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Dietary intakes of polyunsaturated fatty acids and indices of oxidative stress in human volunteers

A Jenkinson[1]*, MF Franklin[2], K Wahle[1] and GG Duthie[1†]

[1]*Rowett Research Institute, Bucksburn, Aberdeen, Scotland and;* [2]*Biomathematics and Statistics Scotland (BioSS), Rowett Research Institute, Bucksburn, Aberdeen, Scotland, UK*

Objective: To assess whether nutritionally-relevant changes in polyunsaturated fatty acid (PUFA) intake alter indices of oxidative stress in human volunteers

Design: A split plot/change over dietary study where half the volunteers consumed a diet containing 5% PUFA (low PUFA) as food energy for 4 weeks and after a 6 week washout period consumed a 15% PUFA (high PUFA) diet for another 4 weeks. The second group of volunteers completed this protocol in reverse. Total fat, carbohydrate, protein and vitamin E contents of the diets were constant.

Subjects: 10 healthy, non-smoking, male volunteers aged 32.6 ± 1.7 y

Results: There was a significant increase in whole blood oxidised glutathione ($P < 0.05$), an index of oxidative stress, after consumption of the high PUFA diet. Moreover, urinary thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation, significantly increased ($P = 0.038$) following consumption of the high PUFA diet and decreased ($P = 0.031$) after consuming the low PUFA diet. However, there was no change in non specific plasma indices of lipid peroxidation, conjugated dienes and TBARS, nor in red cell antioxidant enzymes glutathione peroxidase, glutathione reductase, and catalase. However, superoxide dismutase significantly decreased (13%, $P = 0.018$) after consumption of the low PUFA diet. Total cholesterol increased by 13% ($P = 0.014$) after consumption of the low PUFA diet.

Conclusions: This study indicates that although increasing dietary levels of PUFA may favourably alter cholesterol profiles, the same dietary changes may adversely affect some indices of lipid peroxidation. Care should be taken when providing dietary advice on PUFA intake and an adequate intake of antioxidants to match any increased PUFA may be important for preventing oxidative stress.

Sponsorship: Ministry of Agriculture, Fisheries and Food (MAFF) and the Scottish Office Agriculture, Environment, and Fisheries Department (SOAEFD)

Descriptors: polyunsaturated fatty acids; PUFA; lipid peroxidation; antioxidants; vitamin E; humans

Introduction

Consumption of diets containing polyunsaturated fatty acids (PUFAs) is perceived to be beneficial in the prevention of diseases such as coronary heart disease (Klör *et al* 1997). As a result, although total dietary fat levels have remained constant, intakes of PUFAs in the UK, particularly n-6 PUFAs, have increased to up to 10% of total energy intake at the expense of saturated fatty acids (MAFF 1996). However, PUFAs are highly susceptible to damaging, free radical initiated, lipid peroxidation particularly when intakes of antioxidants such as vitamin E, the major lipid peroxidation chain breaking antioxidant in membranes are low. Increased intakes of PUFAs, without concomitant antioxidant supplementation have been shown to exceed the protective capacity of the antioxidant defence systems and cause myopathies and neuropathies in animals (Arthur, 1982). Moreover, human trials have shown that supplementing the diet with relatively high levels of encapsulated n-3 PUFA increases plasma indices of oxidative stress (Brown and Wahle, 1990; Nair *et al* 1993). This increase could be prevented by increasing α-tocopherol intake (Brown and Wahle, 1990). However, the effect of increasing intake of PUFA by normal dietary means, without use of supplements, is unclear.

Consequently, we have assessed the effect of altering dietary PUFA intake, by manipulating normal components of the Scottish diet and without the use of supplements, on indices of oxidative stress, while concomitantly keeping vitamin E intake at the low levels relevant to the Scottish diet.

Methods

Ten healthy, male non-smokers aged 32.6 ± 1.7 y were recruited, none were taking medication or vitamin and mineral supplements. Basal metabolic rate (BMR) was measured using a ventilated hood system (Deltatrac II, MBM-200, Datex Instrumentation Co. Helsinki, Finland).

Free living subjects participated in a split plot/change over trial where half consumed a diet containing 5% PUFA

*Correspondence: Dr. Alison Jenkinson, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, Scotland, AB21 9SB, UK.

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Table 1 Macronutrient composition of the diets as % total energy of diets

Diet	*Protein*	*Fat*	*CHO*	*Saturated Fat*	*PUFA*	*Monounsaturated Fat*
5% PUFA	16.8 (1.2)	44.9 (2.3)	38.3 (2.2)	24.2 (1.0)	5.5 (0.5)	14.8 (1.4)
15% PUFA	17.2 (1.0)	43.3 (3.2)	39.6 (2.5)	14.8 (2.1)	14.0 (0.5)	14.4 (1.7)

Data are composition of the 7 day rotating menu (mean and s.d), each day analysed in duplicate. CHO = carbohydrate, PUFA = polyunsaturated fatty acids.

as food energy for 4 weeks and after a 6 week washout period consumed a 15% PUFA diet for another 4 weeks (Group A). The other subjects followed an identical protocol except that they consumed the 15% PUFA diet first (Group B).

Blood and Urine Collection and Storage

Blood (up to 50 ml) was removed from an anticubital vein into EDTA vacutainers (Becton Dickinson, France). Blood and urine samples were obtained at weeks- 2, 0, 2, 4 (diet 1), and 10, 12, 14 (diet 2), after an overnight fast. The study was approved by the Joint Ethical Committee of the Grampian Region and the University of Aberdeen, and subjects gave their informed consent in writing.

Blood samples were stored on ice for a maximum of 1 h prior to centrifugation (4°C, 2400 × *g*, 15 min). Plasma was divided into aliquots with 2% (w/v) butylated hydroxytoluene (BHT) added (25 μl per 600 μl plasma), frozen in liquid N_2, and stored at − 70°C. The erythrocytes were also stored at − 70°C after being washed twice with phosphate buffered saline (PBS; pH 7.4) and resuspended in PBS to the original blood volume. Additionally, urine aliquots were stored with BHT (25 μl per 700 μl urine) at − 70°C.

Table 2 Fatty acid composition of the diets

Fatty acid	*7 day mean 5%*		*7 day mean 15%*	
8:0	0.23	(0.14)	0.08	(0.05)
10:0	1.31	(0.14)	0.35	(0.16)
12:0	3.17	(1.45)	1.02	(0.55)
14:0	7.57	(0.60)	2.61	(0.66)
a	0.20	(0.01)	0.07	(0.02)
ai	0.35	(0.03)	0.12	(0.04)
14:1	0.56	(0.04)	0.21	(0.08)
15:0	0.81	(0.05)	0.30	(0.08)
15:1	0.20	(0.01)	0.08	(0.02)
16:0	27.07	(0.49)	18.95	(1.32)
16:1 *trans*	0.75	(0.09)	0.29	(0.05)
16:1 *cis*	1.40	(0.19)	0.82	(0.29)
17:0	0.57	(0.05)	0.28	(0.08)
17:1	0.24	(0.08)	0.18	(0.11)
18:0	11.72	(0.74)	8.45	(1.15)
18:1 *trans*	3.35	(0.42)	3.62	(1.27)
18:1 *cis*	26.19	(1.39)	28.16	(1.24)
18:2 *trans*	0.51	(0.04)	0.41	(0.05)
18:2 *cis*	9.25	(0.77)	28.17	(1.94)
18:3 *gam*	0.08	(0.08)	0.20	(0.04)
18:3	1.17	(0.22)	2.91	(0.37)
19:0	0.29	(0.30)	0.10	(0.02)
20:0	0.29	(0.06)	0.35	(0.05)
20:1	0.29	(0.05)	0.42	(0.10)
20:2	0.46	(0.09)	0.27	(0.06)
20:3	0.08	(0.04)		
20:4	0.11	(0.02)	0.11	(0.04)
20:5	0.09	(0.05)	0.29	(0.39)
22:0	0.17	(0.03)	0.30	(0.04)
22:1				
22:6	0.20	(0.12)	0.26	(0.13)
Total	98.53	(0.64)	99.30	(0.66)
P:S Ratio	0.23		0.95	

Data are composition of the 7 day rotating menu (mean and s.d), each day analysed in duplicate. P:S ratio = polyunsaturated fatty acids: saturated fat ratio.

Dietary Analysis

Diets consisted of normal foods available from local stores and were analysed for protein, following the Foss-Heraus Macro-N instruction manual, and fat and carbohydrate using standard methods (MAFF, 1985; Southgate, 1976). Fatty acid compositions were measured using GC after lipid extraction and preparation of methyl esters (Christie, 1982; Hanson and Olley, 1963). Dietary vitamins C and E were measured by HPLC using previously published procedures (Ross, 1994; Hess *et al*, 1991).

Total fat, protein and carbohydrate levels were constant in both diets (Table 1). However, although monounsaturated fat levels were consistent, the amounts of polyunsaturated and saturated fats expressed as a percentage of energy varied. The ratio of n-6 : n-3 fatty acids and the amounts of trans fatty acids were not different between the diets (Table 2). Amounts of α-tocopherol in both diets were below 10 mg/d (Table 3). There was no difference in the concentration of vitamin C in the high and low PUFA diets (Table 3).

Biochemical Analysis

Lipid peroxidation was estimated by measurement of plasma concentrations of thiobarbituric acid reactive substances (TBARS), using a modified fluorimetric method (Duthie *et al* 1992) with tetramethoxypropane (Aldrich, U.K.) as a standard. Plasma conjugated dienes were measured in hexane extracts as the first derivative of their ultraviolet (UV) spectrum (Hunter and Mohamed 1986). Plasma vitamin E was measured by reverse phase HPLC

Table 3 Vitamin E and Vitamin C levels (mg/d) in the diets

Diet	*α-tocopherol*	*γ-tocopherol*	*Total tocopherol* (α + γ)	Vitamin C
5% PUFA	5.09 (1.02)	3.68 (1.73)	8.77 (2.35)	69.4 (2.9)
15% PUFA	6.92 (2.32)	8.11 (2.51)	15.02 (3.30)	73.8 (2.4)

Data are composition of the 7 d rotating menu (mean and s.d), each day analysed in triplicate.

using a method adapted from Hess *et al*. (1991). Urinary TBARS were also measured by HPLC (Wong *et al* 1987). Total and HDL cholesterol were estimated, using a Kone dynamic discrete analyser and haemoglobin was measured using Drabkins reagent (Sigma, Poole, Dorset, diagnostics procedure no 525). Concentrations of reduced (GSH) and oxidised (GSSG) glutathione were measured using a standard method (Allen and Arthur, 1987). The activity of the following enzymes were also measured using standard procedures; glutathione peroxidase (EC 1.11.1.9) (Paglia and Valentine, 1967); glutathione reductase (EC 1.6.4.2) (Galbraith and Watts, 1980); catalase (EC 1.11.1.6) (Aebi, 1984); and superoxide dismutase (EC 1.15.1.1) (Arthur and Boyne, 1985).

*Statistical Anal*ysis

The trial was arranged as a two period cross-over trial. There were two groups of subjects and two treatments, high (T15) and low (T5) intakes of PUFA. In each period one group received high and one received low PUFA diets but the treatments were switched between periods. Interest centred upon the total change observed over the period that the treatments were administered. For some variables this was assessed as *change = final* value-initial value; for others it was assessed as *change = final value/initial value* (or rather) *change = log(final value/initial value) = log(final value)-log(initial value)*. In each period the mean changes observed for the two treatments were compared using two sample *t*-tests with significance level $P = 0.05$. The results for the two periods were combined as follows: for each subject difference = change for T5 − change for T15 was calculated. These differences were analysed and the mean difference was compared to zero using a single sample *t*-test with error based on the within groups variance and significance level $P = 0.05$. (This test is equivalent to the test of treatment differences given by the analysis of variance for a cross-over trial.) In the tables we report the mean observed changes together with standard deviations.

Table 4 Anthropometric characteristics of the subjects

	Group A	*Group B*	*P*
Age (y)	34.8 (2.8)	29.3 (7.2)	N.S.
Height (cm)	179.4 (2.8)	175.4 (6.6)	N.S.
Weight (kg)	78.3 (6.3)	69.5 (8.0)	N.S.
BMR (kcal)	1741.6 (88.5)	1591.2 (97.6)	N.S.

Data presented are mean (s.d). Group A consists of subjects who consumed the 5% PUFA diet first, then the 15% PUFA diet. Group B subjects followed the reverse procedure. BMR = basal metabolic rate

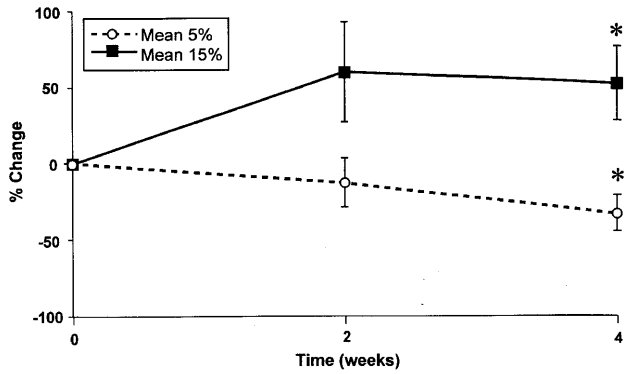

Figure 1 Urinary TBARS (percentage change and s.d.). The results of groups A and B have been combined to show results from the beginning, middle and end of diets. * Significance from baseline $P < 0.05$.

Results

The means ($\pm$ sd) of major anthropometric parameters of group A were similar to group B (Table 4). Urinary TBARS in groups A and B showed a significant increase ($P = 0.038$) following consumption of the high PUFA diet and a significant decrease ($P = 0.031$) following consumption of the 5% PUFA diet (Figure 1). These changes were not reflected by similar changes in concentrations of TBARS and conjugated dienes in plasma over time on either diet (Table 5). Total and reduced glutathione in whole blood did not change over both diets 1 and 2, although there was an increase during the washout period (Figure 2). A significant increase in whole blood oxidised glutathione ($P < 0.05$) was associated with consumption of the 15% diet and this appeared to decline over the washout and 5% diet (Figure 2). Erythrocyte antioxidant enzymes glutathione peroxidase, glutathione reductase and catalase did not change over time on either diet (Table 6). However, red cell superoxide dismutase significantly decreased ($P = 0.018$) following consumption of the low PUFA diet (Table 6). Total plasma cholesterol showed a small but significant increase ($P = 0.014$) in both groups on the 5% PUFA diet with no change on the 15% diets (Figure 3). There was no significant change in HDL cholesterol over time (Figure 4). Plasma α-tocopherol did not change following consumption of the high PUFA diet although there was a small but significant ($P < 0.001$) decrease in α-tocopherol concentrations after intake of the low PUFA diets (Figure 5). However, the plasma γ-tocopherol concentrations did not change after consumption of either the 5 or 15% PUFA diets (Figure 6).

Table 5 Plasma conjugated dienes and TBARS concentrations over time, following consumption of diets

Week	*− 2*	*0*	*2*	*4*	*10*	*12*	*14*
Conjugated Dienes μgHODE equiv/ml							
Group A	15.32 (5.50)	15.77 (6.12)	21.22 (2.35)	21.46 (2.68)	17.63 (6.18)	11.88 (3.60)	13.19 (2.12)
Group B	21.13 (6.11)	15.94 (4.09)	15.18 (4.92)	18.83 (12.23)	24.62 (3.69)	21.04 (12.06)	21.90 (5.75)
Plasma TBARS nmol/ml							
Group A	0.72 (0.11)	0.65 (0.15)	0.73 (0.14)	0.78 (0.17)	0.65 (0.05)	0.79 (0.03)	0.66 (0.12)
Group B	0.76 (0.12)	0.80 (0.21)	0.73 (0.15)	0.63 (0.20)	0.83 (0.30)	0.71 (0.23)	0.73 (0.18)

Data presented are mean (s.d) over time. Group A = 5% then 15% PUFA, Group B = 15% then 5% PUFA diets. TBARS = thiobarbituric reactive substances.

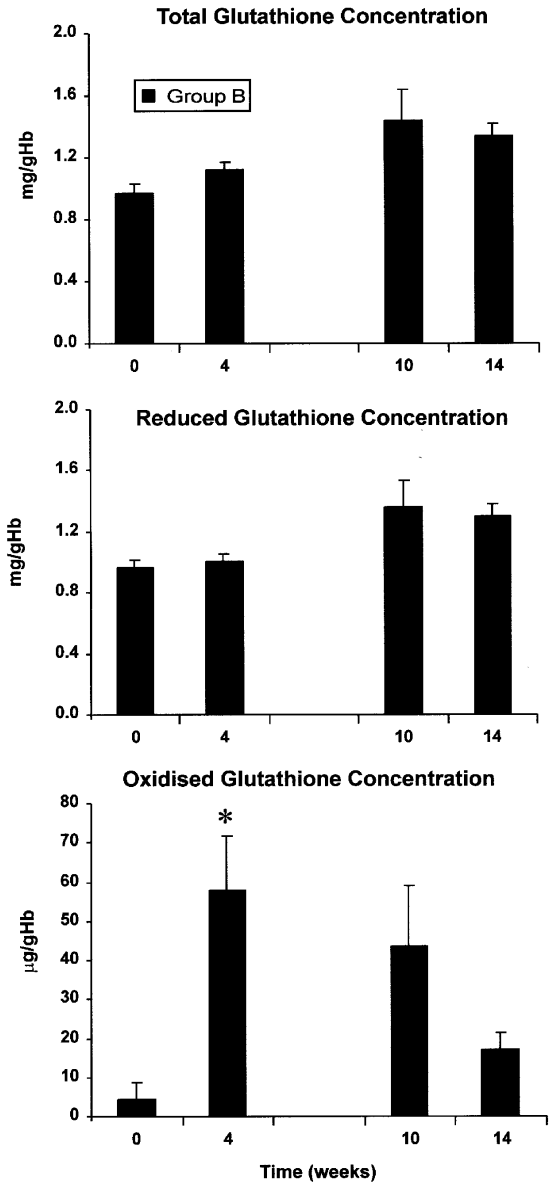

Figure 2 Whole blood total, reduced, and oxidised glutathione concentrations across time following consumption of a high then low PUFA diet (group B). Data presented are mean (s.d) across time. * Significance from baseline $P < 0.05$.

Discussion

These results suggest that some indices of oxidative stress may be modified by altering dietary PUFA intake. Urinary TBARS, an index of oxidative stress, showed a significant decrease in both groups on the 5% PUFA diet suggesting a decrease in oxidative stress. Dhanakoti and Draper (1987) found similar results in rats with a low PUFA diet producing significantly lower amounts of urinary TBARS than high PUFA diets. However, plasma TBARS and conjugated dienes did not significantly change on either diet. These methods are relatively non-specific and may measure products other than those resulting from lipid peroxidation *in vivo* (Halliwell and Chirico, 1993), particularly when assessing dietary changes at nutritionally relevant levels. However, oxidised glutathione (GSSG) in whole blood appears to be a more sensitive marker of oxidative stress because levels significantly ($P < 0.05$) increased after 4 weeks on a high PUFA diet. Moreover, there was an elevated level of GSSG at baseline when starting the 5% PUFA diet indicating that the effect of the earlier 15% diet was continuing through the washout period into the second dietary study. Therefore, the washout period may not have been sufficiently long to prevent the 15% diet from influencing the 5% diet results. Total and reduced glutathione were unchanged on the high PUFA diet and the apparent increase in these levels during the washout period also suggests that the length of the washout period may require to be increased in future studies. The selenium enzyme glutathione peroxidase in red cells did not change on either diet suggesting that the selenium levels in the diets were constant. There was also no change in other antioxidant enzymes, glutathione reductase and catalase, in red cells, possibly suggesting that any dietary imposed oxidative stress was insufficient or over too short a period to induce an adaptive upregulation in enzyme activity. However, superoxide dismutase concentrations did decrease after consumption of the low PUFA diet indicating a possible reduction in oxidative stress.

A small but significant increase in total cholesterol on the 5% PUFA diet may be due to the purported cholesterol raising effects of saturated fats such as palmitic acid (Grundy and Vega, 1988). However, such a small change may also reflect natural day to day variation in total cholesterol levels and as a result have little biological

Table 6 Red cell antioxidant enzyme activity over time, following consumption of diets

Week	*− 2*	*0*	*2*	*4*	*10*	*12*	*14*
Glutathione Peroxidase U/gHb							
Group A	59.80	62.20	59.91	65.36	62.08	62.59	62.45
	(11.28)	(13.04)	(11.15)	(15.28)	(15.60)	(10.11)	(18.37)
Group B	52.34	59.87	60.98	55.76	58.99	61.62	54.94
	(10.76)	(13.31)	(15.78)	(12.72)	(16.88)	(15.04)	(12.68)
Glutathione Reductase U/gHb							
Group A	2.08 (0.61)	2.02 (0.51)	2.15 (0.60)	2.25 (0.66)	1.96 (0.24)	2.45 (0.44)	2.24 (0.66)
Group B	1.91 (0.22)	2.33 (0.09)	2.97 (0.73)	2.52 (0.92)	2.64 (0.62)	2.86 (1.11)	2.45 (0.91)
Catalase k/gHb							
Group A	1904.59	1390.43	2460.65	1988.74	1782.06	1728.28	2107.07
	(290.04)	(277.29)	(134.81)	(829.48)	(519.15)	(321.72)	(269.01)
Group B	1698.31	2257.73	2611.97	1954.83	2076.92	2360.41	2022.74
	(672.52)	(317.99)	(705.91)	(654.53)	(339.86)	(454.58)	(391.71)
Superoxide Dismutase mg/gHb							
Group A	0.32 (0.03)	0.34 (0.04)	0.31 (0.07)	0.33 (0.08)*	0.29 (0.03)	0.30 (0.03)	0.27 (0.02)
Group B	0.29 (0.04)	0.31 (0.04)	0.30 (0.01)	0.32 (0.06)	0.33 (0.02)	0.30 (0.06)	0.24 (0.01)*

Data presented are mean (s.d) across time. Group A = 5% then 15% PUFA, Group B = 15% then 5% PUFA diets.* Significance $P < 0.05$ from baseline — results from groups A and B combined for statistical analysis.

significance. There was no change in HDL cholesterol levels suggesting that, neither the high or the low PUFA diet influenced HDL. Concentrations of plasma α-tocopherol did not change with the high PUFA diet confirming that the levels of dietary α-tocopherol were similar to the normal diet. However, there was a small decrease in plasma α-tocopherol concentrations following consumption of the low PUFA diet. This may be a result of slightly lower dietary α-tocopherol levels in the low PUFA diet which although not significant from the high PUFA diet may, over a period of time, cause a lowering of plasma levels. There was no significant change in plasma levels of γ-tocopherol.

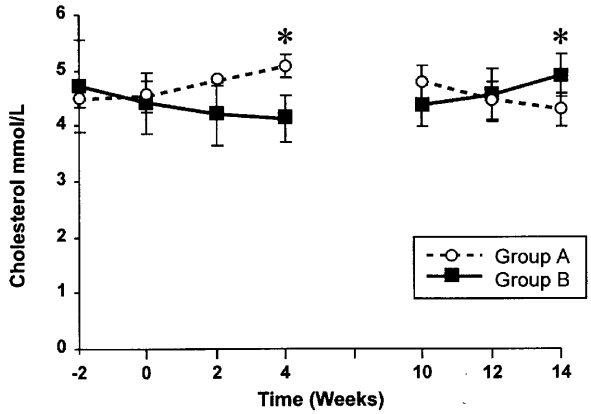

Figure 3 Plasma total cholesterol across time following consumption of diets (Group A = 5% then 15% PUFA, Group B 15% then 5% PUFA). Data presented are mean (s.d) across time. Results from groups A and B combined for statistical analysis. * Significance from baseline $P < 0.05$.

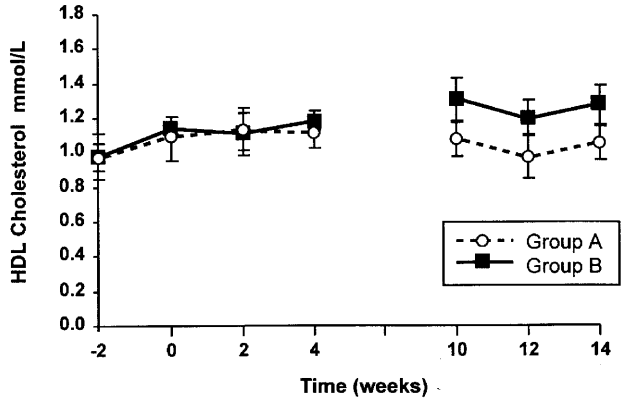

Figure 4 Plasma HDL cholesterol across time following consumption of diets (Group A = 5% then 15% PUFA, Group B 15% then 5% PUFA). Data presented are mean (s.d) across time. No significant changes.

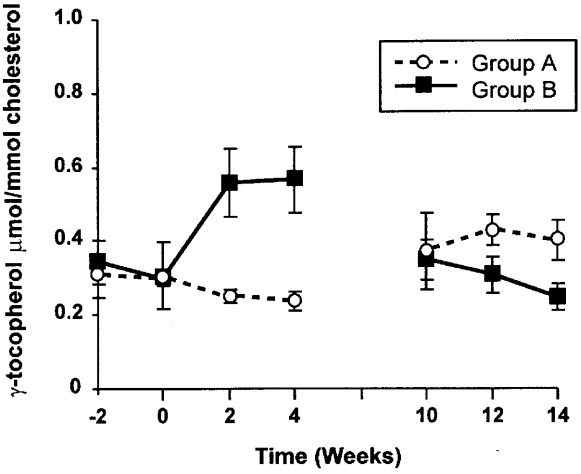

Figure 6 Plasma γ-tocopherol. Means and s.d. across time, following consumption of diets (Group A = 5% then 15% PUFA, Group B 15% then 5% PUFA). Data presented are mean (s.d) across time. No significant changes.

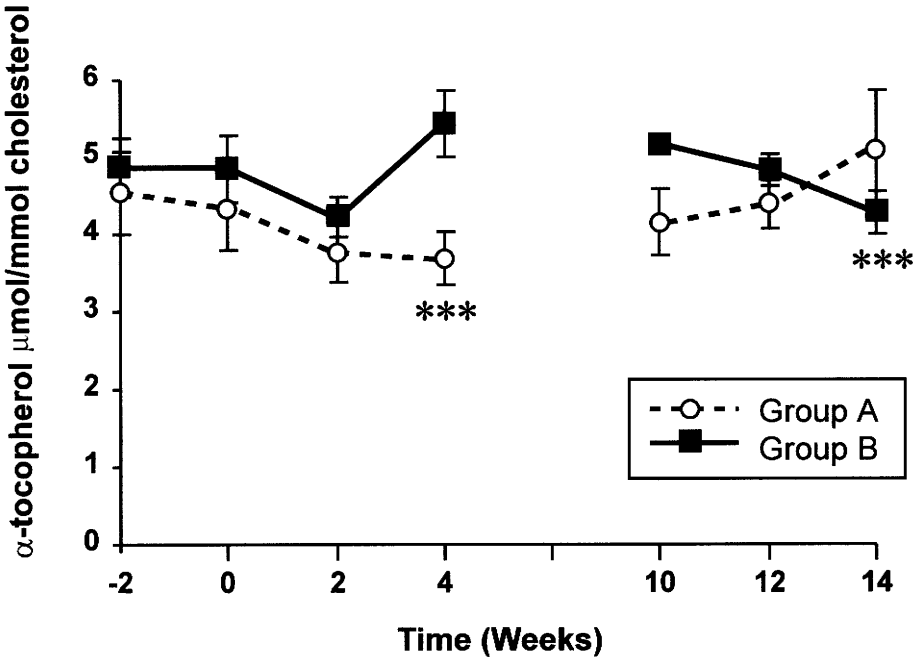

Figure 5 Plasma α-tocopherol. Means and s.d. across time following consumption of diets (Group A = 5% then 15% PUFA, Group B 15% then 5% PUFA). Data presented are mean (s.d) across time. Results from groups A and B combined for statistical analysis. *** Significance from baseline $P < 0.001$.

Conclusion

In conclusion, this study indicated that although alteration of dietary levels of PUFA may favourably modify cholesterol profiles, the same dietary changes may adversely affect some indices of lipid peroxidation. The study also highlights the possibility that a high PUFA diet with low levels of vitamin E is attainable with typical foods and suggests that care should be taken when providing dietary advice. A recommendation to increase vitamin E intake may be required when PUFA intakes are increased. The effect of high and low PUFA diets requires to be investigated further with vitamin E supplementation and longer washout periods.

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References

Aebi H (1984): Catalase in vitro. *Methods in Enzymol.* **105**, 121–126.

Allen KJD and Arthur JR (1987): Inhibition by 5-sulphosalicylic acid of glutathione reductase recycling assay for glutathione analysis. *Clin Chim Acta* **162**, 237–239.

Arthur, J.R. (1982): Nutritional interrelationships between selenium and vitamin E. *Rowett Research Institute Annual* Report **38**, 124–135.

Arthur JR and Boyne R (1985): Superoxide dismutase and glutathione peroxidase activities in neutrophils from selenium deficient and copper deficient cattle. *Life Science* **36**, 1569–1575.

Brown JE and Wahle KWJ (1990): Effect of fish-oil and vitamin E supplementation on lipid peroxidation and whole-blood aggregation in man. *Clin Chim Acta* **193**, 147–156.

Christie WW (1982): *Lipid Analysis* 2nd edn, Oxford: Pergammon Press.

Dhanakoti SN and Draper HH (1987): Response of urinary malonaldehyde to factors that stimulate lipid peroxidation *in vivo*. *Lipids* **22**, 643–646.

Duthie GG, Morrice PC, Ventresca PG, McLay JS (1992): Effects of storage, iron and time of day on indices of lipid peroxidation in plasma from healthy volunteers. *Clin. Chim. Acta,* **206**, 207–213.

Galbraith DA and Watts DC (1980): Changes in some cytosolic enzymes from red cells fractionated into age groups by centrifugation in Ficoll/Triosil gradients. *Biochem. J.* **191**, 63–70.

Grundy SM and Vega GL (1988): Plasma cholesterol responsiveness to saturated fatty acids. *Am. J. Clin. Nutr.* **47**, 822–824.

Hanson SWF and Olley J (1963): Application of the Bligh and Dyer method of lipid extraction to tissue homogenates. *Biochem. J.* **89**, 101.

Halliwell B and Chirico S (1993): Lipid peroxidation: its mechanism, measurement, and significance. *Am. J. Clin. Nutr.* **57** (Suppl), S715–S725.

Hess D, Keller HE, Oberlin B, Bonfanti R, Schuet W (1991): Simultaneous determination of retinol, tocopherols, carotenes and lycopene in plasma by means of high-performance liquid chromatography on reverse phase. *Int. J. Vit. Nut. Res.* **61**, 232–238.

Hunter I and Mohamed J (1986): Plasma antioxidants and lipid peroxidation products in Duchenne muscular dystrophy. *Clin Chim Acta* **155**, 123–132.

Klör HU, Hauenschild A, Holbach I, Schnell-Kretschmer H, Stroh S. (1997): Nutrition and cardiovascular disease. *Eur. J. Med. Res.* **2**, 243–257.

MAFF (1985): *The feedstuffs (sampling and analysis) regulations* (as amended) SI No.1119. London: HMSO.

MAFF National Food Survey (1996): *Annual Report on Household Food Consumption & Expenditure*. London: HMSO, 110 pp

Nair PP, Judd JT, Berlin E, Taylor PR, Shami S, Sainz E, and Bhagavan HN (1993): Dietary fish oil-induced changes in the distribution of α-tocopherol, retinol, and β-carotene in plasma, red blood cells, and platelets: modulation by vitamin E. *Am. J. Clin. Nutr.* **58**, 98–102.

Paglia DE and Valentine WN (1967): Studies on the quantitative and qualitative characteristics of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* **70**, 158–169.

Ross MA (1994): Determination of ascorbic acid and uric acid in plasma by high-performance liquid chromatography. *J. Chromatogr. B. Biomed. Appl.* **657**: 197–200.

Southgate DAT (1976): *Determination of Food Carbohydrates*. London, Applied Science Publishers

Wong S, Knight JA, Hopfer SM, Zaharia O, Leach CN Jr, and Sunderman FW Jr. (1987): Lipid peroxides in plasma as measured by liquid chromatographic separation of malondialdehyde-thiobarbituric acid adducts. *Clin Chem* **33**, 214–220.