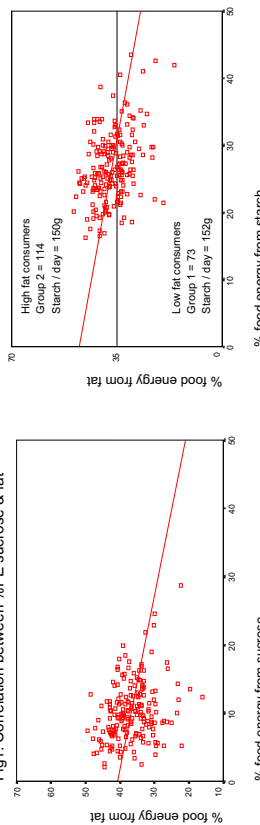Fig1: Correlation between %FE sucrose & fat

Fig 1: $y = 40.6$ (SD 1.1) $- 0.39$ (SD 0.10) x_1, R^2 0.08, $P<0.001$.
Fig 2: $y = 47.5$ (SD 2.1) $- 0.41$ (SD 0.08) x_2, R^2 0.13, $P<0.001$.

Figure 1 confirms a significant inverse correlation between %FE from sucrose and fat. However a stronger inverse correlation between %FE from fat and %FE from starch was found in these subjects (Fig. 2). This supports the hypothesis, and current dietary recommendations, that it may be possible to reduce fat in the diet by increasing starch rather than sucrose. Figure 2 identifies high- and low-fat consumers in this population (defined from a dietary target of <35%FE). The sources of starch (as expressed as a % of total starch) consumed by each group differed. Low-fat consumers chose the highest intakes of breads and breakfast cereals (46% of starch intake compared with 38 % for high-fat consumers). High-fat consumers obtained 35% of starch sources from chips, meat products, savoury snacks, cakes and biscuits, compared with 23% for low-fat consumers.

Increasing %FE from starch may reduce the %FE from fat. Within the context of the whole diet, the sources of starch may be as important as the amount consumed.

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MAFF (1999) *The National Food Survey 1998.* London: HMSO.

Immune function in rural Gambian children is not related to season of birth, birth size or maternal supplementation status. By S.E. MOORE, A.C. COLLINSON and A.M. PRENTICE, *MRC Keneba, The Gambia and MRC International Nutrition Group, London School of Hygiene & Tropical Medicine, 49–51 Bedford Square, London WC1B 3DP*

The subsistence farming existence in rural Gambia is heavily influenced by a monomodal annual rainy season. The annual rains coincide with a 'hungry' season when all adults are forced into negative energy balance. In addition, a hungry season deficit in birth weight is observed. These effects are known to be nutritionally mediated since they are reversed by maternal dietary supplementation (Ceesay *et al.* 1997). Season of birth can therefore be used as a proxy measure of early exposures. This measure has proved robust enough to demonstrate that birth during the annual hungry season predicts premature, mainly infection-related, adult mortality (Moore *et al.* 1997). This finding led to the hypothesis that nutritional or other seasonally related insults (infectious, toxic) during early life may programme long-term immune function.

The current study tested this hypothesis in a cohort of 472 6.5–9.5 year-old rural Gambian children whose mothers had been randomized to a dietary supplement during pregnancy (intervention) or lactation (control). The three main *a priori* hypotheses were that birth weight, season of birth, and supplementation status would predict immune function in these children. Immune function was measured by delayed-type hypersensitivity (DTH) responses (Merieux 'Multitest cell mediated immunity (CMI)' kit), response to T-cell mediated (human diploid cell rabies vaccine) and B-cell mediated vaccination (Pneumovax® 23 valent pnuemococcal capsular polysaccharide vaccine), intestinal permeability (lactulose–mannitol test) and levels of salivary secretory IgA (sIgA). Seasonally varying confounding factors also measured included anthropometry, micronutrient status (plasma zinc, vitamin A, vitamin C and haemoglobin levels), malaria parasitaemia and serum aflatoxin–albumin adduct levels (Moore, 2000). Data were adjusted by month of study, age and gender. Results were considered significant where $P<0.01$. The table shows the key results from this study.

Measure	Birth weight	Season of birth	Supplementation status
DTH (CMI)	NS	NS	Increased response in intervention children (P 0.006)
Pneumococcal vaccination	NS	NS	NS
Rabies vaccination	NS	NS	Increased response in control children (1st dose P 0.024, 2nd dose P 0.005)
Intestinal permeability	NS	NS	NS
Salivary sIgA levels	NS	Increased response in hungry season births (P 0.0018)	NS

None of the measures of immune function were related to the birth weight of the children, and they were not significantly different in the group of children who were born with a low birth weight (<2.5 kg). There were no consistent associations between supplementation status or season of birth and immune function. These findings do not necessarily negate the main hypothesis that immune function can be programmed during early life, as based on the original Kaplan–Meier survival analysis (Moore *et al.* 1997). In the survival plots, it was only after the age of approximately 15 years that the survival of the hungry-season births diverged from that of the harvest-season births. Future studies will therefore continue to explore the 'early programming of immune function' hypothesis at later ages with a focus on the possibility that the defect may be in immunological memory, rather than in early immune responses.

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Responses of piglet tissue composition to increasing maternal intake of long chain n-3 polyunsaturated fatty acids. By J.A. ROOKE, A.G. SINCLAIR, M. EWEN and L.M. BIRNIE, *Animal Biology Division, SAC, Craibstone Estate, Aberdeen AB21 9YA*

Inclusion of marine oils in the diet of the sow has been shown to reduce pre-weaning mortality (Cordoba *et al.* 2000). However, in studies where piglet tissue fatty acid composition has been analysed, improvements in 22:6n-3 content as a result of feeding marine oil to the sow have been accompanied by decreases in 20:4n-6 content (Rooke *et al.* 1999). Birth weight in the human has been positively correlated with 20:4n-6 status (Carlsson *et al.* 1993) and inclusion of marine oil in sow diets has depressed birth weight in piglets (Cordoba *et al.* 2000). Since low birth weight is a risk factor for pre-weaning mortality, the objective of the experiment was therefore to establish an amount of dietary marine oil which would enhance piglet 22:6n-3 status while minimizing reductions in 20:4n-6 status.

Twenty-four pregnant multiparous sows were used in the experiment which began on day 60 of pregnancy. To give four diets, salmon oil was added in increasing amounts (0, 5, 10 and 20 g/kg diet) to a basal diet whose ingredients were chosen to minimize the inclusion of n-3 acids; the diets were made isoenergetic by adding palm oil to each diet to give a total of 20 g oil/kg diet. Diets were offered to the sows in fixed amounts (2.5 kg/day) until parturition. Samples of brain and liver were obtained from piglets at birth (before consumption of colostrum) weighed and fatty acid composition determined.

Salmon oil (g/kg diet)	0	5	10	20	SED	Significance
Fatty acid (g/100g total fatty acids) in piglet						
Brain						
20:4n-6	15.1	14.8	14.4	13.9	0.53	Linear *
22:6n-3	17.8	19.6	20.6	19.8	0.79	Quadratic *
22:6n-3/22:5n-6	6.1	9.9	12.4	13.9	0.61	Quadratic ***
Weight (g/kg)	25.2	28.6	30.6	26.7	1.92	Quadratic *
Liver						
20:4n-6	13.5	13.0	11.7	9.6	1.30	Linear **
22:6n-3	6.8	9.6	9.9	10.0	1.24	Linear *
22:6n-3/22:5n-6	4.3	7.6	9.5	11.5	1.55	Linear ***

SED for nine piglets/diet; *$P<0.05$; **$P<0.01$; ***$P<0.001$.

The greatest increase in piglet liver and brain 22:6n-3 proportions occurred between 0 and 5 g salmon oil/kg diet with only small increases between 10 and 20 g salmon oil/kg diet. In contrast, brain and liver 20:4n-6 proportions declined progressively as the amount of salmon oil fed to the sow increased. In brain, the change in the ratio 22:6n-3:22:5n-6 was greatest between 0 and 5 g salmon oil/kg diet whereas in liver the ratio increased linearly with added salmon oil. In addition, piglet brain weight (g/kg live weight) increased to a maximum at 10 g salmon oil/kg diet.

The optimum amount of supplementary salmon oil in the current experiment, defined as that which gives the greatest response in brain 22:6n-3 proportions with a minimum reduction in 20:4n-6, was 5 g salmon oil/kg diet. This corresponds to an intake of approximately 3 g 20:5n-3 plus 22:6n-3/d or 0.3% Digestible Energy with a n-6:n-3 ratio of 6.8.

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Rooke JA, Bland IM & Edwards SA (1999) *British Journal of Nutrition* **82**, 213–221.

Energy requirements of confined cats estimated by the doubly-labelled water method and indirect calorimetry. By J. DECOMBAZ[1], J. AMBROSE[2], R. RUDNICK[2], G. GREMAUD[1], C. PIGUET-WELSCH[1], D. CHAUSOW[2] and O. BALLEVRE[1], [1]*Nestlé Research Centre, Nestec Ltd, 1000 Lausanne, Switzerland,* [2]*Friskies Product Technology Centre, St. Joseph, MO 64503, USA*

Energy requirements of cats estimated by many investigators led the US National Research Council (1986) to propose 293 kJ/kg per d (70 kcal/kg per d) as a reasonable approximation of maintenance needs. This value may be biased on the high side for two reasons. Firstly, Atwater's factors for metabolizable energy of nutrients (ME) used in the early feeding trials overestimate the true value for cats. Secondly, there may be a confounding effect of physical activity. Previous data (Earle & Smith, 1991; Taylor *et al.* 1995) suggest that the NRC value may be too high. The purpose of this study was to reassess the energy requirements of adult inactive cats using the doubly-labelled water (DLW) method and indirect calorimetry (IC) concurrently.

Four male and four female domestic short-hair cats ranging from 2.7 to 5.2 kg in body weight were individually confined in a calorimetry chamber (Columbus Instruments, Columbus Ohio, USA) for 15 d (23 h/d) after 1 week acclimation. They were fed a commercial wet cat diet of known composition and ME. Energy expenditure (EE) was measured by DLW over the period and in parallel by IC for 12 effective days. ^{18}O and D rates of decay were calculated with the regression method (days 1, 10 and 16) after correction for baseline isotope enrichment. Carbon dioxide production (rCO$_2$) was corrected for nutrient imbalance and EE$_{DLW}$ calculated as before (Ballèvre *et al.* 1994). EE$_{IC}$ was calculated using classic equations (Weir, 1949). Physical activity (PA) was assessed by infra-red movement detection and resting EE (REE) by regression to zero PA. Body fat was determined from total body water by deuterium dilution before and after the period.
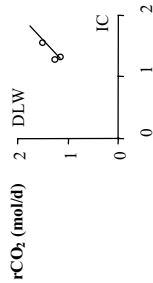
Method	Energy expenditure (kJ/kg per d)	
	Mean	SEM
(n 8)		
DLW	241	19
IC	248	19

Estimates were not significantly different (P 0.42).

Weight gain (1.5 g/kg per d) was negligible, but energy balance was positive ($P<0.001$) as a result of combined fat gain and loss of lean mass, probably in relation to the unusually low level of PA (17% of total EE) in the confined chamber space. EE$_{IC}$ declined between initial and final days ($P<0.05$), indicative of incomplete acclimation (stress and activity). Daily rCO$_2$ obtained by both methods were correlated (r 0.91), with the slope not different from 1 and the intercept not different from 0 (see Figure). Total daily energy expenditures calculated by both methods were not different (see Table) and in good agreement with the mean value from numerous recent prolonged feeding protocols at maintenance (238 kJ/kg per d; Czarnecki-Maulden 1995, personal communication).

These data validate the use of the DLW method in cats and support the contention that the current NRC recommendation for the maintenance of adult inactive cats is about 20% too high.

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Both moderate vitamin A deficiency during pregnancy and birth weight affect piglet immunity. By C. ANTIPATIS[1], J.A. ROOKE[2], M. EWEN[2] and C.J. ASHWORTH[1,2]. [1]Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, [2]Applied Physiology Department, SAC, Craibstone Estate, Bucksburn, Aberdeen AB21 9YA

Maternal vitamin A deficiency during pregnancy retards fetal development and reduces neonatal survival (Antipatis et al. 2000); however, the factors associated with these perinatal deaths are poorly understood. Although vitamin A is known to be important for immune function in non-pregnant animals (Semba, 1999), few studies have addressed the effects of maternal vitamin A status during pregnancy on neonatal immune competence. Individual piglet birth weight is also a major determinant of subsequent survival (Widdowson, 1971). The objectives of this study were to determine the relationship between birth weight and piglet IgG levels, to assess the effects of moderate maternal vitamin A deficiency on piglet birth weight, litter size and IgG levels, and to determine whether low birth weight piglets were particularly susceptible to the effects of inadequate maternal retinol.

Large White × Landrace gilts received 2.3 kg/d of either a vitamin A-sufficient (C; n 5, vitamin A content 8000 IU/kg) or a vitamin A-free (VAF; n 5) diet for one oestrous cycle prior to mating and throughout pregnancy until slaughter 48 h after parturition. Litter size and individual piglet birth weights were recorded and a normal-sized and the smallest (runt) piglet from each litter were sacrificed approximately 12 h after birth. Maternal retinol status was assessed and neonatal liver and plasma retinol and plasma IgG levels were measured.

Maternal plasma (C: 0.25 (SE 0.02) v. VAF: 0.17 (SE 0.02) μg/ml, $P<0.05$) and liver (C: 171.33 (SE 4.31) v. VAF: 105.42 (SE 11.03) μg/g, $P<0.001$) retinol levels were 32 and 38% lower, respectively, in gilts fed the VAF compared with the control diet. Vitamin A deficiency also reduced neonatal plasma and liver retinol (see Table). Total number of piglets born (C: 13.2 (SE 1.3) v. VAF: 13.0 (SE 0.3), $P=0.48$), piglets born alive (C: 98.5 (SE 1.5) v. VAF: 92.5 (SE 2.3)%, $P=0.06$) and live piglet birth weight (C: 1.17 (SE 0.06) v. VAF: 1.28 (SE 0.06) kg, $P=0.19$) were not significantly affected by maternal vitamin A deficiency.

Plasma and liver retinol and plasma IgG levels in piglets from mothers fed a VAF or a Control diet.

| | Control | | | | VAF | | | | Probability* | | |
| | Normal | | Runt | | Normal | | Runt | | | | |
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Diet	Size	Interaction
Plasma retinol (μg/ml)	0.06	0.01	0.06	0.01	0.03	0.01	0.04	0.01	0.01	0.83	0.98
Liver retinol (μg/g)	15.35	2.06	10.48	0.55	7.69	1.64	6.77	1.16	0.001	0.066	0.19
Plasma IgG (mg/ml)	13.95	0.38	8.34	2.09	8.29	1.17	5.02	1.28	0.005	0.005	0.41

*Data were analysed by two-way analysis of variance.

Piglet weights were positively correlated with plasma IgG ($y=0.005+3.007*x$, $R^2=0.30$, $P<0.05$) with the runts having significantly less IgG than their normally grown siblings. Moderate vitamin A deficiency also reduced piglet IgG levels; normally grown piglets born to gilts fed the VAF diet had IgG levels similar to runts born to control mothers. The combination of vitamin A deficiency and reduced size at birth had an additive effect as runts from the vitamin A-deficient group had the lowest plasma IgG levels. These data show that withdrawal of vitamin A from the maternal diet produces a moderate deficiency in gilts and their offspring. Although such deficiency did not significantly alter live piglet birth weight and neonatal survival, it compromised neonatal immunity. The underlying mechanism requires further study, but may be related to effects of vitamin A deficiency on colostrum composition, suckling ability or neonatal gut function.

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Effects of supplementing the maternal diet with vitamins and vaccinating the sow on immunoglobulin G concentrations in piglet plasma. By I.M. BLAND[1], J.A. ROOKE[1], A.G. SINCLAIR[1], V.C. BLAND[1] and S.A. EDWARDS[2]. [1]Animal Biology Division, SAC, Craibstone Estate, Aberdeen AB21 9YA, [2]Department of Agriculture, University of Aberdeen, Aberdeen AB24 5UA

The newborn piglet depends upon a supply of immunoglobulin G (IgG) from colostrum for passive immune protection after birth. A previous study (Bland et al. 2000) in which piglets were prevented from suckling the sow for 8 or 12 h after birth decreased piglet plasma IgG concentrations and therefore reduced passive immune protection. The objective of the present study was to establish whether maternal vaccination or nutrition could change the IgG status of the piglet.

Two different vitamin supplements were included in the diet of the sow from day 60 of gestation (L and H, mg/kg; retinol 1.5 and 4.8, vitamin E 35 and 150, vitamin C 0 and 100, respectively; representing approximately 1 and 3 times NRC (1988) requirements). Sows were also vaccinated 6 and 3 weeks before farrowing against atrophic rhinitis, parvovirus, avian Newcastle disease and E. coli/erysipelas (Y) or E. coli/erysipelas alone (N). Sows were assigned to treatments according to a 2 (L v. H) × 2 (Y v. N) factorial design using a total of twenty-four sows. Sows were induced to farrow on day 114 of gestation. Blood samples were obtained from sows on day 104 and a colostrum sample at farrowing before piglets were allowed to suckle. Blood samples were obtained from piglets (three piglets/sow) at birth and at 4-h intervals until 24 h after suckling commenced. Retinol and μ-tocopherol concentrations in plasma and colostrum samples were analysed by HPLC and total IgG concentrations by ELISA. For piglets, vitamin concentrations were determined on samples pooled within litters, whereas IgG concentrations were determined on an individual piglet basis.

Vaccination had no effect on plasma or colostrum vitamin concentrations. Increasing maternal dietary retinol increased (L v. H) maternal plasma and colostrum retinol concentrations (*mol/l) from 0.39 to 0.74 (SE 0.077) and from 1.22 to 2.76 (SE 0.241) respectively (both $P<0.001$). Piglet plasma retinol concentrations were 0.13 (L) and 0.34 (H) *mol/l (SE 0.047, $P<0.01$) at birth and 0.19 (L) and 0.28 (H) *mol/l (SE 0.021, $P<0.01$) after 24 h. Similarly, increasing maternal dietary vitamin E increased (L v. H) maternal plasma and colostrum μ-tocopherol concentrations (*mol/l) from 1.74 to 2.71 (SE 0.129) and from 10.9 to 20.2 (SE 1.65) respectively. Piglet plasma μ-tocopherol concentrations (*mol/l) were 0.27 (L) and 1.01 (H) *mol/l (SE 0.299, NS) at birth and 1.66 (L) and 3.40 (H) (SE 0.434, $P<0.05$) after 24 h. Neither diet nor vaccination changed sow plasma or colostrum total IgG concentrations (mean values with SEM, 23.5 (1.72) and 88.4 (5.77) mg/ml). However, overall both increasing dietary vitamin concentration and vaccination of the sow increased ($P<0.05$) piglet plasma IgG concentrations (12 h and 24 h values are shown in the Table).

| Vitamins | L | H | L | H | SEM* |
Vaccination	N	N	Y	Y	
Piglet plasma total IgG concentrations (mg/ml) after					
12 h suckling	25.5	37.0	41.9	48.7	4.69
24 h suckling	16.9	19.9	21.6	22.0	4.08

* Analysis of variance.

As neither estimated piglet colostrum, and therefore IgG intakes, nor piglet packed cell volumes were changed by diet or vaccination, the increased piglet plasma concentrations of IgG suggest an increased transfer of colostral IgG from the gut. The mechanisms underlying this increased transfer are unclear but would benefit piglets whose supply of colostral IgG is low, perhaps because of delayed suckling.

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The effect of composition of gain during a winter store period on beef cattle performance. By L. HEASMAN[1], D.G. CHAPPLE[2], K.P.A. WHEELER[2], N.S. PRATHALINGHAM[3] and M.A. LOMAX[3], [1]*ADAS High Mowthorpe, Duggleby, Malton, North Yorkshire YO17 8PG,* [2]*ADAS Rosemaund, Preston Wynne, Hereford HR1 3PG,* [3]*Department of Agriculture, University of Aberdeen, 581 King Street, Aberdeen AB24 5UA*

In the UK, 18-month beef finishing systems are common. Spring-born calves are fed a restricted diet over their first winter, and finished off cheap grazed grass in their second summer. Previous work has focused on the effects of rate of gain during the winter store period on animal performance (Lowman *et al.* 1994) and subsequent carcass composition (Wright & Russel, 1991). However, the effects of the composition of gain (i.e. percentage body fat) during a winter store period on beef cattle performance are unknown. The objective of this study, therefore, was to determine the relationship between the composition of gain during a winter store period and the performance of cattle finishing off grass.

Seventy-two Spring 1998-born steers were offered one of two diets during the winter store period. One diet was high (102–115 % requirements) in metabolizable protein (HMP), to achieve a low fat body composition, whilst the other was low (84–90 % requirements) in metabolizable protein (LMP) to achieve a high fat body composition. To determine whether effects of the composition of gain were influenced by the rate of gain, each diet was offered to achieve target growth rates of either 0.3, 0.6 or 0.9 kg/d over the winter store period. Steers were turned out to graze a ryegrass/clover sward in April 1999, and were slaughtered at a target weight of 450–470 kg between August and November 1999. Body composition in the live animal was measured using a Velocity of Sound (VOS) scanner both at turnout and prior to slaughter, and daily live weight gain (DLWG) over the grazing period was calculated.

| | Diet | Winter growth rate (kg/d) | | | | | |
| | | 0.3 | | 0.6 | | 0.9 | |
		Mean	SEM	Mean	SEM	Mean	SEM
Turnout VOS (μs/cm)*	HMP	6.27	0.006	6.31	0.011	6.34	0.016
	LMP	6.34	0.012	6.36	0.013	6.37	0.021
DLWG at grass (kg/day)	HMP	0.92	0.03	0.93	0.05	0.80	0.02
	LMP	1.05	0.04	0.95	0.04	0.89	0.04
Slaughter weight (kg)	HMP	454	3.7	465	3.5	462	2.5
	LMP	454	6.0	463	2.6	464	3.3
Carcass weight (kg)	HMP	233	2.8	238	1.5	239	1.6
	LMP	238	3.6	238	2.7	247	2.5

* Measured at 10th rib. Reciprocal of velocity (μs/cm) increases with increasing body fat (Fisher, 1997).

VOS results at turnout indicate that body composition was significantly affected both by diet ($P<0.001$) and rate of gain ($P<0.01$) during the store period, with an increase in the proportion of lean tissue being associated with feeding a HMP diet, and lower rates of gain. Growth rates at grass were inversely related to winter gain ($P<0.01$), and were greater in steers fed the LMP diet than those fed the HMP diet ($P<0.01$), irrespective of rate of winter gain. However, slaughter weight of animals fed to gain 0.3 kg/d during the winter was significantly ($P<0.05$) lighter than in steers fed to gain either 0.6 or 0.9 kg/d. Carcass weight increased with increasing rate of winter gain ($P<0.05$), and was also significantly greater in LMP than HMP steers ($P<0.05$). VOS results were similar for all groups at slaughter.

The results suggest that both the rate and composition of gain during a winter store period significantly affect the performance of beef cattle finished off grass in an 18-month system. The greatest rate of DLWG at grass is associated with the lowest rate of winter gain, and this is further improved by feeding a LMP diet during the winter store period. The effects of composition and rate of winter gain on meat quality, however, remain to be determined.

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Effect of anaerobic fermentation of jack bean (*Canavalia ensiformis*) grain, with and without molasses as an additive, on its acceptance by pigs. By R. NAVA-MONTERO[1] and R. BELMAR[2], [1]*CRUPY-Universidad Autónoma Chapingo, Apdo. 50 Cordemex, Yucatán, CP 97310, México,* [2]*Faculty of Veterinary Medicine and Animal Science, University of Yucatán, Apdo. 4–116, Mérida, Yucatán, 97100, México*

The advantages and nutritional qualities of *Canavalia ensiformis* (jack bean), which grows well in Yucatan, are constrained by its content of antinutritional factors (ANF), which particularly affect intake. The principal ANF factors are canavanine (an amino acid) and concanavalin-A (a lectin), although there are others. It has been shown that heat treatments used to reduce the ANF of raw legumes can have detrimental effects on nutritional quality. Fermentation is a process which modifies the biochemical traits of the legumes and can reduce their toxicity and change their organoleptic features. The objective of this experiment was to test the effectiveness of fermentation with molasses as a detoxifying process to enable the use of canavalia beans as feed for growing pigs using a short-term intake trial for assessment.

Canavalia beans were mechanically chopped, soaked in tap water (1 h), and then drained for 8 h to give a final dry matter (DM) of 45%. The product was divided into two parts and 4.5% molasses was added to one portion. Both portions were put into plastic bags, air squeezed out, sealed, and kept for 12 d. The contents were then dried at 60° and ground into flour. Some raw canavalia was also ground. Four diets formulated to ARC standards were prepared, three of which included a canavalia flour at 25% (replacing soya protein), with sorghum, soyabean meal, wheat bran, vegetable oil, vitamins and minerals. The experimental diets were: control (*Ctrl*), fermented canavalia (*FeC*), Canavalia fermented with molasses (*FMC*) and raw canavalia (*Raw*). Twenty-four crossbred pigs, of 43.3 (SD 1.7) kg in individual pens were allocated at random to one of the four diets for a 3-d trial and daily intakes recorded as: day-1, diet (day-1) were similar to those of day-1 with no evidence of compensation. The experimental diets; day-3, the commercial food again.

In *FeC*, acetic and lactic were the main acids produced (15.4 and 8.5 g kg⁻¹ DM, respectively). Canavanine was reduced from 18 to 0.9 g kg⁻¹ DM; haemaglutination reaction (HR) was slightly reduced. With *FMC*, lactic and acetic acids (24 and 7.12 g kg⁻¹ DM) were the main products; HR was completely eliminated and canavanine reduced to 15 g kg⁻¹ DM. The acetic-fermentation pattern was associated with a large reduction in canavanine concentration but with a limited effect on lectins. The lactic-fermentation pattern was associated with the elimination of HR and reduction of canavanine to 83% of its original concentration. This agrees with other trials in our laboratory. Means of cumulative intake of the normal diet on days 1 and 3, and of the experimental diets on day 2 are given in the Table. Intakes of the normal diet (day-2) were accustomed to which the pigs were accustomed: day-2, the experimental diets; day-3, the commercial diet to which the pigs were accustomed: day-2, the experimental diets; day-3, the commercial diet to which the pigs were accustomed. The results suggest

Intake by growing pigs of normal diet (days 1and 3) and cumulative intakes of experimental diets with raw and fermented canavalia (g, fresh basis); day-2 (*n* 4).

	Ctrl	FeC	FMC	Raw	SED
Day-1	1473	1799	1896	2156	91
0.5h	314 a	184 ab	138 b	70.8 b	68
1h	433 a	202 b	138 b	75.8 b	91
1.5h	478 a	202 b	149 b	76.7 b	94
2h	578 a	207 b	150 b	82.5 b	99
4h	784 a	222 b	172 b	109.2 b	110
8h	1050 a	323 b	284 b	131.7 b	124
12h	1505 a	686 b	362 b	175.8 b	140
18h	1707 a	835 b	367 c	176.7 c	149
Day-3	1316	1695	2172	2283	177

Different superscripts indicate significant differences ($P<0.05$).

that both fermentation treatments improved intake over that obtained with the raw canavalia, but only reached statistical significance ($P<0.05$) with the *FeC* treatment by 18 h. Intake of the control diet with no canavalia was substantially better ($P<0.001$).

The reduction of canavanine and possibly other ANF by the *FeC* treatment is the most promising, for it allowed a higher intake than *Raw* and the *FMC* treatments. However intake was still restricted by some factor(s), and it is necessary to focus efforts on procedures that will allow elimination of several ANF at the same time. The short-term trial, which minimizes stress to the pigs, provided a useful screening of methods of treatment preparatory to longer term trials with the most promising treatments.

Effects of diet and length of feeding period on the fatty acid content of the phospholipid fraction of bovine *longissimus dorsi* muscle. By N.J. CHOI[1], A. COOPER[1], M. ENSER[2], J.D. WOOD[2] and N.D. SCOLLAN[1], [1]*Institute of Grassland and Environmental Research, Aberystwyth SY23 3EB, [2]Division of Food Animal Science, University of Bristol, Langford, Bristol BS40 5DU*

The polyunsaturated fatty acid (PUFA) composition of ruminant tissues is affected by differences between the metabolism of linoleic acid (C18:2n-6) and α-linolenic acid (C18:3n-3) both in the rumen and postruminally. This study investigated the effect of different dietary ratios of C18:2n-6/C18:3n-3 provided by full fat soya (high C18:2n-6) or whole linseed (high C18:3n-3), under a high concentrate feeding regime, on the fatty acid composition of beef muscle.

Twenty-eight Charolais steers with an initial live weight of 548 kg (SE 10.0) were randomly allocated to receive one of two dietary treatments for either 60 or 90 d. The diets were forage (mixture of grass silage and straw, 50:50 ratio on a dry matter basis) and one of two experimental concentrates (80% on a DM basis) which were based on wheat and molassed sugarbeet feed with differing fat sources: Diet 1; full fat soya and Diet 2; whole linseed, with C18:2n-6/C18:3n-3 ratios (whole diet) of 6.28 and 0.38, respectively. Diets were formulated so that total dietary oil intake was 7% of DM intake with approximately 3.5% from the experimental test fat. Lipids were extracted from *longissimus dorsi* muscle (Choi *et al.* 2000), phospholipids were separated by silicic acid chromatography and fatty acids analysed by GC.

The amounts of C18:2n-6 and C20:4n-6 were higher in the muscle phospholipids of soya-fed steers, while C18:3n-3, C20:5n-3 and C22:5n-3 were higher after linseed feeding. The latter also resulted in more C18:1 *trans* and CLA but C22:6n-3 was unaffected. C18:2n-6 increased with time on feed (TOF) for the soya diet as did C18:3n-3 on the linseed feed. C18:2n-6 also increased with TOF for the linseed treatment despite the lower intake relative to the soya treatment and an 18-fold difference in the dietary C18:2/C18:3 ratio. Changes in C18:2n-6 and C18:3n-3 in muscle neutral lipids (data not shown) reflected those in the phospholipids and total lipid P:S ratio was unaffected by feed although the n-6:n-3 ratio was higher on soya.

	Soya		Linseed				Significance	
	60 d	90 d	60 d	90 d	SED	Diet	TOF	Interaction
mg/100g muscle (phospholipid)								
18:1*trans*	4.6	4.6	7.1	5.3	0.97	*	NS	NS
CLA	0.71	0.98	1.38	1.58	0.316	**	NS	NS
18:2n-6	83.2	98.1	58.2	71.0	7.71	***	*	NS
18:3n-3	10.1	8.8	26.0	33.7	2.76	***	NS	*
20:4n-6	23.7	28.5	23.6	21.8	2.32	*	NS	NS
20:5n-3	9.7	8.3	14.1	13.9	1.21	***	NS	NS
22:5n-3	14.7	16.1	17.7	19.9	1.32	**	NS	NS
22:6n-3	2.1	1.6	1.7	1.8	0.36	NS	NS	NS
P:S and n-6:n-3 ratios (total fat)								
P:S	0.11	0.10	0.09	0.10	0.014	NS	NS	NS
n-6:n-3	3.33	4.09	1.45	1.33	0.238	***	NS	*

NS>0.05, *P<0.05, **P<0.01 and ***P<0.001, analysis of variance.

This study demonstrates the strong preferential incorporation of n-6 PUFA into beef muscle phospholipids. An 8-fold higher intake of C18:3n-3 and much lower n-6:n-3 ratio in the feed on linseed compared with soya only increased muscle phospholipid C18:3n-3 levels 2- to 3-fold and decreased the ratio of C20:4n-6/C20:5n-3 from 2.9 to 1.6 with small increases in other n-3 PUFAs. Nevertheless, linseed resulted in a balance of fatty acids in beef muscle, which is more in line with current health recommendations.

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Effect of diet on nitrogen partitioning between carcass and individual organs of growing beef cattle fed at similar levels of metabolizable energy intake. By E.-J. KIM[1,2], A. COOPER[1], D.S. PARKER[2], J.M. DAWSON[3], P.J. BUTTERY[3], M.S. DHANOA[1] and N.D. SCOLLAN[1], [1]*Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth SY23 3EB, [2]Department of Biological and Nutritional Sciences, University of Newcastle, Newcastle upon Tyne NE1 7RU, [3]School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD*

An understanding of the dynamics of protein metabolism requires more information on rates of protein accretion. A comparative slaughter experiment was carried out to examine the effect of feeding grass silage alone compared to silage and concentrate at a similar level of metabolizable energy (ME) intake on the partitioning of nitrogen into carcass and other body components.

Eighteen Hereford × Friesian steers were randomly assigned to two dietary treatments: grass silage alone or a mixture of grass silage and a barley/soya concentrate (80:20 DM basis) in the ratio of 60:40 (on a ME basis), and one of three slaughter live weights, 250, 350 or 500 kg. Each animal received 800 kJ ME per kg $M^{0.75}$ per day. At slaughter, the animals were dissected into seven different fractional (F1–F7) components: (1) head, hide, feet and spinal cord, (2) heart, lungs, trachea, spleen, thymus and diaphragm, (3) oesophagus, rumen, reticulum, omasum, abomasum, intestines and pancreas, (4) liver and gall bladder, (5) mesenteric, omental, thymus fat and kidney knob channel fat, (6) penis, empty bladder and left kidney, (7) left half carcass. The total N content of each fraction was measured by the macro-Kjeldahl method. Two-way analysis of variance was used to test the effects of diet and stage of growth, and interactions. The animal performance and chemical composition of the diets were reported by Kim *et al.* (2000).

Actual ME intakes were 791 and 822 (SED 15.6) kJ/kg $M^{0.75}$ per day on silage alone and silage–concentrate, respectively. Nitrogen (N) accretions in the whole body between 250 and 500 kg live weight were linear and no significant differences between diets were detected for total body N at each slaughter point. Although N accretion of heart and lung, and gut tissues declined as the animals grew, the proportional distribution of N in most components was similar at each slaughter point.

	Proportion of total nitrogen (g/kg)							*P*-value	
Slaughter weight (kg)	250		350		500				
Diet	S	S–C	S	S–C	S	S–C	SED	Diet	Slaughter wt
Head, hide, feet (F1)	237	227	231	240	233	235	10.2	NS	NS
Heart, lung (F2)	24.8	23.9	22.5	23.3	20.9	21.9	1.50	NS	0.05
Intestinal tissues (F3)	38.2	36.3	37.0	36.6	32.4	30.0	2.42	NS	0.01
Liver (F4)	17.2	15.9	17.1	16.3	16.5	16.6	0.87	NS	NS
Fat (F5)	9.4	9.9	6.7	9.7	5.4	6.3	2.51	NS	NS
Bladder, kidney (F6)	5.1	5.1	4.8	4.6	4.9	4.2	0.35	NS	NS
Carcass (F7)	668	682	681	670	687	686	13.0	NS	NS
Total body N (kg)	6.0	6.3	8.2	8.2	11.3	11.7	0.48	NS	0.01
Total N concentration (g N/kg slaughter wt)	24.3	25.1	24.4	23.9	23.0	23.4	0.38	NS	0.01

NS, not significant. Interactions between diet and slaughter weight were not significant (residual d.f. 11).

The results for total N deposition support the age-based (weight) decline in the N content of the empty body weight adopted by ARC (1980). Diet did not affect any of the parameters reported. The main difference between feeding all forage, silage compared to silage and concentrate is to increase the rate of tissue accretion as reported by Kim *et al.* (2000), but it does not influence the amount of N in the total body or in various organs and tissues.

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Levels of protein intake in young restricted steers do not affect myofibrillar protein degradation. By N.S. PRATHALINGAM[1], J.R. SCAIFE[1], L. HEASMAN[2], J.R. PARKER[2] D.G. CHAPPLE[3] and M.A. LOMAX[1], [1]*Department of Agriculture, University of Aberdeen, 581 King Street, Aberdeen, AB24 5UA* [2]*ADAS High Mowthorpe, Duggleby, Malton, N. Yorkshire, YO17 8BP and* 3ADAS *Rosemaund, Preston Wynne, Hereford, HR1 3PG*

Young ruminants undergoing normal growth have a higher fractional rate of myofibrillar protein degradation than older animals (Waterlow *et al.* 1978). After a period of feed restriction in young ruminants the rate of myofibrillar protein degradation decreases, playing a key role in total muscle accretion during subsequent compensatory growth. The level of protein fed during the restriction period and the resulting altered carcass composition may affect myofibrillar protein degradation rates and therefore subsequent growth rates. The fractional rate of myofibrillar protein degradation can be estimated using urinary 3-methyl-histidine (3MH) excretion (Harris & Milne, 1980) and creatinine levels to assess total muscle mass (Funabe *et al.* 1996).

In autumn, twenty-eight spring-born Limousin cross steers were allocated to one of two dietary treatments. During winter half the steers (*n* 14) were fed a silage-based diet supplemented with rumen bypass fat (Magnapac) to achieve a diet low in metabolizable protein (LMP) and to attain a carcass high in fat composition. The remaining fourteen steers were fed a diet high in metabolizable protein (HMP) (straw and barley-based diet supplemented with a source of digestible undegradable protein (Sopralin)) to achieve a lean body composition. Throughout the winter store period, steers were individually fed to limit growth rates to 0.6 kg per day. In spring seven animals per treatment were restrained in metabolism crates and urine collections were carried out for 5 d. After urine collection the cattle were pulse bled through a jugular catheter at 15-min intervals for 6 h before being slaughtered. The remaining seven steers from each group were weighed weekly and individually fed on grass and silage *ad libitum* to determine live weight gain and dry matter intakes. In autumn when the remaining steers reached a mean weight of 382 kg, urine and blood samples were taken before slaughter. Urine samples were analysed for 3MH and creatinine. Blood samples were analysed for plasma insulin, IGF-1 and glucose. All statistical analysis was carried out using ANOVA.

Glucose, insulin, plasma IGF-1, creatinine, 3MH and 3MH:creatinine ratios in steers.

Index	Spring HMP Mean	HMP SEM	Spring LMP M	LMP SEM	P	Autumn HMP Mean	HMP SEM	Autumn LMP Mean	LMP SEM	P
Glucose (ng/ml)	4.171	0.183	4.757	0.171	*	3.798	0.230	4.095	0.181	NS
Insulin (ng/ml)	0.649	0.064	1.055	0.115	*	0.793	0.097	0.886	0.084	NS
IGF-1 (ng/ml)	58.31	9.36	62.74	7.32	NS	56.08	6.25	52.96	4.67	NS
Creatinine (g/d)	5.67	0.45	7.04	0.33	*	9.41	0.75	9.86	1.07	NS
3MH (μmol/kgBW)	14.45	2.68	18.95	4.26	NS	27.71	6.05	29.08	7.63	NS
3MH (mg/d)	138.1	28.4	182.6	46.6	NS	333.6	67.9	363.6	93.7	NS
3MH(mg/d)/Creatinine	25.37	6.32	26.30	7.29	NS	38.90	10.20	40.30	12.60	NS

NS Not significant, * *P*<0.05.

In the spring slaughter group animals fed on the LMP diet had significantly higher levels of insulin, glucose and creatinine than animals on the HMP diet. There was no significant difference after feed restriction on subsequent feed intake and growth rates between the two groups. In the autumn slaughter group, however, there was no significant difference between the HMP and the LMP steers in the parameters measured.

The higher levels of insulin in the spring LMP group compared with the spring HMP group are reflected in the respective glucose levels. There was no effect of treatment on myofibrillar protein degradation in the steers. Subsequent feed intake and growth rates remained unaffected by the feed regime during the restriction period. Chemical analysis of the half carcass remains to be determined.

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Effect of rumen fish oil injection on *in vivo* **digestion and fermentation characteristics: differences in animal response.** By V. FIEVEZ, M. DANNEELS and D. DEMEYER, *Department of Animal Production, Ghent University, Proefhoevestraat 10, 9090 Melle, Belgium*

In general, depressed rumen methane (CH₄) production by long chain fatty acids is accompanied by an increased proportion of propionate and a lower rumen fibre digestibility because of a direct toxic effect on methanogens and defaunation (Van Nevel & Demeyer, 1996). With fish oil, this shift from CH₄ to propionate has been confirmed without any effect on ruminal (Keady & Mayne, 1999) or faecal (Doreau & Chilliard, 1997) fibre digestibility. Hence, the effect on rumen fermentation characteristics of fish oil [main fatty acids (% w/w): C₁₄:₀, 7.1; C₁₆:₀, 16.1; C₁₆:₁, 9.5; C₁₈:₁, 12.9; C₂₀:₅, 18.1; C₂₂:₆, 11.8] injection (20 ml, twice a day, 1 h after feeding) through the cannulas of four rumen-fistulated sheep, fed a hay/concentrate (65/35) diet at maintenance level, was studied in a crossover design experiment. Daily DM intake, potential *in sacco* NDF degradation after 48 h incubation, faecal NDF digestibility, rumen particle outflow rate (Cr-hay), protozoa and VFA concentrations, renal excretion of purine derivatives and *in vitro* rumen fermentation pattern (24 h incubation) were determined.

		Sheep A	Sheep B	Sheep C	Sheep D	Mean SD
DM intake (*n* 16)		709	687	888ᵃ	959ᵃ	19
(g/d)	FOI	703	708	720ᵇ	880ᵇ	42
In sacco NDF degradation (48 h) (*n* 6)		54.5	58.2ᵃ	60.9ᵃ	56.4	2.5
(%)	FOI	52.7	45.9ᵇ	50.9ᵇ	56.8	4.7
Outflow rate (*n* 3)		3.54	5.00	4.09	2.28	1.05
(% h⁻¹)	FOI	2.90	2.70	2.93	2.02	0.69
Protozoa (*n* 3)		1.64	1.72ᵃ	1.79	1.37	0.35
(10⁶ ml⁻¹ rumen contents)	FOI	1.87	0.63ᵇ	2.01	1.54	0.40
Renal purine derivate excretion (*n* 3)		4.34	2.61ᵃ	4.38	4.07ᵃ	0.73
(mmol/d)	FOI	3.67	4.24ᵇ	3.81	3.10ᵇ	0.65
In vivo rumen propionate concentration		211ᵃ	227ᵃ	223ᵃ	187ᵃ	7
(*n* 3) (mmol/mol total VFA)	FOI	252ᵇ	282ᵇ	270ᵇ	246ᵇ	16
In vitro CH₄ production (*n* 3)		367ᵃ	269	326	388ᵃ	22
(mmol/mol total VFA)	FOI	293ᵇ	283	305	271ᵇ	35
Apparent faecal NDF digestibility		48.9	50.3	50.9	50.3	4.1
(*n* 3) (%)	FOI	40.9	53.7	51.7	46.3	6.0

ᵃ,ᵇ Different letters indicate significant differences due to fish oil injection (FOI) (*P* <0.05).
n = number of repeated measurements.

Results, shown in the table, indicate that fish oil injection did not alter faecal NDF digestibility. However a lower potential *in sacco* degradability was observed for two sheep. Increased rumen retention, as suggested by the tendency towards a decreased outflow rate, or a shift of the fermentation towards the hindgut (Van Nevel *et al.* 1993) may have compensated for this lower rumen degradability. Fish oil induced higher rumen *in vivo* propionate concentration, suggesting a depression in methanogenesis, as confirmed in incubations *in vitro*. However, CH₄ depression was not associated with defaunation, as reduced protozoal numbers were observed in one sheep only. Except for this partially defaunated sheep, there was a clear tendency for microbial growth to be reduced by fish oil supplementation, as also observed *in vitro* by Hoover *et al.* (1989). The results suggest that fish oil (fatty acids) depress methanogenesis in the rumen and may shift fibre digestion to the lower intestinal tract. The latter effect is associated with reductive acetogenesis, a process also contributing to an overall decrease in animal methanogenesis. Possible negative effects on rumen microbial growth and differences in animal response have to be taken into account when using fish oil in ruminant diets.

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Attitudes to food and handling of nutrition concepts among school-aged children in Guildford. By K.H. HART[1], J.A. BISHOP[1], E. BUCHANAN-BARROW[2] and H. ANTHONY[1], [1]*School of Biological Sciences*, [2]*School of Human Sciences, University of Surrey, Guildford GU2 7XH*

There is evidence that behaviours in childhood continue throughout life (Kelder *et al.* 1994) and the reported link between childhood diet and adult coronary heart disease risk (Willett, 1990) illustrates a need to maximize the quality of nutritional intake in this population. The disparity between nutritional knowledge and behaviour change is likely to be pronounced in this group due to the limitations of cognitive development (Lytle *et al.* 1997) and in this heterogeneous population effective education must be appropriate in context and content. The aim of this study was to determine the attitudes and approaches used by children of different ages, gender and socio-economic status (SES) when dealing with nutrition issues.

114 children from four local schools participated in focus groups investigating parental food rules, and knowledge of diet–disease links and grouping of foods. Two schools were classified as lower and two as higher SES on the basis of their free school meal ratio. Groups were separated by age (7 and 11 years) and gender and systematically sampled from alphabetical class lists. Transcripts from twenty-three focus groups were coded and qualitatively analysed to establish areas of between-group consensus and variance.

An effect of gender was apparent. As well as being more likely to report parental food restriction, girls in both age groups were more likely than boys to correctly identify a diet–disease link, particularly in relation to fattening foods, and were less likely to use taste or preference as an indicator of a food's health value. Differences were also noted between the low and high SES schools, particularly in relation to rules, with those attending a high SES school more likely to be prescribed or restricted foods and less likely to be given free choice than those attending a low SES school. Overall nutritional knowledge and handling of the nutritional concepts were good, although recognition of more advanced ideas such as "moderation" and "variety" was restricted to a small sub-section of the group, primarily older children and those attending a high SES school. However, the older children did not show a consistently better grasp of the nutritional concepts, with 11-year-olds more likely to mention single foods when asked about food groupings, than 7-year-olds. Overall foods were most frequently grouped by nutrients or Balance of Good Health categories (Health Education Authority, 1994), although knowledge in this area appeared to vary greatly between individuals, independent of age or SES. Direct questions provoked greater accuracy than asking children to develop their own food groups and deviations from standard categories were logical and based on sources, meal combinations or primary ingredients.

This investigation demonstrated that the interpretation of nutritional topics differs among children, varying with gender and SES. These findings may affect the way in which any new healthy eating guidelines are accepted and acted upon. Further development of this research will elucidate the extent to which these differences are mirrored in actual consumption, enabling an informed approach to new, effective healthy eating guidelines for school-aged children.

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Dietary supplement use in women: costs and frequency of use. By A. GREENHALGH[1], J. CADE[1] and C. RICHARDSON[2], [1]*Nutrition Epidemiology Group, Nuffield Institute for Health, University of Leeds, 71–75 Clarendon Rd, Leeds LS2 9PL*, [2]*Leeds Metropolitan University, Caverley St, Leeds LS1 3HE*

The use of dietary vitamin and mineral supplementation is reported to be increasing within the UK population with a projected growth rate of 10–14% in the next 3 years (Nesheim, 1999). The supplement market is presently valued at £360 million per year (Euromonitor, 1998). Despite this, little is known about the financial costs of supplement usage.

The reported use of dietary supplements in subjects, aged 35–69 years at baseline, recruited by the UK Women's Cohort Study has been previously reported; 60% of these subjects claimed to take dietary supplements on a regular basis (Kirk *et al.* 1999). The second phase of data collection further explores this data.

To date we have questionnaire data for a total of 2810 subjects. Of those, a total of 1972 subjects (70%) reported taking dietary supplements, with 1580 (80.1%) taking these supplements daily, 100 (5.1%) intermittently and 292 (14.8%) reporting a mixture of daily and intermittent use. The mean number of supplements taken per day was found to be 2.8 (SD 2.1) for the daily supplement users and 0.3 (SD 0.8) for intermittent users. A total of twenty-one different supplements were used by the sample and the most popular supplements were found to be fish-oils (*n* 757), multivitamins (*n* 755) and vitamin C (*n* 684), followed by evening primrose oil (*n* 641) and calcium (*n* 494).

Qualitative data from a random selection of this group (*n* 30) was obtained via a telephone interview. Subjects were asked a variety of questions focusing on the cost, perceived knowledge of vitamins and minerals and rationale for supplement use.

The mean annual expenditure for supplements was £88 per person (range 5–360). Subjects from higher socio-economic groups and those who claimed to have a greater knowledge of supplements and their uses were found to spend significantly more on supplements than those from lower socio-economic groups and those who reported having limited knowledge of supplements (*P*<0.05). The mean duration of supplement use was 6 years (range 0.25–30). Women who took supplements both on a daily and intermittent basis were found to spend significantly more on supplements, to take a greater number of different types of supplements, and to have taken these for longer than either the daily or intermittent users (*P*<0.05). The majority of subjects thought that supplements were expensive. Despite this, those subjects who took supplements on a daily basis claimed that they would take more supplements if they were cheaper, and would rather spend money on supplements than cosmetics, clothes, books or music. The main influence on supplement use in this sample was reported to be the media, followed by the family.

In conclusion, results from this small sub-study suggest that dietary supplements are generally taken on a daily basis and the use of these may be increasing amongst women in the UK, despite the fact that supplements are often viewed as being too expensive.

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Dietary advice and assessment by practice nurses in routine consultations: missed opportunities? By D.L. WARM[1], P.S. LITTLE[2] and J. BARNETT[2], *Institute of Human Nutrition, [2]Primary Medical Care, University of Southampton, Southampton SO16 6YD*

Many of the most common chronic diseases in this country are lifestyle-related and many have been targeted in the recent Government White Paper 'Saving Lives – Our Healthier Nation' (Department of Health, 1999). The White Paper also proposes a reorientation of the NHS such that Primary Health Care will have new responsibilities for Public Health. This in turn will lead to a greater role as a focal point for lifestyle counselling. Although there is doubt concerning the optimal strategies in primary care (Little & Margetts, 1996), there is evidence that practice nurses giving dietary advice can be cost-effective (Wonderling *et al.* 1996). At present it is not clear what dietary advice and assessment are given and when.

We report the frequency and nature of dietary assessment and counselling by eight practice nurses in consecutive consultations in three general practices. Each practice nurse was to complete a list of the first five 'tasks' performed on consecutive patients entering the treatment room over a 1-week period.

During the study period, 669 patients were seen of which 144 (21.5%) were 'lifestyle' patients: i.e. hypertensive (*n* 65), overweight (*n* 28), diabetic (*n* 40) or hypercholesterolaemic (*n* 11) patients.

'Task'	Hypertensive	High cholesterol	Overweight	Diabetic	Total
Weight	16	3	18	12	49
Blood pressure	43	1	3	10	57
Advice on fat	6	3	6	7	22
Advice on salt	7	1	0	1	9
Advice on fibre	0	0	3	0	3
Advice on fruit and vegetables	2	0	3	0	5
Advice on alcohol	5	1	1	0	7
Other dietary advice	3	0	2	9	14
Dietary assessment	3	1	3	1	8

Tasks performed by practice nurses on 'lifestyle patients' in routine consultations.

Of these patients, eight underwent some sort of dietary assessment (see Table), although none was by a recognised validated method, and very few were given any sort of dietary advice. However practice nurses do use opportunities for dietary advice, and most dietary advice and assessment is 'hidden' – occurring as a secondary activity in the consultation. Such activity may not be picked up by normal methods of assigning attendance labels to consultations.

Since those willing to undertake this research are likely to be more interested in lifestyle counselling than most, even the figures quoted in this study are likely to be an overestimate of true activity. One drawback with this study is knowing whether patients had been previously seen for dietary assessment or advice: however, even if these results just apply to patients being followed up for conditions where lifestyle is likely to be important, the level of activity is still low.

This research raises issues about the opportunities taken and missed, and the quality and likely effectiveness of dietary assessment and counselling in primary care. Additionally, it is important to assess what the demand for dietary counselling in Primary Health Care is, and whether it is being met or not.

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Food choice and socio-economic variables in relation to young women's confidence in cooking specific foods. By J.M. LAWRENCE, R.L. THOMPSON and B.M. MARGETTS, *Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD*

The Independent Inquiry into Inequalities in Health (Acheson, 1998) recommended policies to improve the health and nutrition of women of child-bearing age, specifically the development of budgeting and cooking skills.

We previously confirmed a link between cooking skills and food choice (Lawrence *et al.* 2000) but concluded that socio-economic variables needed to be considered. Respondents, 877 women aged 20–34 years taken from a nationally representative sample of 5553 English adults (70% response rate; Margetts *et al.* 1998), were asked if they felt confident about cooking specific types of foods. The odds ratios for high fruit and vegetable intake (fruit and vegetables each day) and low fat intake (less than or equal to 84 g/d) were calculated for those women who were confident about their cooking ability. Women who were not confident about their ability to cook formed the reference category. Total fat and fruit and vegetable content of the diet were assessed using the DINE (Roe *et al.* 1994) questionnaire. The odds ratios were then adjusted for four socio-economic variables (having 'A' levels or higher qualifications *v.* lower level of qualification, being in non-manual *v.* manual employment, home ownership *v.* rented accommodation, and use of a car *v.* no car) using logistic regression.

Confidence in ones ability to cook:	Crude OR (95% CI) for high fruit & vegetable intake	Adjusted OR (95% CI) for high fruit & vegetable intake	Crude OR (95% CI) for low fat intake	Adjusted OR (95% CI) for low fat intake
Oily fish	1.68 (1.28–2.20)	1.56 (1.14–2.13)	1.37 (1.01–1.86)	1.13 (0.80–1.60)
Pulses	1.60 (1.23–2.09)	1.43 (1.05–1.95)	1.60 (1.18–2.19)	1.62 (1.14–2.31)
Pasta	2.11 (1.39–3.20)	1.62 (0.98–2.69)	1.72 (1.03–2.85)	1.43 (0.77–2.65)
Rice	2.60 (1.66–4.06)	1.91 (1.11–3.29)	2.04 (1.18–3.54)	1.84 (0.94–3.62)
Fresh green vegetables	2.26 (1.30–3.93)	1.87 (0.92–3.79)	3.53 (1.50–8.35)	2.91 (1.03–8.22)
Not confident (reference)	1	1	1	1

The table shows that women who were confident about their ability to cook various foods were more likely to eat a high fruit and vegetable diet (odds ratio between 1.60–2.60) and also a low fat diet (1.37 and 3.53). Taking account of socio-economic variables reduced the odds ratios, reducing the apparent effect of confidence in cooking as to whether people had a low fat consumption. Adjusting for socio-economic factors had less of an effect on the relationship between confidence and consumption of a high fruit and vegetable intake.

These results confirm an association between confidence in ability to cook specific foods, healthy eating and socio-economic variables. They suggest that in addition to confidence, socio-economic circumstances have an important impact on cooking ability, particularly for low fat foods. Policies aimed at the development of cooking skills may not be effective in socially deprived areas.

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Roe L, Strong C, Whiteside C, Neil A & Mant D (1994) *Family Practice* **11**, 375–381.

Assessment of knowledge about osteoporosis of postmenopausal women referred for bone densitometry. By J. EARLAND[2], C. WRIGHT[1], J. KLIENMANN[1], L.M. WALLACE[1], J. BARLOW[1] and A. PARSONS[2], [1]School of Health and Social Sciences, Coventry University, Priory Street, Coventry CV1 5FB, [2]Obstetrics and Gynaecology Department, Hospital of St. Cross, Barby Road, Rugby CV22 5XP

Osteoporosis is a major public health problem in the UK. Although peak bone mass is determined in early life, there is evidence that the risk of fractures can be reduced in later life by changing lifestyle factors. These include increasing calcium intakes several years after the menopause (Reid & New, 1997) and carrying out weight-bearing exercise (Rutherford, 1997). As part of a larger study, it was decided to examine existing knowledge about osteoporosis, including dietary factors of a group of women who had been referred for bone densitometry to a hospital outpatient department. Fifty women (response rate 72%) completed the study. Information was collected on lifestyle behaviours, osteoporotic risk factors, medical history and knowledge about osteoporosis, bone scans, HRT and diet. A FFQ for the estimation of calcium intakes was also administered.

The mean age of the sample was 62 years (SD 13 years). Fifty per cent held educational qualifications, of which a third were above O level. Of the sample, 30% were currently working whilst 56% were retired. Regular exercise for health reasons was carried out by 71% of the women. Information about osteoporosis had been received by 38% of the sample, the most common sources being the media, particularly magazines, and their GP. Preliminary results from the FFQ indicated that 74% of the subjects in this study had intakes which were above the RNI set by the Department of Health of 700 mg/d.

The mean knowledge score was 16.6 out of 36 (SD 5.88, range 0–28). The majority of respondents were aware that osteoporosis is more common in women (92%), the elderly (86%) and if present in the mother (56%). The responses to the dietary questions indicated that there was some confusion about the calcium content of low fat dairy products and the role of dairy products in determining plasma cholesterol, as shown below. The percentage of subjects giving each response is shown, with the correct answer in bold.

Statement	False	Don't know	True
Milk chocolate is a good source of calcium	26.0	22.0	**52.0**
There is more calcium in a pint of whole milk than skimmed milk	**50.0**	12.0	38.0
People with raised blood cholesterol can get plenty of calcium from eating cottage cheese	10.0	46.0	**44.0**
People with raised blood cholesterol should not eat dairy products	**24.0**	26.0	50.0
People with raised blood cholesterol can increase their calcium intake by eating low fat dairy products and drinking skimmed milk	16.0	22.0	**62.0**
Dairy foods provide the main source of calcium in the Western diet	6.0	12.0	**82.0**

The following variables were found to be determinants of knowledge: age of the subject, (r –0.285, P 0.045), having an educational qualification (P 0.025) and having received information about osteoporosis (P 0.004). In conclusion, knowledge about osteoporosis amongst this small sample of women was only at a superficial level. It is important that women are fully informed about osteoporosis so that they can make informed choices about accepting treatment and changing lifestyle habits.

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Nutrition in the news! By A. EVES, M. LUMBERS and M. KIPPS, *Food and Health Care Management Research Group, SOMSS, University of Surrey, Guildford GU2 7XH*

Newspapers are an important source of food-related information. There has been criticism of the accuracy with which science topics are reported, particularly in the tabloid press (Pellachia, 1997) and accusations of sensationalism. Chipman *et al* (1996), however, noted that it is a considerable challenge to create messages perceived to be objective and not sensational, and yet maintain the interest of the reader. Hertog & Fan (1995) noted a tendency for journalists to emphasise conflict, controversy and sensationalism, leading to public confusion, distrust of experts and fear. In particular, Smith *et al.* (1997) recognised the crucial role that the media play in the dynamics of food scares. There is a small, but growing, literature on the reporting of food-related issues in the newspapers (The Times, The Telegraph, The Guardian, The Daily Express, The Sun and The Daily Mirror) over a 3-month period from January to April 1998. Each day, including weekends, the number of food-related stories, the subjects covered and the amount of space devoted to these was recorded. Food-related stories were categorized into eight broad topics, and total stories and space over the period were determined.

Number of stories in... (column cm)	The Times	The Daily Mail	The Telegraph	The Sun	The Daily Mirror	The Guardian
BSE	46 (2632)	39 (2447)	51 (2543)	6 (320)	21 (601)	74 (3647)
Agriculture	8 (854)	7 (682)	10 (824)	1 (8)	3 (172)	13 (934)
European Union	6 (164)	8 (230)	6 (487)	3 (228)	3 (20)	4 (377)
Government	6 (411)	5 (294)	7 (401)	1 (5)	5 (3719)	6 (580)
Special diets	22 (1386)	23 (2411)	13 (1024)	5 (425)	41 (6873)	17 (5015)
Food and health	50 (2669)	53 (3710)	38 (2718)	3 (204)	44 (1821)	29 (1743)
Food safety	17 (821)	18 (653)	10 (861)	1 (8)	3 (104)	13 (938)
General	57 (5598)	24 (1681)	25 (1875)	5 (520)	11 (451)	6 (616)
TOTAL	212 (14535)	177 (12108)	160 (10733)	25 (1718)	131 (13761)	162 (13850)

The extent of food-related coverage varied considerably between newspapers. In particular, The Sun carried very few food-related stories. There were also differences in emphasis between newspapers, with The Daily Mirror ascribing 50% of its 'food' column centimetres, and relatively little space (0.8%) and few stories (2%) to 'food safety'. The Times carried 27% of 'food' stories classified as 'General', including restaurant and business profiles in the weekend editions. The 'food and health' category included coverage of suggested links between a wide variety of foods and particular complaints, including a possible link between cancer and alcohol consumption. Some reports suggested that alcohol prevented cancer in general, and others that it might lead to the development of breast cancer in women. The government's anticipated advice on red meat consumption, moderated in this period, received some coverage, most referring to a government U-turn. A large proportion of stories in five of the newspapers related to BSE (between 16 and 46% depending on newspaper). The BSE inquiry was launched in this period, and also the ban on beef on the bone was instigated. In February 1998, the UK government published a major Green Paper (which has since become a White Paper) – *Our Healthier Nation*. Very few stories were devoted to this and, in most cases, very little space (comprising part of the 'Government' category). The exception was the Daily Mirror, which included a 4-page pull-out on the subject. Arguably this could ultimately be of more significance to the public's health and mortality than BSE. It is, however, less newsworthy, illustrating the bias that can occur in reporting through the emphasis given to different issues. Whilst nutritional issues were widely covered in five of the newspapers, many stories, especially in the tabloid press, could be described as 'sensational' or 'bizarre', for instance giving graphic descriptions of eating disorders, or suggesting that diets should be chosen according to blood group.

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Hertog JK & Fan DP (1995) *Communication Research* **22**(5), 545–574.
Pellachia MG (1997) *Public Understanding of Science* **6**(1), 49–68.
Smith AP, Young JA & Gibson J (1997) *Risk Decision and Policy* **2**(11), 41–51.

Probiotics: perceptions and dietetic practice. By C. BRICE, B. McKEVITH and C. SHORTT, *Yakult UK, 12–16 Telford Way, Acton, London W3 7XS*

Scientific publications relating to the use of probiotics in clinical research and practice are steadily increasing (Salminen *et al.* 1998; Naidu *et al.* 1999). Among health professionals, dietitians have shown particular interest in the use of probiotics for the modulation of the gut flora. To date, few studies have evaluated the views of dietitians regarding the therapeutic potential of probiotics.

The present study was designed to assess perceptions, knowledge and application of probiotics among dietitians. A postal questionnaire was sent to 1201 randomly selected UK-registered dietitians. Fifty-two questionnaires were returned as the addressee had moved away. A total of 441 (39%) of the questionnaires were returned and analysed using the Minitab statistical package (version 12.0). Thirty-one questionnaires were incomplete and were not included in the analysis.

The survey revealed that 74% of the dietitians were able to identify the definition of a probiotic. Interestingly, 40% of the respondents indicated that they use probiotics in practice. The data also suggested that those who had been in practice longer were more likely to use probiotics. The conditions that dietitians most commonly recommend probiotic products for were: antibiotic-induced diarrhoea, post-antibiotic therapy and irritable bowel syndrome. The most common reasons given for not applying probiotics in practice were: being unsure of the potential benefits, perceived lack of supporting evidence and a lack of specific product knowledge.

Understanding of the properties and effects of probiotics and lactic acid bacteria was generally of a high standard. However, 57% were unsure whether probiotic bacteria remained viable after transit through the gut – one of the key criteria for a probiotic. Thus, there is scope for further targeted education programmes.

Knowledge statement	Dietitians' Responses (%)		
	Agree	Unsure	Disagree
Probiotics contain live, beneficial micro-organisms	93	6	1
All bacteria are pathogenic	3	10	87
Lactic acid bacteria produce acids and hydrogen peroxide, which contribute to the maintenance of colonization resistance	38	57	5
Lactobacilli occur naturally in the human intestine	64	27	9
Probiotic bacteria remain viable after transit through the gut	37	57	6

In conclusion, our results suggest that UK registered dietitians are interested in dietary modulation of the gut flora and are keen to increase their knowledge of the therapeutic potential of probiotics. Our findings highlight that dietitians are aware of the probiotic concept but specific characteristics and the mechanism of probiotic bacteria are not well understood.

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Salminen SJ, Bouley C, Boutron-Ruault M-C, Cummings JH, Franck A, Gibson GR, Isolauri E, Moreau M-C, Roberfroid M & Rowland I (1998) *British Journal of Nutrition* **80**, S147–S171.

Perception of relationships between diet and cancer and reported dietary changes over the previous five years. By M. LUMBERS[1], A. EVES[1], M. KIPPS[1] and S. GIBSON[2,] [1]*Centre for Food and Health Care Management, School of Management Studies for the Service Sector, University of Surrey, Guildford GU2 7XH,* [2]*Consultant Nutritionist, 11 Woodway, Guildford, Surrey GU1 2TF*

Cancer is now responsible for more deaths in England than are attributable to coronary heart disease (CHD), 25% *v.* 23%, respectively (Department of Health, 1999). However, dietary advice to prevent the development of cancer is less well established and may be less well understood than that for CHD (Department of Health, 1998). As part of a larger study investigating consumers' risk perceptions, we investigated beliefs about diet and cancer risk and reported changes to diet in the previous 5 years. Respondents aged 16 years and over (*n* 1118) in four locations in England (London, Birmingham, Plymouth and Newcastle) completed a face-to-face interview (August 1998). Respondents were asked whether specific dietary factors were associated with an increased, or decreased risk of developing cancer, or 'neither' (see Table 1). They were also asked to what extent they had changed their consumption of key foods in the last 5 years using a 5-point scale from 'never consumed' to 'consume more than used to' (Table 2).

Table 1

	Age group					
% of respondents associating foods with cancer risk	16–30y	30–50y	50–65y	>65y	Total	*P* value*
n	*405*	*455*	*176*	*79*	*1115*	
More fruit & veg (lower risk)	68%	65%	71%	60%	67%	ns
More vitamins (lower risk)	53%	44%	43%	34%	47%	0.002
Less fat (lower risk)	57%	58%	64%	35%	57%	0.0001
Less saturated fat (lower risk)	56%	55%	65%	45%	56%	0.016
More oily fish (lower risk)	46%	50%	54%	33%	48%	0.016
More fibre (lower risk)	56%	58%	61%	45%	57%	ns
Red meat (higher risk)	45%	49%	46%	34%	46%	ns
Alcohol (higher risk)	57%	47%	50%	44%	51%	0.012

* Chi-squared.

Table 2

	Age group					
% of respondents adopting these changes in last 5 years	16–30y	30–50y	50–65y	>65y	Total	*P* value*
n	*405*	*455*	*176*	*79*	*1115*	
More fruit & veg	41%	36%	35%	23%	37%	0.004
Less fat	45%	53%	52%	37%	49%	0.013
Less saturated fat	43%	46%	44%	27%	43%	0.019
More oily fish	12%	11%	14%	8%	11%	ns
More fibre	27%	31%	34%	27%	30%	ns
Less/no red meat	44%	45%	48%	25%	44%	0.005
Less/no alcohol	16%	20%	27%	28%	20%	0.006

* Chi-squared.

With increasing age, respondents were less likely to perceive a link between selected foods and cancer risk or to have made changes in their diets over the previous 5 years, compared with younger respondents. Only around 10% of all respondents stated that prevention of cancer was the reason for specific dietary changes; more cited heart disease or other reasons. These findings emphasise the need to understand better how people perceive health risks from food and how they respond to diet-related questions. Further work will examine differences in perception of risk between men and women, different socio-economic groups and levels of educational attainment.

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Department of Health (1999) *Saving Lives: Our Healthier Nation.* London: The Stationery Office.

Survey of General Practitioners' attitudes to and advice given on peanuts. By L. COLLIS and C. CHAPMAN, *United Biscuits (UK) Ltd., Lane End Road, Sands, High Wycombe, Bucks. HP12 4JX*

Peanuts have historically been seen as a high fat, salty snack which has no regular or essential place in a balanced diet. Peanuts have also received bad press because they can trigger potentially fatal allergic reactions in certain individuals. There is, however, increasing evidence in the US to suggest that consumption of peanuts can help to reduce coronary heart disease risk (Sabate & Fraser, 1994; Hu *et al.* 1998; Kris-Etherton *et al.* 1999) and improve weight loss in the overweight (McManus, 1999; P.M. Kris-Etherton *et al.*, unpublished).

The current study was designed to investigate the level of understanding of the positive role peanuts can play in the diet and the extent to which this is translated into advice given by General Practitioners (GPs) in the UK. Personal interviews were conducted by National Opinion Poll Healthcare's IQCS-approved medical interviewers amongst a random sample of 200 NHS GPs who had qualified since 1955, covering GPs in eight English regions, Scotland and Wales.

More than half of the GPs interviewed said they were hardly ever asked about advice on peanut consumption and 18% declared never to be asked. However, in Scotland, a quarter of the GPs were reportedly asked about peanuts once or twice a month – more than any other area in the survey. When asked about the change in frequency of questions from patients about peanut consumption, most GPs declared a growing interest from patients and this was most apparent again in Scotland. Interestingly, GPs were only recommending avoidance of peanuts to peanut allergy sufferers or young children. GPs did not give advice to avoid peanuts to pregnant women unless there was a history of allergy. The advice to pregnant women is in line with the recent Committee on Toxicity advice (Department of Health, 1998).

When GPs were asked if they had the information they needed to answer questions relating to the consumption of peanuts, 62% felt well equipped, with 74% of Scottish GPs claiming to have sufficient information. It was not asked in this research which sources of information GPs relied upon but previous research has shown that consumers regard health professionals as the most frequently used source of information on healthy eating. In conclusion, therefore, whilst GPs give advice on peanut consumption to the few who ask, proactive education of health professionals could lead to an increase in the consumption of peanuts. Regular peanut consumption has been shown to correlate with lower plasma cholesterol and triacylglycerol levels (Kris-Etherton *et al.* 1999) and peanuts included as part of a weight-loss regime enabled patients to adhere to a diet more rigorously (McManus, 1999). This research therefore points to the opportunity for a public health message surrounding the inclusion of peanuts in a balanced diet. It is also apparent that there have been few peanut intervention studies and certainly few UK-based studies or data until this questionnaire.

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McManus K (1999) *Experimental Biology*, April 1999.
Sabate J & Fraser G (1994) *Current Opinion in Lipidology* **5**, 11–16.

Current public misunderstandings about peanuts as part of a balanced diet: an opportunity for further research and a need for better education? By C. CHAPMAN, *United Biscuits (UK) Ltd, Lane End Road, Sands, High Wycombe, Bucks. HP12 4JX*

There is now little doubt that not only the amount but also the type of fat is important in determining disease risk. Peanuts are a rich source of monounsaturated fat and a recent wave of research suggests that regular consumption of peanuts can help to reduce coronary heart disease risk (Hu *et al.* 1998; Kris-Etherton *et al.* 1999) and improve weight loss in the overweight (McManus, 1999; P.M. Kris-Etherton *et al.*, unpublished). Peanuts also contain antioxidants such as -sitosterol and reseveratrol which have been shown to inhibit cancer growth and may protect against atherosclerosis (Jang *et al.* 1997).

The current study was designed to investigate the level of understanding amongst UK consumers of these positive roles peanuts can play in the diet. Five hundred consumers were interviewed in the street by questionnaire.

Seven out of ten people interviewed were generally aware that unsaturated fats are 'better for you' than saturated fats. 33% of men and 49% of women wrongly believed that peanuts are high in cholesterol and just under a quarter of the adults asked believed that peanuts are high in saturated fat. When asked to rank a number of foods in order of perceived saturates content, there was clear confusion as to the sources of such fats. In fact less than half the sample were able to complete the task correctly. In almost every case, women estimated that the content of saturated fats, unsaturated fats and salt is much higher than the men would think. Over a quarter of adults also believed that the salt content of peanuts is high.

Despite the lack of understanding of peanuts as a whole food which can provide many positive attributes to a balanced diet, 20% of men and 13% of women said they eat nuts 2–3 times a week and a lower proportion of those interviewed claimed never to eat nuts. Those not consuming nuts said it was because they didn't like them (29%), they thought they were fattening (7% men, 27% women) or they were allergic to them (4%).

This study shows that there are a number of misconceptions in the UK surrounding the fat, cholesterol and salt content of peanuts. The reality is that peanuts can make a big impact on the saturated:unsaturated fatty acid ratio if eaten as part of a low-fat diet because they are so high in monounsaturated fatty acids. A standard 50-g portion of peanuts contains less salt than two slices of wholemeal bread and peanuts are cholesterol-free. Numerous studies in the US are coming out in support of peanuts playing a beneficial role in terms of disease protection. To date there is a paucity of both UK data and intervention studies. The current study highlights a research opportunity and the need for a public health awareness campaign promoting the positive attributes of peanuts. There is a need for intervention studies incorporating peanuts into the diet and investigating the potential health benefits.

Hu FB, Stampfer MJ, Manson JE, Rimm EB, Colditz GA, Rosner BA, Speizer FE, Hennekens CH & Willett WC (1998) *British Medical Journal* **317**, 1332–1333, 1341–1345.
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