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The effects of ageing, vitamin D and growth hormone on ectopic bone formation in the rat. By KEVIN CASHMAN[1] and JAMES C. FLEET[2], [1]*Department of Nutrition, University College, Cork, Ireland,* [2]*USDA Human Research Center on Aging, Tufts University, Boston, USA*

Data from animal (Sontag, 1992) and cell-culture models (Tsuji *et al.* 1990) are consistent with the hypothesis that bone formation is reduced with ageing and this contributes to the imbalance between formation and resorption that leads to bone loss. The reasons for this phenomenon are largely unknown. Bone formation can now be studied *in vivo* using a model of subcutaneous (sc) implantation of demineralized bone particles and recombinant human bone morphogenetic protein-2 (rhBMP-2), which is an effective stimulator of ectopic bone formation in young rats. The objective of the present study was to examine the effects of ageing on ectopic bone formation in this rat model and to determine if vitamin D or growth hormone (GH) influences this age-associated reduction in bone formation.

Demineralized bone implants containing 5 μg rhBMP-2 were inserted subcutaneously into female Spraque-Dawley rats aged 1, 3 and 16 months for 12 d. In addition, two subgroups of the 16-month-old animals received either GH (100 μg/100 g body weight per d) or 1,25-dihydroxycholecalciferol ($1,25\text{-}(OH)_2D_3$) (5 ng/100 g body weight per d). At the end of the implantation period, rats were killed and implants removed and processed for histology (bone formation scoring) and RNA analysis (osteocalcin mRNA expression). Blood was also obtained by cardiac puncture and serum analysed for $1,25\text{-}(OH)_2D_3$ and insulin-like growth factor-I (IGF-I).

					Serum			
			Bone formation score*		$1,25\text{-}(OH)_2D_3$ (pg/ml)		IGF-I (ng/ml)	
Age (months)	Treatment	*n*	Mean	SE	Mean	SE	Mean	SE
1	-	5	100[a]	18.1	65.1[a]	4.5	905.3[a]	32.2
3	-	5	52.4[b]	10.9	28.7[b]	4.8	943.0[a]	36.7
16	-	8	17.7[c]	4.2	16.4[b]	4.5	737.8[b]	39.3
16	GH	8	44.0[b]	9.4	28.8[b]	5.2	1117.1[a]	38.2
16	$1,25\text{-}(OH)_2D_3$	8	39.9[bc]	5.3	105.0[c]	5.2	790.4[b]	66.7

[abc] Mean values within a column with unlike superscript letters were significantly different, $P<0.05$ (ANOVA).

* Results are expressed relative to the amount of bone formation that occurred in the 1-month-old rats given implants.

Bone formation induced after 12 d of sc implantation decreased with increasing age from 1 - 16 months. Osteocalcin mRNA levels of implants also declined in ageing animals (data not shown). There was also a progressive decline in serum levels of the two important Ca- and bone-regulating agents ($1,25\text{-}(OH)_2D_3$ and IGF-I) with ageing. Treatment with either GH or $1,25\text{-}(OH)_2D_3$ during the 12 d implantation period restored bone formation in the 16-month-old rats to levels approaching that in 3-month-old rats. These studies show that ageing blunts rhBMP-2-induced bone formation in rats but that this can be partially reversed by treatment with $1,25\text{-}(OH)_2D_3$ or GH.

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

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Tsuji, T., Hughes, F.J., McCulloch, C.A.G. & Melcher, A.H. (1990). *Mechanisms of Ageing and Development* **51**, 121-132.

The effect of copper sulphate supplementation on fibrinolytic factors in healthy subjects. By M. S. FAUGHNAN, M. BONHAM, A. MCKEOWN, J. M. O'CONNOR, E. TURLEY, J. COULTER, W. S. GILMORE and J. J. STRAIN, *Human Nutrition Research Group, University of Ulster, Coleraine, BT52 1SA.*

Haemostasis involves a careful balance between coagulation and fibrinolysis which is essential for normal wound repair and healing. In CHD these processes appear imbalanced, with reduced fibrinolytic activity playing a key role (Marckmann, 1995). Cu deficiency has been implicated in the aetiology of CHD (Strain, 1994). In the present study, the effect of 3 mg $CuSO_4$ supplementation on the fibrinolytic factors, tissue plasminogen activator (tPA) and plasminogen activator inhibitor 1 (PAI-1), was investigated. Twenty seven healthy individuals (fourteen females, thirteen males), aged 20-40 years participated in a double-blind crossover design trial. Treatment and placebo periods were of 6 weeks duration. Fasting blood samples (20 ml) were taken at the beginning of the study and at the end of the treatment and placebo periods. tPA and PAI-1 were measured by ELISA methods. The putative Cu indices measured were platelet (plt) cytochrome c oxidase (CCO), leucocyte (WBC) CCO, WBC superoxide dismutase (SOD), erythrocyte SOD, plasma caeruloplasmin protein and oxidase activity. All indices, except caerulpolasmin protein, were measured using automated enzymatic methods. Plasma caeruloplasmin protein was measured by an immunoturbidimetric method.

Although Cu supplementation (3 mg $CuSO_4$/d) tended to increase pltCCO ($P=0.06$, paired *t* test), there was paradoxically a significant decrease in WBC SOD ($P<0.001$, paired *t* test). No other putative indices of Cu status were affected by treatment.

	Baseline		3 mg $CuSO_4$		Placebo	
	Mean	SEM	Mean	SEM	Mean	SEM
tPA (ng/ml)	5.6	0.6	7.3*	0.8	6.7	0.8
	n 18		*n* 17		*n* 18	
PAI-1(ng/ml)	47.8	8.2	36.8	6.6	36.6	7.2
	n 22		*n* 17		*n* 17	

Significantly different from baseline * $P = 0.01$ (paired *t* test)

The Table shows the effect of Cu supplementation on the fibrinolytic factors. Levels of tPA were increased and levels of PAI-1 were decreased after supplementation. This effect was seen with little effect on putative Cu status. The decrease in WBC SOD may have resulted from the anti-inflammatory effect of Cu decreasing the requirement for SOD in WBC.

These findings suggest that $CuSO_4$ supplementation has a stimulatory effect on fibrinolysis. This may lead to a more favourable balance within the haemostatic system towards fibrinolysis.

This study is funded through the EC 4th Framework (FAIR) Programme (FOODCUE:CT95-0813) and the Ministry of Agriculture, Food and Fisheries (MAFF), UK.

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Teas and wines as potentially important sources of dietary antioxidants; an *in vitro* comparison study. By I.F.F. BENZIE[1] and J.J. STRAIN[2], [1]*Department of Health Sciences, The Hong Kong Polytechnic University, Kowloon, Hong Kong,* [2]*Human Nutrition Research Group, University of Ulster at Coleraine, BT52 1SA*

Risk of chronic disease may be decreased by improved antioxidant status. This may be achievable by increased intake of plant-based dietary agents, such as wine, tea and herbs, as these are rich in polyphenolic flavonoids (Halliwell, 1996; Strain & Benzie, 1997). In the present *in vitro* study, the antioxidant (reducing) power of red and white wines, and cooled, filtered infusions (50 g/L) of several types of Chinese tea (*Camellia sinensis*) was measured using a recently developed automated method for antioxidant (reducing) power, the FRAP assay (Benzie & Strain, 1996). Results were expressed as µmol ferric reducing/antioxidant power: the FRAP value. The antioxidant (reducing) power of freshly prepared aqueous solutions of ascorbic acid (vitamin C) and of commercially available 'fresh' orange juice was also measured.

Results showed that the FRAP value of tea varied from 228-960 µmol/g dried tea leaves, with green tea being most active and Pu Erh (black) tea least active. The FRAP value of red wine ranged from 14000-26000 µmol/l; white wine, 2328-3192 µmol/l; that of orange juice was 3000 µmol/l. Table 1 shows the typical antioxidant content of stated quantities of these beverages, as they would be drunk, with that of 1 g of pure ascorbic acid, a powerful antioxidant of established importance, as reference.

Beverage	Amount	Antioxidant power (µmol)
Black tea (10 g/L)	200 ml	500 - 900
e.g Pu Erh, Pu Li		
'Semi-fermented' tea (10 g/L)	200 ml	1000 - 1300
e.g Oolong		
Green tea (10 g/L)	200 ml	2000
Red wine:		
e.g. Merlot, Shiraz, Cabernet Sauvignon*	150 ml	2900 - 3700
White wine:		
e.g.Reisling, Sauvignon Blanc, Chardonnay	150 ml	380 - 520
'Fresh' orange juice	200 ml	600
Ascorbic acid	1 g	11364

* Mean of two wines, one Chilean, one South African

Tea is a potentially rich dietary source of antioxidants. This is particularly true of green tea, the form of tea most commonly drunk in Asia and North Africa. The polyphenolic content of green tea is high owing to the inactivation of polyphenol oxidase by the steaming of tea leaves soon after picking. 'Semi-fermented' tea, commonly drunk in China, is steamed 1-2 h after picking. Black tea, the form most commonly drunk in the West, is left for 6 h before steaming, and has a concomitantly lower polyphenolic content. Red wine, the fermentation mixture of which contains grape skins, and to a lesser extent white wines, which are mde from fermented grape juice, are also rich in antioxidants.

Whether the antioxidant power in teas and wines is bioavailable, and the question of the clinical utility of increasing antioxidant defence through increased intake of such dietary agents remain to be confirmed. The results of this study will be useful, however, in performing bioavailability studies, since pre- and post-ingestion changes in the FRAP value of biological fluids, such as plasma, can be monitored. It also remains to be confirmed if all constituent antioxidants are bioavailable, or if there are 'key' constituents which are more bioactive than others and which could, therefore, be selectively used to enhance antioxidant status more effectively.

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Is dietary consumption of fruit and vegetables reflected in the plasma carotenoid profiles of older people? By Y. CARROLL, B. CORRIDAN and P.A. MORRISSEY, *Department of Nutrition, University College Cork, Ireland*

Increasing evidence suggests that the consumption of fruit and vegetables high in carotenoids is inversely associated with the risk of CHD and cancer.

The aims of the present study were to document dietary consumption of carotenoids and plasma carotenoids in a group of elderly people and to investigate the strength of the relationship between dietary carotenoid intake and plasma carotenoid levels in this group of people in Ireland. Dietary carotenoid intake of fifty-four volunteers aged over 65 years was assessed by 7d diet records (DR) and a food frequency questionnaire (FFQ). Dietary carotenoids were quantified by reference to a comprehensive database compiled from an extensive literature search. Fasting blood samples were obtained at screening and on the day after completing the diary. Plasma carotenoid levels were analysed in triplicate by reverse-phase HPLC with two on-line U.V./Visible detectors connected to a Millenium software package.

	Males *n* 25								
Nutrient	Diet records (µg/d)		FFQ (µg/d)		DR *v* FFQ	Plasma (nmol/l)		DR *v* plasma	FFQ. *v* plasma
	Mean	SD	Mean	SD	r	Mean	SD	r	r
α-Carotene	812	651	1217	1375	0.25	122	81	0.32	0.70†
β-Carotene	3099	2060	5277	4607	0.23	472	222	0.04	0.34
β-Cryptoxanthin	116	153	295	346	0.62††	117	96	0.46*	0.54†
Lycopene	2092	1809	2026	2211	0.12	91	69	0.14	0.47*
Lutein+Zeaxanthin	778	320	1877	1462	0.40	187	73	0.07	0.39
Total Carotenoids	6899	4624	10693	8475	0.27	992	395	0.11	0.55*

* P < 0.05, † P < 0.002, †† P < 0.0001

	Females *n* 29								
Nutrient	Diet records (µg/d)		FFQ (µg/d)		DR*v* FFQ	Plasma (nmol/l)		DR *v* plasma	FFQ. *v* plasma
	Mean	SD	Mean	SD	r	Mean	SD	r	r
α-Carotene	973	530	1223	797	0.14	166	94	0.11	-0.14
β-Carotene	3358	1447	5496	3035	0.24	553	254	-0.09	0.12
β-Cryptoxanthin	108	160	317	338	0.48	123	62	0.52	0.65†
Lycopene	2285	2367	4615	5368	0.28	111	61	0.32	0.44
Lutein+Zeaxanthin	1015	463	2120	1277	0.44**	223	73	0.14	0.10
Total Carotenoids	7739	3245	13773	8823	0.33	1177	436	0.27*	0.34

* P < 0.05, ** P< 0.02, † P < 0.002,

The predominant dietary carotenoids were β-carotene and lycopene by both dietary methods. β-Carotene and lutein plus zeaxanthin were the predominant plasma carotenoids. FFQ gave higher estimates of dietary carotenoid intake than DR. The strongest correlation between dietary and plasma carotenoids existed for β-cryptoxanthin in both males and females. Significant correlations between dietary intake based on FFQ and plasma concentrations were also observed for α-carotene and lycopene in males. We conclude that positive associations between dietary and plasma carotenoids were evident with both dietary tools but the strength of the association varied according to the dietary method and sex.

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Carotenoids and immune response in elderly people. By BERNICE M. CORRIDAN, MAURICE P. O'DONOHUE and PATRICK A. MORRISSEY, *Department of Nutrition, University College, Cork, Ireland*

The elderly are a group at risk for decreased immune responses, including reduced lymphocyte proliferation and interleukin-2 (IL-2) production. Several studies have shown that supplementation with antioxidant nutrients improves immune responses in the healthy elderly (Santos *et al.* 1996), but other studies have shown no effect. The immuno-enhancing effects of β-carotene may be independent of its conversion to vitamin A. The aim of the present study was to clarify whether supplementation with the carotenoids, lycopene or β-carotene, could enhance the immune function of healthy elderly subjects living independently in the community.

Fifty-two volunteers, aged 65 years and older, were randomly assigned to groups supplemented with 15 mg lycopene, β-carotene or placebo daily for 12 weeks. Whole blood was incubated with markers for lymphocyte subsets which were counted by flow cytometry. Lymphocyte proliferation was measured by incorporation of [^{3}H]thymidine in peripheral blood mononuclear cells (PBMC), following stimulation with 2.7 μg phytohaemagglutinin (PHA)/ml. Supernatant fractions of PBMC stimulated with PHA were assayed for IL-2 and interleukin-4 (IL-4) by ELISA. ANCOVA with treatment group as the factor and baseline values of the variable as the covariate, was used to test for post-supplementation differences between the groups.

	Placebo group (n 18)		Lycopene group (n 17)		β-Carotene group (n 17)	
Total T lymphocytes	76.2	6.1	71.2	18.5	72.4	10.6
Total B lymphocytes	10.0	3.3	11.6	5.3	11.2	5.0
Natural killer cells	9.7	5.4	12.4	7.9	12.7	8.2
T helper/inducer lymphocytes	48.5	11.7	46.3	11.9	42.8	13.4
T suppressor/cytotoxic lymphocytes	15.8	7.2	16.6	11.4	14.7	6.8
T helper/inducer : T suppressor/cytotoxic lymphocytes	4.30	3.22	4.31	3.11	3.43	1.70
Lymphocyte proliferation (counts/min)	42 379	21 436	56 317	31 772	50 732	33 167
IL-2 (pg/10^6 cells)	101	132	99	52	90	76
IL-4 (pg/10^6 cells)	19.8	17.4	31.2	35.7	26.0	24.1

Plasma β-carotene and lycopene concentrations increased significantly in response to supplementation. In healthy elderly volunteers, the concentrations of circulating leucocytes, monocytes, lymphocytes and their subsets were not changed by supplementation with lycopene or β-carotene. Lymphocyte proliferation and production of the cytokines IL-2 and IL-4, were also unaffected by supplementation. These results indicate that in this healthy elderly population, moderate supplementation with lycopene or β-carotene has no beneficial or adverse effects on immune status.

This project is funded by EC AIR programme (project no AIR2-CT93-0888)

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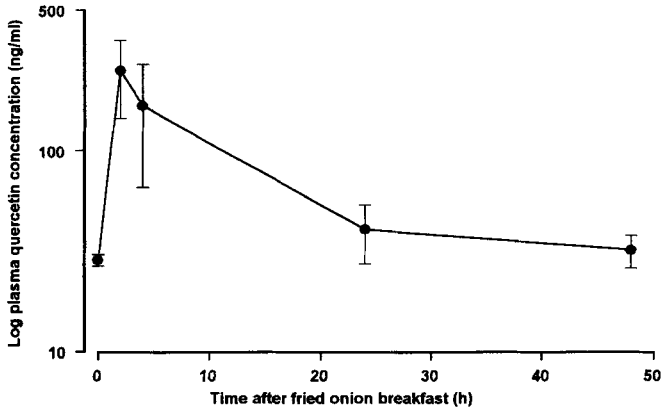
Quercetin levels in plasma and resistance of plasma to oxidation after fried onion breakfast. By G.T. MCANLIS[1, 2], J. MCENENY[2], J. PEARCE[1] and I.S. YOUNG[2], [1]*Department of Food Science, The Queens University of Belfast, Newforge Lane, Belfast BT9 5PX,* [2]*Department of Clinical Biochemistry, The Queens University of Belfast, Royal Victoria Hospital, Belfast BT12 6BJ*

Flavonoids are a group of polyphenols produced naturally by plants. They occur in fruit and vegetables and are therefore an integral part of the human diet. Several epidemiological studies have linked increased flavonoid consumption to lower risk of heart disease and strokes (Hertog *et al.* 1993). *In vitro* studies have shown that the main dietary flavonoids, especially quercetin, can protect LDL from oxidation, a process considered to be important in the pathogenesis of atherosclerosis (De-Whalley *et al.* 1990). In spite of increasing *in vitro* evidence there is still little known about flavonoid effects *in vivo*.

The aim of the present study was to assess the potential of dietary flavonoids to reduce the oxidation of LDL *in vivo*. Five volunteers consumed 225 g fried onions (a rich source of quercetin) for breakfast. Blood samples were taken at 0, 2, 4, 24 and 48 h. Quercetin levels in the plasma were measured using an HPLC / fluorescence detection technique (Hollman *et al.* 1996). Quercetin levels increased from baseline (28.42 (SD 1.89) ng/ml) to a peak at 2 h (248.42 (SD 103.9) ng/ml) before decreasing again to baseline levels after 24 h.



Plasma resistance to Cu-induced oxidation was also measured as a way of assessing its total antioxidant capacity (Nyyssonen *et al.* 1997). The duration of the lag phase is indicative of the resistance to oxidation. There was no significant change in lag times of the plasma after ingestion of the onion suggesting no significant change in total plasma antioxidant capacity. This study shows that dietary flavonoids can be absorbed but not at high enough levels to reduce the susceptibility of the plasma to oxidation.

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Effects of cholesterol oxidation products on DNA content and membrane bound enzyme activities in Chinese hamster ovary cells. By R.M. SISK, A.M. WILSON and N.M. O'BRIEN. *Department of Nutrition, University College Cork, Republic of Ireland*

Cholesterol has a fundamental role in the formation and function of cellular membranes. However, it can readily oxidize to form biologically active cholesterol oxidation products (COP). Detected at low levels in many foods and in plasma and tissues of human subjects (Emanuel *et al.* 1991), COP have the capacity to inhibit cell growth and proliferation. COP have also been reported to influence membrane properties by altering the rate of cholesterol biosynthesis or by their direct insertion into cellular membranes (Kupferberg *et al.* 1991). The objectives of the present study were to investigate the cytotoxic nature of cholesterol and COP *in vitro*, and to determine their effect on DNA content and membrane bound enzyme activities.

Chinese hamster ovary (CHO) cells were maintained in a humidified atmosphere of CO_2 (50 ml/l) at 37° in Ham's F12 medium. The cells were cultured in the absence or presence (5-50 μM) of cholesterol and the cholesterol oxidation products cholestan-3β-5α-6β-triol (cholestan-triol) or β-epoxide for a 24 h period. Cytotoxicity was determined by the neutral-red uptake assay. A concentration of 30 μM cholesterol and COP was selected for use in all subsequent experiments. Differential centrifugation was used to isolate the various enriched membrane fractions. The nuclear (P1), mitochondrial/lysosomal (P2) and microsomal (P3) enriched fractions were obtained by centrifugation at 600 *g*, 1500 *g* and 100 000 *g* respectively. The cytosolic fraction present in the remaining supernatant (S3) fraction was also retained for analysis. Each fraction was analysed for DNA content, acid phosphatase, glucose-6-phosphatase and lactate dehydrogenase activity. Control values were arbitrarily assigned a value of 100%. The results are expressed as percentage marker activity compared with the control.

Test compound	DNA (%)		Acid Phosphatase (%)		Glucose-6-phosphatase (%)		Lactate dehydrogenase (%)	
(30 μM)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Cholesterol	105.29	6.64	98.28	5.35	111.57	3.44	96.37	6.79
β-Epoxide	88.01	4.76	87.39	6.34	110.11	1.61	78.86	3.52
Cholestan-triol	62.12	4.65	59 57	3.30	108.19	1.76	37.10	3.59
LSD (P<0.05)	10.98		9.01		NS		13.46	

Statistical analysis was by one-way ANOVA, followed by LSD, least significant difference. *n* 6 for treatments.

Cholesterol was found to be non-toxic to CHO cells, however supplementation with cholestan-triol and β-epoxide resulted in a significant reduction in cell viability (results not shown). DNA content and marker enzyme activities were unaffected following supplementation with cholesterol. The addition of COP to the growth media of CHO cells resulted in a significant suppression in DNA content, acid phosphatase and lactate dehydrogenase activity relative to that of control cells (P<0.05). However, glucose-6-phosphatase activity was not influenced by either cholestan-triol or β-epoxide. The findings of this study demonstrate that COP are cytotoxic to CHO cells *in vitro*. The toxic nature of COP may be due, in part, to their direct incorporation within cellular membranes.

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

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Flavonoids: their protective role in cholestan-3β-5α-6β-triol induced toxicity *in vitro*. By S.A. AHERNE, A.M. WILSON and N.M. O'BRIEN, *Department of Nutrition, University College Cork, Republic of Ireland*

Fruit and vegetables contain many bioactive components which may possess anticarcinogenic and cardioprotective properties (Rice-Evans *et al.* 1996). Examples of these potentially beneficial compounds are the flavonoids, which are found widely among plants and plant products. However, cholesterol oxidation products (COP) are also present in the diet and are known to influence negatively the health status of the individual (Guardiola *et al.* 1996). These dietary toxicants (COP) are found predominantly in cholesterol-rich processed foods. The aim of the present study was to determine the ability of certain flavonoids and butylated hydroxytoluene (BHT, a synthetic antioxidant) to protect against COP-induced toxicity *in vitro*. The COP used was cholestan-3β-5α-6β-triol (triol).

Chinese hamster ovary (CHO) cells were cultured in Ham's F12 medium and maintained in a humified atmosphere at 37° and enriched with CO_2 (50 ml/i). In the initial study, the growth medium was supplemented with either 0-50 μM triol or 0-100 μmol/l of the flavonoids, rutin, quercetin and myricetin or BHT. Cytotoxicity was measured by two separate assays, namely the neutral-red uptake assay and lactate dehydrogenase release assay. Triol at 15 μmol/l resulted in cell viability of 75%; 5 μM - rutin, quercetin, or myricetin and 100 μM - BHT resulted in cell viability of 100%. These concentrations were used in subsequent studies where the cells were exposed to medium containing triol in the presence or absence of the flavonoids or BHT for a period of 24 h. Lipid peroxidation, as indicated by thiobarbituric acid reactive substances (TBARS) and the activities of the endogenous antioxidant enzymes catalase (CAT, *EC* 1.11.1.6), superoxide dismutase (SOD, *EC* 1.15.1.1) and glutathione peroxidase (GSH-Px, *EC* 1.11.1.9) were measured.

	Endogenous antioxidant enzymes						Lipid peroxidation	
	Catalase (units/mg protein)		Superoxide dismutase (units/mg protein)		Glutathione peroxidase (units/mg protein)		TBARS (nmol MDA/mg protein)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	7.16	0.23	3.37	0.06	4.72	0.28	1.04	0.17
Triol	2.97	0.25	2.73	0.11	2.21	0.18	2.29	0.01
Triol + rutin	5.24	0.20	3.78	0.07	4.37	0.37	1.81	0.12
Triol + myricetin	6.80	0.73	3.69	0.06	2.88	0.51	1.67	0.11
Triol + quercetin	3.22	0.16	3.87	0.12	3.28	0.41	1.52	0.12
Triol + BHT	5.97	0.49	3.88	0.04	4.05	0.39	1.96	0.25
LSD (P<0.05)	1.54		0.56		1.12		0.49	

MDA, malondialdehyde. Statistical analysis was by one-way ANOVA, followed by LSD, least significant difference; *n* 4 for treatments.

Triol significantly reduced antioxidant enzyme activity and increased the extent of lipid peroxidation in the CHO cells (P<0.05). The addition of the flavonoids or BHT to triol-supplemented media returned SOD activity back to control levels in the cells. Rutin, myricetin and BHT restored CAT activity levels in the appropriately supplemented cells, whereas quercetin had little influence. Rutin and BHT resulted in GSH-Px activity returning to control levels, however, quercetin and myricetin showed no significant effect. The flavonoids also reduced the extent of triol-induced lipid peroxidation in this cellular model. These results suggest that certain flavonoids may play a role in reducing COP-induced toxicities *in vitro*.

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

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Rice-Evans, C.A., Miller, N. J. & Paganga, G. (1996). *Free Radicals in Biology and Medicine* **20**, 933 - 956.

Effects of caffeine on performance, metabolism and reaction times during endurance exercise in man. By JOHANNA McLOUGHLIN, BERNARD DONNE and J. F. ANDREWS, *Department of Physiology, Trinity College, Dublin 2, Republic of Ireland*

This study investigated the effects of a high dose of caffeine on performance and substrate metabolism, in addition to considering the effect of caffeine on reaction times during prolonged exercise. Ten male subjects (age 22.6 (SE 1.5) years, weight 72.5 (SE 2.5) kg, height 1.77 (SE 0.01) m, caffeine intake 440 (SE 61) mg/d, VO_{2max} 75 (SE 2.4) $ml.kg^{-1}.min^{-1}$), cycled to exhaustion at a load equivalent to 80% VO_{2max} on two occasions. Consecutive tests were separated by 7 d. Exercise at 80% VO_{2max} on a weight loaded cycle ergometer (Monark) commenced 60 min following the administration of 9 mg/kg body mass of either caffeine (CF) or placebo (CT) in a subject blinded design. Respiratory exchange ratio (RER) was determined from on-line expired gas analysis and reaction times to an auditory stimulus were recorded. Data were analysed using paired Student's *t* test.

Endurance time was significantly ($P<0.05$) improved in the CF trial (59.2, SE 9.7 min) compared with the CT trial (44.4, SE 8.5 min). Non-esterified fatty acid concentration (NEFA) in blood samples taken from the antecubital vein pre-treatment, pre-exercise (60 min post-treatment) and at exhaustion were analysed using an enzymic colorimetric assay (Boehringer Mannheim Biochemica). NEFA level was significantly ($P<0.001$) elevated pre-exercise (0.41, SE 0.03 v. 0.25, SE 0.02 mM) and at exhaustion (0.75, SE 0.04 v. 0.42, SE 0.03 mM) after CF ingestion compared with CT. Mean RER was significantly ($P<0.001$) reduced during exercise in the CF trial (0.86, SE 0.01) compared with the CT trial (0.88, SE 0.01). These results are indicative of enhanced fat metabolism, supporting the hypothesis that CF induces increased lipolysis thereby reducing glucose oxidation from glycogen stores to delay fatigue (Costill *et al.* 1978; Graham & Spriet, 1991). Neural stimulatory effects of CF were demonstrated by reaction time tests performed at rest and every 6 min during the exercise to exhaustion. Reaction times were significantly ($P<0.05$) reduced throughout the CF trial compared with the CT trial. Mean difference was about 20% at rest, gradually decreasing as exercise progressed, to a minimum of about 4% after 39 min.

The results of this study suggest that caffeine mediates its ergogenic effect on exercise performance by altered substrate availability, which in turn may be effected by neuronal excitability.

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The effects of altered fat and carbohydrate availability on some peripheral indices of central fatigue during cycling exercise in trained humans. By Y.P. PITSILADIS[1]*, I. DAVIDSON[2], I. SMITH[3], A. STRACHAN[1] and R.J. MAUGHAN[1], [1]*Department of Environmental and Occupational Medicine,* [2]*Department of Molecular and Cell Biology,* [3]*Department of Surgery, University Medical School, Aberdeen AB25 2ZD*

In a recent study, the peripheral indices of central fatigue measured were unrelated to exercise performance and perception of effort (Pitsiladis *et al.* 1996). The small increases in plasma non-esterified fatty acids (NEFA) seemed responsible for the equally small changes in the free tryptophan (Trp):branched-chain amino acid (BCAA) ratio. The present study was conducted to investigate the relationship between these peripheral indices of central fatigue, exercise performance and perception of effort, using a high-fat meal followed by the administration of heparin to increase the circulating levels of NEFA before exercise. Following determination of maximum O_2 uptake (VO_2max) and a familiarization period, six trained cyclists participated in a diet-exercise regimen lasting 9 d and comprising three cycling tests to exhaustion. A work load was selected that would result in fatigue after approximately 100 min at an ambient temperature of 10°. The first exercise test was a control trial and was preceded by a period during which a normal diet (mean 56 (SD 7) % energy carbohydrate (CHO)) was consumed. Following this exercise bout, a prescribed high (70 % energy) CHO diet was consumed for 3.5 d. Following this diet, a second exercise test was performed; one of two meals was consumed 4 h before this test (70 % energy CHO meal or 90 % energy fat meal). The second exercise test was followed by a further 3.5 d on the high-CHO diet. At 4 h before the third test, subjects consumed the other meal. Heparin was administered intravenously 30 min (1000 U), 15 min (500 U), and 0 min (500 U) before exercise on the fat trial. Subjects were assigned to the two meals in randomized order. Blood samples were obtained at rest, at 15 min intervals during exercise and at exhaustion. Expired gases were collected every 15 min during exercise. Ratings of perceived exertion (RPE) were obtained every 10 min until exhaustion. Statistical analysis was carried out using two-factor ANOVA for repeated measures followed by Student's *t* test for paired data where appropriate. Time to exhaustion during the control trial was 105.6 (SD 12.1) min (data from this trial were not included in any statistical analysis). Time to exhaustion increased from 118.2 (SD 12.4) min on the CHO trial to 127.9 (SD 12.1) min on the fat trial ($P=0.001$). A higher plasma NEFA concentration was found at rest and at exhaustion on the fat trial; only on the CHO trial was there an increase in plasma NEFA over time. No difference between trials or over time was found in the plasma free Trp and in the free Trp:BCAA ratio while there was a modest 40 % increase in the total Trp:BCAA ratio at exhaustion and only on the fat trial. The total Trp:BCAA ratio was higher at rest on the CHO trial while there was no difference between trials at exhaustion. Only at the end of exercise on the fat trial was total plasma Trp higher than at rest. Total plasma Trp at rest and plasma BCAA from 30 min onwards were higher on the CHO trial; BCAA were not different over time. No difference in serum prolactin (Prl) concentration was found between trials at rest (0.5 (SD 0.5) nmol/L and 0.4 (SD 0.3) nmol/L on the fat and CHO trials respectively). Serum Prl was higher on the fat trial at 60 min of exercise (0.9 (SD 0.6) nmol/L compared with 0.4 (SD 0.1) nmol/L) and at exhaustion (1.5 (SD 0.8) nmol/L compared with 1.0 (SD 0.6) nmol/L). Only on the fat trial was the Prl concentration higher during exercise compared with the resting level (the percentage increase in individual subjects ranged between 36 and 922 %). While no difference in RPE was found between trials, there was an earlier rise in RPE on the fat trial. No difference in total CHO oxidation was found between trials. These results suggest that the peripheral indices of central fatigue measured were unrelated to exercise performance and perception of effort. The greater Prl response and the finding of a more rapid rise in RPE on the fat trial does not preclude central neurotransmitter involvement during exercise on this trial. However, the performance results would suggest that this central component was unlikely to be the cause of fatigue. These results are consistent with the hypothesis that substrate availability limits exercise capacity during this type of exercise in a cold environment.

This study had local ethics committee approval.

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Diurnal and diet-induced changes in skeletal muscle function in normal subjects. By A. CUNLIFFE[1], D. GOLDHILL[2], O. OBEID[1] and J. POWELL-TUCK[1], *[1]Department of Human Nutrition, [2]Anaesthetics Unit, St Bartholomew's and the Royal London School of Medicine and Dentistry, London E1 2AD*

We have previously shown that there are diurnal changes in voluntary work output during wrist ergometry in normal subjects tested at hourly intervals through the working day (Cunliffe *et al.* 1997[a]). Moreover the macronutrient composition of a meal was found to affect work capacity and fatiguability (Cunliffe *et al.* 1997[b]). The present study was designed to assess the involvement of motivation and other central factors in these changes. Two experiments were conducted in which the force frequency characteristics and relaxation rate of the adductor pollicis in response to supramaximal ulnar nerve stimulation (UNS) were examined as measures of involuntary muscle function (Edwards *et al.* 1977). In the first experiment (Diurnal) ten healthy volunteers (six male, four female), with a mean age 30.6 years and mean BMI 22.9 kg/m^2, were tested at 2 h intervals through the working day. Force of contraction was measured at stimulation frequencies of 10, 20 and 30 Hz with a pulse width of 100 μs at approximately 50 mA. In the second experiment (Dietary), five overnight fasted volunteers (three male, two female) with a mean age 29.56 years and mean BMI 23.2 kg/m^2, were tested in the same manner with UNS before, and then at hourly intervals for 4 h after ingesting 200 ml maltodextrin solution (1672 kJ).

			Time (h)						
Experiment	Test		0	1	2	3	4	6	8
Diurnal:	F_{10}:F_{30} (%):	Mean	29.3	-	36.6	-	32.2	31.1	32.1
		SE	1.24		2.22		1.85	1.81	3.20
	MRR (%):	Mean	15.2	-	10.5	-	11.1	10.8	11.5
		SE	3.10		0.40		0.28	0.65	0.68
Dietary:	F_{10}:F_{30} (%):	Mean	44.3	43.7	43.9	50.6	43.8		
		SE	2.13	1.61	2.64	3.14	2.58		
	MRR (%):	Mean	8.66	9.59	8.65	11.7	11.4		
		SE	0.11	0.68	0.28	1.65	1.59		

The Table shows the results as the means for maximum force measured at stimulation of 10 Hz expressed as a percentage of that measured at 30 Hz (F_{10}:F_{30}) and the means for muscle relaxation rate (MRR; calculated from the maximum slope of the initial phase of relaxation and expressed as percentage force fall/10 ms) at 20 Hz as a measure of muscle fatigue. ANOVA indicated no significant difference in force production or in the relaxation rate of the adductor pollicis muscle as a result of time of day, or as a result of consuming a pure carbohydrate meal. These findings indicate that the acute changes in voluntary functional capacity previously recorded were probably due to effects at a central level and not due to changes in the peripheral apparatus responsible for the contraction at the level of muscle itself. Functional assessment of fatigue state therefore will yield most information if it includes tests of volitional and non-volitional capacity thereby revealing the contribution of central and peripheral components of fatigue. In the clinical arena this may aid in the more accurate assessment of the efficacy of nutritional or other interventions.

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The effect of prolonged exercise on protein metabolism in top triathletes during carbohydrate ingestion. By ANTON J.M. WAGENMAKERS, DAPHNE L.E. PANNEMANS, ASKER E. JEUKENDRUP, ANNEMIE P. GIJSEN, JOAN M.G. SENDEN, DAVID HALLIDAY and WIM H.M. SARIS, *Department of Human Biology, NUTRIM, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands*

The aim of the present study was to investigate whether endurance exercise in the laboratory, simulating conditions usually only seen in field competition, leads to net protein catabolism. Eight male Dutch top triathletes were investigated first during 4 h of rest, then during 6 h of exercise (starting with cycling-1 for 2.5 h at 50% VO_{2max}, then running for 1 h at 11 km/h and ending with 2.5 h cycling-2 at 50% VO_{2max}) and finally during 4 h recovery. Subjects continuously ingested carbohydrate drinks (0.8 g/min) during the entire 14 h experiment as athletes during competion in the field always ingest carbohydrates during exercise of this duration and intensity. The studies were started after overnight fasting with a primed continuous infusion of L-[1-^{13}C]leucine, L-[$^2H_5$]phenylalanine (with L-[$^2H_4$]tyrosine prime) and [$^{15}N_2$]urea as tracers. This multiple tracer approach was chosen as discrepancies exist in previous literature on the catabolic nature of exercise. Breath and blood samples were taken for measurement of $^{13}CO_2$ enrichment by isotope ratio mass spectrometry and plasma α-ketoisocaproic acid, phenylalanine, tyrosine and urea tracer enrichment by GC-MS. A stable isotope steady state was present during the last 2 h of the resting periods, and during the last 1 h of cycling-1 and -2. Protein synthesis, degradation and oxidation and urea production were calculated from the tracer infusion rates and steady state enrichments according to published standard procedures.

	Protein oxidation (mg protein/kg per h)		Protein synthesis (mg protein/kg per h)		Protein degradation (mg protein/kg per h)		Urea production (μmol/kg pr h)	
Tracer	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Leucine	(*n* 6)		(*n* 6)		(*n* 6)			
rest	26	7	173	14	200	11	NA	
cycling-1	71*†	25	123*†	26	194	5	NA	
cycling-2	68*†	21	124*†	22	191	6	NA	
recovery	25	4	155	14	181*	15	NA	
Phenylalanine	(*n* 8)		(*n* 8)		(*n* 8)			
rest	13	2	163	17	176	17	NA	
cycling-1	17	3	153	13	170	16	NA	
cycling-2	19	5	154	15	172	19	NA	
recovery	15	6	147	36	162	41	NA	
Urea							(*n* 8)	
rest	NA		NA		NA		518	110
cycling-1	NA		NA		NA		413*†	92
cycling-2	NA		NA		NA		346*†	79
recovery	NA		NA		NA		293*	62

NA, not applicable. *Significantly different from rest, $P<0.01$ (2-tail). †Significantly different from recovery, $P<0.01$.

With leucine as tracer oxidation was 2- to 3-fold higher during exercise than in the resting periods, while protein synthesis was lower. Protein degradation did not change during exercise and decreased during recovery. Net protein balance (degradation - synthesis) was 2- to 3-fold more negative during exercise than during rest and recovery. With phenylalanine as tracer protein oxidation, synthesis and degradation were similar at rest, during exercise and during recovery. Urea production was significantly lower during exercise and recovery than at rest. Diurnal changes in urea production may underlie this decrease in this 14 h study.

It is concluded that either the leucine or phenylalanine method is not valid during exercise. The lack of increase of urea production seems to point to a methodological problem with the leucine method. The data, therefore, suggest that prolonged demanding exercise with carbohydrate ingestion, as athletes practice during competition, in contrast to the general belief does not lead to increased net protein catabolism.

Muscle glycogen utilization during mild and severe exercise, hyperglycaemic with and without insulin. By H. MOHEBBI[1], M.A. KEEGAN[1], I.T. CAMPBELL[1], C.T. BEST[1], D.A. SPERRY[1], D.P. MACLAREN[1] AND A. McARDLE[2]. [1]*University Department of Anaesthesia, Withington Hospital, Manchester M20 2LR and* [2]*University Department of Medicine, Royal Liverpool Hospital, Liverpool L69 3BX*

Muscle glycogen is utilized during severe exercise (70% VO_2max). Even hyperglycaemia with blood glucose "clamped" at 10.8 mmol/l during 2 h cycling at 70% VO_2max did not affect glycogen utilisation (Coyle *et al.* 1991). The present study was undertaken to determine whether insulin and hyperglycaemia would affect muscle glycogen utilization during mild (40% VO_2max) and severe (70% VO_2max) exercise.

Eight male subjects (aged 26.2 (SD 7.4) years; height 1.75 (SD 0.03)m; weight 71.4 (SD 6.0) kg) gave informed consent. The study was approved by the local Ethics Committee. They were randomized to cycle on four occasions, each for 120 min, twice at nominally 40% VO_2max and twice at 70% while 200 g/l D-glucose solution was infused intravenously at rates designed to maintain blood glucose at 10 mmol/l on one occasion at each intensity; insulin was also infused, concurrently with the glucose, at 40 mU/m^2/per min. The glucose and insulin were started 30 min before the start of exercise. A muscle biopsy was taken immediately at the end of exercise from the vastus lateralis for glycogen assay, from between four and six subjects as follows: 40% VO_2max, n = 6; 40% with insulin (40%I), n = 5; 70%, n = 5; 70%I, n = 4. All subjects attended the laboratory on two other occasions, once for measurement of VO_2max, once for a non-exercised muscle biopsy.

In the event subjects rode at 42.0 (SD 3.0) % glucose alone; 40.7 (SD 2.9) % glucose and insulin; 66.1 (SD 7.1) % glucose alone and 69.1 (SD 4.9) % glucose and insulin, of their VO_2max. Average blood glucose during each of the studies is given in the Table. Muscle glycogen was expressed as μmol glucosyl units/mg protein and is presented as a percentage of the non-exercised value.

	40%I		40%		70%I		70%	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Blood glucose (mmol/l)	10.0	0.2	10.1	0.2	9.9	0.4	10.0	0.1
Muscle glycogen (% non-exercise)	81.3	5.4	61.9	5.0	43.6	14.3	40.9	10.9

In all instances muscle glycogen concentrations were significantly lower after exercise than the non-exercised value (40%, 70% and 70%I, P<0.001; 40%I, P<0.03). At 40% VO_2max muscle glycogen concentration was significantly lower with glucose alone compared with glucose and insulin (P<0.03). At 70% VO_2max insulin made no difference to glycogen utilization (P= 0.854). Muscle glycogen at 70%VO_2max, with or without insulin, was lower than at 40% but the difference was not significant (P=0.086).

It is concluded that, in the presence of hyperglycaemia, insulin infused intravenously at 40mU/m^2/per min attenuates muscle glycogen utilization during mild exercise but not during severe exercise.

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Exogenous carbohydrate oxidation from ingested drinks during prolonged exercise in a cold environment in man. By S.D.R. GALLOWAY[1]*, S.A. WOOTTON[2], J.L. MURPHY[2] and R.J. MAUGHAN[1], [1]*Department of Environmental and Occupational Medicine, University Medical School, Foresterhill, Aberdeen AB9 2ZD and* [2]*Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD*

Several authors have suggested that the carbohydrate (CHO) content of ingested drinks should be high during exercise in a cold environment as substrate provision is relatively more important than rehydration (Hargreaves, 1991; Maughan & Noakes, 1991). In the present study we examined the oxidation of exogenous glucose from a range of CHO/electrolyte beverages of differing concentrations during prolonged exercise in the cold (10.0 (SD 0.2) °). Six healthy male volunteers (age 28 (SD 4) years) performed four rides to exhaustion on an electrically braked cycle ergometer at approximately 80 % of VO_2max. Dietary intake was the same on the 2 d before each trial and subjects were asked to refrain from consuming foods naturally enriched with ^{13}C (e.g. commercial sports drinks, maize and maize-starch based products) throughout the study period. The four trials were performed 1 or 2 weeks apart after an overnight fast and at the same time of day. In each trial subjects ingested a bolus volume of fluid (7.14 ml/kg) immediately before exercise and additional fluid volumes (1.43 ml/kg/) every 10 min during exercise. The fluids ingested were either a flavoured water control (0%) or CHO/electrolyte beverages with glucose concentrations of 20 g/L (2%), 60 g/L (6%) and 120 g/L (12%). The beverages were labelled with [U-^{13}C]-glucose (99.2 Atom Percent Excess: 0.05 g/L). Expired air was collected for 1 min into Douglas bags at rest and every 15 minutes during exercise. Substrate oxidation was estimated from VO_2 and respiratory exchange ratio (Consolazio *et al.* 1963). Enrichment of $^{13}CO_2$ in breath was measured by isotope ratio mass spectrometry. Exogenous oxidation of substrate was calculated over each 15 minute period according to the method of Mosora *et al.* (1976) and values were corrected for any change in background ^{13}C production in the control trial.

There was a tendency for exercise capacity to be less (P=0.08) on the 0% trial (84.6 (SD 4.1) min) than on the trials where CHO was given: 2% (102.2 (SD 14.0) min); 6% (101.1 (SD 15.4) min); 12% (95.1 (SD 12.1) min). There were no significant main effects of CHO ingestion on the estimated rates of total CHO (P=0.99) or fat (P=0.99) oxidation. The oxidation of exogenous glucose in each 15 minute period was not different (P=0.21) between trials but there was a tendency for the oxidation to be highest in each 15 minute interval in the 2% trial and lowest in the 12% trial. The mean difference in exogenous glucose oxidation between the 2% and 12% trials in each 15 min period was 2.13 (range 0.54 - 2.82) g. Cumulative exogenous glucose oxidation in the first 75 min of exercise was not significantly different (P=0.22) between trials with mean oxidation values of 26.2 (SD 13.1), 20.5 (SD 8.5) and 15.6 (SD 7.5) g for the 2%, 6% and 12% trials respectively.

The present work revealed that there was no significant difference in the amount of exogenous glucose oxidation between trials regardless of the ingested drink CHO content. This may be due either to slower gastric emptying and intestinal absorption characteristics of the more concentrated CHO beverages or to a limitation in skeletal muscle glucose uptake and oxidation at this exercise intensity. These findings indicate that the inclusion of a high concentration of glucose in ingested fluids appears unwarranted during exercise of this intensity and duration in a cold environment.

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Metabolic and performance-related responses during endurance exercise following high-fat and high-carbohydrate meals. By HELENA A. WHITLEY[1], S. M. HUMPHREYS[1], I. T. CAMPBELL[2], M. KEEGAN[2], T. JAYANETTI[2], D. SPERRY[2], D. M. MACLAREN[3], T. REILLY[3] and K. N. FRAYN[1], [1]*Oxford Lipid Metabolism Group, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE,* [2]*University Department of Anaesthesia, Withington Hospital, Nell Lane, Manchester M20 8LR and* [3]*Centre for Sport and Exercise Sciences, Liverpool John Moores University, Mountford Building, Byrom Street, Liverpool L3 3AF*

We studied the effects of pre-exercise meal composition on metabolic and performance-related variables during endurance exercise. Eight well-trained cyclists (VO_2 max 65.0 - 83.5 ml/kg per min) were studied on three occasions after an overnight fast. In a random order, subjects were given isoenergetic meals containing carbohydrate (CHO), protein (P) and fat (F) in the following amounts (g per 70 kg body weight): high-carbohydrate meal, 215CHO, 26P, 3F; high-fat meal, 50CHO, 14P, 80F, and on one occasion they were studied after an overnight fast (No meal). At 4 h after consumption of the meal, subjects cycled on a 'Kingcycle trainer' for 90 min at 70% of their VO_2max, followed by a 10 km time-trial.

The high-carbohydrate meal resulted in significant decreases in blood glucose (P <0.05), plasma non-esterified fatty acids, plasma glycerol, plasma chylomicron-triacylglycerol and plasma 3-hydroxybutyrate concentrations (P <0.01) during exercise when compared with the high-fat trial. This was accompanied by an increase in plasma insulin (P <0.01), plasma adrenaline and plasma growth hormone concentrations (P <0.05) during exercise. The rate of fat oxidation was 56 % higher and carbohydrate oxidation 20 % lower during the first 15 min of endurance exercise in the high-fat trial when compared with the high-carbohydrate trial.

Meal	Time to completion (s)		Mean power (W)		Maximum power (W)		Maximum heart rate (beats/min)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
No meal	874	16.9	279	11.1	303***	11.3	187*	3.1
High-fat meal	854	13.0	290	16.2	357	18.1	191	3.0
High-CHO meal	878	15.5	276	11.6	309***	13.0	187*	3.2

Significantly different from high-fat meal: *P <0.05, ***P< 0.001.
The mean power was calculated as the average power output during the 10 km time-trial. The maximum power was calculated as the maximum power output during the final 60 seconds of the 10 km time-trial. n 8 for all three meals.

The Table shows that time to complete the 10 km performance test was reduced by approximately 20 s in the high-fat trial, although this effect was not statistically significant. Correspondingly, average power output during the 10 km trial tended to be greater in the high-fat trial. Additionally, maximum power output during the final 1 min of the 10 km time-trial was significantly greater and maximum heart rate significantly higher following the high-fat meal.

These findings suggest that a pre-exercise meal high in fat and moderate in carbohydrate and protein, favours a high work output during the finishing phase of an endurance performance test. Whether this is related to alterations in substrate availability remains to be determined.

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Influence of carbohydrate-electrolyte beverage feedings during recovery, on subsequent substrate utilisation during exercise: the use of naturally enriched [^{13}C] glucose. By J.L.J. BILZON[1], J.L. MURPHY[2], A.J. ALLSOPP[1], S.A. WOOTTON[2] and C. WILLIAMS[3], [1] *Institute of Naval Medicine, Gosport PO12 2DL,* [2] *Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD,* [3] *Department of Physical Education Sports Science & Recreation Management, Loughborough University, Loughborough LE11 3TU*

Carbohydrate (CHO) feeding following strenuous exercise has been shown to enhance endurance performance during a subsequent bout of activity (Fallowfield *et al.* 1995; Wong *et al.* 1995). However, the optimal pattern of feeding and amount of CHO ingested during the recovery period required to maximize performance remains unclear. In the present study naturally enriched [^{13}C] glucose, in the form of maltodextrin was used to compare the effects of feeding different amounts of CHO following an initial exhaustive treadmill run on subsequent running performance and substrate metabolism. Eleven male subjects performed two trials separated by an interval of at least 7 d. In each trial they ran at 60% VO_2max (in a room temperature of 35°) to fatigue or until aural temperature reached 39° (T1) and then again (T2) after a 4 h recovery period (REC). In REC of both trials subjects consumed 50 g maltodextrin (Maxijul Powder, SHS Ltd, Liverpool; −10.48‰ ^{13}C) in the form of a 75 g/l carbohydrate-electrolyte (CHO-E) beverage (667 ml) immediately after T1. The subjects then consumed either (i) the same quantity of CHO-E (HI-CHO) or (ii) an equivalent volume of a CHO-free electrolyte beverage (LO-CHO) at hourly intervals for 4 h. Expired air was collected every 15 min during exercise into Douglas bags for determination of total CHO and fat oxidation by indirect calorimetry; exogenous CHO oxidation was estimated from $^{13}CO_2$ enrichment determined by isotope ratio mass spectrometry (Europa Scientific Ltd, Crewe). The results of T2 are shown in the Table.

T2 run	CHO oxidation (g/45 min)						Fat oxidation (g/45 min)	
	Total †		Exogenous ‡		Endogenous §		Total †	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
HI-CHO	134.2*	6.6	69.3*	3.0	64.9	5.6	10.0*	2.0
LO-CHO	78.5	7.2	19.3	2.1	59.2	6.4	33.1	2.7

* Mean values were significantly different from LO-CHO (P<0.01, ANOVA); † calculated using Frayn (1983); ‡ calculated from breath $^{13}CO_2$ excretion; § Total CHO − Exogenous CHO.

Performance times during the first run were 59.5 (SE 5.8) min and 40.2 (SE 3.4) min in the second run of the HI-CHO trial whereas the corresponding times during the LO-CHO trial were 59.9 (SE 5.8) min and 41.4 (SE 2.4) min. There were no differences in endurance running performance between conditions during T1 or T2. Endogenous CHO oxidation was not spared by repeated CHO feedings. Exogenous CHO oxidation was greater and conversely fat oxidation lower at each time point during T2 following repeated feedings. In this model exercise performance was limited by an inability to thermoregulate rather than substrate availability *per se*. However, more of the CHO consumed remained unoxidized on completion of the second task during HI-CHO (130.7 (SE 3.0) g) compared to LO-CHO (30.7 (SE 2.1) g: P<0.01), and may influence subsequent performance.

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Perceived barriers to healthy eating among nationally-representative samples of European adults. By MARY KEARNEY, JOHN M. KEARNEY and MICHAEL J. GIBNEY, *Institute of European Food Studies, Trinity College, Dublin 2, Republic of Ireland.*

There is evidence to suggest that dietary guidelines are achieved by only a small percentage of the general population (Hulshoff *et al.* 1993; Ministry of Agriculture, Fisheries and Food, 1994). The promotion of healthy eating may be more effective if nutrition educators are aware of the factors which the general public perceive as preventing them from eating healthy diets. The objective of the present study was to identify perceived barriers to healthy eating among Europeans. Quota-controlled nationally-representative samples of European adults (aged 15 years upwards) from each member state of the European Union (EU) completed an interview-assisted face-to-face questionnaire on attitudes to food, nutrition and health. The subjects were asked to select any factors from a list of twenty-two which they believed prevented them from eating a healthy diet. In total, 14 331 adults completed the questionnaire. Data were weighted for population size when examining EU averages. The most frequently selected barriers were "irregular work hours' (24%; ranging from Germany 12% to Luxembourg 41%), "giving up favourite foods" (23%; Spain 15% - Luxembourg 43%); "willpower " (18%; Germany and Italy 10% - Ireland 31%) "busy lifestyle" (17%; Germany 6% - Sweden 27%) and "price" (15%; Italy 7% - UK 23%). Overall, only 7% of EU subjects mentioned that "lack of knowledge" prevented them from eating healthy diets and only 8% said that "experts keep changing their minds" was a barrier. The variation between EU demographic groups was less than that between member states. In the EU the difference males and females were small, although in each member state significantly more males than females mentioned that "irregular work hours" was a barrier and in eleven member states "other people's preferences" was a barrier for significantly more females than males. In almost all member states, as age increased the relative importance of time factors ("irregular work hours" and "busy lifestyles") decreased while the importance of time increased as the level of education increased. In total 21% EU subjects reported "no difficulty" in trying to eat healthily, varying from 7% in Luxembourg to 32% in Germany and in all member states older subjects were the most likely to report no difficulty. The survey also identified another important barrier to the promotion of healthy eating. Subjects were asked to indicate their level of agreement/disagreement (on a four point Likert scale) with the statement "I do not need to make changes to the food I eat, as it is already healthy enough". In total, 71% of EU subjects agreed with the statement suggesting that the majority of European adults do not perceive a need to alter their eating habits, and that they do not see dietary guidelines as personally relevant. This suggests a need for more targeted nutrition education messages. Given that time and taste factors are the most important perceived barriers, nutrition educators need to ensure that healthy eating is viewed positively in terms of taste and convenience. Because of the variation between countries and demographic groups a uniform pan EU strategy to tackle perceived barriers to healthy eating is unlikely to be effective.

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Prevalence of undernutrition and weight loss changes during the course of hospitalization among patients admitted to two Dublin hospitals. By C. CORISH[1], P. FLOOD[2], S. MULLIGAN[3] and N.P. KENNEDY[1], *[1]Department of Clinical Medicine, Trinity College Dublin, [2]Department of Clinical Nutrition, St. James's Hospital, Dublin and [3]Department of Nutrition and Dietetics, Meath Hospital, Dublin, Ireland*

A prevalence of undernutrition of 40% among 500 patients on admission to hospital was reported in a recent study from Dundee (McWhirter & Pennington, 1994). The purpose of the present study was to assess the prevalence of undernutrition in patients admitted among all specialities to two Dublin hospitals. The criteria for definition of undernutrition were those used in the Dundee study.

Data were collected on 594 patients of which 569 were analysed to calculate prevalence of undernutrition. The mean prevalence of undernutrition was shown to be 11%, forty-three (7.6%) were mildly undernourished, fourteen (2.5%) were moderately undernourished and five (0.9%) were severely undernourished. Contrary to expectations, a higher prevalence of undernutrition was not found in patients over the age of 65 years (9.6%) by comparison with those aged less than 65 years (11.7%). A higher prevalence of undernutrition was found in the lower socio-economic groups (11.4%) by comparison with the higher socio-economic groups (9.1%). No difference was found in the prevalence of undernutrition between males (10.7%) and females (11.1%).

	Dublin study		Dundee study	
	No. malnourished	No. assessed	No. malnourished	No. assessed
General medicine	19 (11%)	165	46	100
General surgery	11 (9%)	123	27	100
Respiratory medicine	5 (25%)	20	45	100
Medicine for elderly	4 (15%)	26	43	100
Orthopaedic surgery	4 (9%)	43	39	100
Miscellaneous medicine	12 (16%)	73		
Miscellaneous surgery	7 (6%)	119		

Unintentional weight loss of more than 10% of body weight in the 6 months before admission occurred in sixty-four (11% of 549) patients by comparison with 13% in the Dundee study. A further twenty-nine (5%) patients had an unintentional weight loss of more than 5% of body weight in the month before admission. Of the sixty-two undernourished patients, fifty-seven had data on weight before admission, twenty-one (37%) lost more than 10% body weight over the previous 6 months while a further nine (16%) lost more than 5% over the previous month.

Only twenty-five (40%) of the sixty-two undernourished patients were referred for nutritional support. A larger proportion of those moderately undernourished (*n* 9, 64%) and severely undernourished (*n* 3, 60%) by comparison with those mildly undernourished (*n* 13, 43%) were referred. An additional four undernourished patients received regular nutritional supplements from the nursing staff. Twenty-five (67%) of the thirty-seven undernourished patients not referred were discharged in less than 7 d. Twenty-two undernourished patients were reassessed on discharge. Ten patients gained weight (six referred), three remained a stable weight (none referred) while nine lost weight (6 referred). Of patients not referred, one who gained weight and one who maintained a stable weight received regular nutritional supplements from the nursing staff.

Weight loss during the hospital stay occurred in 118 (62%) of 189 patients; fifteen of these dropped into a lower BMI category. None of the undernourished patients moved into a worse category of undernutrition. Only two patients of normal weight became underweight but nineteen of normal weight lost more than 1 BMI unit (which implies a clinically significant weight loss) over the course of the hospital stay. In the ninety-three patients with clinically significant weight loss before admission (i.e. >10% over 6 months or >5% over 1 month), thirty continued to lose weight in hospital, five moving into a lower BMI category, sixteen losing more than 1 BMI unit.

In conclusion, a lower prevalence of undernutrition and a higher referral for nutritional support was observed in this study by comparison with the Dundee study. However, the proportion of patients who lost weight in hospital was similar in both studies. The number of undernourished patients discharged into the community without nutritional intervention or advice is a cause for concern.

C. Corish is the recipient of a studentship sponsored by Abbott Laboratories (Irl.) Ltd.

McWhirter, J. & Pennington C.R. (1994). *British Medical Journal* **308,** 945-948.

Daily energy expenditure in free-living children: comparison of Caltrac motion sensors with the doubly-labelled water method. By L.C. GREENE[1], M.B.E. LIVINGSTONE[1], A.F. McGLOIN[1], S.E. WEBB[1], S.A. JEBB[2] and A.M. PRENTICE[2], [1]*Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA and* [2]*Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH*

The role of childhood physical activity for long-term health status has been difficult to evaluate because of the lack of socially acceptable, inexpensive, objective and low-interference techniques for estimating habitual total energy expenditure (TEE) (Livingstone, 1994).

In the present study the validity of the Caltrac motion sensor was assessed in twenty-three (eight female, fifteen male) healthy, free-living children aged 6-8 years by comparing results of Caltrac TEE measured over seven consecutive days with results from simultaneous measurements of TEE over 10 d by the doubly-labelled water (DLW) method. BMR of each child was measured before the assessment of free-living TEE for 35 min by open circuit indirect calorimetry under standardized conditions.

	Group (*n* 23)		Male (*n* 15)		Female (*n* 8)	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	6.6	0.7	6.7	0.7	6.5	0.8
Weight (kg)	24.7	6.1	24.6	5.2	24.8	7.9
Body fat (%)	19.6	9.1	16.3	6.7	25.7	10.1
BMR (kJ/d)	4702	569	4858	545	4410	505
DLW TEE (kJ/d)	7418	1162	7831	1179	6643	644
Caltrac TEE (kJ/d)	7065	1572	7585	1601	6090	985
DLW AEE (kJ/d)	2716	924	2973	1010	2233	487
Caltrac AEE (kJ/d)	2524	1224	2792	1415	2021	516

Mean Caltrac TEE was lower than the corresponding DLW TEE, however, this difference was not significant (paired *t* test, NS). Individual Caltrac TEE discrepancies ranged from -26.6 to +41.9% (-1851 to +3066 kJ/d), with eleven values lying within +/-10% of DLW TEE estimates. The degree of agreement between DLW and Caltrac TEE was assessed by comparing the difference between the two methods with their mean. The 95% confidence limits of agreement (mean difference in TEE +/- 2 SD) were -2904 to +2198 kJ/d. Day-to-day activity related energy expenditure (AEE) was calculated for the Caltrac data by subtracting BMR from TEE. Caltrac AEE levels increased during the weekend but these were not significantly greater than week-day Caltrac AEE (paired *t* test, NS).

Although individual estimates of Caltrac TEE lacked precision, the Caltrac motion sensor is a useful epidemiological tool for assessing free-living energy expenditure and provides an objective assessment of children's activity patterns at population level.

Livingstone, M.B.E. (1994). *Proceedings of the Nutrition Society* **53**, 207-221.

Day-to-day and meal-to-meal variation in energy intake in children. By AILEEN F. MCGLOIN[1], M. BARBARA E. LIVINGSTONE[1], LUCY C. GREENE[1], SANDRA E. WEBB[1], SUSAN A. JEBB[2], and ANDREW M. PRENTICE[2], [1]*Human Nutrition Research Group, University of Ulster, Coleraine, BT52 ISA ,* [2]*Dunn Clinical Nutrition Centre, Hills Rd, Cambridge CB2 2DH*

The role of short-term regulation of energy intake (EI) in children in the long-term maintenance of energy balance is poorly understood. Despite apparently erratic food preferences and patterns of eating, children seem to be able to maintain normal health and growth in the long-term. The extent to which childrens' energy intake at one meal can influence intake over successive meals has been assessed in very young children under controlled protocols using weighed dietary records (WDR) and in free-living conditions using 24 H recalls (Birch et al. 1991; Shea et al. 1992). This present study aimed to examine variation in meal-to-meal EI and day-to-day EI in school-aged children to assess the level of short-term auto-regulation of EI in this age group.

Subjects were thirty healthy children (17 M , 13 F), aged 5-8 years, weight 25.3 (SD 6.0, range 18.4-43.9) kg, height 1.25 (SD 0.07, range 1.14-1.38) m. Parents of each child completed a WDR of their child's food intake for seven consecutive days. Each day was then divided into six eating occasions. These were pre-breakfast snacks and breakfast, mid-morning snacks, lunch, afternoon snacks, dinner, evening snacks and supper. Mean energy intake for the group was 7351 (SD 1433) kJ/d. The following Table shows the intra-individual variation in EI for the group.

Variation	CV (%)	Min	Max
Total EI day-to-day	55.8	44.5	140.0
Between meals within days	80.4	41.0	179.0
Between same meals day-to-day	86.5	49.0	125.0
Snacks day-to-day	112.0	44.0	211.0
Main meals day-to-day	51.0	23.0	148.0

Mean total daily EI was more tightly regulated from day-to-day than for either meals within days or between the same meals from day-to-day (paired *t* test $P < 0.01$). Variation in EI at snack eating occasions (mean EI 659 (SD 250) kJ/d was significantly higher than at meal times (mean EI 1775 (SD 420) kJ/d. In order to assess whether any energy auto-regulation of EI had occurred, we proposed a null hypothesis that there was no auto-regulation of EI. That is, when correlation between one meal and the next is zero. In this case, the CV for the observed total daily EI (55.8%) was significantly less than would be expected if no auto-regulation of energy existed (67.9%) ($P < 0.01$). This suggests that short-term negative feedback has occurred over a 24 H period. Compensation at subsequent meals for high or low energy intake at previous meals may be responsible. High variation in snack EI suggests that snacks may contribute a key role in the auto-regulation of EI in children. These findings are consistent with previous studies of variability in EI in children (Shea et al. 1992) and show some evidence of a mechanism of short-term energy regulation by children. However, to what extent other factors such as food preferences, food availability, and particularly parental control of childrens' eating behaviour, may undermine longer term appetite control needs to be considered.

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Changes in plasma lipid profiles in response to the fat content of an evening meal persist during the following day. By R.A. HENDERSON, G.E. VIST and R.D.E. RUMSEY, *Department of Biomedical Science, University of Sheffield, Sheffield S10 2TN*

The effects of the fat content of an evening meal on plasma levels of free fatty acids (FFA) and triacylglycerols (TAG) in response to tolerance tests performed the following morning were investigated. With the approval of the local ethics committee, ten healthy male subjects were provided with a high-fat meal (HFM, 62% fat by energy) and a low-fat meal (LFM, 13% fat by energy) on two occasions each. Meals comprised a pasta-based main course followed by a dessert made with either skimmed milk or cream; a high-energy (sucrose) drink was given with LFM so that the two meals were isoenergetic (3520 kJ). An oral fat tolerance test (OFTT, 84 ml dairy cream, 40 g fat) was performed 12h after the evening meal, once after HFM and once after LFM. Similarly, an oral glucose tolerance test (OGTT, 100 g glucose; HighCal Beechams) was performed on one occasion after each meal. Arterialized venous blood samples were collected before and at intervals after ingestion of the tolerance test materials and analysed for concentrations of FFA and TAG. Statistical analysis was by repeated measures ANOVA followed by paired *t* tests where appropriate.

	Low-fat meal		High-fat meal		Significance
	Mean	SD	Mean	SD	
Fasted FFA concentration (mmol/l)	0.42	0.11	0.52	0.16	*P* 0.0460
FFA slope value in first 15 min	-0.29	0.32	-0.71	0.53	*P* 0.0018
Fasted TAG concentrations (mmol/l)	1.10	0.42	0.91	0.43	*P* 0.0006

There were significant differences between plasma concentrations of FFA before ingestion of either tolerance test with plasma FFA concentrations higher after HFM (Table). Initially, there was a significantly greater total decrease from baseline in plasma FFA after OGTT compared with OFTT (P 0.0093) with the mean decrease from baseline after OGTT as 0.29 (SD 0.16) mmol and 0.16 (SD 0.13) mmol after OFTT. This difference was presumably due to an enhanced immediate postprandial insulin response after ingestion of glucose. The initial rate of decrease in plasma FFA was, however, significantly faster subsequent to HFM compared with LFM irrespective of the composition of the tolerance test (Table). After this initial decrease, plasma FFA concentrations rose in both OFTT and OGTT, but FFA concentrations were significantly higher 90, 120 and 240 minutes ($P<0.05$) after OFTT (after both meals). There were, however, no significant differences between plasma levels of FFA, slope values or areas under curves when comparing responses after HFM and LFM for the remainder of the sampling period when considering OGTT and OFTT separately.

As with FFA, there were significant differences between fasted levels of plasma TAG although in the case of TAG, levels were significantly higher after LFM (Table). After OFTT there was a rapid equalization of TAG levels which rose 1 h after ingestion of OFTT with no significant differences in TAG levels after the commencement of this rise. However, after OGTT, differences in plasma TAG levels due to the previous meal were maintained with mean plasma TAG levels 0.22 (SD 0.04) mmol higher after LFM than after HFM for the duration of the 6 h period ($P<0.05$).

It is concluded that there are changes in plasma FFA and TAG the morning after a high-fat meal. Consumption of dairy cream rapidly eliminates the differences caused by the previous meal. Equalization of FFA levels also occurs after ingestion of glucose but levels of TAG remain different. These changes in plasma lipid profiles may influence other metabolic responses to subsequent feeding and confirm the need to control pretest meals in clinical and research situations.

Baseline variability of non-esterified fatty acids (NEFA). By CARA A. PRIOR, HELEN M. ROCHE, and MICHAEL J. GIBNEY, *Unit of Clinical Nutrition, Department of Clinical Medicine, Trinity Centre for Health Sciences, St James' Hospital, Dublin 8, Ireland*

In post-absorptive states such as an overnight fast there is marked day-to-day variability in plasma NEFA in normal subjects (Roche & Gibney, 1997). The greater the fasting value of NEFA, the greater the rate and the extent of fall in NEFA concentration following a meal. Recent studies have proposed a link between elevated plasma NEFA concentration and increased risk of CHD. However, despite the acknowledged importance of plasma baseline NEFA concentration in determining the postprandial response, relatively little attention has been paid to the role which chronic dietary factors play in both the intra- and inter-individual fasting plasma NEFA variation. In the present study a standard test meal was consumed by ten healthy free-living volunteers, aged 19-25 years, on three occasions following 3 d on a background diet (habitual, higher-fat(additional 10% of habitual energy derived from fat) or higher-carbohydrate(additional 10% of habitual energy derived from carbohydrate)). Blood samples at baseline and at 20 min intervals thereafter were taken over an 80 min period for analysis of plasma NEFA, triacylglycerol, glucose and insulin.

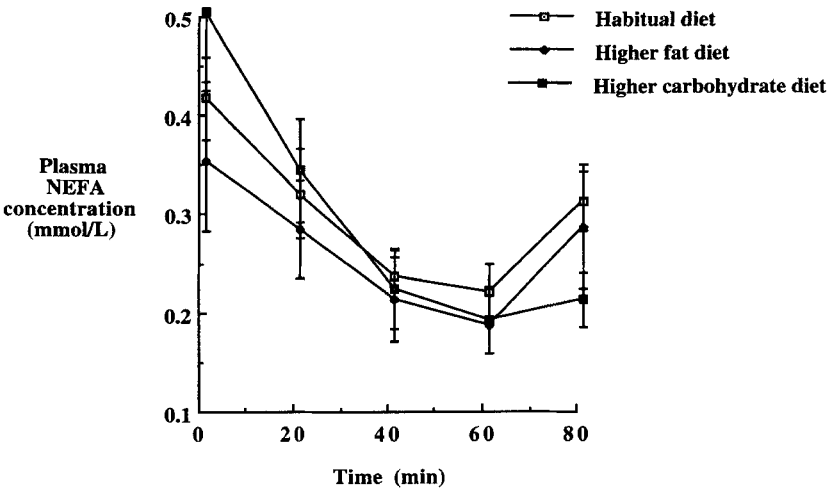

Fig.1 shows the postprandial plasma NEFA concentrations on the three study occasions. Fasting NEFA concentrations were not significantly different following the three background diets (habitual, higher-fat or higher-carbohydrate; $P = 0.073$). However plasma NEFA concentrations were significantly reduced during the postprandial response ($P \leq 0.0001$) and fasting concentrations were found to be significantly correlated with the change in the plasma NEFA concentration observed postprandially (R^2 0.787, $P \leq 0.0001$).

These findings demonstrate that significant differences in postprandial plasma NEFA concentrations, as a direct result of quantitatively small changes in fasting plasma NEFA levels, can be achieved through a modest alteration of diet composition during the 3 d period before testing. Studies which fail to control adequately for the influence of recent dietary composition on the postprandial lipaemic response, risk introducing yet another variable into an already complex situation.

Roche, H.M. & Gibney, M.J. (1997). *British Journal of Nutrition* **77**, 537-549.

The administration of a β3-adrenergic agonist decreases leptin expression in a diet-induced obesity model. By B. BERRAONDO, G. FRÜHBECK, A. MARTI, M.P. FERNANDEZ-OTERO and J.A. MARTINEZ, *Department of Physiology and Nutrition, University of Navarra, Pamplona, Spain.*

Obesity is a prevalent disorder characterized by a chronic imbalance between energy intake and expenditure. In this context, the *ob* gene product, leptin, and β3-adrenoceptors are known to be involved in the regulation of energy utilization (Mantzoros *et al.* 1996). The aim of the present experimental trial was to study the effects of Trecadrine®, a molecule with β3-adrenergic receptor affinity (Barrionuevo *et al.* 1996), on body composition and white adipose tissue leptin expression in a cafeteria-diet-induced obesity model.

Twenty-four female Wistar rats weighing about 160 g, were divided in two groups. One group (control) was fed *ad libitum* on a standard laboratory pelleted diet and water and the second group (obese) was fed *ad libitum* on a fat-rich high-energy diet (cafeteria diet) and water for 40 d as previously reported (Berraondo *et al.* 1995). After this period, rats fed on the cafeteria diet were divided into two new groups, which continued to be fed on cafeteria diet up to day 75, but Trecadrine® (1mg/kg per d) was orally administered by intubation to one group (obese+β3), while the obese and control groups received a placebo. Fat content was measured by using a non-invasive electromagnetic technique (EM-SCAN Model SA-2) and leptin expression from white adipose tissue was determined by using a reverse transcription polymerase chain reaction method. *Ob* mRNA bands were quantitated by densitometry (Gel Doc 1000 UV, Bio-Rad).

	Control (*n* 8)		Obese (*n* 8)		Obese+β3 (*n* 8)	
	Mean	SE	Mean	SE	Mean	SE
Final body weight (BW)	263.8	10.0	312.4 **	11.0	290.0^{+}	10.0
Fat content (g/kg BW)	50.7	7.60	162.8***	5.00	65.9^{+++}	5.30
Gastrocnemius muscle (g/kg BW)	6.40	0.30	5.40*	0.20	6.30^{+}	0.30
Leptin expression (leptin:β-actin mRNA ratio)	0.90	0.17	2.77*	0.73	0.90^{++}	0.14

Mean values were significantly different from controls: $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ (Mann-Whitney).
Mean values were significantly different from obese: $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ (Mann-Whitney).

Body weights and fat content were increased in those experimentally induced obese rats fed on the fat-rich diet for 40 d in relation to lean controls (results not shown). On the other hand, oral Trecadrine® administration to cafeteria-fed animals (1 mg/kg per d) for 35 d significantly decreased final body weight, fat content and white adipose leptin mRNA, while muscle proportion was increased as compared with the non-treated obese rats. Moreover, rats given orally administered Trecadrine® were not significantly different from control lean rats regarding white adipose leptin expression and other measurements (body weight, fat and muscle content).

Furthermore, data obtained from obese rats treated with Trecadrine® showed an apparent repartitioning effect by increasing gastrocnemius muscle weight (1.82 *v.* 1.68 g) at the expense of fat (19.1 *v.* 50.8 g), which has been found with other adrenergic agonists. The effects of Trecadrine® on *ob* mRNA could be due to a direct action of the β3-adrenergic receptors activation on leptin expression and/or simply reflect the reduction in fat content (Trayhurn *et al.* 1996). It can be concluded that leptin overexpression in a diet-induced obesity model is reduced by the administration of a β3-adrenergic agonist, which may have implications in obesity management.

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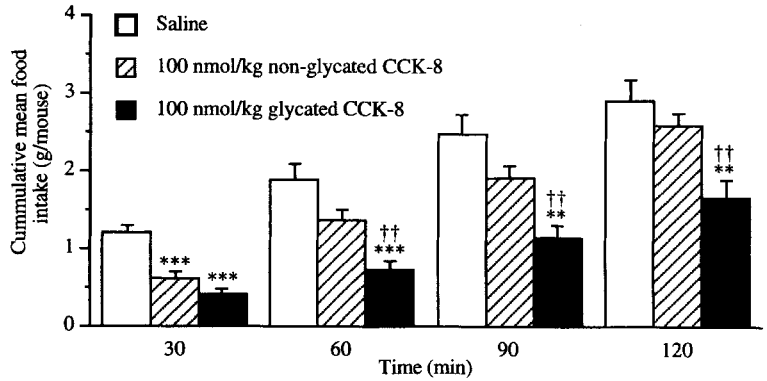
Effects of glycated and non-glycated cholecystokinin-8 on appetite control in mice. By F.P.M. O'HARTE, C.M.N. KELLY, M. MOONEY and P.R. FLATT, *School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA*

Structural modification can alter the half-life and biological activity of peptide hormones. The present study investigated the effect on food intake in mice of post-translational modification of the neuropeptide hormone cholecystokinin-8 (CCK-8) by glycation. CCK is considered to function as an important regulator of satiety. Monoglycated CCK-8 (1228.4 Da, 100μg) was prepared by incubation with 220 mM-D-glucose in 10 mM-sodium phosphate buffer (pH 7.4) with a 1000-fold molar excess of $NaBH_3CN$ relative to CCK-8 and purified by reversed-phase HPLC (O'Harte *et al.* 1996). Primary structure determination by automated peptide sequencing analysis indicated that the amino terminal Asp^1 residue was modified by glycation.

The ability of non-glycated and glycated CCK-8 to inhibit food intake was investigated in 7-12-week-old male Swiss TO mice. Mice were gradually habituated (standard breeding diet, Trouw Nutrition, Belfast) to a reduced voluntary food intake period of 2 h (10.00 - 12.00 hours) per d over a 3-week period, before beginning experimental studies. On day 0 of the study mice (*n* 16) were injected intraperitoneally with physiological saline (10 ml/kg, control) and voluntary food intake monitored 30, 60, 90 and 120 min post injection. On day 1, mice (average weight 23.4 (SE 2.83) g, *n* 8) were injected intraperitoneally with either non-glycated or glycated CCK-8 (100 nmol/kg dissolved in saline) and food intake monitored up to 2 h. Non-glycated CCK-8 significantly reduced ($P<0.001$) food intake in mice up to 30 min after its administration but failed to significantly reduce intake at 60, 90 and 120 min compared with controls. Glycated CCK-8 reduced food intake in mice at 30, 60, 90 and 120 min compared with controls ($P<0.01$ - $P<0.001$) as well as significantly reducing intake compared with non-glycated CCK-8 at 60, 90 and 120 min ($P<0.01$). Food intake results are shown in the Fig.



Significant difference indicated by $^{**}P<0.01$, $^{***}P<0.001$ compared with saline and $^{††}P<0.01$ compared with non-glycated CCK-8.

This study demonstrates that CCK-8 is a potent short-term inhibitor of food intake and that structural modification of this peptide at the amino terminal Asp^1 residue leads to enhanced satiating activity. This may be due to the increased resistance of the glycated CCK-8 to aminopeptidase degradation in the circulation (Migaud *et al.* 1996), thus prolonging its half-life and biological activity *in vivo*.

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Effects of Exercise Training on Total Plasma Cholesterol Concentrations and Rates of Cholesterol Synthesis. By M.L. THOMASON, P.W. WATT, A. PATEL and M.J. RENNIE, *Department of Anatomy & Physiology, Dundee DD1 4HN.*

Cardiovascular disease is still the biggest killer in the Western world accounting for some 50% of all deaths (McCardle *et al.* 1994). Both elevated plasma cholesterol concentrations and a sedentary lifestyle have been shown to be risk factors for the development of this disease (Miller 1987; Powell *et al.* 1987). As it is known that individuals who exercise habitually have lower plasma cholesterol concentrations, it was our aim to investigate the possibility that exercise training lowers plasma cholesterol via a reduction in *de novo* cholesterol synthesis. Healthy male volunteers (*n* 10) aged between 35 and 55 years took part in a 3-month aerobic exercise programme training four times per week for 30 min each session. Rates of cholesterol synthesis (FSR Chol) and plasma total cholesterol concentrations were determined before and after the training period, the former using stable isotope methodologies (Kinter *et al.* 1988). Vo_2max and percentage body fat were also measured. Subjects were refed hourly after an overnight fast, with a nutritionally complete liquid diet plus 0.059 mmol [1-^{13}C] acetate/kg per h given orally. Sulfamethoxazole (SMX) was administered orally at the start of the study as a 400 mg bolus dose. Venous blood samples and urine samples were taken before dosing and hourly thereafter over the 6 h study period. Lipids were extracted from the plasma using solvents, separated by TLC then subjected to combustion isotope ratio mass spectrometry to determine ^{13}C:^{12}C ratio. Urinary acetyl-SMX enrichments were obtained using GC-mass spectrometry and were taken to represent enrichment of the hepatic percursor pool for cholesterol synthesis.

	Vo_2max (ml/kg per min)		Weight (kg)		Body fat (%)		Plasma cholesterol (mmol/l)		FSR Chol (%/d)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Untrained (*n* 10)	38.01	0.83	80.6	3.4	25.35	1.35	5.27	0.23	5.65	0.51
Trained (*n* 10)	43.38***	1.49	80.8	3.4	24.76	1.14	4.44**	0.23	4.43**	0.52

Mean values were significantly different from untrained state, ** P <0.01, *** P <0.001 (paired Student's t-test.)

The results demonstrate that the exercise training programme brought about a significant increase in aerobic fitness accompanied by a decrease in both plasma cholesterol concentrations and rates of cholesterol synthesis, without significantly altering body composition.

In conclusion, aerobic exercise training appears to lower plasma total cholesterol concentrations independently of changes in body composition, which may be mediated by a reduction in *de novo* cholesterol synthesis.

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The decrease in postprandial lipaemia evident after a bout of exercise is independent of substrate utilization during the exercise. By D. MALKOVA, R. J. BOWNESS, S. L. HERD and A. E. HARDMAN, *Sports Nutrition and Exercise Biochemistry Research Group, Loughborough University, Leics LE11 3TU*

The reduction in postprandial lipaemia after exercise could reflect replenishment of intramuscular stores of triacylglycerol (TAG) (Annuzzi *et al.* 1987). We used acipimox to suppress mobilization of non-esterified fatty acids (NEFA) from adipose tissue during moderate exercise in an attempt to increase skeletal muscle utilization of endogenous TAG (Head *et al.* 1993). Postprandial lipaemia was determined three times, i.e. after a day of minimal physical activity (control), after a bout of exercise preceded by ingestion of acipimox and after a bout of exercise preceded by ingestion of a placebo.

Twelve normolipidaemic men aged 21 - 36 years, with BMI 24.0 (SD 2.5) kg/m^2, participated. Each trial was conducted over 2 d. On day 1 of each exercise trial subjects ran on a treadmill at 60% of maximal O_2 uptake for 1.5 h, starting 2.5 h after a light lunch. At 1.5 h before the run, they ingested either acipimox (1.7 mg/kg body mass) or an identical capsule containing 5 mg glucose as placebo. Venous blood samples obtained before ingestion of acipimox or placebo and immediately before, during and after the run were analysed for NEFA and lactate. Fat and carbohydrate oxidation were estimated using indirect calorimetry. On day 2 of each trial a fat tolerance test was conducted. Blood samples were obtained after a 12 h fast and at 0.5, 1, 2, 3, 4, 5 and 6 h after consumption of a test meal (1.2 g fat, 1.2 g carbohydrate/kg body mass, 67% energy from fat, 29 % from carbohydrate). Plasma was analysed for TAG, glucose and NEFA and serum for insulin. Comparisons between trials were made using the Friedman two-way ANOVA by ranks.

Acipimox depressed plasma NEFA before, during and after running (pre-run 46 (SE 4) *v.* 89 (SE 14) μmol/l, end run 69 (SE 11) *v.* 504 (SE 95) μmol/l, 30 min post-run 129 (SE 25) *v.* 891 (SE 142) μmol/l; all $P < 0.01$). Total lipid oxidation during running was lower after acipimox, compared with placebo (21.2 (SE 2.8) *v.* 36.6 (SE 6.7) g, $P < 0.05$) but total energy expenditure was unchanged (4829 (SE 184) *v.* 4859 (SE 198) kJ). Blood lactate concentration was low and did not differ between acipimox (1.29 (SE 0.23) mmol/l) and placebo (1.24 (SE 0.25) mmol/l) trials.

	Fasting TAG (mmol/l)		Fasting NEFA (mmol/l)		Fasting insulin (μIU/ml)		Total lipaemic response (mmol/l.h)		Incremental lipaemic response (mmol/l.h)		Total insulinaemic response (μIU/ml.h)	
Trial	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	0.92	0.13	0.45	0.07	10.4	1.0	8.77	1.17	3.25	0.56	128	9
Ex-acipimox	0.83	0.11	0.81*	0.10	11.8	0.9	6.81**	0.81	1.84*	0.24	130†	10
Ex-placebo	0.79	0.11	0.67*	0.06	9.6	0.7	6.95**	0.97	2.19*	0.47	110**	9

Significantly different from control * $P<0.05$, ** $P < 0.01$. Significantly different from Ex-placebo † $P< 0.05$.

The Table summarizes fasting and postprandial responses on day 2. Exercise reduced lipaemia but manipulation of substrate metabolism during exercise did not influence the magnitude of this effect. Total energy expenditure therefore appears to be a more important determinant of exercise-induced decreases in lipaemia than the relative contributions from fat and carbohydrate metabolism. The postprandial insulin response was higher when exercise was preceded by acipimox, however, and this could have influenced our findings through an effect on lipoprotein lipase activity.

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Effect of a pre-exercise bar rich in β-glucan on gastrointestinal transit time and blood glucose. By V. RIBORDY, I. MEIRIM, C. PIGUET-WELSCH, A. THELIN-DOERNER and J. DECOMBAZ, *Nestlé Research Centre, Nestec Ltd, Vers-chez-les-Blanc, CH-1000 Lausanne 26, Switzerland*

The ingestion of carbohydrate (CHO) foods and beverages at appropriate times before exercise can attenuate hypoglycaemia and delay fatigue. Slow release carbohydrates, in particular, may provide useful energy without causing large variations of blood glucose concentration (Thomas *et al.* 1991). If the food contains fibre, the magnitude of the glycaemic response is related to the viscosity of the soluble fibre present (Wood *et al.* 1994). How fast viscosity develops after ingestion is, however, unclear.

The present study examined the effect of meals containing equal amounts of CHO (72 g, 1 g/kg) and water (300 ml), but different amounts of a cereal bar containing the soluble fibre β-glucan, on gastrointestinal transit time and on blood glucose shortly after the onset of exercise (see Table). Well-trained athletes received three treatments on different occasions : (1) BAR: all of the CHO from the test bar, 13.9 g β-glucan; (2) BARDEX: two-thirds of the CHO from the bar (9.2 g β-glucan) and one third from maltodextrin; and (3) DEX: all of the CHO as maltodextrin (reference treatment).

The meals were ingested in two successive parts with an interval of 10 min. The solid part (test bar) was eaten first (time : -10 min). The liquid part (water or dextrin solutions), labelled with tracers (deuterated water 6.5 g, paracetamol 0.5 g, free [^{13}C]glucose 0.2 g) was ingested next (0 min). At +15 min, tracers' concentrations were measured in blood (deuterium, paracetamol) and expired air ($^{13}CO_2$). At +30 min, the subjects started to run on a treadmill (3.7 litres O_2/min) for 0.5 h.

Meal	Time (min)	BAR		BARDEX		DEX		
solid part...	-10	72 g CHO as bar		48 g CHO as bar		-		
liquid part (300 ml)†...	0	water		24 g CHO (8%) (90 mOsm/kg)		72 g CHO (24%) (337 mOsmol/kg)		
		Mean	SE	Mean	SE	Mean	SE	*n*
Tracers								
Deuterium (δ ‰)‡	+15	847**	115	782**	79	375	70	7
Paracetamol (mg/l)‡	+15	7.2***	0.9	6.7***	0.5	2.2	0.4	11
$^{13}CO_2$ (Atom %)‡	+15	1.101**	0.003	1.092**	0.001	1.087	0.001	11
Venous glucose (mmol/l)								
pre-exercise	+28	5.6	0.3	6.4	0.5	6.4	0.4	11
in exercise	+45	4.4*	0.3	2.9	0.2	3.3	0.3	6

Mean values were significantly different from DEX : *P<0.05, **P<0.01, ***P<0.001.
†With tracers added. ‡Measured in blood (deuterium, paracetamol) or expired air ($^{13}CO_2$).

Blood glucose immediately before exercise (+28 min) was similar in all treatments. However, insulin was much lower after BAR (mean value 19 (SE 3) μU/ml) than either DEX (38 (SE 5) μU/ml) or BARDEX (36 (SE 3) μU/ml) (P<0.01). At 15 min after the exercise onset (+45 min), mild hypoglycaemia (i.e. ≤3.5 mmol/l) was observed with the two latter meals, but not after BAR. These data show a slower rate of carbohydrate delivery to the gut when the cereal bar was eaten, but this effect did not extend to the combination of cereal bar and dextrin. On the other hand, digestive assimilation of the liquid part of the meals was much faster after BAR and BARDEX than after DEX, as assessed by all three tracers. Therefore, both meals with the test bar delivered fluid to the gut at a faster rate than the concentrated dextrin solution.

Together, these results show that a cereal bar rich in β-glucan dispenses glucose more gradually than glucose polymer solutions. However, the gastric emptying of the liquid bolus ingested on top of it was not slowed down, suggesting that viscosity did not develop in the stomach within 10 min to include the liquid phase.

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Iron status and habitual physical activity in UK adolescent girls. By RUTH ASH and MICHAEL NELSON, *Department of Nutrition and Dietetics, Kings College London, Campden Hill Road, Kensington, London W8 7AH*

Fe-deficiency anaemia (IDA) and Fe deficiency (ID) are common among UK adolescent girls (Nelson *et al*, 1993, 1994). Reduced haemoglobin (Hb) levels are associated with decreased physical activity. One consequence of IDA and ID in adolescent girls may be the establishment of poor physical activity patterns, with implications for future adult health.

Girls (*n* 539) aged 11.5 - 15.5 years, attending three comprehensive schools in greater London, took part in IDA/ID screening in either February 1995 or February 1996. An age and height-matched sample of 138 girls, classed either: IDA(*n* 47) on the basis of capillary measurements of Hb (<120 g/l) and packed cell volume (PCV< 37%); ID(*n* 38) on the basis of raised Zn protoporphyrin (>700 μg/l); or Fe replete (IR *n* 53), were selected for a 10 week, double- blind, Fe/placebo intervention. They completed a dietary assessment and measurements at baseline, T_1 (March 1995 or 1996) and post intervention, T_2 (June19 95 or 1996). Measurements included: height and weight, venous blood counts and serum ferritin (SF) and an assessment of physical activity (habitual activity questionnaire (HAQ)).

The HAQ was completed by 138 girls at T_1. The mean time spent in physical activities (TSPA) was 43 (*SD* 26) min/d; the median 39 min/d.

Table 1. *Time spent in physical activities by quintile of total activity time and activity type*

Activity quintile (min/d)	<15 (*n* 27)			16-30 (*n* 24)			31-45 (*n* 32)			45-60(*n* 22)			>60 (*n* 33)		
	%	Mean	SD	%	Mean	SD	%	Mean	SD	%	Mean	SD	%	Mean	SD
Activity Type															
Walking to/from school	7	45	35	50	85	42	67	142	53	86	173	83	82	185	114
School games/PE	100	44	23	100	66	30	100	89	40	100	95	42	100	99	46
Other school sport	11	43	29	19	54	50	28	59	31	23	115	58	36	194	122
Activities outside school	26	46	17	50	72	32	72	89	67	82	124	90	91	286	147
Daily mean (TSPA)	100	9	4	100	23	4	100	38	4	100	53	5	100	83	15

There were no differences in: age (13.5 (*SD* 1.3) years); height (1.58 (*SD* 0.1) m); weight (51.4 (*SD* 9.9) kg); BMI (20.5 (*SD* 3.3) kg/m^2); Hb (125 (*SD* 14) g/l); PCV (35.7 (*SD* 3.3) %); or SF (24 (*SD* 12.7) μg/l) by activity quintile and no difference in TSPA by Fe status (IDA,ID,IR). Girls were divided into two activity groups: low activity (TSPA<median) and high activity (TSPA≥ median). There were small, non- significant, differences between the low and high activity groups in: Hb 123 (*SD* 14) *v.* 126 (*SD* 13) g/l (two-tailed *t* test, *P*=0.299); and SF 21.7 (*SD* 3.4) *v.* 26.2 (*SD* 3.2) μg/l (two-tailed *t* test, *P*=0.054).

A total of 114 girls completed an HAQ at T_2. Mean TSPA was 49 (SD 36)min/d. The difference in TSPA (T_2 TSPA - T_1 TSPA) was 7 (*SD* 36) min/d. There was no significant effect of treatment (Fe or placebo), nor of treatment by Fe status, on difference in TSPA (MANOVA, *P* = 0.420).

Table 2. *Difference in time spent in physical activities by treatment and Fe status*

		Fe (*n* 59)			Placebo (*n* 55)		
		IDA (n 24)	*ID (n 15)*	*IR (n 20)*	*IDA(n 16)*	*ID(n 14)*	*IR(n25)*
Difference in TSPA (min/d)	Mean	6	12	3	-2	8	13
	SD	28	36	25	33	14	37

Lower mean Hb and SF levels were found for girls classified in the low activity group, but this was not significant. The significant increase in TSPA between March and June, probably due to seasonality, was not found for the IDA girls who received the placebo. Poor iron status may play a part in the decline in physical activity levels in girls over their secondary school years (Sallis, 1993).

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Iron status in a group of sportsmen and women belonging to the National Institute of Physical Education (Spain). By ANA M. REQUEJO[1], ROSA M. ORTEGA[1], SONSOLES YSART Y ALVAREZ DE TOLEDO[1],ELENA QUINTAS[1], ANA MARÍA LÓPEZ-SOBALER[1], PEDRO ANDRÉS[1], MARÍA JESÚS GASPAR[2] and GUADALUPE GARRIDO[3]. *[1]-Departamento de Nutrición, Facultad de Farmacia, Universidad Complutense, 28040-Madrid (Spain), [2]-Departamento de Fisiología del Ejercicio, Instituto Nacional de Educación Física (INEF), Madrid (Spain), [3]-Servicio de Análisis Clínicos, Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Madrid (Spain).*

Iron deficiency is a frequent problem amongst those who practice sport, especially amongst women (Haymes *et al.* 1989; Weight *et al.* 1992).

An investigation was made into the Fe status of a group of forty-five young sportsmen and women (thirty-two men and thirteen 13 women, BMI 23.3±2.8 kg/m^2) aged 19-26 who practiced an average of 4h sport/d as part of their studies. The sports practiced by subjects included basketball, swimming, volleyball, hockey, athletics, fencing, soccer, gymnastics, aerobics and cycling. About 30% subjects also spent their leisure time practising sports.

Food intake was monitored using a 5-day weighed food record which included a Sunday. All foods consumed were transformed into their energy and nutrient values using Food Spanish Composition Tables (Departamento de Nutrición, 1994a) and intakes compared to those recommended for Spanish population (Departamento de Nutrición, 1994b).

Following a 12 h fast, blood samples were taken for measurement of erythrocyte count, haemoglobin level, packed cell volume, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), MCH concentration (MCHC) and serum Fe concentration. The results are given in the Table.

	Men (*n* 32)		Women (*n* 13)	
	Mean	SD	Mean	SD
Fe intake (mg/d)	19.9	6.0	15.1*	4.4
Contribution to RI (%)	180.4	52.9	84.1*	27.3
Intekes less than RI (%)	3.1		61.5	
Erythrocytes ($10^6/\mu$l)	5.13	0.3	4.52*	0.27
Haemoglobin (g/l)	155	8	136*	6
Packed cell volume (%)	44.4	2.9	39.8*	1.6
MCV (μ^3)	84.7	11.9	88.2	4.1
MCH (pg)	30.2	2.2	30.1	1.4
MCHC (%)	34.9	1.9	34.1	1.0
Serum iron (μg/l)	1191	912	1018	311

RI, recommended intake.
*Mean values were significantly different from those for men, $P<0.05$.

Women subjects showed significantly lower Fe intakes than did men subjects. Women subjects also showed significantly lower numbers of erythrocytes, and lower haemoglobin and packed cell volume levels than did their male counterparts. However this could not be clinically significant. Nonetheless, intake of Fe-rich foods should be monitored and, if necessary, increased.

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Lack of effect of long-term antioxidant supplementation on incidence of upper-respiratory-tract infections in athletes. By MIKAEL FOGELHOLM [1], TOMMI VASANKARI [2], PATRIK BORG [3], RIIKKA KATILA [4] and TIMO TUOMI [4], [1] *The UKK Institute for Health Promotion Research, Tampere, Finland, [2] Department of Physiology, University of Turku, Finland, [3] Department of Applied Chemistry and Microbiology, Division of Nutrition, University of Helsinki, Finland, [4] Department of Exercise Biology, University of Jyväskylä, Finland*

Antioxidant vitamins are needed for optimal immunological function. Heavy physical strain has been proposed to increase the use of antioxidants. If antioxidant requirements are not covered by dietary intake, immune function might be hampered. Some years ago, Peters *et al.* (1993) showed that vitamin C supplementation (600 mg/d) reduces the incidence of upper-respiratory-tract (URT) infections in endurance athletes during 2 weeks after an ultra-marathon race. In the present study, we investigated the hypothesis that long-term (8 months) oral antioxidant supplementation reduces the incidence of URT infections in athletes during their normal training regimen.

The study design was randomized and placebo controlled. Seventy-five athletes (ten females, sixty-five males), aged 15 to 30 years (mean: 19 years), completed the study. The participants were national standard adult or young ball-games athletes. Forty-nine took a daily oral supplement containing 1000 mg vitamin C (ascorbic acid), 294 mg vitamin E (α-tocopherol) and 90 mg ubiquinone (coenzyme Q_{10}) (ANT group). The remaining thirty-six athletes took a placebo (PLA group). The subjects kept a daily record of their training volume, URT infection symptoms and effects of infections on training. The mean number of recording days was 216 (sd: 27). All subjects with les s than 100 recording days were excluded from the analyses. During the study, dietary antioxidant intakes were assessed from a 4 d food record. The main outcome variables were the proportion of days with URT infection symptoms (fever, runny nose, sneezing, sore throat, coughing), and the proportion of days with training affected by URT infection. The group differences for URT infection symptoms were tested by the Mann-Whitney test; all other variables were statistically evaluated by Student's *t* test.

The median (range in parentheses) incidence (as % of recording days) of days with URT infection symptoms was not significantly different between the groups: fever: ANT 0.4 (0 - 6.1) %, PLA 0.5 (0.0 - 4.8) % (P=0.20); runny nose: ANT 15.4 (0.0 - 46.0) %, PLA 7.0 (0.0 - 23.9) % (P=0.08); sneezing: ANT 2.3 (0.0 - 21.9) %, PLA 1.6 (0.0 - 15.2) % (P=0.29); coughing: ANT 2.1 (0.0 - 20.6) %, PLA 1.3 (0.0 - 30.6) % (P=0.68), sore throat: ANT 4.4 (0.0 - 22.0) %, PLA 3.6 (0.0 - 22.7) % (P=0.23). Like the URT infection symptoms, the dietary intake of vitamin C (ANT: mean 172 (sd 98) mg/d, PLA 138 (sd 63) mg/d, P=0.13) or vitamin E (ANT 13.0 (sd 4.5) mg/d, PLA 11.6 (sd 3.6) mg/d, P=0.20), or the amount of sports training (ANT 94 (sd 24) min/d, PLA 89 (sd 26) min/d, P=0.30) were not different between the two groups.

Neither the background variables (age, sex, training, dietary intake) nor the main outcome variables (URT infection symptoms) were significantly different between the treatment and placebo groups. The findings do not support the hypothesis that long-term oral antioxidant supplementation reduces the incidence of URT infections in national standard, ball-games athletes. However, the results might be different in athletes with higher incidence of URT infections, with lower dietary intake of antioxidant vitamins, or with heavier physical strain.

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Nutrition, physical activity and immunocompetence in an aged Spanish group. By P. VARELA, P. GARCÍA-GARCÍA, R. M. ORTEGA, E. QUINTAS and A. M. REQUEJO, *Centro Mixto Instituto de Nutrición y Bromatología (CSIC-UCM), Facultad de Farmacia, Ciudad Universitaria, 28040 Madrid, Spain*

It is well known that ageing is linked to nutritional status impairment and to reduced immune capacity. Moreover the elderly usually have a sedentary life style that may contribute to deterioration of the situation. On the other hand, the effects of physical activity on immunity are controversial, and in the elderly, there is scant information about the relationship between nutritional status, physical activity and immunocompetence. Therefore, the aim of the present study was to investigate the relationship between these aspects in an aged non-institutionalized group belonging to Comunidad de Madrid.

A total of 119 elderly people (men and women) were studied and divided into two groups: (1) sedentary (physical activity under 2 h/d) and (2) active (physical activity (mainly walking) over 2 h/d). Each group was divided into two subgroups depending on BMI: (A) normal weight (BMI: 20-28 kg/m^2) and (B) overweight (BMI: 28-30 kg/m^2). Intakes of energy, Zn and vitamins A and E and plasma levels of these vitamins were measured. Similarly, total leucocyte and lymphocyte counts (Coulter counter), lymphocyte subsets CD3, CD4, CD8, CD19 and CD57 (flow cytometry) and CD4:CD8 and CD3:CD19 ratios were assessed. Immunoglobulins IgA, IgG and IgM as well as C3 and C4 complement factors were also determined by simple radial immunodiffusion.

	SEDENTARY (*n* 61)				ACTIVE (*n* 58)			
	Normal weight (*n* 29)		Overweight (*n* 32)		Normal weight (*n* 26)		Overweight (*n* 32)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Energy (kJ)	6437.9	1609.4	7806	1231	7575.5*	1645.8	6666.3†	1329.1
Zn (mg)	7.8	2.8	9.0	1.9	8.8	2.6	8.4	2.7
Vitamins E (mg)	4.2	1.5	5.2	2.4	5.2	2.4	5.05	1.84
Leucocytes (cells/mm^3)	5800	1500	6700†	1700	6400	1600	5600*†	1200
Lymphocytes (cells/mm^3)	1931	430	2292	663	1943	446	2036	514
CD3 (cells/mm^3)	1239	386	1398	360	1217	22	1334	390
CD4 (cells/mm^3)	739	207	830	239	675	181	798†	191
CD19 (cells/mm^3)	151	58	203†	73	138	52	182†	68
CD4:CD8	2.2	0.7	2.2	0.8	1.9	0.5	2.2†	0.6
IgA (mg/dL)	194.4	11.8	241	117.7	273.4*	86.4	183.6†	56.9

* Significantly different from sedentary: *$P<0.05$; † Significantly different from normal weight: $^{\dagger}P<0.05$ (Student's t test).

Energy, Zn and vitamin E intakes in both groups were lower than recommended, whereas their immunological variables showed normal mean values. Energy intake and plasma IgA level were higher in the active aged and were BMI-dependent. Active subjects with higher BMI present a lower leucocyte count than the sedentary subjects with equal BMI; moreover, the BMI effect was different depending on the activity. Lymphocyte and lymphocyte subsets CD3, CD4 and CD19 were influencied by BMI, independently of physical activity. It might be concluded that the nutritional status in the elderly, showed a impairment situation. Specific immunity was directly influencied by BMI. Physical activity effects were only observed in energy intake and IgA plasma level.

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The effect of acute dietary creatine supplementation upon indices of renal, hepatic and haematological function in human subjects By DEAN A. SEWELL, TRISTAN M. ROBINSON, ANNA CASEY and PAUL L. GREENHAFF, *Department of Physiology and Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, NG7 2UH.*

Current interests in creatine (Cr) as a nutritional supplement include its use as an ergogenic aid (Casey *et al.*, 1996) and its potential therapeutic role in conditions of cardiovascular insufficiency (Gordon *et al.* 1995). The improvement in exercise performance brought about by Cr ingestion has been positively associated with the extent of muscle Cr accumulation (Casey *et al.* 1996), which can be markedly increased when Cr is ingested with carbohydrate (CHO; Green *et al.* 1996). Despite its apparent widespread use amongst athletes, there is little published information concerning the effects of Cr supplementation on indices of renal, hepatic and haematological function. The aim of the present study, therefore, was to obtain information relating to such indices in young, healthy, adult subjects, before and after 5 d of Cr, Cr and CHO or placebo supplementation.

Twenty-six healthy, young, adult subjects took part in the study which had ethical committee approval. Subjects were divided into four experimental groups who ingested one of the following supplements, four times daily for 5 d: 5 g Cr monohydrate plus 1 g glucose (Cr, *n* 6); 6 g glucose (Glucose, *n* 6); 5 g Cr followed by 500 ml CHO drink (185 g/l simple sugars, Cr + CHO, *n* 7) and 500 ml CHO drink (CHO, *n* 7). Venous blood samples for analyses (see Table) were taken before the start of the supplementation period, on the day following supplementation (Cr + CHO and CHO groups) and 6 weeks following supplementation (Cr and Glucose groups).

Measurements were at all times within the normal range for each index. Changes from pre- to post-supplementation within group are shown in the Table.

	Cr + CHO		CHO		Cr		Glucose	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Sodium (mmol/l)	1	0	1	1	2	0	2	1
Potassium (mmol/l)	-0.1	0.1	0.3	0.1	-0.1	0.1	0.0	0.1
Urea (mmol/l)	-0.6	0.4	-1.4	0.2	1.2***	0.3	-0.4	0.2
Creatinine (μmol/l)	24*	10	-3	2	2	2	3	2
Gamma glutamyl transferase (U/l)	-1	1	0	1	1	2	0	1
Alkaline phosphatase (U/l)	5	4	0	2	-1	2	-3	3
Alanine aminotransferase (U/l)	-1	1	3	2	3	4	2	1
Albumin (g/l)	-1	1	0	0	-2	1	-2	0
Bilirubin (μmol/l)	-3	1	-4	1	-3	1	-2	1
Haemoglobin (g/l)	-0.4	2.1	-1.6	1.3	-1.7	2.7	-4.5	2.3
Leucocytes (x 10^9/l)	0.4	0.4	1.3	0.6	0.4	0.2	0.0	0.6
Platelets (x 10^9/l)	15	12	10	10	-27	14	-9	11

Mean values were significantly different from those for the corresponding placebo group; *$P < 0.05$, ***$P < 0.001$ Student's *t*-test).

The increase in serum creatinine concentration which was apparent on the day following Cr + CHO supplementation but not 6 weeks post Cr supplementation reflects an increased rate of muscle Cr degradation (Hultman *et al.* 1996). Presumably the small increase in serum urea concentration 6 weeks following Cr supplementation occurred as a consequence of an increase in hepatic arginine availability, which resulted from a reduction in the rate of endogenous Cr synthesis. We conclude that acute Cr supplementation (i.e. 4 x 5 g/d for 5 d) poses no obvious health risk to healthy young adults.

This work was supported by the Defence Research Agency and Experimental and Applied Sciences

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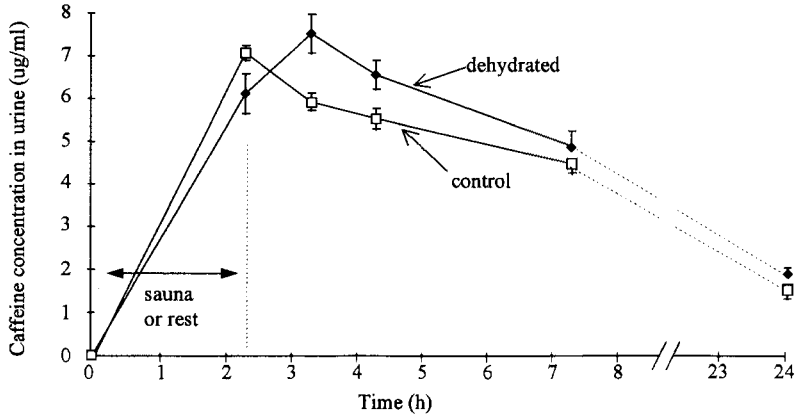
Urinary caffeine concentration after coffee consumption and heat dehydration. By A. CHAMBAZ and J. DECOMBAZ, *Nestlé Research Centre, Nestec Ltd, Vers-chez-les-Blanc, CH-1000 Lausanne 26, Switzerland*

Caffeine-containing beverages are frequently consumed by sportsmen in order to stimulate performance during competition. The intentional use of caffeine for this purpose is presently prohibited and the International Olympic Committee has set an upper limit of 12 mg caffeine/l urine to differentiate between use and abuse. It is argued that in some people this limit can be reached with only two to three cups of coffee. Recently it has been proposed that the limit should be decreased, because enhancement of physical performance has been demonstrated (Graham & Spriet, 1991) at doses of caffeine that produce urinary concentrations lower than the legal threshold.

Many factors have been implicated in the large variability in urinary caffeine excretion after coffee consumption, one of which is the effect of dehydration due to physical exercise. However, variations in the hydration state of the body (a situation most common in athletics) have not been adequately studied.

The objectives of the present study were (1) to determine peak urinary caffeine concentrations following ingestion of a "very strong" coffee solution (6.4 mg caffeine/kg lean body mass); (2) to test the effect of passive dehydration (3% lean mass) on peak values and their variability.

The consumption of coffee (one bolus, 300 ml, time 0) was immediately followed by a sauna (80°, bouts of 10-20 min, total duration 2 h 30 min, *n* 10 non-athletic males, 66-89 kg, 26-43 y). Mean weight loss was 1.7 kg. In the control situation, the same coffee was given but the volunteers remained resting at ambient temperature. Caffeine was measured by HPLC.



Results showed that (1) mean peak concentration was 7.6 mg/ml in the dehydrated condition (control 7.1 μg/ml, not significantly different); (2) acute dehydration resulted in slower kinetics of caffeine elimination: concentration was reduced during the sauna, but then peaked in the following hour. The variability at peak was higher when dehydrated (range 6.1-10.2 μg/ml, CV 17%) than in the normal state (6.1-8.0 μg/ml, CV 8%).

In contrast with other metabolites, a drastic reduction in urine flow does not concentrate caffeine in the urine. Therefore, there is no indication that mean peak caffeine concentration in urine will be higher in the dehydrated state than in the normal state. However, in spite of a large elimination in the sweat (15 (SE 1) mg, 4.3% of dose) and because of greater variability, the risk that a single individual reaches a high value is greater with heat dehydration.

Graham, T.E. & Spriet, L.L. (1991). *Journal of Applied Physiology* **6**, 2292-2298.

Changes in measures of fatigue in normal subjects following meals with a high protein or fat content. By A. CUNLIFFE[1], I. LAMPLOUGH[2], R. OBRA[2], O. OBEID[1] and J. POWELL-TUCK[1], [1]*Department of Human Nutrition, St Bartholomew's and the Royal London School of Medicine and Dentistry, London E1 2AD,* [2]*Department of Nutrition and Dietetics, King's College London, London W8 7AH*

We have previously investigated the diurnal changes in central and peripheral components of fatigue in healthy volunteers (Cunliffe *et al.* 1997[a]). The present study employed the same techniques (visual analogue scale (VAS), flicker fusion frequency (FFF), wrist ergometry (WE) and grip strength (GS)) to assess the acute effect of diet with different macronutrient content on fatigue state. Healthy volunteers (4M, 3F) with a mean age of 28.5 years and mean BMI of 23.3 kg/m^2 were recruited into the study. After an overnight fast subjects were tested before and then at hourly intervals for 4 h after drinking one of two isoenergetic (1672 kJ) liquid meals, (with a minimum of 1 week between test diets): (1) High protein (HP) where 50%, 25% and 25% of energy was supplied as protein, carbohydrate, and fat respectively; (2) High fat (HF) with 15%, 20% and 65% energy as protein carbohydrate and fat respectively. The meals were of similar volume and made up of maltodextrin (Maxijul), a long-chain triacylglycerol emulsion (Calogen) (gifts from SHS) and whey protein concentrate (Promod, Abbot). The Table gives results for mean percentage changes for each test at hourly intervals.

Test	Diet	Time (h) 1 Mean	1 SE	2 Mean	2 SE	3 Mean	3 SE	4 Mean	4 SE
VAS	HF	3.5	15.2	309	236	145.6	43.6	342	198
	HP	-0.23	9.38	12.87	7.65	46.8	25.5	94.9	45.9
FFF	HF	-0.62	0.69	-1.91	1.14	-2.06	1.19	-3.79	1.97
	HP	1.04	1.32	2.05	0.75	0.74	1.01	0.91	1.31
GS	HF	-1.27	1.97	2.22	1.76	0.40	1.75	-1.26	2.96
	HP	1.11	2.38	3.30	4.02	1.38	5.75	0.07	4.69
WE	HF	8.4	10.5	6.1	14.8	13.8	23.0	10.6	13.6
	HP	-7.15	4.51	-8.72	7.50	-16.4	6.86	-6.71	5.34

Subjective fatigue as measured by the VAS was significantly increased by the high fat meal compared with the high protein meal (ANOVA; $P<0.05$). This was associated with an increase in objective central fatigue, as indicated by the decrease in FFF scores of the high fat meal compared with that of the high protein meal (ANOVA; $P<0.001$). With respect to peripheral voluntary function there was no significant difference between the groups in terms of brief maximal voluntary effort as reflected by GS results. However, endurance work, as indicated by the work output during WE, of the high protein group was found to be lower than that of the high fat group (ANOVA; $P<0.02$). It is interesting to note that the changes in central fatigue, as reflected by the subjective (VAS) and objective (FFF) assessment, were not accompanied by similar changes in the voluntary peripheral tests (GS, WE). The increase in central fatigue following a high fat meal was associated with an improvement in endurance, although that of brief maximal voluntary contractions was not affected. This divergence in effect is similar to that noted in a previous study, where a pure fat meal led to smaller decrements in work done during WE than did a control meal (Cunliffe *et al.* 1997[b]). Thus, the different components of fatigue seem to be controlled by different mechanisms.

Cunliffe, A., Obeid, O.A,. Powell Tuck, J. (1997[a]) *Proceedings of the Nutrition Society* (In the Press).
Cunliffe, A., Obeid, O.A,. Powell Tuck, J., Obra, R. & Lamplough, I. (1997[b]) *Proceedings of the Nutrition Society* (In the Press).

The response of energy intake and macronutrient balance to manipulation of physical activity levels in lean men. By M.B. GILSENAN, P.R. MURGATROYD, F.E. LEAHY, G.R. GOLDBERG and A.M. PRENTICE, *MRC Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH*

The prevalence of obesity has virtually doubled between 1980 and 1991 during a period when the average household's food intake has fallen (Prentice & Jebb, 1995). This implies an even more profound fall in energy expended in physical activity. Laboratory-derived evidence supporting this proposal is as yet limited. The aim of the present study was to examine the effects of physical activity on energy and macronutrient balance and to investigate whether subjects modify their habitual energy intake in response to an imposed level of physical activity.

Seven healthy, habitually sedentary, weight-stable male subjects (age 37 (SD 6) years; weight 69.06 (SD 7.43) kg; BMI 21.14 (SD 1.88) kg/m^2) were studied twice by whole-body indirect calorimetry. On each occasion subjects spent an equilibration day in our metabolic suite during which energy intake (EI) was prescribed as 1.35 x BMR and physical activity was minimized. After their evening meal they entered a whole-body calorimeter where they remained for 61 h. Within the calorimeter a fixed activity protocol was followed. This included either three 40 min periods of cycle ergometer exercise (75 W) (EX) or no exercise (NoEX) on each day. The order of the treatments was randomized. Subjects were provided with three large meals (35:52:13 % energy from fat:carbohydrate:protein) each day from which they were asked to eat *ad libitum*. Food consumption was covertly recorded. The study was approved by the Dunn Nutrition Unit Ethical Committee.

Protein oxidation was calculated from urinary N excretion. Fat and carbohydrate (CHO) oxidation rates were calculated from non-protein O_2 consumption and CO_2 production. Energy expenditure (EE) was calculated as the sum of the macronutrient oxidation rates. Results from the EX and NoEX protocols were compared using paired *t* tests.

	Exercise		No Exercise		Difference
MJ	Mean	SD	Mean	SD	
Energy intake	11.91	3.24	11.13	3.25	-0.78
Energy expenditure	11.91	0.62	8.90	0.66	-3.01***
Energy balance	0.01	2.97	2.24	3.36	2.23***
Fat oxidation	4.48	1.38	3.19	1.23	-1.29***
Fat balance	-0.29	2.44	0.73	2.17	1.02**
CHO oxidation	6.10	1.40	4.41	0.95	-1.69***
CHO balance	0.03	0.85	1.30†	1.28	1.27***

Significant difference between Exercise and No Exercise, ** P<0.002;*** P<0.001.

Physical activity levels (PAL; 24 h EE/BMR) in the calorimeter were: EX 1.65 (SD 0.09) and NoEX 1.24 (SD 0.08). Despite the difference in the level of activity imposed in this study there were no significant differences in EI between treatments over the 2-d period of the manipulation. Subjects' *ad libitum* EI on the EX protocol closely matched their EE. Doubly-labelled water data (Black,1996) indicate that PAL for this group of habitually sedentary subjects would be close to 1.6, suggesting that subjects may have elected to eat at their habitual free-living level. Consequently, subjects were in substantial positive energy balance on the NoEX protocol. This is reflected in the macronutrient balances. Both fat and CHO were close to balance on the EX protocol while both were substantially positive on the NoEX protocol.

We conclude that on both treatments subjects ate to a level conditioned by their free-living lifestyle, and did not significantly modify this to compensate for the reduced level of energy expenditure on the NoEX protocol. The findings of this short study are consistent with the suppositions that exercise may facilitate the maintenance of energy balance and that a reduction in habitual activity may lead to weight gain through inadequate compensatory reductions in energy intake.

Black, A.E. (1996). *Nutrition Reviews* **54**, 170-174.
Prentice, A.M. & Jebb, S.A. (1995). *British Medical Journal* **311**, 437-439.

The effect of exercise on subsequent feeding behaviour. By J.H. LAVIN, N.W. READ, J. NWAJIAKU, P.R. STAFFORD and S.J. FRENCH, *Centre for Human Nutrition, University of Sheffield, Northern General Hospital, Sheffield S5 7AU*

The role of carbohydrate (CHO) utilization in the regulation of feeding behaviour was investigated in two studies. It has been proposed that high-intensity exercise predominantly utilizes carbohydrate as an energy source while low-intensity exercise mainly utilizes fat (Coyle, 1995). Therefore exercise was prescribed at 30% and 70% VO_2max on separate days; in the first study, subjects also underwent a no-exercise control session. In both studies, the length of the exercise session was manipulated to provide an equivalent total work-load and exercise was initiated such that both sessions were completed at the same time on the different test days. Seven and nine normal weight, male volunteers (22-30 y) took part in studies 1 and 2 respectively. In study 1, subjects were presented with a buffet-style selection of lunch food items 1 h after the end of the exercise sessions and *ad-libitum* food intake was covertly recorded.

	Control		30% VO_2max		70% VO_2max	
	Mean	SE	Mean	SE	Mean	SE
Energy (MJ)	3.02	0.47	4.34*	0.41	4.15*	0.29
CHO (g {% of energy})	76.0 {44.5}	10.3 {3.3}	102.9* {42.3}	7.9 {3.1}	95.8 {48.3}	11.0 {4.8}
Fat (g {% of energy})	29.0 {39.0}	5.7 {3.2}	42.6* {43.6}	4.9 {2.4}	35.6 {39.9}	4.6 {2.8}
Protein (g {% of energy})	25.2 {14.8}	5.8 {1.3}	34.4* {15.0}	4.5 {0.9}	29.8 {14.7}	4.4 {1.1}

* Significantly different from control, $P<0.05$

Energy intake was higher following exercise sessions compared with control but that there was no difference in energy intake between the two exercise sessions. Subjects ate significantly more of all of the macronutrients in the 30% VO_2max condition compared with control, but no differences were seen when these were expressed as a percentage of total intake. Furthermore there were no differences in macronutrient intake between the two exercise conditions.

Study 2 was designed to investigate more closely the relationship between exercise intensity and feeding, and to relate this to changes in blood glucose levels. Subjects were able to select from a variety of foods available over 6 h following the end of the exercise session. Food consumed was measured every 30 min during this period and finger-prick blood samples were taken for determination of blood glucose concentration throughout the experiment.

	30% VO_2max		70% VO_2max	
	Mean	SE	Mean	SE
Energy (MJ)	8.57	2.1	8.55	1.51
CHO (g {% of energy})	254.7 {51.8}	52.3 {3.8}	284.7 {48.3}	45.9 {3.2}
Fat (g {% of energy})	92.3 {35.7}	33.2 {3.9}	90.4 {38.6}	21.2 {3.7}
Protein (g {% of energy})	68.4 {12.5}	16.6 {1.3}	64.8 {13.2}	8.8 {0.8}

In this study there was no significant difference in total energy or macronutrient intake between the two exercise conditions. Upon closer inspection, there were no differences in cumulative intake from the analysis of consecutive 30 min intake measurements ($F = 0.51$; $P = 0.895$). These results confirm the finding of study 1, showing that at these intensities and periods of exercise, there are no differences in energy or macronutrient intake. However, the exercise loads were not sufficient to cause a significant difference in blood glucose. Hence it is possible that more intense exercise, or exercise for a longer period, may be required to elicit changes in glucose utilization and feeding behaviour.

This work was supported, in part, by the Sugar Bureau.

Coyle, E.F. (1995). *American Journal of Clinical Nutrition* **61** Suppl., 968S-979S.

Comparison of the short-term effects of exercise on food hedonics and food consumption in dietary restrained and unrestrained females. By ANNE LLUCH[1,2], NEIL A. KING[1], ZARA J. LIPSEY[1] and JOHN E. BLUNDELL[1], *[1]BioPsychology Group, Department of Psychology, University of Leeds, Leeds LS2 9JT and [2]INSERM U308, 38 rue Lionnois, 54000 Nancy, France*

In theory, exercise should provide an effective method of weight control since it induces a negative energy balance by increasing daily energy expenditure. Nevertheless, the usefulness of exercise in the prevention and treatment of obesity is still controversial. Behavioural characteristics of subjects including their appetite control (i.e. dietary restraint, referring to the extent that individuals exert conscious control over their eating and body weight) and exercise behaviour are likely to affect food compensation following exercise.

To explore this issue, we conducted two studies in normal-weight, regularly exercising females, defined as unrestrained (U) and restrained (R) eaters by the Three Factor Eating Questionnaire (Stunkard & Messick, 1985). In a 2 x 2 repeated measures design, the effects of a bout of high-intensity exercise (cycling 50 min, 70% VO_2 max) and exposure to foods varying in macronutrient composition (high-fat (HF) *V.* low-fat (LF)) on food consumption were assessed in thirteen U and twelve R women. Energy intake (EI) of the lunch was calculated by weighing the food before and after consumption. Post-meal hedonic ratings were completed after lunch. Comparisons between U and R subjects were performed, using repeated measures ANOVA, independent *t* tests and Pearson correlation analyses.

There was a main effect of lunch type (HF *V.* LF) on EI following exercise and rest ($F[1,23]=111.2$; $P<0.001$) in both R and U females : EI increased during both HF conditions compared with the LF. There was a positive relationship between restraint scores and EI on the rest (r 0.54; $P<0.01$) but not on the exercise conditions (r 0.09; NS). Exercise significantly increased hedonic ratings of pleasantness, tastiness and palatability of the foods served at lunch ($F[1,23]=19.6$; $P<0.001$; $F[1,23]=11.5$; $P<0.01$; $F[1,23]=8.9$; $P<0.01$ respectively). For the pleasantness ratings, nutrient x group ($F[1,23]=5.6$; $P<0.05$) and nutrient x group x exercise ($F[1,23]=4.1$; $P=0.05$) interactions were found. Exercise enhanced the pleasantness of foods in the HF and LF conditions in U and R subjects respectively.

		Rest-LF		Rest-HF		Exercise-LF		Exercise-HF	
		U	R	U	R	U	R	U	R
EI (MJ)	Mean	2.72	3.23*	4.48	5.12	2.99	2.93	4.88	5.17
	SD	0.55	0.55	0.78	0.99	0.50	0.58	0.99	1.14
Pleasantness (mm)	Mean	78.6	70.9	70.8	84.9*	79.9	82.5	81.2	88.2
	SD	15.7	12.9	15.9	14.6	12.5	11.1	14.6	13.3

Significant difference between R and U, * $P<0.05$

These results show that female restrained and unrestrained eaters respond similarly to exposure to high-fat foods and experience "passive overconsumption". Therefore, there is the tendency for high-fat food selection to overcome energy deficit of physical activity. Exercise raised the perceived pleasantness of foods in restrained and unrestrained eaters, but did not induce a drive to eat in spite of the energy deficit. These results suggest that in restrained women (dieters), exercise could be used advantageously to control appetite.

Supported by the Biotechnology and Biological Sciences Research Council (F02501) and Institut National de la Santé Et de la Recherche Médicale.

Stunkard, A. J. & Messick, S. (1985). The Three-Factor Eating Questionnaire to measure dietary restraint, disinhibition and hunger. *Journal of Psychosomatic Research* **29**,71-83.

Food choice at a buffet meal 1 h after physical exercise. By J. DECOMBAZ, C. BOVIER and G. REUTELER, *Nestlé Research Centre, Nestec Ltd, Vers-chez-les-Blanc, CH-1000 Lausanne 26, Switzerland*

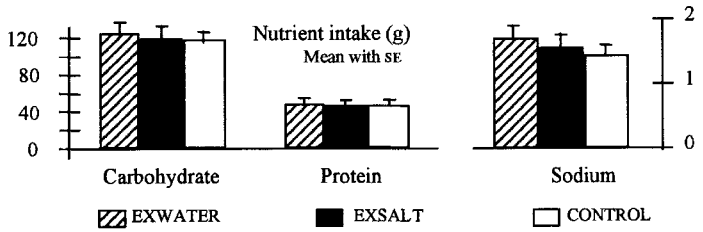
Little is known about the short term ingestive regulation of nutrient deficits. Physical exercise induces rapid and sizeable deficits in carbohydrate (CHO) and Na. Replenishment of these nutrients is critical for the recovery of performance capacity and, normally, balance is re-established within 1 d as a result of food ingestion. A specific appetite for salt after Na depletion has recently been reported in man (Takamata *et al.* 1994). No specific appetite for CHO has been demonstrated, but endurance athletes have occasionally been reported to consume more CHO than sedentary people. In addition, Verger *et al.* (1994) have suggested an increased preference for protein following exercise.

The present study investigated the spontaneous intake of nutrients in the first meal following exercise. The hypotheses under test were (a) that exercise-induced deficits in CHO and Na are followed by spontaneous and subconscious increases in consumption of these nutrients at the next meal; (b) that the specific appetite for protein reported by Verger *et al.*(1994) is in fact a response to a need for salt which is high in many protein foods.

Fifteen subjects (8F, 7M) participated in three trials : (1) EXWATER : exercise + water + meal; (2) EXSALT : exercise + salted water (NaCl 4.5 g/l) + meal; and (3) CONTROL: rest + meal. The first two trials (crossover) included 2 h of supervised exercise (heart rate 120-160 beats/min, sweat loss 1.1 kg). The volume of sweat lost was entirely made up immediately after the exercise with the indicated beverage. After 1 h, a buffet lunch was offered *ad libitum*. In the third trial, the buffet was served with no prior exercise.

The buffet consisted of fifty-one familiar food items. In order to prevent a bias in nutrient selection, salty, neutral and sweet tasting items were offered in all combinations across protein-rich and carbohydrate-rich dishes. Each dish was attributed a score for the intensity of sweet and salty tastes by an independent panel of tasters. Nutrient intake was calculated from food tables and, for Na, by food analysis.

Water retention was improved in EXSALT compared with EXWATER (urine production until the end of the meal: 192 *v.* 410 g, $P<0.001$).



No significant difference was found between the three treatments (EXWATER, EXSALT, CONTROL) for, respectively, energy (4.5, 4.3, 4.2 MJ), CHO (126, 120 and 119 g), protein (49, 47 and 47 g), total water (784, 861 and 750 g) and Na (1.71, 1.58 and 1.45 g) intakes, as well as for the amount of "sweetness" and "saltiness" consumed, as quantified by the sum of products (weight eaten x taste score) for each food.

In conclusion, sizeable disturbances in CHO and Na balance induced by exercise do not appear to be sensed. Correction mechanisms take longer than one meal, or deficits need to be larger than in the present study. The results do not support the concept that, during the first meal after physical exercise, there is a specific appetite for the depleted nutrients or for proteins.

Takamata, A., Mack, G.W., Gillen, C.M. & Nadel, E.R. (1994). *American Journal of Physiology* **266**, R1493-R1502.
Verger, P., Lanteaume, M.T. & Louis-Sylvestre, J. (1994). *Appetite* **22**, 159-164.

The influence of place of residence on the intake of energy and nutrients in a group of Spanish soccer players of lower division teams. By ROSA M. ORTEGA[1], ANA M REQUEJO[1], MARCELA GONZÁLEZ-GROSS[1], ANA MARÍA LÓPEZ-SOBALER[1], ELENA QUINTAS[1], PEDRO ANDRÉS[1], and MIGUEL ANGEL HERRADOR[2]. [1] *Departamento de Nutrición, Facultad de Farmacia, Universidad Complutense, 28040-Madrid, Spain.* [2] *Servicios Médicos del Real Madrid. Spain.*

The aim of the present investigation was to compare the differences in the energy and nutrient intakes of soccer players with respect to whether they lived in their own homes (H) or in hostels (HL). A 7-day weighed food intake record was used to measure the intake of energy and nutrients in forty-six soccer players aged between 17 and 21 years (BMI 22.8±1.4 kg/m^2) . This time period was chosen in order to follow a complete training cycle and at least one competitive match. Foods consumed were transformed into energy and nutrient values (Departamento de Nutrición, 1994a) and intakes compared to those recommended (Departamento de Nutrición, 1994b).

	Players who lived in their own homes (*n* 32)			Players who lived in hostels (*n* 14)		
Daily intake	Mean	SD	(%)	Mean	SD	(%)
Energy (MJ)	14.7	3.1		13.9	3.7	
Carbohydrates (g)	396.3	106.0		351.5	106.8	
Proteins (g)	150.5	31.0	(0)	145.1	35.8	(0)
Lipids (g)	157.1	39.9		157.4	41.5	
Fibre (g)	24.3	6.9	(28.1)	23.8	8.0	(35.7)
Calcium (mg)	1293.6	508.4	(9.4)	1052.4	462.0	(50)
Iron (mg)	19.2	3.7	(6.3)	18.8	4.5	(21.4)
Iodine (μg)	391.4	192.0	(0)	318.7	149.7	(0)
Magnesium (mg)	378.0	79.9	(65.6)	360.2	108.4	(71.4)
Zinc (mg)	17.8	4.2	(28.1)	17.7	4.5	(28.5)
Thiamine (mg)	1.9	0.4	(3.1)	1.9	0.5	(7.1)
Riboflavin (mg)	2.5	0.6	(18.8)	2.3	0.7	(28.6)
Niacin (mg)	54.2	10.8	(0)	50.1	12.2*	(0)
Pyridoxine (mg)	2.6	0.4	(12.5)	2.3	0.5*	(35.7)
Folic acid (μg)	222.0	46.1	(21.9)	172.8	41.0	(64.3)
Vitamin B_{12} (μg)	13.5	13.7	(0)	9.3	5.0*	(0)
Vitamin C (mg)	153.3	69.2	(0)	93.3	25.8*	(14.3)
Vitamin A (μg)	1758.7	1680.4	(12.5)	889.4	231.5	(28.6)
Vitamin D (μg)	5.7	5.8	(28.1)	4.8	3.0	(28.6)
Vitamin E (mg)	7.6	2.2	(90.6)	7.4	4.6	(92.9)

*Mean values were significantly different from those for players who lived in their own homes, $P<0.05$
The percentage of players with an intake lower than that recommended is shown in parentheses.

The results of the present study show a more satisfactory nutritional status for players who lived in their own homes. These subjects showed significantly greater intakes of pyridoxine, folic acid, vitamin C and vitamin A , than those who lived in hostels. The percentage of intakes lower than those recommended was higher amongst players living in hostels.

A high percentage of players had intakes of Mg, Zn riboflavin, pyridoxine, folic acid and vitamin E that were lower than those recommended. While the energy profiles of the diets of both groups of players were imbalanced (excess intake of energy from fats and proteins and insufficient from carbohydrates), those of players who lived in hostels were worse. 8.3% of H subjects and 100% of HL subjects showed energy intakes from lipids at above 35% of total energy intake; 59.4% of H subjects and 92.9% of HL subjects showed energy intakes from carbohydrates at less than 45% of total intake.

Given that dietary imbalances can influence the health and physical performance of soccer players, it would seem advisable to monitor their diets, especially of those who live away from their families.

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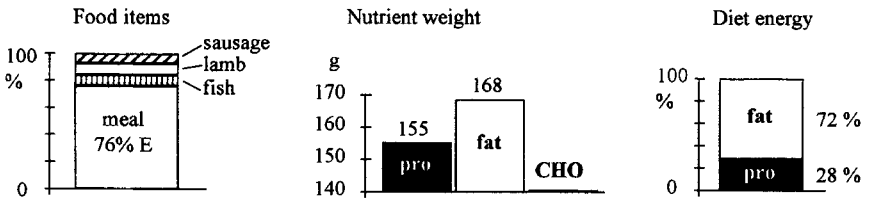
Diet and food intake of sled dogs during a multi-stage race. By J. DECOMBAZ[1], M. JAMBON[1], R. WRIGHT CHAMPAINE[2] and O. BALLEVRE[1], [1]*Nestlé Research Centre (Friskies Research), Nestec Ltd, Vers-chez-les-Blanc, CH-1000 Lausanne 26, Switzerland and* [2]*Champaine Enterprises, Fairbanks, AL, USA*

To sustain high levels of energy expenditure in sled dog competitions lasting several days without decline in performance, the energy demands must be matched by a correspondingly high energy intake. Experienced mushers have developed feeding methods, sometimes empirically, to achieve success. In the present field study, the diet fed to one such team of dogs by their musher was investigated.

The Alpirod is a 10 d sled dog race, often over hilly terrains, across the Alps. A team of thirteen Alaskan huskies was studied in 1995. Their sled covered about 65 km/day at a speed of 20 km/h and ranked 6th among the fifty-three starters. One meal (mash with raw lamb, beef or duck meat, eggs, maize oil and a small proportion of dry dog feed) was given in the evening. A snack (occasionally two depending on the distance covered during the day) consisting of a piece of frozen white fish, lamb or sausage was given either during the run or on arrival. The meal was weighed to the nearest gram, the snacks were estimated from the size (large, medium, small) of the pieces. Freeze-dried samples were analysed for gross energy (GE), N and fat. Carbohydrates (CHO) were calculated by difference between measured GE and the GE of protein (pro) and fat. Metabolizable energy (ME) was calculated using availability factors for wet canned dog food.

Body weight was maintained (initial 19.0 (SE 1.8) kg; final 19.5 (SE 1.6) kg).



ME was 8.74 (SE 0.9) MJ/d (0.95 MJ/kg$^{0.75}$). Using a value of 142 kJ/kg per d for the BMR of dogs of this size, ME was 3.2-fold higher than minimal requirements. Snacks amounted to 0.24 of daily energy intake. The mean energy density of the diet was 23 MJ/kg, higher than commercial formulations for working dogs. Carbohydrate intake was not significantly different from zero.

The transition from sustenance rations of the average dog (up to 50% dietary CHO) to the performance ration of endurance trained dogs (as low as 0%) is in sharp contrast with the chosen diet of human athletes making comparable efforts. Ultra-marathoners ingested 57% (30 d, 65 km/d, Décombaz *et al.* 1990) to 94% (5 d, 915 km, Rontoyannis *et al.* 1989) carbohydrate energy. Reasons for this species difference (fat loading *v.* carbohydrate loading) probably include anatomic, adaptive, metabolic and mental components such as: (1) the shorter gut of the husky, their lower amylolytic capacity and their proneness to diarrhoea which support the advantage of a high digestibility fat diet; (2) the risk of exertional rhabdomyolysis with carbohydrates; (3) the larger contribution of fat oxidation at "moderate" intensity relative to the exceptional aerobic reserve (Reynolds *et al.* 1994); (4) mitochondrial adaptations to chronically high fat diets (Taylor *et al.* 1994); (5) gluconeogenic ability and, perhaps, (6) a reduced mental motivation of the animal for anaerobic work.

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Determining consumers preferences using sensory evaluation. By J. M. MURRAY[1], C. M. DELAHUNTY[1], P. A. MORRISSEY[1], M. HENRY[2] and J. BOGUE[2], *[1]Department of Nutrition and [2]Department of Food Economics, University College, Cork, Ireland*

Sensory quality is an important determinant of food and beverage choice. The science of sensory evaluation is the only means of measuring sensory characteristics of foods as perceived by consumers. In order to build a predictive model for consumer preference of cheese, descriptive and preference tests (which use trained assessors and "naive" consumers respectively) were applied to the measurement of eight Cheddar-type cheeses (premium, two medium mature, vegetarian, retail brand, farmhouse, light and vintage). Using Quantitative Descriptive Analysis (QDA) the flavour, texture and appearance of the samples were measured objectively in a modern sensory laboratory using fifteen trained assessors and a predetermined vocabulary of 33 terms. Analysis of the data by Principal Components Analysis (PCA) revealed the relationships between cheeses and descriptive terms. Premium and vintage Cheddars scored highly for "acidic" and "mouldy" flavour and "mouthcoating" and "moist" texture, whereas vegetarian and retail brand were characterized as having "cheddary" and "balanced" flavour and "firm" and "rubbery texture". Light Cheddar-type was different in sensory character to all other samples; it was described as having a "rancid" flavour and a "rubbery texture". In parallel with this, 100 "naive" consumers were asked to scale the samples on a nine-point hedonic scale for overall acceptability. The premium Cheddar was most preferred with a mean score of 6.34 while the least preferred was the light Cheddar-type which had a mean score of 3.89. Data were analysed using hierarchical cluster analysis, and five clusters were found by grouping consumers with similar acceptability scores. (Table)

Cluster	n	Premium	Medium mature	Vegetarian	Retail brand	Medium mature	Farm-house	Light	Vintage
1	24	5.62	3.75	4.75	5.37	3.67	4.42	2.75†	6.50*
2	31	6.74*	4.26	6.42	6.19	6.19	4.61	5.58	3.10†
3	14	7.86*	7.71	5.64	7.00	6.86	7.71	6.07	5.57†
4	16	6.37	7.19*	4.75	4.62	5.94	3.94	2.25†	3.50
5	15	5.20	7.27	3.93	3.60	3.00	7.33*	1.93†	4.20
All	100	6.34*	5.54	5.27	5.16	5.16	5.30	3.89†	4.49

* " Winner" products.
† "Loser" products.

An internal preference map was then obtained by PCA which illustrated individual consumer preference. In order to relate descriptive and preference measurements an external preference map was constructed using Partial Least Squares regression. The model illustrated the relationship between consumers' preferences and sensory character, and the regression coefficients indicated how well preference could be predicted from sensory variables. Best prediction was obtained for clusters 4 and 5. This model may be used to predict whether these consumers will like or dislike other cheeses.

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Comparative diet and lifestyle study between Irish and West Virginian women By G. NOLAN, S. FRIEL and C. KELLEHER, *National Nutrition Surveillance Centre, Department of Health Promotion, University College Galway, Republic of Ireland*

Following the establishment of links between the Department of Community Medicine, West Virginia University, U.S.A. and the Department of Health Promotion, University College Galway it was proposed that a joint project be carried out to compare diet and exercise patterns of women in Ireland and West Virginia.

Both areas have very similar demographic characteristics and are essentially rural with many small communities isolated from centres of population and industry. The people of Ireland and West Virginia suffer predominantly from the same major chronic diseases -heart disease and cancer.

A comparison was carried out of diet and lifestyle of similar women's organisations in Ireland (Irish Countrywomen's Association) and West Virginia (West Virginia Extension Homemakers Clubs). The aim of the study was to determine the dietary intake and lifestyle habits of the women in both countries; and to asess their knowledge and attitudes in relation to diet, lifestyle and health. A postal self-administered questionnaire was sent to 400 women in each country and an overall response rate of 56% was achieved.

Results showed that 44% of Irish women were in the overweight category as compared with 33% of West Virginian women, (both based on Irish cut off limits B.M.I 25-30) while a further 8% of Irish women were obese compared with 30% of those in West Virginia (both based on Irish cut off limits B.M.I 30+) Some 45% of West Virginian women and 50% of Irish women had modified their diet during the previous year due to concern with a healthy diet. The next most important reason for diet modification was to treat overweight.

While 60% of West Virginian women drank low fat milk, only 33% of their Irish counterparts did likewise. The vast majority of both groups of women cooked with vegetable oils (92%) and used low fat methods to cook vegetables.(93%)

In both countries 75% of women engaged in exercise as a method to improve health or fitness,or as a means weight control (55%) and stress reduction (51%) Walking (40%) followed by gardening (22%) were the most frequent forms of exercise in both groups. The two main barriers to participating in exercise were 'lack of time due to family commitments'(36%) , followed by 'lack of interest'(22%). Overall the results showed that the women have some similar characteristics in terms of eating and exercise patterns. While they also have a similar degree of overweight, obesity was a greater problem in American women and self reported indications are that lifestyle changes are more likely in this group. Based on the results of this comparative study it may be possible to design similar lifestyle intervention programmes for the Irish and American women.

Process evaluation of a health promotion educational resource material for children: Galway Health Project. By M. O'DONNELL [1], U. FALLON [2] and C. KELLEHER [2], [1] *Western Health Board and* [2] *University College Galway, Republic of Ireland*

A health promotion intervention targeting 8-15-year-old children (n130) took place in general practice as part of The Galway Health Project (1995). The objectives of this research study were to produce a 'tailor made' health promotion resource material, to standardize the 10 min. intervention process using the resource and to evaluate the effectiveness of this resource. A wallchart folded "accordion style" was developed. The format consisted of six instructional steps and simulation questions (Iammarino *et al.* 1980). The content covered diet, exercise and smoking. Sixteen general practices were recruited to participate. Practices were randomized using a factorial design to use either an opportunistic or recall system. Subjects were children on the general practitioner (GP) registers. The intervention was provided by a research nurse (RN) in half the practices and by the GP in the other half (Galway Health Project, 1995). The evaluation research used methodological triangulation. Six GP and two RN were interviewed using a structured questionnaire. Children (*n*130) who received the intervention by August 1996 were forwarded a postal questionnaire; questionnaire A for the 8-11-year-old and questionnaire B for the 12-15-year-old children. Thirty children were invited to attend two focus groups. Response rates were; 100% from GP and RN (6 M, 2 F), 63% from the children's postal survey (37 M, 46 F; 68.7% from 8-11 year age group, 31.3% from 12-15 year age group) and 64% from the focus groups (10M, 10F; age group 8-11 years).

Results indicated that all practitioners found the resource assisted in standardizing the intervention by using the step process and that a 10 min. intervention was appropriate for general practice. The majority of children (97.6%, *n* 81) found it easy to read and 96.4% (*n* 80) read the wallchart at home. However 66.3% (*n* 55) of children did not measure their height and 56.6% (n 47) did not complete a 24 h food record as home activities. Overall nutrition knowledge and attitudes of practitioners and children compared favourably with other studies (Hopper & Barker, 1995). However, the 'increase dietary fibre' message required further clarification. Practitioners may not be familiar with the practical sources of fibre as 50% thought the wallchart did not promote this message and 56% of children were unaware of the intervention message to eat more of the bread, cereal and potato group.

This study indicated that a resource designed for the general practice setting and targeting children was required. In future interventions practitioners need to show the children how to complete the home activities. They also need to know of the commonly consumed food sources of fibre.

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Diet diaries and difficulty with the "three R's" in a 43-year-old British birth cohort. By G.M. PRICE[1], A.A. PAUL[1] and M.E.J. WADSWORTH[2], [1]*MRC Dunn Nutrition Unit, Milton Rd, Cambridge CB4 1XJ and* [2]*MRC National Survey of Health and Development, Department of Epidemiology and Public Health, University College London Medical School, London WC1E 6BT*

As part of an investigation into bias in low-energy reporting in a large survey (Price *et al.* 1997), we wished to establish whether subjects' confidence or ability in reading, writing or arithmetic might have any association with their response to our request for dietary information.

The Medical Research Council National Survey of Health and Development is a prospective survey of a socially stratified sample of people born in England, Wales or Scotland in March 1946, comprising information on many aspects of the members' lives. At age 43 years, 3262 survey members were asked at interview: "Do you have any difficulties in your day-to-day life with: (a) reading? (b) writing or spelling? or (c) sums and calculations?" At the end of each interview a 2 d dietary recall was taken and the subject was asked to keep a diet diary in household measures for the subsequent 5 d, with postal return.

Of the men, 3.4% reported that they had difficulty with reading, 11.2% with writing or spelling and 4.1% with sums and calculations. The corresponding proportions in women were 1.7, 5.1 and 4.5% respectively. The perceived difficulties could have had any cause. Although significantly associated as expected with education and social class, 10 - 20 % of subjects reporting these problems had attained A-levels and higher or were currently in social class II, while 15 - 30 % managed comfortably on their current income.

Among those interviewees who submitted a diet diary ("responders"; 70.4% overall), men reporting difficulty with writing or spelling ("write/spell") were half as likely as other men to be low-energy reporters (LER, defined as having energy intake (EI)/BMR<1.10)(Pearson χ^2 7.4; P=0.007). When added to a logistic regression model of factors predicting LER among responders including education, social class and current BMI, "write/spell" was a significantly negative predictor (P<0.01) with BMI as positive predictor (P<0.0001). This was a surprising result since literacy or numeracy problems would be expected to contribute to insufficient information in a dietary record, and hence low EI/BMR.

Among female responders, the only association between the "three R's" and low-energy reporting was a tendency for those having difficulty with sums and calculations to be more likely to be LER (Pearson χ^2 4.4, P=0.036). However, when other factors were similarly controlled in logistic regression analysis, numeracy difficulties were not significantly predictive of LER.

The unexpected result in the men was explained by an analysis of all interviewees. The Table shows the significantly varying distribution of percentage of male interviewees with or without "write/spell" according to type of diary response (Pearson χ^2 20.6, *df* 2; P<0.0001), compared with a non-significant (NS) effect in women (Pearson χ^2 2.4; *df* 2; P=0.3). Fifty-eight cases were excluded due to missing values.

	Proportion of men (%)			Proportion of women (%)		
Difficulty with writing / spelling...	Yes (*n* 186)	No (*n* 1436)	All (*n* 1622)	Yes (*n* 81)	No (*n* 1501)	All (*n* 1582)
No diary	42.9	29.1	30.6	33.3	28.4	28.6
Diary yielding EI/BMR <1.10	5.5	14.7	13.7	23.5	19.6	19.8
Diary yielding EI/BMR ≥ 1.10	51.6	56.2	55.7	43.2	52.0	51.6

Men with perceived writing problems were less likely to provide a diet diary at all while those who did were less likely than other men to report EI/BMR<1.10. In contrast, women with these difficulties were slightly (NS) less likely to return a diet diary but also slightly more likely to be LER.

Price, G.M., Paul, A.A., Cole, T.J. & Wadsworth, M.E.J. (1997). *British Journal of Nutrition* 77, 833-851.

Use of the Irish household budget survey for food availability estimation. By S. FRIEL, C. KELLEHER and the DAFNE II GROUP*, *National Nutrition Surveillance Centre (NNSC), Department of Health Promotion, University College Galway, Republic of Ireland*

Household budget surveys are routinely collected in almost all European countries primarily as an economic measure. These surveys regularly collect a variety of data including food-related information and sociodemographics of a large nationally representative number of households. The HBS is used for weighting purposes for the consumer price index (CPI) and the data for 1987, which will be discussed in the present paper, were recorded to determine the pattern of household expenditure at that time in order to update the weighting basis of the CPI.

The pan-European concerted action data food networking (DAFNE) project has been developing a standardized food data bank using HBS to estimate food availability from various European countries. Comparability across countries should be enhanced due to the degree of standardization in the HBS data collection by the National Statistics Offices. In the initial stages of the DAFNE project a new food coding system was devised into which each country's foods were fitted since each participating country recorded various levels of food grouping. In the Irish HBS data 134 food groups existed within which there were differing levels of aggregation. These were disaggregated using estimates of food item consumption from the 1990 nutrition survey, micro surveys and food balance sheet data.

The Irish data are collected primarily as expenditure and it was necessary therefore, if the data were to be fitted into the DAFNE model, that they should be converted into quantities. Disaggregation of the Irish HBS data resulted in 186 food items for which retail prices per unit weight were required. Various sources were identified which supplied retail prices per unit weight including the CPI section of the Central Statistics Office (CSO) and the Consumers Association of Ireland Regional Price Survey of 1990. No food organizations or retailers responded to the price request. The remaining retail prices were obtained by surveying the shelves of four Galway city supermarkets in 1996. All prices collected were adjusted to those of 1987 using the CPI. There now existed a list of 186 food items with 1987 retail prices per unit weight. Conversion of the Irish HBS expenditure data in quantities was performed using a model based on level of food grouping, expenditure data and retail price.

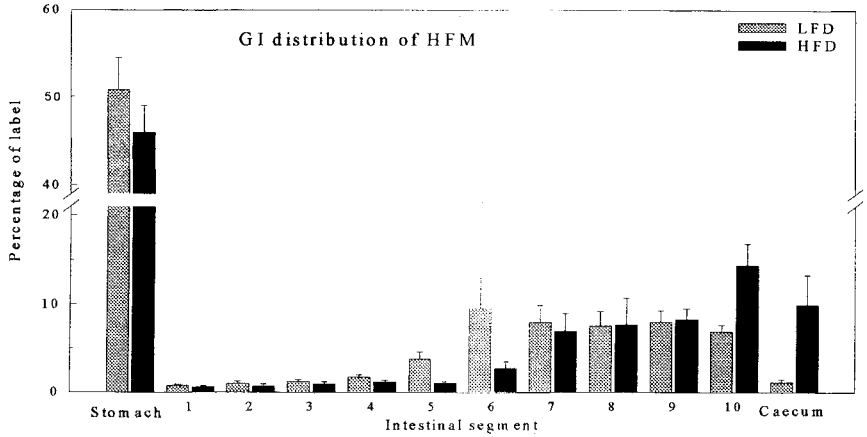
Twelve foods in the dataset are traditionally recorded by the CSO as both expenditure and quantity and were used by the NNSC as an initial internal validation mechanism for the price conversions estimation of quantities. The Wilcoxon signed rank test showed no significant difference overall between the estimated quantities and those published for twelve foodstuffs as shown in the Table. Although the internal validation showed good agreement for the majority of items there are a number of individual food items for which substantial differences exist between estimated and published results. Extensive investigation into the errors associated with the price conversions is on-going.

	Expenditure (£ / week)	Retail price (£ / g)	Estimated quantity (g / week)	Published quantity (g / week)
White Bread	2.603	0.715 / 800	2912.4	3030.1
White Flour	0.276	0.84 / 2000	657	640
White Self Raising Flour	0.061	0.834 / 2000	146	142
Wholemeal Flour	0.075	0.879 / 2000	171	163
Fresh Milk	5.153	0.489 / 1000	10538	6123
Eggs	0.94	1.069 / 600	527.6	526.7
Butter	1.63	2.822 / 1000	577.6	630.61
Margarine	0.369	0.13 / 100	283.85	287.38
Lard/ cooking fats	0.067	0.26 / 320	82.46	59.93
Potatoes	1.601	0.295 / 1000	5427.12	7402.47
Sugar	0.758	0.704 / 1000	1077	1080
Oatmeal	0.098	0.79 / 1000	124	135

* Greece: A. Trichopoulou, T. Vassilakou and P. Thriskos; Luxembourg: A. Schmitt; Norway: E. Mork and K. Trygg; Spain: O. Moreiras; United Kingdom: M. Nelson and S. Paterakis

Gastrointestinal transit in rats adapted to a high-fat diet. By A. SHAFAT and R.D.E. RUMSEY, *Department of Biomedical Science, University of Sheffield, Sheffield S10 2TN*

The gastrointestinal (GI) transit of a meal is closely regulated by its nutrient composition. Specifically, the presence of fat prolongs stomach-to-caecum transit time in a dose-dependent manner (Murray *et al.* 1987). The present study investigated whether high-fat feeding alters the gastrointestinal delay caused by a high-fat meal in adult rats. Twenty-six Sprague-Dawley rats were allocated to two groups and fed *ad libitum* on either a low-fat diet (25g olive-oil/kg, LFD), or a high-fat diet (430g olive-oil/kg, HFD). Animals were weighed and their food intake was recorded daily. After 18 d, rats were fasted for 18 h and half the animals in each group intubated with a low-fat meal (10g/kg fat; LFM), and the other half with a high-fat meal (300g/kg fat; HFM), labelled with ^{99}Tc-tin colloid. Rats were killed by chloroform inhalation 4 h after intubation and the GI tract was dissected out immediately. The distribution of the meal along the GI tract was determined using a dedicated gamma-counter and computer (Brown *et al.* 1987). Results were analysed using two-way ANOVA. Significance level was $P<0.05$.



The average daily energy intake was higher in the HFD group (476 SE 31 *n* 14 *V.* 203 SE 9 *n* 12 kJ/d per rat, $P<0.001$) although weight gain was not significantly different between the two dietary groups. In both dietary groups the HFM was significantly delayed in the stomach, had a more proximal geometric centre and had reduced caecal content compared with a LFM. Feeding HFD for 18 d reduced the ability of the GI tract to delay a HFM in the stomach ($P<0.03$). HFD also reduced the ability of the ileo-caecal valve to delay the entry of label into the caecum in response to fat ($P<0.02$). As a result of the gastric and caecal differences, and the different intestinal distribution, the proximal shifting of the geometric centre in response to increased fat in the meal was significantly reduced ($P<0.001$). There were no significant differences in the transit of the LFM between the two dietary groups. Dameto *et al.* (1991) showed faster transit of an inert meal in rats on a cafeteria dietary regimen from weaning. The results of the present study show the importance of the fat component of the test meal in adult animals fed on a uniform high-fat diet.

In conclusion, rats adapted to a high-fat diet have reduced ability to delay the transit of a high-fat meal along the gastrointestinal tract, while the transit of a low-fat meal is unaffected.

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Carotid atheromatous plaques, intimal-medial thicknesses and plasma homocysteine levels in asymptomatic post-menopausal women. By C.P. JAMIESON[1], O.A. OBEID[1], E.R.E. DENTON[2], E.P. MORRIS[3], J. POWELL-TUCK[1] and J. RYMER[3], *[1]Department of Human Nutrition, St Bartholomew's and the Royal London School of Medicine and Dentistry, London E1 1BB, [2]Department of Radiology and [3]Hormone Replacement Therapy Research Unit, Guy's Hospital, London SE1 9RT*

The elevation of plasma homocysteine is an independent risk factor for ischaemic coronary vascular disease (Robinson *et al.* 1995). An increased common carotid artery posterior intimal-medial thickness (CIMT) is an indicator of preatheromatous disease (Sidhu and Desai, 1997). CIMT measurements greater than 1.00 mm are usually accepted to be abnormal (Salonen and Salonen, 1993). The present study was conducted to investigate the relationship between plasma homocysteine, CIMT and the presence of carotid plaques. Fifty-two post-menopausal women (mean BMI 26.1, range 19.2- 39.0 kg/m^2) with no symptomatic carotid arterial disease underwent B-mode grey-scale ultrasound to assess the presence of common carotid plaques together with measurement of CIMT. Blood (non-fasting) was taken from each patient and the plasma homocysteine concentration was assayed by HPLC (Ubbink *et al.* 1991). Information was recorded from each individual with respect to smoking status, past IHD and the presence of a family history (FH) of such disease. The study was approved by the district ethical committee.

	Homocysteine > 14 μmol/L	Homocysteine < 14 μmol/L
No of patients	10	42
Median CIMT (mm)	0.76	0.68
No with plaques (%)	8 (80)	18 (43)*
No with IHD (%)	2 (20)	12 (29)
No with FHof IHD (%)	7 (70)	27 (64)
No current smokers (%)	2 (20)	9 (21)

*Statistically significantly different from the group with homocysteine > 14 μmol/l, $P < 0.05$ by χ^2 test.

The overall results revealed no significant correlation between CIMT and plasma homocysteine concentration. There were trends of increasing median CIMT measurements and homocysteine levels between the groups of never, ex and current smokers respectively (median CIMT= 0.74, 0.78, 0.80 mm, median plasma homocysteine= 11.56, 12.38, 12.64 μmol/l respectively). These trends did not reach statistical significance.

Previous studies have identified an upper limit of normal plasma homocysteine of approximately 14 μmol/l (Robinson *et al.* 1995). The Table compares the group with a plasma homocysteine level above that limit with that with a level below it.

There was an association between the presence of carotid arterial plaques and a raised plasma homocysteine level ($P<0.05$) but no significant correlation overall between plasma homocysteine and the CIMT measurement. However, it is unclear whether there is an association between plasma homocysteine levels above the threshold of 14 μmol/l and abnormally raised CIMT measurements.

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Plasma homocysteine shows no response to altered dietary methionine intake. By M. WARD[1], H. McNULTY[1], B. O NEILL[1], J.M. McPARTLIN[2], J.J. STRAIN[1], D. WEIR[2] and J. M. SCOTT[3], *[1]Human Nutrition Research Group, University of Ulster, Coleraine BT52 ISA and Departments of [2]Clinical Medicine and [3]Biochemistry Trinity College, Dublin, Ireland*

Moderately elevated plasma homocysteine is an established independent risk factor for cardiovascular disease (Boushey *et al.* 1995). The principal dietary source of homocysteine is the essental amino acid methionine which is found in foods rich in protein of animal origin. Folate, vitamin B_6 and vitamin B_{12} all play key roles in homocysteine metabolism, and supplementation with one or all of these vitamins at varying doses has been shown to lower plasma homocysteine concentrations in both healthy and hyperhomocysteinaemic subjects (Ueland *et al.* 1992). The relationship between dietary methionine and plasma homocysteine, however, is less clear.

In order to investigate the effect of dietary methionine intake on plasma homocysteine concentration, Thirteen male subjects aged 19 - 29 years were selected from an initial screen of fifty subjects on the basis of having the highest habitual dietary methionine intake (assessed using a food frequency questionnaire). These subjects participated in a 2-week cross over intervention trial. Following a baseline period during which diet was recorded, subjects were randomly divided into two groups to recieve a low-methionine intervention diet (ID) for 1 week followed by usual diet (UD) for a further week or vice versa. Low methionine (i.e. high-carbohydrate) foodstuffs were provided for the duration of the intervention period, along with detailed dietary advice (both oral and written) on foods to be avoided as well as those allowed freely. The dietary manipulation was designed to minimize nutrient differences between ID and UD. Subjects were asked to complete 7 d food diaries at baseline and during intervention and these were analysed using the nutritional analysis program Comp-Eat. Fasting blood samples collected at baseline and at the end of weeks 1 and 2 were analysed for plasma homocysteine by HPLC.

	Baseline		ID		UD		*P*
Variable	Mean	SD	Mean	SD	Mean	SD	
Dietary							
Methionine (mg/d)	1947[a]	679	407[b]	83	1969[a]	639	<0.001
Haematological							
Plasma homocysteine (μmol/l)	7.01	1.84	7.09	1.90	8.08	2.30	0.3754

[ab]Values with different superscripts were significantly different (ANOVA)($P<0.05$;LSD).

Responses to intervention are shown in the Table. Methionine intake decreased significantly on ID as compared with UD with a mean decrease of 1562 mg/d calculated for the group. No significant differences in plasma homocysteine concentration were observed in response to changes in dietary methionine. These findings support earlier work which showed that plasma homocysteine did not respond to supplementary methionine until levels equivalent to four times the usual methionine intake were reached (Ward *et al.* 1996). Thus, plasma homocysteine concentration appears to be unaffected by alterations in methionine within normal dietary ranges.

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The thermolabile 5,10-methylenetetrahydrofolate reductase (MTHFR) gene, mild hyperhomocysteinaemia and B-group vitamins. By J.V. WOODSIDE[1], D.L. HARMON[2], J.W.G. YARNELL[1], D. MCMASTER[1], I.S. YOUNG[1], E.E. MCCRUM[1], K.F. GEY[3], A.S. WHITEHEAD[2] and A.E. EVANS[1], [1]*School of Clinical Medicine, The Queen's University of Belfast, Grosvenor Road, Belfast BT12 6BJ,* [2] *Depatment of Genetics and Biotechnology Institute, Trinity College, Dublin, Republic of Ireland and* [3] *Department of Biochemistry, University of Berne, Switzerland*

Elevated plasma total homocysteine (tHcy) is accepted as an independent risk factor for premature vascular disease. There are several possible causes, both genetic and environmental in origin. The contribution of homozygosity for a common thermolabile mutation in the MTHFR gene to the hyperhomocysteinaemic phenotype has been quantified in a group of working men aged 30-49 years (*n* 625). Serum folate and cobalamin concentrations were also measured and their relationship with tHcy status and MTHFR genotype was assessed (Harmon *et al.*, 1996).

The homozygous thermolabile genotype was observed in 48, 36 and 23% of the top 5, 10 and 20% of individuals respectively ranked by tHcy levels, compared with a frequency of 11.5% in the study population as a whole, establishing that the mutation is a major determinant of tHcy levels at the upper end of the range. Thermolabile homozygotes had a 9.7-fold risk of being in the top 5% of the tHcy distribution, compared with non-thermolabile homozygotes. Serum folate and, less strikingly, cobalamin were observed to vary with genotype, being lowest in thermolabile homozygotes.

In a separate study, fifty men with a homocysteine level >8.34 μmol/l were given supplementation with B-group vitamins (1 mg pteroylglutamic acid, 7.2 mg pyridoxine, 0.02 mg cyanocobalamin) over an 8-week period. While the numbers studied were not adequate to reach statistical significance, a trend in folate response was observed between the three genotypes, with thermolabile homozygotes showing the biggest increase in serum folate (36%) after 8 weeks, compared with heterozygotes and non-thermolabile homozygotes, despite all three genotypes having similar folate levels at the start of the supplementation period.

Table 1 *Baseline measurements of homocysteine, folate and vitamin B_{12} by MTHFR genotype in subjects randomized to B vitamins*

	Genotype					
	+/+ (*n* 16)		+/- (*n* 26)		-/- (*n* 15)	
	Mean	(95% CI)	Mean	(95% CI)	Mean	(95% CI)
Baseline tHcy	11.18	(8.30,15.05)	10.34	(9.40,11.37)	9.09	(7.92,10.44)
Baseline folate	9.23	(7.42,11.47)	8.71	(7.46,10.17)	10.68	(8.51,13.40)
Baseline cobalamin	214	(179,256)	265	(224,313)	237	(182,309)

Table 2 *Percentage change in homocysteine, folate and vitamin B_{12} concentrations between week 0 and week 8 by MTHFR genotype in the subjects randomized to B vitamins*

	Genotype					
	+/+ (*n* 14)		+/- (*n* 23)		-/- (*n* 13)	
	Mean	(95% CI)	Mean	(95% CI)	Mean	(95% CI)
% Change in tHcy	-31	(-41,-18)	-34	(-39,-28)	-26	(-35,-15)
% Change in folate	308	(216,440)	326	(260,410)	420	(320,552)
% Change in cobalamin	161	(134,193)	142	(120,168)	161	(139,186)

$$\text{Difference in response between genotypes} = \frac{(\%\text{ change in -/-}) - (\%\text{ change in +/+})}{(\%\text{change in +/+})} \times 100$$

These findings suggest that the MTHFR thermolabile genotype is a major determinant of tHcy levels in the general population, that this is related metabolically to dietary intake of two B-group vitamins, pteroylglutamic acid and cyanocobalamin, and that possession of the allele may affect response to B vitamin supplementation.

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Differences between smokers and non-smokers in the intestinal absorption of carotenoids. By M.E. O' NEILL and D.I. THURNHAM, *Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA*

Dietary carotenoids may be protective against both heart disease and cancers (Gey, 1993). Most studies have traditionally been carried out on ß-carotene, however there is growing interest in the non pro-vitamin A carotenes since work has shown that these may have better antioxidant properties than ß-carotene (Chopra et al. 1993). Smokers have been shown to have lower circulating levels of carotenoids than non-smokers (Thurnham, 1990), and as yet the reason is unknown.

Twenty-two healthy volunteers (eleven females, eleven males), ten smokers (5M, 5F) and twelve non-smokers (6M, 6F) consumed specific encapsulated carotenoids, ß-carotene (40 mg), lutein (31.2 mg) or lycopene (38 mg), with a 42 g fat meal devoid of carotenoids and vitamin A although retinol palmitate (50,000IU) was added to quantify triacylglycerol-rich-lipoprotein (TRL) clearance when lutein and lycopene were tested. Fasting and subsequent hourly blood samples were collected for 8 h. Carotenoids in plasma and the TRL fraction (density < 1.006 kg/l) were measured by HPLC (Thurnham et al. 1988). Postprandial response curves of the carotenoids and retinyl esters in the TRL fraction were used to measure intestinal absorption of carotenoids in smokers and non-smokers.

The Table shows the area under the curve (AUC), or absorptive capacity for the different carotenoids by smokers and non-smokers for all sexes. No differences were observed between the sexes in the TRL response so all data were combined. In non-smokers, difference in the AUC occurred between lutein and ß-carotene ($P<0.05$), likewise in smokers differences were observed between lutein and ß-carotene ($P<0.01$) and lutein and lycopene ($P<0.05$). The amounts of carotenoids given should have saturated absorptive capacity in all cases, however even if lycopene and lutein are corrected to ß-carotene values, lutein were still lower than ß-carotene in smokers ($P<0.05$).

(nmol.h/l)	*Lutein*			*Lycopene*			*Total ß-Carotene* [1]		
	n	Median	Range	*n*	Median	Range	*n*	Median	Range
Smokers	10	32.2*	9.0 - 41.8	10	74.3	40.0 - 300.2	10	177	77.5 - 276
Non-smokers	10	96.0	68.5 - 177.3	10	133.0	112 - 204.0	12	201	149 - 283

* Median value was significantly difference from that of non-smokers ($P < 0.05$)

[1] Total ß-carotene = ß-carotene + conversion to retinyl palmitate

Large variations between individuals in their absorptive capacity for the carotenoids are shown in the Table. A difference in the intestinal absorption of carotenoids between smokers and non-smokers only existed for lutein ($P<0.05$). These results suggest that there are differences between smokers and non-smokers in the intestinal uptake and clearance of the hydrophilic carotene, lutein, from the TRL fraction.

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Effect of dietary fibres on vitamin A, vitamin E and folate status in rats. By J. GIBBS and P.J.A. SHEEHY. *Department of Nutrition, University College, Cork, Ireland*

Current national nutrition policies aimed at reducing the incidence of chronic diseases emphasise reducing fat and refined carbohydrate intake and their replacement by complex carbohydrates (starches and dietary fibre) from increased fruit and vegetable consumption. However, many people are unable or unwilling to alter their diet in a way that the advice envisages, and this has created an opportunity for the development of foods and drinks supplemented with dietary fibre (Southgate, 1997). A new generation of dietary fibres from fruit and vegetable processing is available for use as food ingredients. These fibres have appealing technological properties while allowing a fibre claim to be made on food labels. Some of their biological effects (e.g. on cholesterol binding) have received considerable attention. In contrast, little is known about their effects on vitamin status. The objective of the present study was to evaluate the effect of incorporating fibres from apple, barley, peas and wheat (at a practical inclusion level of 50 g/kg diet) on vitamin A, vitamin E and folate status in rats.

Commercial washed, dried and milled fibres from apple, barley, pea and wheat were obtained from Sofalia, Tour Roussel, Paris, France. Fifty-five weanling male Sprague-Dawley rats were divided into five groups of eleven rats of equal mean weight. One group was given AIN-76 purified diet, which contained cellulose (50 g/kg diet). Other groups were given the same diet except for replacement of the cellulose by the apple, barley, pea or wheat fibres. Rats were given feed and water *ad libitum*. After 56 d, rats were fasted overnight and killed by cardiac puncture under urethane anaesthesia. Retinol and α-tocopherol concentrations in plasma and liver were measured by HPLC (Bieri *et al.*, 1979) (following saponification in the case of the liver assays). Plasma folate concentration was measured by microbiological assay (Scott *et al.*, 1974).

	Fibre type									
	Cellulose		Apple Flavilla F145		Barley Sofabran F168		Pea Sofalite F179		Wheat Sofabran F146	
Vitamin	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Retinol:										
μg/ml plasma	0.33	0.02	0.33	0.01	0.33	0.03	0.31	0.01	0.33	0.02
μg/g liver	109	6.08	112	5.06	106	3.03	122	5.47	110	5.32
α-Tocopherol:										
μg/ml plasma	10.2[a]	0.71	9.21[ab]	0.65	11.0[a]	0.72	7.54[b]	0.51	9.90[a]	0.61
μg/g liver	31.1	1.28	33.1	1.54	30.8	1.58	31.7	1.98	32.1	2.18
Folate:										
ng/ml plasma	77.8	2.64	80.0	2.60	79.6	4.63	79.8	2.44	88.0	3.34

[ab] Mean values within a row not sharing a common superscript letter were significantly different ($P < 0.05$).

There were no significant differences in mean body weight, stomach, small- and large-intestinal weights or food intake between groups. Plasma α-tocopherol concentrations in rats fed with pea fibre were significantly ($P < 0.05$) lower than those of rats given cellulose, barley or wheat fibres. However, there were no significant differences in plasma or hepatic retinol, hepatic α-tocopherol or plasma folate concentrations between groups. The results indicate that at the level of inclusion employed in this study, incorporation of these fibres in the diet had minimal effects on vitamin status in rats.

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Effect of different dietary fat sources on immune function in healthy males and females. By P. M. KIRK [1,2], B. M. HANNIGAN [2], V. J. McKELVEY-MARTIN [2] and J. J. STRAIN [1]. [1]*Human Nutrition Research Group and* [2]*Cancer and Ageing Research Group, University of Ulster, Coleraine BT52 1SA*

Previous studies using animals compared the effects of sunflower seed and olive-oil-rich diets on various indicators of immune function. It was found that while dietary polyunsaturated fatty acids such as those in sunflower seed oil predisposed mice to suppression of certain T-cell mediated reactions, diets rich in monounsaturated fatty acids (i.e. from olive oil) did not (Bennett *et al.*, 1987). The effects of these two oils on human immune function however, have not been well defined. The aim of the present study, was to examine the influence of fatty acid supplementation on the T-cell activity of healthy men and women. Twenty-four subjects (ten male, fourteen female) aged 20-40 years were randomly assigned to a 16-week double-blinded crossover study of olive oil *v.* sunflower seed oil supplementation, fed during two 8-week periods. The supplements (25g/d) were given in the form of a fat-modified spread (containing 60% olive or sunflower seed oil) and/or a fat-modified milk-based drink (based on skimmed milk powder with 3% olive or sunflower seed oil). Fasting blood samples were separated into constituent cells and plasma. The ability of T-cells to proliferate was estimated by their incorporation of [^{3}H]-thymidine into newly synthesized DNA.

	Baseline		Sunflower oil supplement		Olive oil supplement	
Sex	Mean	SEM	Mean	SEM	Mean	SEM
Male (*n* 10)	5.5	1.0	4.4	1.0	4.9	1.1
Female (*n* 14)	6.0	0.9	10.4*	1.9	7.6	1.8

* Females significantly different from males on sunflower seed oil diet, $P<0.05$ (2-tail).

The Table shows counts per minute of ^{3}H in lymphocytes exposed to the T-cell mitogen phytohaemagglutinin (PHA), with four replicates per blood sample. There was a significant difference ($P<0.05$, independent t test) in T-cell proliferation between males and females while on the sunflower seed oil supplemented diet. The higher level in females may reflect better immune status, or alternatively, the documented increased risk of females developing certain autoimmune diseases such as rheumatoid arthritis (Hannigan, 1994). There was no significant difference for males or females in T-cell activity either between the baseline and supplemented diet periods or between the two supplemented diets. These results indicate that although differences were noted between males and females, there was no significant alteration in lymphocyte function for either sex.

The findings of this study suggest that a moderately high intake of either polyunsaturated or monounsaturated fats in the diets of healthy men and women will not suppress immune function.

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Induction of alkali labile lesions by oxidised derivatives of cholesterol in Chinese Hamster Ovary cells as measured by the Comet assay. By J.A. WOODS and N.M. O'BRIEN, *Department of nutrition, University College Cork, Cork, Republic of Ireland.*

Although cholesterol itself has been reported to be non-toxic and to have no mutagenic activity, this has not been the case for its oxidation products, as mixtures of sterol hydroperoxide and epoxide derivatives have been reported to be mutagenic *in vitro* (Smith & Johnson, 1989). In the present study we examined the effect of five cholesterol oxidation products on the generation of strand breaks and alkali labile lesions such as apurinic sites, the incorrect repair of which can lead to mutation. The activity of these compounds was investigated in Chinese Hamster Ovary (CHO) cells using the single cell gel electrophoresis assay (Comet assay) which measures the extent of DNA strand breaks in individual cells (Woods *et al*, 1997). The extent of DNA strand breaks in oxysterol-treated cells was measured as the fold difference relative to untreated control cells. Cell survival was measured in parallel using the MTT assay. The effects of incubation with the five oxysterols after 24 h can be seen in Figs. 1 and 2.

Fig.1. DNA strand breaks following incubation with oxysterols for 24 h.

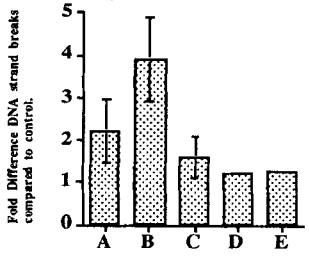
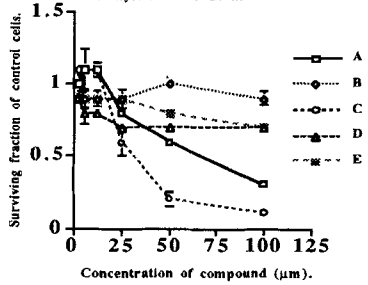


Fig. 2. Cell survival following incubation with oxysterols for 24 h.



A: 25-Hydroxycholesterol, **B**: 7-Ketocholesterol, **C**: Cholestan-3β,5α,6β-triol, **D**: Cholesterol-5α,6α-epoxide, **E**: 7β-Hydroxycholesterol

None of the compounds tested increased the level of DNA alkali labile lesions after a 1 h incubation, implying that these compounds are not directly genotoxic under these experimental conditions. However an increase in strand breaks was observed after a 24 h treatment with both 7-ketocholesterol and 25-hydroxycholesterol. This elevation in DNA damage is most probably as a result of cell death and supports recent data concerning the ability of these compounds to downregulate an important cellular protein which is thought to prevent apoptosis (Nishio & Watanabe, 1996). Oxysterols could prove to be important tools in elucidating the mechanism of apoptosis, which is an essential process for normal development. Dysfunctions in the apoptotic process have been implicated in major human diseases such as cancers.

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Randomised controlled trial of supplementation with Efacal *v.* calcium on bone mineral density in post-menopausal women. By E. JOAN BASSEY[1], JULIE J. LITTLEWOOD[1], and BRENDA E. REYNOLDS [2] [1]*University of Nottingham Medical School, Clifton Boulevard, Nottingham NG7 2UH* [2]*Scotia Pharmaceuticals Plc, Portsmouth Rd, Guildford,GU3 1NA,*

Efacal, which is used as a dietary adjunct, is a preparation of evening primrose oil (EPO), marine fish oil and Ca. It contains two essential fatty acids (EFAs), namely, ∂linolenic acid and eicosapentaenoic acid. EFAs are precursors for intermediaries in bone metabolism and fish oil enhances Ca uptake from the gut compared to EPO or sunflower oil in rats on controlled diets (Kruger *et al.* 1995). It was therefore hoped that Efacal would improve bone mineral density (BMD) in women and a randomized controlled trial was conducted. Permission for the study was obtained from the Medical School Ethics Committee and each woman gave written informed consent.

Total body BMD (TBBMD) was assessed using dual energy X-ray absorptiometry (Lunar DPX-L) which yields areal density in g/cm^2. It is a highly reliable technique with a coefficient of variation of < 1% when groups of subjects were retested after 2 weeks in our laboratory. Healthy post-menopausal women aged 50-65 years were recruited by postal invitation, and screened. Exclusion criteria included confounding drug therapy, BMI above 32 or below 18 kg/m^2, or TBBMD outside 2 SD of age-matched norms. The women were checked for oestrogen status and assessed initially for TBBMD on two occasions 1 month apart and for background diet using a validated food frequency questionnaire for Ca (Ramsdale *et al.* 1994). The mean of these two initial measurements was used as the baseline. Fifty seven women were then randomly assigned to two treatment groups.

For the next 12 months each woman was required to consume ten capsules per d in divided doses with food. Total daily dose for the Efacal group was 1.0 g Ca, 4.0 g evening primrose oil and 440 mg of marine fish oil or for the placebo group 1.0 g Ca. The capsules were supplied and compliance checked monthly in personal interviews. Dropout was 26% and occurred mainly due to ill health not associated with the treatment.

Forty two women completed the study with a mean consumption of capsules of nine per d. Initial mean values for treatment and placebo groups respectively were for background Ca intake 937 (SE 249) and 999 (SE 246) mg per d and for TBBMD 1.114 (SE 0.017) and 1.136 (SE 0.017) g/cm^2. There were no significant differences between the groups. After 12 months supplementation both groups showed a rise in background Ca intake of about 10 % and a.significant decrease in TBBMD of -0.008 (SE 0.002) for treatment and -0.013 (SE 0.003) g/cm^2 for placebo (p<0.001). Losses of this magnitude are expected in oestrogen deplete women of this age.

The loss of TBBMD was 0.005 g/cm^2 less in the treatment than the placebo group, but the difference between the groups was not significant (p = 0.2; Student's t test for unpaired means). Power calculations show that this small difference between groups would become significant if it were found in a trial of 300-400 women. Possible confounding variables such as changes in body mass and initial BMD were examined as covariates but they had no effect on the non-significant outcome of this study.

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The effect of dietary calcium intake on pyridinium crosslinks of collagen in the rat. By ANNETTE CREEDON, ALBERT FLYNN and KEVIN CASHMAN, *Department of Nutrition, University College, Cork, Ireland*

Urinary pyridinium crosslinks of collagen, pyridinoline (Pyr) and deoxypyridinoline (Dpyr), are considered to be specific and sensitive biochemical indices of bone resorption (Eyre, 1992). They may have considerable potential for monitoring changes in rates of bone resorption and how such changes are influenced by diet. Dietary Ca restriction has been shown in animal studies to reduce bone mineral content, which may be due, in part, to an increased rate of bone resorption. The objective of the present study was to determine the effect of dietary Ca intake on urinary excretion of pyridinium crosslinks of collagen in a rat model.

Twenty-four 6-week-old male rats, Wistar strain, average weight 131 g, were randomized into three groups (*n* 8/group), housed individually in metabolism cages and adapted for 2 weeks to an AIN-76 diet containing Ca at the recommended level (5 g Ca/kg). The rats were then placed on AIN-76 diets containing either 2 (low), 5 (control) or 50 (high) g Ca/kg for a further 3 weeks. Pyridinium crosslinks (Pyr and Dpyr) were measured, following acid digestion, by an HPLC method (Colwell *et al.* 1993) in 24 h urine samples taken for three consecutive days each week of the study and in femurs at the end of the study. Femur Ca content was measured using atomic absorption spectrometry after dry ashing.

Dietary Ca (g/kg)	2		5 (Control)		50	
	Mean	SE	Mean	SE	Mean	SE
Final body wt (g)	257[a]	8	246[a]	11	249[a]	4
Femur Ca (mg/g dry wt)	173.1[a]	2.0	186.1[b]	5.0	186.1[b]	2.9
Urinary Pyr (nmol/d)*: week 1	9.2[a]	0.3	9.5[a]	0.6	8.7[a]	0.6
week 2	15.6[a]	1.0	10.2[b]	0.8	10.7[b]	1.3
week 3	14.6[a]	0.9	11.4[b]	0.7	10.9[b]	0.8
Urinary Dpyr (nmol/d)*: week 1	11.1[a]	0.6	10.5[a]	0.6	11.3[a]	0.5
week 2	15.3[a]	1.0	10.8[b]	0.9	12.2[b]	1.3
week 3	16.6[a]	1.0	11.3[b]	0.8	11.9[b]	0.6
Femur Pyr (nmol/g dry wt)	249[a]	21	258[a]	20	239[a]	18
Femur Dpyr (nmol/g dry wt)	367[a]	17	429[a]	21	363[a]	20

[a,b,c] Mean values within a row with unlike superscript letters were significantly different $P<0.05$ (ANOVA).

* Mean values for 3 d collections for weeks 1, 2 and 3 of the study.

Increasing dietary Ca content to 50 g/kg had no effect on the concentration of Ca or crosslinks in bone or on urinary crosslink excretion. Reducing dietary Ca content to 2 g/kg reduced the concentration of Ca, but not pyridinium crosslinks, in bone. During weeks 2 and 3 there was a significant increase in urinary excretion of Pyr and Dpyr in the low Ca group. In conclusion, dietary Ca restriction increased the rate of bone resorption which may have contributed to the reduced bone Ca concentration which was observed. However, the concentration of pyridinium crosslinks in bone was not affected by the low Ca intake.

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The combined effect of exercise and dietary modification on the fitness level and body composition of cardiac rehabilitation patients. By S.M. KING, S. DAVID, H. NEWTON, M. MAGUIRE, F. RAFFERTY and J.H. HORGAN. *Department of Cardiology, Beaumont Hospital, Beaumont Road, Dublin 9, Republic of Ireland*

The importance of both diet and exercise in cardiac rehabilitation has been well documented (Balady, *et al.*, 1994). The aim of the exercise programme in cardiac rehabilitation is to improve exercise tolerance, through improvements in cardiac function and changes in body composition. Diet, especially dietary fat and carbohydrate content, is known to influence body composition. The importance of dietary composition for improved performance is well recognized in the area of sports nutrition (Wilmore, 1996). The precise role of diet during the exercise programme of cardiac rehabilitation is not well defined. The aim of the present study was to examine the role of diets of varying macronutrient composition on body composition and exercise tolerance during a 10-week exercise programme.

Thirty male coronary artery bypass graft (CABG) patients aged 45-70 (mean 58) years were recruited. Baseline measurements included an exercise stress test (EST) using the Bruce protocol (Bruce *et al.*, 1973), a 7 d diet history using photographic atlas and body composition analysis using bioelectrical impedance analysis. Based on the results of the EST, patients were prescribed exercises at 60-70% VO_{2max}, thrice weekly over a 10 week period. Patients were randomly assigned to one of three dietary interventions: diet A (20% energy as fat, weight maintenance), diet B (30% energy as fat, weight maintenance) or diet C (30% energy as fat, weight reducing). Dietary advice was given to each subject in both written and oral format in a dietetic outpatient setting. Diets were followed for the duration of the exercise programme. Compliance was assessed by through a weekly weight check and full dietary assessment at wk 5 of the intervention period. All baseline measurements were repeated post-trial. Results are shown in the Table.

	Group A				Group B				Group C			
	Pre-Trial		Post-Trial		Pre-Trial		Post-Trial		Pre-Trial		Post-Trial	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fat intake (%)	28.9	9.2	21.6*	4.4	31.1	7.7	29.8	6.0	28.0	8.4	22.1*	3.3
CHO (%)	48.9	8.3	53.5	8.3	46.3	4.1	49.5	4.2	46.5	15.0	50.6	9.0
Energy (MJ)	8.0	0.7	6.1*	2.4	9.7	2.7	9.0	4.3	10.4	4.2	7.7*	2.1
BMI	26.7	2.3	26.6*	2.6	26.5	2.7	26.2	2.7	29.3	3.0	28.6*	3.4
Body Fat (%)	28.1	2.5	26.1*	3.4	27.6	3.1	26.4*	2.6	29.8	3.0	27.8*	4.1
LBM (%)	77.0	4.7	79.1*	5.9	76.1	5.5	78.2*	5.4	75.9	6.0	76.9	6.9
Duration EST	6.4	1.8	10.2*	1.4	7.4	2.1	8.8	3.8	6.0	2.5	9.2*	1.4
Heart Rate(6 mins)	147.0	12.0	126.6*	13.9	150.0	13.2	132.3	24.8	142.0	15.7	130.4	10.9

* mean values were significantly different from the corresponding pre-trial values, $P<0.05$ (paired *t* test)

Subjects who followed a very-low-fat, high-carbohydrate, weight-maintenance diet showed a significantly increased duration in EST post-trial ($P<0.05$, Scheffe test). In addition there was a significant decrease in heart rate ($P<0.0005$). This was coupled with a significant increase in LBM and decrease in body fat in group A. These combined improvements were not achieved by either of the other two intervention groups.

The results of this study would suggest that dietary composition during an exercise programme has a significant effect on cardiac function of cardiac rehabilitation patients.

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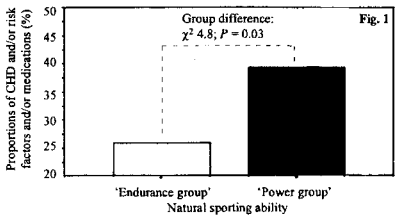
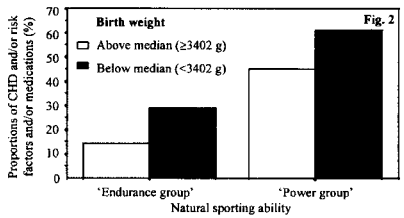
Natural sporting ability, birth weight and predisposition to cardiovascular disorders: study of British former soldiers. By THANG S. HAN and MICHAEL E.J. LEAN, *University Department of Human Nutrition, Glasgow Royal Infirmary, Glasgow G31 2ER*

Several studies have suggested that features related to the metabolic syndrome may be associated with muscle metabolism which tends to rely predominantly on the glycolytic pathway, as opposed to the oxidative pathway (Lillioja & Bogardus, 1988; Rebuffé-Scrive *et al.* 1988; Krotkiewski *et al.* 1990; Mårin *et al.* 1994). Athletes who specialize in endurance sports, e.g. marathon, tend to have a high proportion of slow twitch (type 1) oxidative muscle fibres, while those who specialize in power sports, e.g. sprinting, tend to have a high proportion of fast-twitch (type 2) glycolytic muscle fibres (Åstrand & Rodahl, 1986). We tested the hypothesis that people with a natural ability in 'power sports' (a presumed marker for fast-twitch muscle fibre predominance) might have increased risks of CHD compared with those with a natural ability in 'endurance sports' (a presumed marker for slow-twitch muscle fibre predominance), and that early growth as reflected by birth weight might influence these relationships. We performed a retrospective self-reported study of 231 (response rate 46.2%) male former soldiers, aged 34-87 years, who had undergone a course in physical training in the Army School of Physical Training in Aldershot (Hants), on the basis of their interest in physical activity and probable relative homogeneity in terms of health, fitness, dietary and other lifestyle behaviours.

The proportions who had CHD, defined as angina and/or coronary angioplasty and/or coronary artery bypass graft and/or heart attack were 18.7% of 107 men in the 'power group', compared with 9.7% of 124 men in the 'endurance group' (χ^2 3.9, $P = 0.05$). The proportions with CHD and/or risk factors and/or medications for these conditions rose to 39.3% in the 'power group', compared with 25.8% in the 'endurance group' (χ^2 4.8, $P = 0.03$) (Fig. 1). There were fifty-two men in the 'endurance group' and forty-nine men in the 'power group' who provided a reported birth weight (excluding ten men who were born prematurely). Birth weight related negatively to CHD (β -0.18, $P = 0.38$), and to CHD and/or risk factors (β -0.31, $P = 0.03$). Logistic regression analysis (adjusted for age, education, current BMI, physical activity, smoking, alcohol drinking, frequency of consumption of meat, carbohydrate foods, and raw vegetables) showed that men in the 'power group' with birth weights below median were more likely (odds ratio 10.7, 95% CI: 1.8-61.4, $P < 0.01$) to develop CHD and/or risk factors, compared with men in the 'endurance group' with birth weights above median (Fig. 2).





Men with a natural ability in 'power sports' are at increased risk of developing cardiovascular disorders when compared with men with a natural ability in 'endurance sports'. 'Small' birth weight has an independent additive effect. These findings suggest the hypothesis that a predominance of fast twitch, glycolytic muscle fibres, presumably of genetic origin, predisposes to cardiovascular disorders.

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Weight-and-diet concerns in Finnish female and male athletes. By MIKAEL FOGELHOLM and HANNELE HIILLOSKORPI, *The UKK Institute for Health Promotion Research, P.O.B 30, FIN-33501 Tampere, Finland*

Many athletes assume that success is associated with low body weight or fat content. The recent concern has been that an increased pressure for body-weight loss may lead to eating disturbances or even clinical eating disorders. While some studies indicate increased prevalence of eating disorders in certain groups of female athletes, the data are inconsistent (Sundgot-Borgen, 1994). Very little is known about eating disorders in male athletes. Therefore, the aim of the present study was to compare weight-reduction techniques, weight-and-diet concerns and other factors related to a risk for eating disorders, in female and male athletes, and in untrained controls.

Factors related to eating disorders were studied in five groups of female (n 173) and male (n 190) athletes, and in female (n 79) and male (n 61) controls. The subjects' age ranged from 14 to 40 years. The data were collected by a 121-item questionnaire, which included the eating disorders inventory (EDI) (Garner *et al.* 1983). The group differences were tested by the Kruskall-Wallis test, and by post hoc Mann-Whitney comparisons.

The sum of drive-for-thinness and body-dissatisfaction subscales (called weight-and-diet concerns, WDC) in the EDI was higher ($P<0.05$) in female controls (median 5.0; range 0 - 35) than in endurance athletes (median 0.0; range 0 - 19). The respective median values of the remaining female athlete groups were between 1.0 (ball-games) and 2.5 (aesthetic). The highest individual WDC results were found among the aesthetic and weight-class athletes. The male groups' WDC results did not differ from each other (range of median values: 0.0 (aesthetic) to 6.0 (ball-games), $P=0.08$), or from females ($P=0.62$). The preferred weight loss (discrepancy between present and preferred weight) in the female controls (median: -4.0 kg) was larger ($P<0.05$) than in aesthetic (-2.0 kg), power (-2.0 kg), endurance (-2.0 kg) and weight-class athletes (-1.0 kg). Males, on average, did not want to lose weight (different from females, $P<0.001$).

The prevalence of weight-reduction attempts (85%) in female weight-class athletes was higher ($P<0.05$) compared with endurance and ballgame athletes, and the controls (29-58%). The prevalence of weight reduction with rapid techniques (dehydration), among the female participants, was clearly highest in the weight-class athletes (78%) ($P<0.05$). The occurrence of unsupervised weight reduction was similar in all female groups (19-30%, $P=0.67$). The weight-reduction patterns in males resembled those of the female participants, that is, both the overall frequency (93%) and the frequency of rapid weight reduction (79%) was highest in the weight-class athletes ($P<0.05$). The occurrence of unsupervised weight reduction was higher in speed athletes v. weight-class and ballgame athletes ($P<0.05$).

The main outcome variable, sum score of body-dissatisfaction and drive-for-thinness subscales confirmed the earlier finding that the risk for eating disorders is dependent on the kind of athletic event (Sundgot-Borgen, 1994). However, the claim that some female athlete groups are at greater risk than untrained controls was not supported by the present study. The fact that preferred weight change was highest in control females was in harmony with the present results. However, some obvious high-risk cases among the aesthetic and weight-class athletes underscore the potential problem on an individual level.

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The effects of sodium ingestion on heat acclimation responses. By A. J. ALLSOPP[1], R. M. SUTHERLAND[2] and S. A. WOOTTON,[2] *[1]Institute of Naval Medicine, Gosport PO12 2DL, [2]Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD*

The increased loss of sweat Na upon exposure to heat constitutes a threat to overall Na balance with consequences for fluid balance and susceptibility to heat illness. The reference nutrient intake for Na (70 mmol/d) is based on the likely sweat secretion of individuals living in a temperate climate, who are moderately active. In hot conditions, however, supplementation may be advisable until acclimatized (Department of Health, 1991). Acclimatization occurs following repeated heat exposure, and results in greater Na retention by the sweat glands, a process controlled by aldosterone. However, the secretion of aldosterone is inhibited by increased dietary Na (Taylor *et al.* 1943), suggesting that salt supplementation may counter this adaptive response. The aim of this present study was to determine whether dietary manipulation of Na would influence the process of heat acclimation.

Thirty-three male subjects (aged 27 (SD ± 6) years; mean BMI 25 (SD ± 3) kg/m^2) were confined to an environmental chamber at a temperature of 25° for 3 d, followed by 5 d at 40° from 08.00 to 18.00 hours (25° from 18.00 to 08.00 hours). Na intake was prescribed as follows: high (HNa 340 mmol/d; *n* 7); moderate (MNa 170 mmol/d; *n* 9); low (LNa 70 mmol/d; *n* 9); restricted (MLNa 170 mmol/d for 3 d reduced to 70 mmol/d thereafter; *n* 8). Body weight was assessed daily, whilst Na losses were estimated (from urinary, faecal and sweat washdown collections) on days 4 and 8. Plasma aldosterone was determined by radio immunoassay, and the rise in aural temperature (T_{au}) was recorded whilst the subjects performed a light stepping exercise task for 1 h each day.

On the first day of heat exposure a Na deficit was observed in the MLNa (-47 (SE 12) mmol) and LNa (-19 mmol (SE 9) mmol) groups. This was significantly (P<0.01) attenuated to -7 (SE 4) mmol and +2 (SE 5) mmol respectively on the last day of exposure. Mean plasma aldosterone concentrations were similar on the first day of the trial. For the LNa group aldosterone concentration increased from 412 (SE 41) to 638 (SE 70) pmol/l before heat exposure; this response was potentiated further on heat exposure (day 6: 1461 (SE 355) and 591 (SE 76) pmol/l for LNa and MNa respectively; P<0.05). In contrast, the aldosterone response of the HNa group was attenuated (day 6: 351 (SE 33) pmol/l; NS). Body weight was reduced (P<0.01) in the heat in all four conditions by 0.9 (SE 0.2) %, indicative of a negative fluid balance. This reduction occurred at a faster rate (P<0.05) for the MLNa group (compared with the other twenty-five subjects). In addition, the mean fall in T_{au} (during exercise) associated with acclimation over the heat exposure was attenuated (P<0.05) in the MLNa group compared with the other twenty-five subjects. These changes in body weight and T_{au} were numerically dissimilar (NS) for the LNa and MLNa groups.

Compared with the "normal" (MNa) condition, supplementation (HNa) did not enhance the acclimation process as indicated by a similar loss of body weight and reduction in T_{au}. Restriction of Na intake (MLNa) incurred a marked Na deficit, a greater rate of weight loss and an impaired thermoregulatory response. Prior reduction of Na intake (LNa), however, resulted in a potentiation of the aldosterone response on heat exposure, and improved acclimation responses (T_{au} and relative weight change) compared with the restricted (MLNa), but not the MNa condition. Taken overall, these results suggest that dietary Na supplementation is of no benefit whereas Na restriction impairs heat acclimation. Ingestion of a low Na intake before heat exposure, however, assists Na balance to limit the effects of deficiency upon fluid balance and thermoregulation.

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Net and unidirectional water fluxes from four oral rehydration solutions perfused into the intact human jejunum. By J.B. LEIPER and R.J. MAUGHAN, *Department of Environmental and Occupational Medicine, University Medical School, Foresterhill, Aberdeen AB24 2ZD*

Perfusion of short segments of human intestine has been used to develop improved formulations of oral rehydration solutions (ORS). However, the standard perfusion model gives no indication whether the effectiveness of a solution is due to differences in uptake or efflux of water across the intestinal mucosa. With approval from the local ethics committee, we examined net absorption and unidirectional water fluxes using a perfusion technique in healthy male volunteers who had their body water pool labelled with deuterium oxide (D_2O). Eight subjects consumed 20 g D_2O in the evening and then fasted overnight on two occasions separated by 7 d. Next morning the subjects were intubated with a perfusion tube incorporating a mixing and test segment. Subjects were perfused with two different ORS on both occasions with the perfusion order randomized. Net water uptake was calculated from changes in the polyethylene glycol concentration of the mixing and test segment aspirates (Whalen *et al.* 1966), mucosa-to-lumen water efflux was estimated from the changes in the deuterium content of these aspirates using the formula of Soergel *et al.* (1968) and lumen-to-mucosa water influx was determined by adding together net water transport and mucosa-to-lumen flux rates. The ORS perfused were commercially available glucose-electrolyte solutions (GES). Following passage through the 150 mm mixing segment, mean glucose concentration (mmol/l) of aspirates of solution A was 62 (SD 15), B was 80 (SD 16), C was 182 (SD 29) and D was 169 (SD 34); mean Na concentration (mmol/l) was 87 (SD 15), 78 (SD 18), 60 (SD 14) and 53 (SD 10) respectively. Mean osmolalities of the mixing segment aspirates of the test solutions are shown in the Table.

	Osmolality (mosmol/kg)		Net water absorption (ml/cm per h)		Mucosa-to-lumen water flux (ml/cm per h)		Lumen-to-mucosa water flux (ml/cm per h)	
Solution	Mean	SD	Median	Range	Median	Range	Median	Range
A	241	22	11.5	7.4-36.7	2.8	1.4-13.0	17.0	12.3-38.1
B	286	9	6.1	2.7-13.1	10.9	7.7-14.0	17.0	11.3-23.6
C	308	18	4.8	2.4-17.0	11.3	7.1-13.4	16.6	11.3-25.4
D	342	14	1.2	-3.5- 4.2	13.7	10.8-32.8	14.7	10.1-33.3

In the 300 mm test segment, net water absorption was faster from solution A (Table) than from solutions B (P= 0.01), C (P= 0.05) or D (P= 0.001). While ORS B and C promoted similar rates of water uptake (P= 0.83), there was essentially no net water flux from the hypertonic solution D (P= 0.14). Mucosa-to-lumen water flux was slower when solution A was perfused than when ORS B (P= 0.018), C (P= 0.018) or D (P= 0.003) was perfused. Mucosa-to-lumen water flux was similar when ORS B and C were perfused (P= 1.00) which was slower than when solution D was perfused (P= 0.041). Lumen-to-mucosa flux was similar for all the test solutions (P= 0.65). This study reaffirms the finding that moderate hypotonicity potentiates water absorption from ORS compared with isotonic or hypertonic ORS, and suggests that the increase in net water uptake is due mainly to a decrease in the mucosa-to-lumen efflux rather than to an increase in the lumen-to-mucosa water uptake. It is possible, but unlikely, that the apparent differences in efflux of water between the perfused solutions was due to proportionally faster rates of absorption of the deuterium tracer than of water from the test segment.

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The effect of drink composition on deuterium accumulation in men with spinal cord lesion. By D. BALL[1], I.G. CAMPBELL[1], J.B. LEIPER[2] and R.J. MAUGHAN[2], *[1] Exercise and Sport Science, Manchester Metropolitan University, Alsager ST7 2HL, [2] Environmental and Occupational Medicine, University Medical School, Aberdeen AB25 2ZD*

Rehydration is an important factor for the wheelchair athlete when living, training and competing in a hot climate. Athletes with spinal cord lesion can sweat at rates of up to 1 litre/h during exercise and must therefore ingest fluids to maintain euhydration. With local Ethics Committee approval five male subjects volunteered for the present study. Three subjects had a history of spinal cord injury between T7 and T11; the remaining two subjects had spina bifida. The subjects' mean age and body mass were 29 (SD 9) years and 65.8 (SD 14.7) kg.

On three separate occasions, 5 d apart, each subject reported to the laboratory following an overnight fast, and ingested 500 ml of one of three beverages; sugar-free flavoured water, an 80 g/L glucose-electrolyte (GE) solution and a 160 g/L-GE solution. Deuterium oxide, a tracer for water, was added to each drink at a dose of 71.4 mg/kg body mass. Beverages were administered in randomized order. Arterialized-venous blood samples were taken before ingestion and at intervals up to 75 min after consumption. Blood samples were analysed for blood glucose and blood deuterium (^{2}H) content. Deuterium accumulation in the circulation was measured by infrared spectrophotometry following vacuum distillation of the blood samples. Data were analysed by Kruskall-Wallis test and by post-hoc Mann Whitney test.

Before ingestion of the beverages, median blood glucose concentration was similar across all conditions. The concentration of blood glucose remained constant at 5.08 (range 4.56-5.49) mmol/l throughout the water trial. However, 10 min after ingesting both the 80 g/L and 160 g/L-GE solutions blood glucose was significantly elevated compared with water ingestion ($P<0.012$). The concentration of blood glucose remained elevated compared with the water trial until 60 min after ingestion ($P<0.026$). At 75 min after beverage ingestion the concentration of blood glucose was similar between all trials.

The median rate of ^{2}H accumulation in the circulation was faster ($P<0.012$) in the water trial 10.0 (range 4.5-12.4) ppm/min compared with the 160 g/L-GE solution (2.2 (range 1.2-3.4) ppm/min). In the 80 g/L-GE trial the rate of ^{2}H accumulation was 4.0 (range 3.4-9.8) ppm/min; this was not different from either the water or 160 g/L-GE trial. The maximum median concentration (Cmax) of ^{2}H was 209 (range 175-238) ppm with water ingestion, 180 (range 109-223) ppm during the 80 g/L-GE solution and 143 (range 96-163) ppm after ingesting the 160 g/L-GE solution. The Cmax of ^{2}H was higher during the water trials ($P<0.012$) than the 160 g/L-GE trials but not the 80 g/L-GE trial; no difference in Cmax was observed between the 80- and 160 g/L-GE trials. The time to reach Cmax was significantly shorter after water ingestion than either the 80 g/L-GE ($P<0.07$) or 160 g/L-GE solution ($P<0.009$). These data demonstrate that water uptake, as determined by ^{2}H accumulation, is faster with water ingestion than with a concentrated GE solution. The rate of water uptake, although not significantly different, tended to be faster with an 80 g/L-GE solution compared with a 160 g/L-GE solution. High glucose concentrations in beverages are sufficient to slow the rate of gastric emptying and can result in the net secretion of water into the gut; these two factors could account for the slower rates of water uptake following GE ingestion than with water. There was no evidence from the present data to suggest that water uptake and carbohydrate availability are compromised by a low-level spinal cord lesion.

A comparison of the daily water turnover rates in independent and dependent elderly. By J. PHILLIMORE[2], J. LEIPER[3], W.R. PRIMROSE[1], C.S. PRIMROSE[2], and R. MAUGHAN[3], *[1]Grampian Healthcare NHS Trust, Woodend Hospital, AB15 6LS, [2]Associate Faculty of Nursing, Midwifery and Community Studies, The Robert Gordon University, AB25 2XG and [3]Department of Environmental and Occupational Medicine, University Medical School, Aberdeen AB25 3NN*

Maintaining optimal water balance is essential for homeostasis but is adversely affected by the process of ageing (Kositzke, 1990). The consequences of these disturbances can lead to difficulties with drug metabolism, gastrointestinal problems, altered mental state and, if uncorrected, death (Hoffman, 1991). The present research examines a number of variables of daily water turnover (DWT) in the elderly (over 65 years), including dependency, physical ability and mental function.

Two elderly populations were investigated. A community group: nine males and fifteen females, average age 77 (range 69 - 88) years, who lived in private residences and cared for themselves (n 24); and a dependent group (n 19), who were residents in nursing homes and long-stay wards: five males and fourteen females, average age 82 (72 - 93) years. All volunteers were screened to assess physical function (using the Barthel index) and mental ability (using the abbreviated mental test), renal function and suitability. On the evening before the study commenced, subjects ingested 10.0002 g deuterium oxide (D_2O). They obtained a sample of urine for the seven subsequent mornings at approximately the same time each morning. During this period at least three 24 h urine collections were also obtained. All subjects were requested to maintain their usual pattern of eating, drinking and daily lifestyle. Six subjects in each group were taking low dose diuretics.

Following vacuum distillation of the morning urine sample, the D_2O concentration of the aqueous fraction was determined in duplicate by infra-red spectroscopy. This concentration was used to estimate average DWT assuming that average total body water content remained constant for each subject. Average daily urine output was calculated from the 24 h urine collections including the sample volume used for D_2O measurement. Average daily non-urine water loss (NUWL) was estimated as the difference between the calculated water turnover for that day minus the volume of urine excreted over the same period.

	Community		Dependent		
	Median	Range	Median	Range	P
DWT (litres/d)	2.1	1.0 to 3.6	1.5	0.9 to 2.7	0.002
Daily urine loss (litres/d)	1.7	0.7 to 3.3	1.1	0.6 to 2.7	0.012
NUWL (litres/d)	0.4	-0.3 to 1.4	0.3	0.0 to 0.9	0.32

The Table shows that the community subjects had a significantly (P=0.002) faster water turnover rate than the dependent group. Average median daily urine loss was also greater (P=0.012) in the community subjects than in the dependent volunteers. These results indicate that the dependent subjects did not drink as much as the community volunteers and there are a number of possible explanations for these results. The level of physical function, measured by the Barthel index, was higher in the community group (mean 19.83) than the dependent group (mean 12.79, P<0.001), and may indicate an association between physical function and the ability to maintain adequate hydration. Mental ability was significantly higher in the community group (P=0.001). Subjects in the dependent group relied on nurses and ancillary workers to prepare their drinks, to feed them liquid in some cases, and toilet them. This may have had a bearing on the difference found.

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