Proceedings of the Nutrition Society

Abstracts of Original Communications

A joint meeting of the Nutrition Society and the Institute of Food Science and Technology was held at the Royal College of Physicians, London on 21 February 2001, when the following papers were presented.

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α-Tocopherol supplementation does not prevent acute alcohol-induced heart muscle damage. By M. KOLL[1,2], V.B. PATEL[2], R.A. SHERWOOD[2], H.K. SEITZ[3], U.A. SIMANOWSKI[3], P.J. RICHARDSON[4], T.J. PETERS[2] and V.R. PREEDY[1], [1]*Nutrition & Dietetics,* [2]*Clinical Biochemistry and* [4]*Cardiology, King's College, London, SE1 and* [3]*Department of Medicine, Salem Medical Centre, Heidelberg, Germany*

Although low to moderate alcohol ingestion may be cardio-protective, excessive alcohol ingestion, i.e. greater than the guidelines from the Royal Colleges, is clearly damaging. Heart muscle defects occur in 36% of alcoholics with an alcohol intake of 60 g/d for 20 years or more (7 kg ethanol/kg body weight in a lifetime)(Fernandez-Sola *et al.* 1997). The mechanism of alcohol-induced heart muscle damage is unknown. It has been suggested that α-tocopherol (AT) is cardio-protective (Nordmann, 1994). The aim of this study was to investigate whether AT is protective against acute and chronic ethanol injury.

Rats (approx. 0.1 kg body weight) were supplemented with AT (30 mg/d/kg bw in Intralipid carrier (i.p.)) for 4 weeks. Controls were treated with isovolumetric Intralipid carrier. We showed increased AT-levels in plasma of At-supplemented rats. On the final day, animals were given a bolus of ethanol (75 mmol/kg bw i.p.) or isovolumetric saline (0.15 mol/l NaCl). After 2.5 h, the rats were killed. The groups were as follows: (A) Control (carrier) + saline; (B) Control (carrier) + ethanol; (C) AT + saline; (D) AT + ethanol. Plasma cardiac troponin-T (cTnT), a marker of cardiac damage, was measured by enzyme immunoassay.

	Pretreatment	Treatment	cTNT-levels (μg/l)	Significance
(A)	Intralipid	Saline	0.08±0.01	
(B)	Intralipid	Saline	0.16±0.04	*v.* (a) P<0.001
(C)	Intralipid + AT	Ethanol	0.07±0.03	
(D)	Intralipid + AT	Ethanol	0.18±0.07	*v.* (c) P<0.001

Data presented as mean ± SEM; *n* 5–7. All rats were fed an alcohol diet for 4 weeks. Other differences were not significant.

In addition, studies showed that chronic ethanol feeding for 4 weeks (ethanol as 35% of total calories) had no effect on circulating cTnT, indicating adaptation. AT supplementation in these rats had no effect on cTnT release. The supposition that adaptation occurred was supported by the observation that when chronic ethanol-fed rats had their ethanol removed overnight, an acute bolus of ethanol the following day increased cTnT as shown in the following table.

	Pretreatment	Treatment	cTNT-levels (μg/l)	Significance
(A)	Intralipid	Saline	0.07±0.01	
(B)	Intralipid	Ethanol	0.21±0.04	*v.* (a) P<0.05
(C)	Intralipid + AT	Saline	0.08±0.01	
(D)	Intralipid + AT	Ethanol	0.20±0.03	*v.* (c) P<0.05

All data presented as mean ± SEM; *n* 5–7. Other differences were not significant.

These results show that pre-treatment with AT does not prevent heart damage induced by acute ethanol.

Fernandez-Sola J, Estruch R & Urbano-Marquez A (1997) *Addiction Biology* **2**, 9-17.
Nordmann R (1994) *Alcohol & Alcoholism* **29**, 513-22.

The effects of a nitric oxide donor and a nitric oxide synthase inhibitor on the ethanol-induced reduction of jejunal protein synthesis. By R. RAJENDRAM[1,2], J.S. MARWAY[2], D. MANTLE[3], T.J. PETERS[1] and V.R. PREEDY[2], *Departments of* [1]*Clinical Biochemistry,* [2]*Nutrition, King's College, London, Stamford Street, London SE1 8WA and* [3]*Department of Neurochemistry, Newcastle General Hospital, Newcastle NE4 6BE*

Ethanol induces pathological changes in the jejunum which may be explained by changes in protein synthesis (Marway *et al.* 1993). The pathogenesis is not well understood, but changes in nitric oxide (NO) may contribute. To investigate this, rats were exposed to ethanol (75 mmol/kg bw; i.p.; 2.5 h) with or without the NO donor S-nitrosoglutathione (SNOG; 25 mg/kg bw) 0.5 h prior to ethanol. Comparative studies were made with the NO synthase (NOS) inhibitors *N*-ω-nitro-L-arginine methyl ester (NAME; 25 or 100 mg/kg) and *N*(G)-nitro-L-arginine (NNA; 100 mg/kg). Controls were injected with 0.15 mol/l saline. Fractional rates of protein synthesis (i.e. k_s, % protein renewed per d); were measured in the jejunal mucosa and serosa *in vivo* with [^{3}H]phenylalanine (Garlick *et al.* 1980). Data (as mean ± SEM) were as follows:

	Jejunal mucosa				Jejunal serosa			
Group	k_s[1]	Difference[2]	*P* value	n	k_s[1]	Difference[2]	*P* value	n
1. Control	169±7			9	83±3			9
2. Ethanol	146±6	–14%	*P*<0.05	10	73±3	–12%	*P*<0.05	10
3. NAME (25mg/kg)	153±6	–10%	NS	5	71±3	–15%	*P*<0.05	5
4. NAME (100mg/kg)	87±32	–49%	*P*<0.001	3	36±20	–57%	*P*<0.001	3
5. NNA	144±18	–15%	NS	3	65±5	–22%	*P*<0.05	3
6. SNOG	163±6	–3%	NS	9	84±5	+1%	NS	9
7. Ethanol+NAME (25mg/kg)	121±12	–28%	*P*<0.001	7	56±4	–33%	*P*<0.001	7
8. Ethanol+SNOG	132±6	–22%	*P*<0.001	9	63±4	–24%	*P*<0.001	9

[1]k_s, % protein renewed/day. [2]Difference as percentage of control. NS, not significant. Other differences were as follows:
Jejunal mucosa: 2 *v.* 8, –10% NS; 4 *v.* 8, –20% *P*=0.001; 2 *v.* 7, –17% *P*<0.05; 3 *v.* 7, –21% *P*<0.01.
Jejunal serosa: 2 *v.* 8, –14% *P*<0.05; 4 *v.* 8, –25% *P*<0.001; 2 *v.* 7, –23% *P*<0.005; 3 *v.* 7, –20% *P*<0.05.

Both NAME (25 mg/kg) and NNA reduced k_s in the serosa but not in the mucosa. However, administration of a higher dose of NAME (100 mg/kg) resulted in reduction of k_s in both the mucosa and the serosa. As both NNA and NAME reduced jejunal k_s, these effects on protein synthesis represent responses to NOS inhibitors in general rather than specific reactions to either agent. These observations suggest that NO facilitates protein synthesis in the jejunum *in vivo*. This has been suggested in other tissues, for example skeletal muscle (Fryburg, 1996) but never before in the jejunum. Furthermore, the data suggests that the jejunal mucosa is less sensitive to NOS inhibitors than the serosa and that mucosal protein synthesis may be regulated differently from protein synthesis in the serosa. This is as expected, given that mucosal cells are continually shed and replaced, whilst serosal turnover is slow.

In accordance with previous studies (Marway *et al.* 1993) acute ethanol administration inhibited protein synthesis in the jejunum. Pre-dosage with NAME potentiated the ethanol-induced reduction of k_s in both the mucosa and the serosa of the jejunum. Pre-dosage with SNOG potentiated ethanol-induced reduction of k_s in the serosa, but did not alter the effects of ethanol on the mucosa. The specific radioactivity of free phenylalanine in acid-soluble fractions of tissue homogenates (s_i) was increased 15% (P<0.005) in the jejunal mucosa in the group treated with SNOG alone. The serosal s_i was reduced by 9% (P<0.05) in the group treated with ethanol alone. All other effects on k_s in the jejunum were not due to changes in precursor enrichment, i.e. s_i was not altered (data not shown for brevity). These data suggest that NO is not involved in the ethanol-induced reduction of protein synthesis in the jejunum. However, disruption of NO may enhance ethanol toxicity. Other mechanisms may be involved in ethanol toxicity in the jejunum, for example free radical damage.

Fryburg DA (1996) *Journal of Clinical Investigation* **97**, 1319–1328.
Garlick PJ, McNurlan MA & Preedy VR (1980) *Biochemical Journal* **192**, 719–723.
Marway JS, Rodrigues LM, Griffiths JR & Preedy VR (1993) *Alcohol* **28**, 521–528.

Malondialdehyde–protein adducts in Type I and Type II fibre predominant muscles of rats exposed to alcohol and cyanamide. By O. NIEMELA[1], S. PARKKILA[1], M. KOLL[2], T.J. PETERS[3] and V.R. PREEDY[2], [1]*Department of Clinical Chemistry, Anatomy and Cell Biology, University of Oulu, FIN-90220 Oulu, Finland and Departments of* [2]*Nutrition and Dietetics and* [3]*Clinical Biochemistry, King's College, London SE1*

Excessive or high concentrations of the acute ingested alcohol has to be detoxified by the liver but causes alcohol-induced muscle disease (AIMD) which encompasses a spectrum of disorders characterized by reduced muscle strength and structural and biochemical changes, including reductions in the synthesis of myofibrillary proteins. Type II (glycolytic, fast-twitch, anaerobic) fibres are particularly sensitive whereas Type I fibres (oxidative, slow-twitch, aerobic) are relatively protected. The pathogenic mechanisms are unknown although it is possible that protein-adduct formation may be a contributory process. To test this hypothesis, we analysed skeletal muscles from Male Wistar rats (approx 0.15 kg body weight) treated with a standard dose of ethanol (75 mmol/kg body weight; i.p.) with or without the acetaldehyde dehydrogenase inhibitor cyanamide (0.5 mmol/kg body weight; i.p.). The treatments included a pre-treatment stage of 30 min duration followed by a treatment stage of 2.5 h duration. Controls were injected with saline (0.15 mol/l NaCl). The groups (pretreatment + treatment) were as follows: (a) saline + saline; (b) cyanamide + saline; (c) saline + ethanol; (d) cyanamide + ethanol. At the end of the study, the soleus (Type I fibre-predominant) and plantaris (Type II fibre-predominant) muscles were dissected and analysed for malondialdehyde–protein adducts by immuno-histochemistry. Adduct density scores (on a visual scale of 0–5) were as follows (mean ± SEM; *n* 7–8):

			Malondialdehyde-protein adduct levels (Adduct density scores)			
			Soleus		Plantaris	
	Pretreatment	Treatment	Mean	SEM	Mean	SEM
(a)	Saline	Saline	0.91	0.07	1.19	0.15
(b)	Cyanamide	Saline	1.29	0.19	1.57	0.25
(c)	Saline	Ethanol	1.69	0.26***	1.84	0.24*
(d)	Cyanamide	Ethanol	1.59	0.18**	2.53	0.26****†

Superscripts pertain to differences from group (a) for a particular muscle: **P*<0.05; ***P*<0,025; ****P*<0.01; *****P*<0.001; Differences between (c) and (d): †*P*<0.05. Other differences were not significant (*P*>0.05).

This is the first report of increased muscle malondialdehyde–protein adduct levels in ethanol-dosed rats. Adduct formation is enhanced when acetaldehyde levels are raised with cyanamide pre-dosing, indicative of greater oxidative stress. The increased protein-adduct formation in the plantaris compared to the soleus may reflect, or be responsible for, the enhanced susceptibility of this muscle during alcohol exposure.

Skeletal muscle protein content is reduced in male and female rats by a novel high-fat/low-carbohydrate alcohol diet. By R.J. HUNTER[1], K.O. LINDROS[2], H.A. JARVELAINEN[2] and V.R. PREEDY[1], [1]*Department of Nutrition and Dietetics, King's College School of Medicine and Dentistry, Bessemer Road, London SE5 9P3 and* [2]*Alcohol Research Center, National Public Health Institute, Box 719 00101, Helsinki, Finland*

Skeletal muscle myopathy due to alcoholism occurs in between one- and two-thirds of all chronic alcohol abusers, and is a major cause of morbidity (Martin *et al.* 1985; Urbano Marquez *et al*. 1989). This alcoholic myopathy is characterized by a decrease in protein synthesis and content resulting in atrophy of muscle fibres (Preedy *et al.* 1998). Alcohol feeding regimens currently utilize a high carbohydrate diet to produce a model of alcohol-induced pathology, and it has been suggested that carbohydrate loading may contribute towards the pathology. A novel feeding regimen utilizing a high-fat/low-carbohydrate diet has recently been shown to cause liver histological changes which surpass those seen in traditional high carbohydrate feeding regimens (Lindros *et al.* 1998). This study aimed to investigate the effects of this high-fat/low-carbohydrate diet on skeletal muscle protein and RNA content. Also, it has been noticed clinically that women are more susceptible to the toxic effects of alcohol than men are (Urbano-Marquez *et al*. 1995). To test the hypothesis that female rats are more susceptible than males to the toxic effects of alcohol on skeletal muscle, male and female rats were included in this study.

The four groups: (1) males fed alcoholic diet *ad libitum*; (2) males pair-fed to group (1); (3) females fed alcoholic diet *ad libitum*; (4) females pair-fed to group (3).

Rats were treated for 10 weeks with a nutritionally adequate high-fat/low-carbohydrate liquid diet containing 16% of calories as protein, 44% as fat, and 5.5% as carbohydrates (or 40% in controls) and 34.5% as ethanol (Lindros *et al.* 1998). Male and female alcoholic groups consumed the same dose of alcohol per kg body weight, and at the time of death blood ethanol levels were found to be the same. Results were analysed using two-way ANOVA, all data presented as mean and SEM for 7–10 observations.

After 10 weeks, alcohol caused a 15% reduction in plantaris protein content in males compared with 17% in females, a 19% reduction in gastrocnemius total protein content in both males and females, and a 21% decrease in gastrocnemius myofibrillary protein content in males compared with 24% in females (all changes: alcohol $P<0.05$; gender $P<0.05$; interaction NS). Alcohol also caused a decrease in plantaris RNA content of 19% in males compared with 28% in females, and a decrease in gastrocnemius RNA content of 19% in males and 27% in females (both changes: alcohol $P<0.05$; gender $P<0.05$; interaction NS). Inspection of RNA:protein ratio for the plantaris muscle revealed a decrease in male groups, from 3.17 (SEM 0.03) in controls to 3.01 (SEM 0.06) in the alcohol group, and the female groups from 3.06 (SEM 0.02) in controls to 2.66 (SEM 0.09) in the alcohol group (alcohol $P<0.05$; gender $P<0.05$; interaction $P<0.05$). There was no effect of alcohol or any interaction on any parameter for soleus.

Thus the data show that chronic ethanol feeding using this novel high-fat/low-carbohydrate diet induces a type II myopathy, with a reduction in plantaris total protein content, and both total and myofibrillary protein content in gastrocnemius, but not soleus (type I predominant). Alcohol also caused a reduced RNA content in the affected muscles which may indicate a reduced synthetic potential, although there are no data on the changes in muscle RNA turnover in this model. The decrease in the RNA:protein ratio in the alcohol groups showed a significant interaction between the effects of alcohol and gender, demonstrating that female rats were more susceptible to the toxic effects of alcohol. In conclusion, this novel high-fat/low-carbohydrate alcohol diet induced pathological changes in skeletal muscle biochemistry to which females were more susceptible than males.

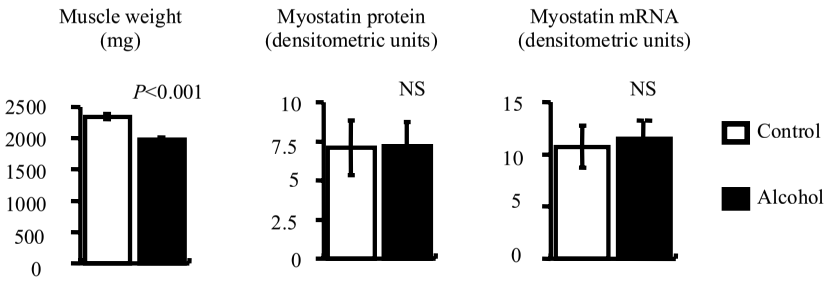
Lindros KO & Jarvelainen HA (1998) *Alcohol and Alcoholism* **33**, 347–353.
Martin F, Ward K, Slavin G, Levi J & Peters TJ (1985) *Quarterly Journal of Medicine* **55**, 233–251.
Urbano Marquez A, Estruch R, Navarro Lopez F, Grau JM, Mont L & Rubin E (1989) *New England Journal of Medicine* **320**, 409–415.
Preedy VR, Reilly M, Mantle D & Peters TJ (1998) In *Free Radicals and Skeletal Muscle*, pp. 283–293 [AE Reznick, DL Packer, CK Sen, JO Holloszy and MJ Jackson, editors]. Basel: Birkhauser Verlag.
Urbano-Marquez A, Estruch R, Fernandez-Sola J, Nicolas JM, Pare JC & Rubin E (1995) *JAMA* **274**, 149–154.

Skeletal muscle myostatin expression is unaffected by alcohol. By R.J. HUNTER[1], M. WEHLING[2], R. RAJENDRAM[1], J. TIDBALL[2] and V.R. PREEDY[1], [1]*Department of Nutrition and Dietetics, King's College, London and* [2]*Duchenne Muscular Dystrophy Research Center, University of California, Los Angeles, California, USA*

Skeletal muscle myopathy due to alcoholism is a major cause of morbidity. This alcoholic myopathy is characterized by a decrease in protein synthesis and lean muscle mass (Preedy *et al*. 1998). Myostatin, also called growth and differentiation factor-8 (GDF-8), is part of the transforming growth factor-β (TGFβ) superfamily. Mutation of the myostatin gene results in excessive muscular development in cattle and mice (Grobet *et al.* 1997). This indicates that myostatin is a negative regulator of skeletal muscle growth, and its expression has been shown to increase in disuse atrophy that occurs following hindlimb unloading in rats (Wehling *et al*. 2000). This study aimed to evaluate the hypothesis that myostatin expression is increased in alcoholic myopathy.

To test this, two groups of male Wistar rats were investigated: group (1) was fed an alcoholic diet *ad libitum*; group (2) was pair fed to group (1). Rats were fed a nutritionally complete diet containing approximately 15% of dietary energy as protein, 18% as fat, 32% as carbohydrates (67% in controls), and 35% as ethanol. After 46–48 d, gastrocnemius muscle was removed and weighed. Western blotting was performed to measure myostatin content, and Northern blotting was performed to measure myostatin mRNA levels (all data *n* 7–10 observations).

The gastrocnemius wet weight was significantly reduced from approximately 2.4 g in the control group to 2.0 g in the group fed alcohol ($P<0.001$), confirming that this feeding regimen induced an alcoholic myopathy. Western blotting showed that gastrocnemius myostatin content was not affected by alcohol. Northern blotting showed that myostatin mRNA was also unchanged in rats fed alcohol, indicating that the synthetic potential of this protein was also unchanged.



In conclusion, this is the first investigation into the expression of myostatin in the muscle of rats fed alcohol. This data suggests that despite the involvement of myostatin in other forms of muscle wasting, altered myostatin expression does not play a role in the pathogenesis of alcoholic myopathy.

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Preedy VR, Reilly M, Mantle D & Peters TJ (1998) In *Free Radicals and Skeletal Muscle*, pp. 283–293 [AE Reznick, DL Packer, CK Sen, JO Holloszy and MJ Jackson, editors]. Basel: Birkhauser Verlag.

Wehling M, Cai B & Tidball JG (2000) *FASEB J* **14**, 103–110.

The association of docosahexaenoic acid with reduced heart and other chronic diseases in China suggests a new hypothesis linking the cell membrane as the site of initiation. By Y.Q. WANG[1], M.A. CRAWFORD[1], J.S. CHEN[2], J.Y. LI[2], K. GHEBREMESKEL[1], T.C. CAMPBELL[3], W.X. FAN[2], R.S. PARKER[3] and J. LEYTON[4], [1]*Institute of Brain Chemistry and Human Nutrition, University of North London, London,* [2]*Chinese Academy of Preventive Medicine, Beijing, China,* [3]*Division of Nutrition Sciences, Cornell University, Ithaca, New York, USA and* [4]*Medicine Branch, National Cancer Institute, Rockville, USA*

There is good evidence implying that the balance between the *n*-3 and *n*-6 essential fatty acids is important for cardiovascular and immune system function. The data on mortality from heart disease and certain cancers, from studies on the Eskimoes and Japanese, suggested that fish consumption is protective (Marcovina *et al.* 1999). The American Heart Association and the Committee on Medical Aspects in the UK are both now recommending the consumption of two fish meals a week as a means of prevention (COMA, 1994; Krauss *et al.* 2000). Moreover, the anti-arrythmic effect of docosahexaenoic acid (DHA) has recently been described and provides a mechanism for its cardio-protection (Leaf *et al*. 1999).

We participated in an epidemiological survey of diet, lifestyle and disease mortality in sixty-five rural counties of China, which was conducted in the 1980s (Chen *et al*. 1990). The study established a comprehensive database on disease patterns, nutritional intake and blood biochemistry, including plasma and red cell fatty acid composition. The traditional Chinese diet is low in fat (about 15% energy). Nevertheless, there is regional variability in the amount and type of fat consumed and the pattern of chronic diseases.

We re-analysed our database to investigate whether fish consumption and DHA protects against cardiovascular disease (CVD) and other chronic diseases, as seen in the Western countries with high-fat diets.

Fish consumption in the Chinese counties correlated with the levels of DHA in RBC (r 0.640, $P<0.001$), plasma selenium (r 0.467, $P<0.001$) and glutathione peroxidase (r 0.333, $P<0.01$). Levels of RBC DHA showed a significant negative association with plasma triacylglycerols (r –0.400, $P<0.001$). We found a significant inverse correlation between docosahexaenoic acid and chronic disease mortality for one-third of about three dozen chronic diseases assessed in this survey. Of particular note was the strong inverse association between DHA and mortality from cardiovascular disease, such as a combination of hypertensive, CHD and myocardial infarction (r –0.288, $P<0.05$), rheumatic heart disease (r –0.479, $P<0.01$).

These findings are consistent with studies on CVD and chronic diseases in high-fat-diet Western countries. This common, preventive effect could relate to the nature of the cell membrane. The plasma membrane is the first point of contact between the essential nutrients and other dietary factors and the cell. As the data we present refers to the red cell membrane, these results suggest that distortion of the cell membrane composition may be a primary cause or a shared common feature of the initiation and pathogenesis of chronic disease.

Chen JS, Campbell TC, Li JY, Peto R, *et al.* (1990) *Diet, Lifestyle and Mortality in China: a Study of the Characteristics of 65 Chinese Counties*. Oxford: Oxford University Press / Ithaca (NY): Cornell University Press / Beijing: People's Medical Publishing House.

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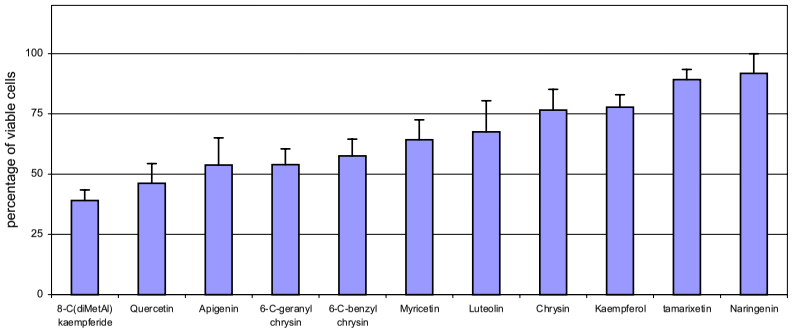
Relationships between the structure of dietary flavonoids and their effect on colonic cell proliferation. By F. DEPEINT, J.M. GEE and I.T. JOHNSON, *Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA*

A wide variety of plant-derived compounds are normally present in the human diet or are consumed for medicinal reasons. It has been shown that these phytochemicals can modulate epithelial cell proliferation (Hara *et al*. 1999) and apoptosis in the intestinal mucosa. In the present study, a range of flavonoids was screened to determine their effects on the viability of a human colonic adenoma cell line (HT29). About forty different compounds were tested *in vitro* using a 96-well plate culture technique and neutral red staining to examine the relationship between structure and cell viability.

Plates were inoculated with 1.5×10^4 cells per well in a volume of 200 µl, and incubated under 5% CO_2 in humidified air for 24 h. The medium (DMEM containing 5% fetal bovine serum) was then replaced with supplemented DMEM containing the test compound at a concentration of 50 µM (dissolved in DMSO; final concentration 0.5%) and incubation continued for a further 24 h. During this stage the growth medium was supplemented with 200 µM ascorbic acid to prevent flavonoid degradation. Viability was assessed by the subsequent uptake of neutral red dye, followed by cell fixation, washing and lysis. Optical densities of the resulting solutions were read in an automatic plate reader.



Effect of flavonoids on cell viability: Twenty-one of the thirty-six compounds tested showed some activity; representative data for eleven are plotted here. The most active compounds were flavonols and flavones. All the glycosides tested gave a negative result and are not included on the histogram.

Flavonoids in general appeared to be relatively weak inhibitors of cell proliferation; however, five of the thirty-six compounds tested (one flavonol, three substituted flavonols and one flavone) reduced viability by at least 50%. As previously described (Depeint *et al*. 2000), hydroxyl substitutions did not produce a clear structure–function relationship. Inhibition of cell growth induced by flavonoids was reduced by methyl or glycosyl substitution, whereas C-prenylation of the flavonoid backbone in position 6 or 8 (6-C-geranyl chrysin and 8-C(1,1-dimethylallyl) kaempferide), increased activity.

Experiments are in progress to determine the extent to which the effect of these compounds on cell viability are due to an inhibition of mitosis or an induction of apoptosis, and to explore the relationships between molecular structure (i.e. position and nature of substitution) and effects on cell signalling pathways. Preliminary results with quercetin have shown that this flavonol induced a dose-dependent arrest of the cell cycle during S phase, at concentrations of 50 and 100 µM, with no evidence of apoptosis.

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Cell ferritin content as a measure of iron uptake into Caco-2 cells from food digests. By S. PARIAGH[1], P. SHARP[2], E.K. LUND[1] and S.J. FAIRWEATHER-TAIT[1], [1]*Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA and* [2]*The University of Surrey, Guildford, Surrey, GU2 7XH*

Iron deficiency anaemia is a prevalent nutrient disorder of which certain groups of people, such as adolescent girls, have been identified as most at risk. In recent years a cell culture method using Caco-2 cells has been developed as means of studying mechanisms of iron absorption into the epithelial cells of the small intestine. Previous work has suggested that iron uptake into Caco-2 cells can be assessed in terms of ferritin content (Glahn *et al.* 1998). However, it is unclear as to whether a linear relationship exists between cell iron content and ferritin formation when cells are exposed to different iron sources.

We performed two experiments that focused on this issue using the highly differentiated Caco-2 TC7 cell line, which is physiologically very similar to the small intestinal villous epithelium. Cells were grown in 24-well tissue culture plates to confluency, before replacement of the culture medium with an iron-free medium to reduce the iron content of the cell, so as to maximize iron absorption across the mucosal cell membrane. A transwell was then inserted above the cells into which was placed a food digest which had been treated with pepsin and pancreatin. The base of the transwell allowed diffusion of iron into the media above the cells. The uptake of ^{55}Fe into cells when added to one of three different protein digests: cooked beef steak, cooked chicken breast or a gluten-based vegetarian alternative, was compared to the ferritin content in the cell. It was found that the ^{55}Fe uptake from the non-meat substitute was significantly lower (0.18 (SEM 0.03) pmol/well per h) compared with either beef (0.47 (SEM 0.03) pmol/well per h) or chicken (0.63 (SEM 0.05) pmol/well per h; $P<0.001$). However the ferritin content of the cell was not significantly different between treatments.

In the second experiment the ferritin content of the cells was compared to the concentration of iron (10–100 µmol/l) in the medium above the cells. In this case a linear correlation between haem iron concentration and ferritin content was observed but, within the concentration range tested, iron sulphate had no effect on cell ferritin. Interestingly, the lowest haem iron concentration (10 µmol/l) was associated with maximal ferritin content.

These results show that the presence of meat increases the uptake of non-haem iron into intestinal epithelial cells but that ferritin content may not be a good marker of iron absorption.

We would like to thank A. Hine (Heinz Foods) for the vegetarian meat substitute and J. Atkinson (Kerry Ingredients) for financial support.

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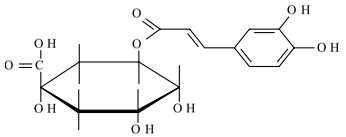
Implications of feruloyl esterase activity in colonic health. By C.B. FAULDS[1], D. COUTEAU[2], G.W. PLUMB[1], G.R. GIBSON[3] and G. WILLIAMSON[1], [1]*Nutrition, Health and Consumer Science Division, Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA,* [2]*Europol'Agro, 9 Boulevard de la Paix, 51097 Reims Cédex, France and* [3]*Food Microbial Sciences Unit, School of Food Biosciences, The University of Reading, Reading RG6 6AP*

Hydroxycinnamic acids, such as caffeate, ferulate, and chlorogenic acid (caffeoyl-quinic acid), are widely consumed in a Western diet, coffee and cereals being some of the richest sources. Ferulic acid occurs ester-linked to the pectin side chains in spinach and to the hemicellulose in cereals. Due to their distinct physiochemical properties (e.g. higher degree of hydrophilicity), they may exhibit different *in vitro* antioxidant properties, such as free radical scavenging, and have demonstrated anticarcinogenic, anti-inflammatory, antimicrobial, antiviral and other cellular effects. However, ingested hydroxycinnamate esters reach the large intestine essentially unaltered (Plumb *et al.* 1999). Free hydroxycinnamates have been identified in plasma and urine, so they must be absorbed (Spencer *et al.* 1999). However, esterified compounds have not been detected, and so a de-esterification reaction must occur either in the gastrointestinal tract or within the gut mucosal cells. If this reaction occurs in the colon, it must be performed by esterases produced by the indigenous microflora. Feruloyl esterases are produced by a wide range of bacteria and fungi, including genera found in the digestive tract of animals (Williamson *et al.* 1998). To date, there is little information on organisms able to hydrolyse chlorogenic acid, one of the commonest dietary ester-linked phenolic compounds. This study was aimed at identifying bacterial species responsible for the release of natural antioxidants, such as hydroxycinnamic acids, in the human large intestine. The ability to do this and the rate of release of such compounds by endogenous gut microbiota would influence the extent of processing of phenolic-containing food required to achieve sufficient *in vivo* health-beneficial effects.

Chlorogenic acid



After anaerobic growth of human faecal bacteria on a chlorogenic acid-based medium, thirty-five isolates were recovered and screened for cinnamoyl esterase activity (Couteau *et al.* 2001). Six isolates released ferulic acid, from its ethyl ester in a plate-screening assay, and were identified through genotypic characterization (16s rRNA sequencing) as *Escherichia coli*, *Bifidobacterium lactis* and *Lactobacillus gasseri*. Activity against chlorogenic acid was essentially intracellular. Released caffeic acid was not degraded further by these isolates. Chlorogenic acid was not toxic to the total faecal culture at levels up to 14 mM.

The results show that certain human gut bacteria, including some already recognized as potentially health-promoting (i.e. *Bifidobacterium* and *Lactobacillus*), may be involved in the release of bioactive hydroxycinnamic acids in the human colon. This beneficial effect complements the activities of hydroxycinnamates and their derivatives shown through *in vitro* testing, and indicates that phenolic acids could exert their biological activities in the colon.

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Effects of processing and storage on the antioxidant activity in vegetables. By K.J. HUNTER and J.M. FLETCHER, *Unilever Research, Colworth, Sharnbrook, Bedfordshire MK44 1LQ*

There is a large amount of published data on individual antioxidant levels in food. However, to understand the impact of a food containing many species of antioxidant, it may be desirable to have an estimate of a food's total antioxidant activity (TAA). We measured the TAA of a range of fresh and processed vegetables using a modification of the ferric reducing ability of plasma (FRAP) assay first described by Benzie & Strain (1996). In the modified assay, water-soluble antioxidants were extracted from the tissue using trichloroacetic acid and lipid-soluble antioxidants were extracted from the residue using tetrahydrofuran and methanol. The water- and lipid-soluble activities were summed and TAA expressed in ascorbate equivalents (AE; amount of ascorbic acid giving equivalent activity) per g wet weight (gWW). The ascorbic acid content of the water-soluble extract was assayed enzymatically (Foyer *et al.* 1995).

The TAA of commercially available frozen vegetables was assayed. Spinach was particularly high in TAA antioxidant activity (6.16 µmol AE.$(gWW)^{-1}$, SD 1.76 µmol AE.$(gWW)^{-1}$). The activity of peas was 1.63 µmol AE.$(gWW)^{-1}$ (SD 0.39 µmol AE.$(gWW)^{-1}$), green beans contained 1.33 µmol AE.$(gWW)^{-1}$ (SD 0.20 µmol AE.$(gWW)^{-1}$) and carrots contained 0.88 µmol AE.$(gWW)^{-1}$ (SD 0.12 µmol AE.$(gWW)^{-1}$). Ascorbic acid accounted for 33–50% of the TAA of the vegetables.

To determine the effects of processing, commercially available fresh, frozen, canned and bottled peas and spinach were assayed. Frozen peas contained 80% of the activity observed in fresh peas, whereas canned and bottled peas contained only 40% of the activity of fresh. Frozen spinach also contained 80% of the activity observed in fresh spinach, with 40% of the fresh activity observed in canned spinach. Vitamin C levels in frozen vegetables were equivalent to those seen in fresh, but reduced to approximately 30% in the canned and bottled peas and 10% in canned spinach.

To assess the effects of storage on antioxidant activity, fresh peas and spinach harvested and stored at ambient (20°) and chill (4°) temperatures. The TAA of the stored samples were assayed over time.

Total antioxidant activities in stored vegetables

	Peas		Spinach	
Time (d)	Ambient	Chilled	Ambient	Chilled
0	1.81–2.60	1.81–2.60	7.17–8.17	7.17–8.17
7	2.01–2.32	2.37–2.40	3.90–4.15	4.95–4.99
14	nd	1.09–1.30	nd	4.88–6.28
21	nd	1.12–1.64	nd	2.27–3.36

Values (in µmol AE.$(gWW)^{-1}$) are the means of duplicates from two independent determinations. nd = not determined. Vegetable samples inedible.

The TAA in peas and spinach decreased after 7 d of ambient and chilled storage. In both peas and spinach, the ascorbic acid content also declined on storage; in spinach, the ascorbic acid content was not detectable in ambient samples after 3 d and in chilled samples after 21 d. Although some ascorbic acid remained in ambient and chilled peas, levels declined to 55% and 35% of starting levels by 7 and 21 d, respectively.

This work confirms that vegetables contain a nutritionally significant amount of TAA. A large proportion of the antioxidant activity is destroyed when vegetables are canned or bottled, but the apparent loss on freezing is small, particularly in peas. The contribution of ascorbic acid to the TAA of peas was relatively large, whereas its contribution in spinach was relatively small, although the ascorbic acid content was still greater in absolute terms than in peas. This study shows that TAA is lost on the storage of fresh vegetables after harvest, mirroring the loss of ascorbic acid demonstrated previously (Favell, 1998).

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Effect of final core temperature and days of storage on retention of vitamin B_6 and vitamin B_2 in *sous-vide* processed pork roast. By A. LASSEN, *Danish Veterinary and Food Administration, Institute of Food Research and Nutrition, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark*

The *sous-vide* process involves slow cooking of vacuum packaged products with storage at refrigeration temperature prior to reheating for serving. Advantages of the process include an extended shelf-life, convenience and fewer personnel in a food service operation. Also, the nutritional quality of the *sous-vide* products is claimed to be superior. This has in fact been shown for ascorbic acid (Lassen *et al.* 1999). However, with regard to vitamin B_1 there seems to be no difference between traditional and *sous-vide* cooking (Lassen *et al.* 2000).

The purpose of this study was to investigate the retention of vitamin B_6 and vitamin B_2 during *sous-vide* processing. In one experiment (1) pork neck was *sous-vide* cooked with varying internal temperatures. In a second experiment (2) lean pork loin was *sous-vide* cooked and stored refrigerated for a period of 14 d. Each experiment was replicated three times, and all analyses were carried out in duplicate.

A substantial variation in the retention of vitamin B_6 was found when *sous-vide* cooking the pork neck to different internal temperatures (Experiment 1). Cooking to 72° internal temperature resulted in retention of 82 (SD 10) % of the vitamin B_6. Increasing the internal temperature to 85° and 92° decreased the retention of vitamin B_6 to 67 (SD 7) % and 56 (SD 5) %, respectively. Keeping the meat warm at 92° internal temperature for a subsequent 3½ hours did not cause further losses of vitamin B_6.

In Experiment 2, the meat was cooked to an internal temperature of 72°, cooled down quickly, stored for 1, 3 or 14 d at 5° and reheated in a microwave oven. After *sous-vide* cooking, 77 (SD 19) % of vitamin B_6 was retained in the meat, and in addition 14(SD 2) % was retained in the meat juice. After storage at 5° for 1 d and subsequent reheating, the retention of vitamin B_6 in the meat dropped to 57 (SD 11) %. The following storage for up to 14 d did not further affect the vitamin content in the meat.

In conclusion, the highest retention of vitamin B_6 was found in the freshly *sous-vide* prepared meat, cooked to 72° internal temperature (retention 77–82%). A greater destruction of vitamin B_6 was found when the meat was (1) cooked to a higher internal temperature (retention 56% at 92° internal temperature) or (2) chilled, stored refrigerated for 1–14 d, and subsequently reheated (retention 50–57%).

The vitamin B_2 content was relatively stable and independent of the internal temperature and time of storage (retention 80–84% in Experiment 1 and 86–90% in Experiment 2).

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Lassen A, Kall M & Hansen K (2000) In *Proceedings of the 8th International Congress on Engineering and Food*, April 9–13, Puebla, Mexico.

A natural seed oil rich in *n*-6 and *n*-3 fatty acids. By Y.Q. WANG, K. GHEBREMESKEL and M.A. CRAWFORD, *Institute of Brain Chemistry and Human Nutrition (IBCHN), University of North London, London N7 8DB*

Epidemiological and experimental studies have established that cardiovascular disease and diabetes (insulin resistance) are linked with high intakes of saturated fat. These findings have led to a significant increase in the utilization of plant seed oils for dietary purposes in the last three decades. Since most of these oils and their margarines are rich in *n*-6 fatty acids and devoid of *n*-3, the enhanced consumption may have resulted in an imbalance at a membrane level and chronic diseases (Okuyama *et al*. 1997; Hibbeln, 2001). In order to reduce these risks, it necessary to find plant oil that contains balanced proportions of the two essential fatty acid families.

We have found a natural seed oil, CY911, which is richer in the *n*-6 and *n*-3 fatty acids than other similar products in the market. The plant in which it is found is a grass that has been used for traditional medicine and food additives in China without any toxic effects for a long time. The fatty acid composition of this oil, as identified by GC-MS/MS and others, is given below.

Main fatty acids (%)*	CY911	Corn oil	Canola oil	Soya oil	Sunflower oil	Vegetable oil, blended	PUFA Margarine	Evening primrose oil	Linseed oil
C16:0	5.0	11.3	4.2	10.7	6.2	5.3	12.5	5.6	5.3
C18:0	2.0	2.1	1.5	3.8	4.3	5.0	5.3	1.7	3.3
C18:1*n*-9	12.3	29.4	57.6	20.8	20.2	29.4	17.2	10.4	16.6
C18:2*n*-6	20.2	50.4	19.7	51.5	63.2	23.2	29.9	68.8	15.0
C18:3*n*-6	14.6	0	0	0	0	0.1	0	8.1	0.1
C18:3*n*-3	29.7	0.9	9.6	7.3	0.1	6.5	3.5	0.1	53.1
C18:4*n*-3	13.6	0	0	0	0	0	0	0	–
C20:1*n*-9	1.1	0.3	1.2	0.2	0.1	0.3	0.1	0.1	0.6
n-6:*n*-3	0.8	56.0	2.1	7.1	632	3.6	8.5	769	0.3

* Data from RSC and MAFF (1998) *Fatty Acids, Supplement to McCance & Widdowson's The Composition of Foods*. London: RSC & MAFF and IBCHN (1997) *Food Base*. London: IBCHN.

The oil has high levels of linoleic and α-linolenic acids and comparable amounts of the *n*-6 and *n*-3 families (*n*-6:*n*-3=0.8). Moreover, it contains appreciable amounts of γ-linolenic and stearidonic acids. The presence of the latter two fatty acids would be beneficial for individuals with impaired desaturase activity.

We believe that CY911 may help avoid the imbalance that is often precipitated by high consumption of oils limiting in one of the essential fatty acid families. Nevertheless, it has a high unsaturation index and a special attention would have to be taken in extraction and processing to minimize peroxidation.

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Okuyama H, Kobayashi T & Watanabe S (1997) *Progress in Lipid Research* **35**, 409–457.

Cooking and the fatty acid composition of herring, farmed salmon and rainbow trout. By D.C. BITSANIS, P. LUMUMBA, M. LEIGHFIELD, K. GHEBREMESKEL and M.A. CRAWFORD, *Institute of Brain Chemistry and Human Nutrition, University of North London, 166–222 Holloway Road, London, N7 8DB*

Oily fish is a rich source of the long-chain, *n*-3 fatty acids. The importance of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in health through the regulation of diverse physiological processes is well established (Connor, 2000; Innis, 2000; Nestel, 2000).

The aim of this preliminary study was to determine the effect of frying and grilling on the fatty acid composition of commonly consumed fish species. Thirty-six samples of raw whole fish or fish fillets were purchased from different outlets in London. Whole fish was cleaned, gutted and filleted. Herring, farmed salmon and rainbow trout were grilled under a gas grill and fried in sunflower oil. Lipid from homogenized raw and cooked fish were extracted in chloroform:methanol (Folch & Sanley, 1957) containing internal standard C17. Fatty acid methyl esters were separated and quantified by gas chromatography. Fish and cooked fish were analysed for their fat and fatty acid composition.

Main fatty acid composition of raw (g/100g food), fried and grilled fish (g/100g edible food)

Fatty acids	Herr. Raw	Herr. Fried	Herr. Grilled	Farmed Salmon Raw	Farmed Salmon Fried	Farmed Salmon Grilled	Rain. Trout Raw	Rain. Trout Fried	Rain. Trout Grilled
	(*n* 4)	(*n* 3)	(*n* 3)	(*n* 5)	(*n* 4)	(*n* 4)	(*n* 5)	(*n* 4)	(*n* 4)
C16:0	3.00	2.44	4.50	1.90	1.42	2.17	0.66	0.54	0.70
C16:1	1.02	0.67	1.43	0.85	0.54	0.92	0.27	0.17	0.29
C18:0	0.77	0.64	0.74	0.36	0.35	0.42	0.11	0.17	0.13
C18:1	2.86	3.64	5.65	2.33	1.90	2.53	0.74	0.88	0.81
C20:1	3.44	2.23	3.94	1.34	0.84	1.41	0.36	0.24	0.38
C22:1	5.17	3.23	5.99	1.67	1.06	1.71	0.40	0.27	0.44
C18:2*n*6	0.27	5.51	0.48	0.56	1.74	0.58	0.17	1.38	0.20
C20:4*n*6	0.12	0.06	0.19	0.07	0.05	0.08	0.02	0.02	0.03
C18:3*n*3	0.14	0.11	0.18	0.22	0.14	0.23	0.06	0.03	0.06
C20:5*n*3	1.13	0.73	1.58	0.90	0.59	0.99	0.24	0.15	0.25
C22:5*n*3	0.14	0.10	0.23	0.44	0.29	0.48	0.08	0.05	0.08
C22:6*n*3	2.00	1.01	3.08	2.09	1.51	2.49	0.80	0.50	0.81

Oily fish is rich in fat and contains high proportions of EPA and DHA. The levels of saturates, monoenes and *n*-3 fatty acids varied among herring, farmed salmon and rainbow trout. Grilled fish contained higher amounts of PUFA than raw and fried fish due to the loss of water during cooking. The residual oil after grilling had higher levels of EPA and DHA than the residual leftover oil after frying (data not shown), suggesting that grilling has a more pronounced effect on the lipid composition of fish. These are preliminary data extracted from an ongoing project and the sample size is small. Nonetheless, the differences are sufficiently large to indicate that the method of cooking alters the fatty acid composition of fish as eaten together with the amount of EPA and DHA consumed per unit of food.

Connor WE (2000) *American Journal of Clinical Nutrition* **71**, 171S–175S.
Folch JL & Sanley GHS (1957) *Journal of Biological Chemistry* **226**, 497–509.
Innis SM (2000) *American Journal of Clinical Nutrition* **71**, 238–244.
Nestel PJ (2000) *American Journal of Clinical Nutrition* **71**, 228–231.

Calculated energy intake *versus* anthropometric measures as a predictor of the need for dietary change in children with cystic fibrosis. By T. THOMAS[1] and M. LAWSON[2], [1]*Dietetic Department, Great Ormond Street Children's Hospital NHS Trust, London WC1N 3JH and* [2]*Childhood Nutrition Research Centre, Institute of Child Health, London WC1N 1EH*

Dietitians frequently use a diet history as a basis for recommending quantitative or qualitative changes to patients' usual food intake. Obtaining a history is tedious and time-consuming for the dietitian and the patient; translating the information into nutrient intakes is liable to a number of inaccuracies (Cameron & van Staveren, 1988) and comparisons of individual intakes with Dietary Reference Values will only provide a guide to nutritional adequacy (Department of Health, 1991).

Some children with cystic fibrosis (CF) are known to have increased nutritional requirements and may suffer from growth failure (Anthony *et al*. 1998). Our Centre uses a 3-d food diary completed annually to assess nutrient intake; measures of height and weight are made at each clinic visit. Compliance with completing the food diary prior to attending the clinic is about 65%. The clinical decision to increase energy intake is made using a both anthropometric and dietary intake data. In this study the aim was to test the usefulness of a calculated diet history compared with measures of height and weight alone in predicting the need for dietary change.

Dietary change recommendations were studied in 100 children with CF attending an outpatient review clinic. All children had height and weight measured and a diet history (65 × 3-d diary, 35 × 24-h recall) calculated. The mean energy intake was 128% of the EAR for age (range 66–300%). The mean BMI *z* score was –0.17 (range –4.89 to +1.82).

Results:	No diet change	Increase energy intake	Totals
BMI *z* score <–0.5	17	15	32
BMI *z* score >–0.5	62	6	68
Energy intake <100% EAR	8	10	18
Energy intake >100% EAR	71	11	82
Either < 100% EAR or BMI < –0.5	23	18	41
EAR >100% and BMI > –0.5	56	3	59
Totals	79	21	100

A total of twenty-one out of 100 children were recommended to increase their energy intake. An intake of <100% EAR for energy for age, obtained by diet history, identified ten out of twenty-one children recommended to change; giving a sensitivity of 48% and a positive predictive value of 56%. A BMI *z* score of <–0.5 (25–50 centile for age) identified fifteen out of twenty-one children who were recommended to change; the sensitivity of the method was 71% and the positive predictive value was 65%. Forty-one children had *either* a low intake *or* a low BMI and eighteen were recommended to change their diet. Using either criterion did not significantly improve the positive predictive value (44%) although sensitivity increased to 86%.

Whilst the diet history is a valuable tool for predicting nutrient deficiencies in selected groups, calculation of BMI is a more sensitive method of identifying children who need to increase their energy intake than a diet history. In patient groups with increased nutritional requirements, routine height and weight measurements and calculation of BMI predicted the need for nutritional intervention without the need for patients to complete and dietitians to calculate a formal diet history.

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Department of Health (1991) *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom.* London: The Stationery Office.

Accuracy, reliability and linearity of air displacement plethysmography in body composition research. By A.L. COLLINS and H.D. McCARTHY, *Nutrition Research Group, School of Health and Sports Science, University of North London, 166–220 Holloway Road, London N7 8DB*

Hydrostatic weighing is the widely accepted reference method for measuring body volume and hence body composition by densitometry. This has recently been challenged by developments in air displacement plethysmography, manifested as the 'BodPod' Measurement System (McCrory *et al.* 1995). Since the original communication regarding the BodPod (Dempster & Aitkens, 1995), the validation studies performed by the manufacturers have yet to be confirmed independently.

A selection of known fixed volumes ranging between 10 and 150 litres was developed using water-filled jerrycans to represent a range of human body volumes. Measurement of this range was repeated five times on different days. The density of the jerrycans was accurately determined by the titration method, and then weighed, completely filled with water to give an accurate, known volume. To relate this to human measurement, repeated measurements on the same day were performed on a total of thirty-four subjects (twenty-six female, eight male) ranging in BMI between 17.9 and 37.2 kg/m^2.

Across the range of known volumes, mean percentage error, calculated as [(observed – actual)/actual × 100], was found to be –0.09%. This level of underestimation was greater than previously quoted (–0.07%; Dempster & Aitkens, 1995) and would correspond to a measurement error of the magnitude of 0.5% body fat. However, the percentage error associated with volumes below 20 litres corresponded to a greater underestimation (0.40%), which would translate to an error of the magnitude of 2.5% body fat. This finding highlights the potential inappropriateness of the BodPod in measuring small body volumes, such as in infants. In terms of reproducibility, for all volumes above 40 litres, the coefficients of variation (CV) were below 0.1%, whereas at volumes below 40 litres this variability increased (CV 0.14–0.36%). A body volume below 40 litres would be representative mainly of children and this points towards a need for caution when measuring this population group using the BodPod. Indeed, previous studies in children using this system have demonstrated a measurement bias (Dewit *et al*. 1998; Nunez *et al*. 1999), probably due to a minor calibration problem of the instrument at these volumes. In light of this, our recommendation is that, at the very least, repeat measurements should be performed on subjects who are below the age of 13 years or below a weight of 40 kg.

Following repeated measurements in human subjects, the mean trial-to-trial coefficients of variation were 0.18, 3.41 and 2.76% for volume, measured lung volume, and percentage body fat, respectively. The variability in volume measurements in human subjects is markedly higher than that shown for the known volumes, and is probably due to additional factors such as attire and body hair, which will affect measurement due to adiabatic air. Nevertheless, the resultant variation shown in body fat measurement only corresponds to a variability of 0.7% body fat between measurements, and appears to be predominantly due to differences observed in lung volume measurements between measure one and two. Indeed, following a paired sample *t*-test, although there was found to be no significant difference between measurements one and two for weight, volume, or percentage body fat (P>0.50), there was a small but significant difference (2.5%) between repeated measurements of lung volume (P 0.014). This difference could be due to familiarization of the subject with the lung volume procedure, and may caution the need for more consistency in the measurement of this parameter which could have introduced further error into the body fat estimation.

These results further highlight the relatively high reproducibility and accuracy of the BodPod Measurement System for measurement of volume, and hence body composition. However, these results also emphasize the potential limitations of this method and aspects of its use, which will have a bearing on confidently obtaining reliable results.

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A comparison of bone mineral density and body composition between anorexics, bulimics and healthy controls. By A.L. COLLINS[1], D.M. REID[1], H. MILLER[2], G. McNEILL[1] and B. DURHAM[3], [1]*Department of Medicine & Therapeutics, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD,* [2]*Royal Cornhill Hospital, Aberdeen AB25 2ZH and* [3]*Department of Biochemistry, University of Liverpool, Liverpool L69 3BX*

It has been documented that the clinical state of anorexia nervosa can give rise to significantly diminished total bone mineral density (BMD) (Lennkh *et al.* 1999) and that this can increase the risk of morbidity and mortality to bone diseases (Carmichael & Carmichael, 1995). However, little evidence exists as to the separate effects of the eating disorder bulimia nervosa on BMD. Anorexia nervosa has also been shown to adversely affect body composition, mainly by depleting body fat stores, but again, the effects of bulimia nervosa on body composition are less clear (Mathiak *et al.* 1999). In this investigation, a small sample of female anorexics (n 6), bulimics (n 5) and healthy slim controls (n 10) undertook whole body scans by dual-energy absorptiometry (DXA) using a Norland XR-36 densitometer to obtain values of total BMD. In addition, biochemical measures of bone turnover were measured in serum and fasted overnight urine samples for all subjects. Body composition was measured in all participants both by DXA and independently by bioelectrical impedance (BIA). The descriptive data and significant differences (P<0.05) between the three subject groups were as follows.

	Healthy range	Anorexics (n 6)		Bulimics (n 5)		Controls (n 10)	
		Mean	SD	Mean	SD	Mean	SD
Age (years)		29.8	6.6	26.0	8.1	25.9	5.7
Height (m)		1.61	0.07	1.63	0.04	1.67	0.06
Weight (kg)		45.3*†	5.6	53.1	6.7	58.6	6.0
BMI (kg/m^2)	(18.5–25.0)	17.5*†	1.1	21.3	2.1	21.0	2.4
% body fat (BIA)		11.7	4.9	18.7	7.7	19.0	7.3
% body fat (DXA		19.2†	3.4	25.7	4.3	22.0	5.8
total BMD (g/cm)	(0.960–1.350)	0.852*†	0.062	0.941	0.052	1.007	0.063

* Significantly lower than controls (P<0.05) following one-way ANOVA,
† Significantly lower than bulimics (P<0.05) following one-way ANOVA.

BMD was significantly diminished in the anorexics, both compared with the controls (P 0.002) and with the bulimics (P 0.018). Although there appeared to be a reduced mean BMD in the bulimic group, this difference was not significant. Perhaps any apparent difference might have been more successfully detected had the subject numbers been larger, even so, these differences may be more due to previous history of anorexia in the bulimia group as suggested elsewhere (Iketani *et al.* 1995). Nevertheless, it was found that levels of carboxyterminal propeptide of type I procollogen in serum (a marker of bone formation) was significantly lower in the bulimic group compared with controls (P 0.02), although this level was within the reference range. In terms of body composition, according to DXA, the anorexics had a significantly lower percentage body fat compared with the bulimics (P 0.018), but not significantly less than the controls, probably due to limitations in sample size and methodology. Significant differences in body fat were not seen with BIA measured.

These results further illustrate the observation from previous studies that anorexia nervosa exhibits detrimental effects on total BMD (Lennkh *et al.* 1999). The inability of this study to fully show significantly depleted body fat stores in anorexics by both methods, may, in part, be due to the small sample size, the applicability of both BIA and DXA in this population, or the fact that those anorexics who volunteered tended to be older and more progressed in their out-patient recovery. Other studies have demonstrated this difference in body composition more successfully, but have still not fully determined differences between bulimics and controls (Mathiak *et al.* 1999).

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