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Cardiovascular Research 59 (2003) 955–962

Cardiovascular Research

www.elsevier.com/locate/cardiores

The effects of dietary fatty acid supplementation on endothelial function and vascular tone in healthy subjects

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Received 10 December 2002; accepted 9 April 2003

Abstract

Objective: Evaluation of the effects of supplementation of n-3 and n-6 fatty acids on vascular tone and endothelial function in healthy men and women aged 40 to 65 years. **Methods:** In a double-blind, randomised, placebo controlled study, 173 healthy volunteers took one of six oil supplements for 8 months. Supplements were placebo, oleic acid rich sunflower oil, evening primrose oil, soya bean oil, tuna fish oil, and tuna/evening primrose oil mix. Endothelium-dependent and independent vascular responses were measured in the forearm skin using laser Doppler imaging following iontophoretic applications of acetylcholine and sodium nitroprusside, respectively. **Results:** Acetylcholine, but not sodium nitroprusside responses were significantly improved after tuna oil supplementation ($P=0.02$). Additionally, there were significant positive correlations between acetylcholine responses and n-3 fatty acid levels in the plasma and erythrocyte membrane phospholipids after tuna oil supplementation. No significant changes in vascular response were seen after supplementation with any of the other oils. **Conclusions:** Fish oil supplementation has a beneficial effect on endothelial function, even in normal healthy subjects. Modification of the diet by an increase of 6% in eicosapentaenoic acid and 27% in docosahexaenoic acid (equivalent to eating oily fish 2–3 times/week) might have significant beneficial effects on cardiovascular function and health.

Keywords: Acetylcholine; Endothelial function; Microcirculation; Nitric oxide; Vasoconstriction/dilation

1. Introduction

The prevalence of coronary heart disease in Scotland is very high and possibly linked to diet [1]. Coronary heart disease has been described as Scotland's national disease and ways of reducing its incidence are therefore important. The manipulation of essential fatty acids within the diet might have profound long-term effects on the development of cardiovascular disease. This concept is now supported by an extensive literature, including epidemiological, interventional and clinical studies [2], but began in studies of Greenland and Alaskan Eskimos, where (n-3) fatty acid intake from marine sources is high and there is a lower mortality rate from ischemic heart disease, compared with European and North American populations [3]. Cross-sectional studies from the Netherlands [4] and the United States [5], and secondary prevention intervention trials [6,7] indicate that a modest diet of fish (2 times/week) or fish oil consumption is associated with a reduction in cardiovascular events and mortality.

The protective effect of fish is related to high concentrations of n-3 polyunsaturated fatty acids (PUFAs) in the form of eicosapentaenoic acid (EPA, $C_{20:5}$ n-3) and docosahexaenoic acid (DHA, $C_{22:6}$ n-3). These fish-derived long-chain n-3 PUFAs have anti-inflammatory and

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Time for primary review 30 days.

0008-6363/03/$ – see front matter
doi:10.1016/S0008-6363(03)00395-X

anti-thrombotic effects through prostaglandin, leukotriene and cytokine pathways [8]. Recent studies have shown that arterial endothelium-dependent dilation is improved by dietary supplementation with marine n-3 fatty acids in patients with hypercholesterolemia [9,10]. The role of diet in endothelial function has recently been reviewed by Brown and Hu [11]. In the recent study by Leeson et al. [12] in normal young adults no relationship was found between circulating levels of n-3 fatty acids (derived from normal dietary variation) and endothelial function in the whole group, but a positive association was present only in those subjects who smoked or had higher levels of insulin, glucose or triglycerides. To date, no studies have examined the effects of dietary supplementation of n-3 and n-6 fatty acids on vascular function in normal healthy subjects. The objective of the current study therefore, was to evaluate the effects of dietary achievable supplementation of n-3 and n-6 fatty acids on microvascular blood flow and endothelial function in healthy subjects.

2. Methods

This was a single-centre, 8-month, randomised, double blind, placebo-controlled study in the Department of Medicine and Cardiovascular Epidemiology Unit at Ninewells Hospital, Dundee, Scotland during January 1997 to April 1999. Volunteers gave written, informed consent to participate, and the Tayside Committee on Medical Research Ethics approved the protocol. The investigation conforms with the principles outlined in the Declaration of Helsinki. Two hundred and six healthy, non-smoking subjects were enrolled. After initial screening and baseline visit, subjects were randomised to one of six oil supplementation groups. On the study days at baseline and at approximately 8 months, subjects arrived fasted. Blood was taken for fatty acid analysis, and vascular reactivity tests were performed.

2.1. Composition of oil supplements

The supplements consisted of peppermint-flavoured 20% oil–water emulsions, containing 100 mg tocopherol antioxidant/daily 50 ml dose: Placebo oil (Scottish diet control): 10 g/day containing 25% soya bean oil and 75% fractionated coconut oil. This mix reflects the standard daily fatty acid intake of the Scottish population (Horrobin, personal communication, 1996). Oleic acid-rich sunflower oil: 10 g/day to provide an approximate 20% increase in dietary oleic acid intake ($C_{18:1}$ n-9). Evening primrose oil (EPO): 5 g/day+5 g/day placebo oil. The EPO provided 400 mg/day of γ-linolenic acid (GLA, $C_{18:3}$ n-6), which would more than double usual intake. Soya bean oil: 5 g/day+5 g/day placebo oil. This was estimated to provide an approximate 30% increase in dietary linolenic acid ($C_{18:2}$ n-6) intake. Tuna fish oil: 5 g/day+5 g/day placebo oil. The tuna oil consisted of 6% eicosapentaenoic acid (EPA, $C_{20:5}$ n-3) and 27% docosahexaenoic acid (DHA, $C_{22:6}$ n-3), which were, respectively, estimated to increase usual intake by about 200 and 400%. This is equivalent to regular eating of oily fish 2–3 times/week. EPO/tuna oil mix: 5 g/day of each oil. Table 1 shows the fatty acid composition of the six oil supplements (mg/10 g oil, equivalent to 50 g emulsion).

2.2. Dietary assessment and monitoring procedures

Usual dietary intake was assessed using a food frequency questionnaire [13] at baseline and endpoint. At regular 6–8 weekly supplement re-supply visits, the volume of supplement returned was measured for compliance, weight measured and any dietary change monitored with a 24 h dietary recall. The level of physical activity was assessed by questionnaire and documented as active, moderate or inactive during work and leisure time. The number of times per week engaged in physical activity for ≥20 min that resulted in breathlessness and sweating was also noted. Any changes in level of physical activity and general health were discussed and noted. Recorded food intakes were assessed for adequacy by expressing total energy intake as a ratio to calculated basal metabolic rate (<1.2 indicates low energy reporting). UK food composition tables were used to estimate daily total fat, saturated fatty acids, total PUFAs and n-3 and n-6 PUFAs intake, and the percentage change in fatty acid intakes

Table 1
Content of key fatty acids in the supplement oils in the six different groups

Fatty acid (mg/10 g emulsion)	Placebo	Oleate	EPO	Soya	Tuna	EPO/tuna
Palmitic acid $C_{16:0}$	64	119	79	171	174	274
Stearic acid $C_{18:0}$	20	75	24	25	53	56
Oleic acid $C_{18:1}$	166	1448	146	327	206	188
Linoleic acid $C_{18:2}$	231	318	666	622	223	418
Linolenic acid $C_{18:3}$	34	7	22	112	27	10
Gamma linolenic acid $C_{18:3\gamma}$	0	2	66	0	3	78
Arachidonic acid (AA) $C_{20:4}$	–	–	–	–	16	14
Eicosapentaenoic acid (EPA) $C_{20:5}$	–	–	–	–	35	35
Docosahexaenoic acid (DHA) $C_{22:6}$	2	0	0	0	188	197

Values are means.

were calculated from their own dietary records, for each individual.

2.3. Measurement of vascular responses

Endothelium-dependent and endothelium-independent responses to iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP), respectively, were measured in the forearm skin as previously described [14]. ACh (Sigma, St. Louis, MO, USA), and SNP (David Bull Laboratories, Warwick, UK) were made up to 1% solutions in deionized, sterile water. Experiments were performed in a quiet, temperature controlled laboratory set at 22–23 °C. Subjects were given a 30 min equilibration period and were seated comfortably with the arms supported at heart level. A direct iontophoresis electrode chamber (internal diameter 20 mm, height 7 mm) was fixed to the skin with adhesive tape midway between the volar surface of wrist and elbow and filled with 2 ml of solution. The indifferent electrode was placed around the wrist to complete the circuit. When an electrical potential difference is established, ions of the drug migrate across the skin, and the dose delivered is therefore a product of the magnitude and duration of current. We used a current of 100 μA for drug delivery, which does not cause non-specific electrical effects with the electrodes we used. ACh and SNP were iontophoresed for a total of 150 s using anodal and cathodal currents, respectively to give a total charge (current×time) of 15 milliCoulombs (mC).

Skin perfusion was measured using a laser Doppler imager (moorLDI, Moor Instruments, Axminster, UK). A 2 mW helium–neon laser scans the surface of the skin, and light back scattered from moving erythrocytes is shifted in frequency by an amount proportional to their velocity. For each scan, the computer builds up a colour-coded image representing skin perfusion in arbitrary units. The recorded images were analysed using dedicated image-processing software (moorLDI 3.1, Moor Instruments). For each drug the peak perfusion value was divided by the baseline value to give a ratio representing change in perfusion.

2.4. Lipid extraction and fatty acid analysis

Fatty acid compositions of plasma total phospholipid and erythrocyte sphingomyelin, phosphatidylcholine, phosphatidylserine and phophatidylethanolamine were measured using an in house gas chromatography method [15]. In brief, blood samples were separated into total plasma phospholipids and individual erythrocyte and platelet phospholipids by extraction and thin-layer chromatography. Phospholipid samples were methylated to produce fatty acid methyl esters. Fatty acid methyl ester samples were analysed using a Unicam 610 series gas chromatograph equipped with a flame ionisation detector, a capillary column and a 610 series autosampler.

2.5. Statistical analysis

The SPSS statistical package was used for all statistical analyses. Data that were not normally distributed were analysed using non-parametric tests. Baseline differences in vascular responses amongst the groups were analysed using the Kruskal–Wallis test. Differences in vascular responses to ACh and SNP before and after supplementation were compared using the Wilcoxon test. Correlations were performed using the Spearman Rank test. Significance was acknowledged if the probability of a type-1 error was less than 5% ($P<0.05$).

3. Results

The final study population consisted of 118 males (age 52±7 years [mean±S.D.]), body mass index 26.6±2.8) and 55 females (age 56±5 years, body mass index 25.2±3). All females were confirmed postmenopausal of at least >2 years. The number taking hormone replacement therapy (11) was split equally amongst the six groups (Table 2). Forty-eight subjects were former smokers, all having stopped ≥2 years. There were no significant differences in distribution of these between the groups. No significant differences were observed in physical activity

Table 2
Baseline characteristics of subjects in the six groups

	Placebo	Oleate	EPO	Soya	Tuna	Tuna/EPO
Age (years)	52±1	54±1	53±1	53±1	55±1	55±1
Male/female	20/8	20/10	23/8	19/9	19/9	17/11
No. on HRT	2	1	1	2	2	3
BMI	25.9±3.3	26.2±3.2	25.1±2.5	26.0±2.5	25.5±2.4	26.3±2.7
Cholesterol (mmol/l)	5.6±0.2	5.6± 0.2	5.0±0.1	5.6±0.2	5.6±0.2	5.6±0.2
RBC×10^{12}/l	4.67±0.09	4.62±0.08	4.51±0.06	4.68±0.09	4.66±0.08	4.57±0.05
WBC×10^{9}/l	5.36±0.01	5.21±0.28	5.23±0.26	5.32±0.28	5.13±0.19	5.31±0.26
Haemoglobin (g/dl)	14.6±0.3	14.3±0.2	14.6±0.2	14.1±0.2	14.3±0.2	14.4±0.2
Haematocrit (%)	42.6±0.8	43.1±0.7	41.9±0.6	42.4±0.6	42.7±0.7	43.3±1.1

Values are means±S.E.M. There were no significant differences amongst the six groups in any parameter.
HRT=Hormone replacement therapy.

between groups, or over the course of the study. Numbers were lost from the total consented by non-attendance at the first visit, and subsequent dropout. Reasons for dropout included dislike of the supplement, stomach problems, moving away, or perceived side effects such as weight gain. Weight change amongst the six groups did not differ significantly and averaged +0.9 kg, which was not a significant increase over baseline weight. Total cholesterol was not significantly different amongst the six groups before supplementation (Table 2) and did not change in any group after supplementation (range 5.19–5.72 mmol/l). Similarly, there were no significant differences amongst the six groups in any of the parameters described in Table 2, or any changes in these measurements after supplementation.

3.1. Compliance

Mean (±S.D.) daily supplement consumption was 49.9±3.0 ml/day, with a minimum of 37.1 ml/day and maximum of 58.1 ml/day. Changes in both plasma phospholipid fatty acids and vitamin E/cholesterol ratio after oil supplementation were also used to assess compliance. Supplementation with each oil elicited the changes expected in the plasma phospholipid fatty acids both in terms of the increase in the major fatty acids or fatty acid metabolite of interest (Table 3). This, taken with the observed increase in vitamin E/cholesterol ratio suggests that in general the subjects complied with their oil supplement regime over the course of the study.

3.2. Baseline vascular responses

Vascular responses to ACh and SNP before supplementation were not significantly different amongst the six groups ($P=0.289$ and $P=0.913$, respectively) (Table 4). There were no significant correlations between fatty acid levels and vascular responses within the six groups.

3.3. Vascular responses after oil supplementation

Using multivariate analysis for all augmented fatty acids and vascular responses after supplementation in the whole study group, we found significant positive correlations between the peak ACh response and: EPA and DHA in phosphatidylcholine ($r=0.23$, $P=0.007$ and $r=0.24$, $P=0.006$, respectively) and EPA in phosphatidylethanolamine ($r=0.23$, $P=0.009$).

Table 4 shows that there were no significant differences in ACh and SNP responses before and after placebo, oleate, EPO, soya and EPO/tuna supplementation, although there was a trend towards a decrease in SNP responses after placebo supplementation ($P=0.06$).

In contrast there was a significant augmentation in responses to ACh, but not SNP, in the tuna-supplemented group ($P=0.02$) (Table 4). ACh responses before supple-

Table 3
Changes in selected plasma phospholipid fatty acids (% of total fatty acids) and vitamin E/cholesterol ratio ($\times 10^{-3}$)

Oil group	Fatty acid or vitamin E	Before supplementation	After supplementation	*P* value
Placebo	Linoleic acid	20.30±7.95	21.20±7.43	0.039
(*n*=28)	DHA	2.47±1.99	2.13±1.72	0.004
	Vitamin E/cholesterol	4.22±1.86	7.10±3.85	0.0001
Oleate	Oleic acid	8.38±2.78	9.35±4.25	0.005
(*n*=30)	DHA	2.94±2.92	2.33±2.26	0.0001
	Vitamin E/cholesterol	4.23±2.25	6.75±3.45	0.0001
EPO	DHLA	2.48±1.99	3.04±1.99	0.0001
(*n*=31)	DHA	2.39±2.38	2.03±2.12	0.058
	Vitamin E/cholesterol	4.29±1.99	6.61±3.98	0.0001
Soya	Linoleic acid	19.64±7.95	21.30±7.56	0.04
(*n*=28)	DHA	2.75±2.65	2.21±1.86	0.002
	Vitamin E/cholesterol	4.34±2.25	7.48±4.91	0.0001
Tuna	DHLA	2.51±1.33	1.85±1.46	0.0001
(*n*=28)	EPA	0.95±1.06	1.90±1.33	0.0001
	DHA	2.67±2.52	4.60±2.12	0.0001
	Vitamin E/cholesterol	4.28±2.25	6.22±2.38	0.0001
Tuna/EPO	DHLA	2.61±1.33	2.56±1.46	0.67
(*n*=28)	EPA	0.79±0.93	1.48±1.06	0.0001
	DHA	2.41±1.46	4.62±2.79	0.0001
	Vitamin E/cholesterol	4.22±2.38	6.34±2.65	0.0001

Values are means±S.D.
EPA: Eicosapentaenoic acid; DHA: docosahexaenoic acid; DHLA: dihomo γ-linoleic acid.

Table 4
Peak vasodilator responses to acetylcholine and sodium nitroprusside before and after supplementation

		Peak acetylcholine response			Peak sodium nitroprusside response		
Placebo	Before	5.64	(3.67–6.80)	$P=0.16$	4.00	(2.67–7.16)	$P=0.06$
($n=28$)	After	4.67	(3.18–5.72)		3.71	(2.41–4.65)	
Oleate	Before	5.47	(4.32–6.54)	$P=0.11$	4.00	(3.33–4.82)	$P=0.95$
($n=30$)	After	5.97	(5.11–7.44)		3.92	(2.39–5.62)	
EPO	Before	5.81	(5.07–7.14)	$P=0.19$	4.65	(3.19–5.86)	$P=0.29$
($n=31$)	After	6.64	(4.99–8.69)		3.96	(3.26–6.77)	
Soya	Before	5.22	(4.34–7.04)	$P=0.43$	3.89	(2.43–6.03)	$P=0.38$
($n=28$)	After	5.65	(4.11–7.63)		4.57	(2.12–6.38)	
Tuna	Before	5.00	(3.38–5.87)	$P=0.02$	4.32	(2.9–5.16)	$P=0.35$
($n=28$)	After	5.85	(4.77–7.37)		3.55	(3.04–4.44)	
EPO/tuna	Before	5.26	(4.48–7.12)	$P=0.304$	4.57	(3.42–5.50)	$P=0.53$
($n=28$)	After	6.18	(4.79–7.22)		4.45	(3.17–6.06)	

Values are medians (interquartile range), and are expressed as the ratio of the peak response over baseline perfusion.

mentation were slightly lower (but not significantly) in the tuna-supplemented group compared with the other groups. This was due to two subjects having relatively small peak responses to ACh (2.45 and 2.97). Exclusion of these two subjects from the statistical analyses still produced a result showing a significant augmentation in ACh response after supplementation with tuna oil ($P=0.04$). Fig. 1 shows the changes in ACh responses in individual subjects. From this it can be seen that responses increased in 71% of subjects (20 out of 28).

In the tuna-supplemented group there was a weak positive correlation between the peak ACh response and total plasma n-3 fatty acids ($r=0.42$, $P=0.032$). In the erythrocyte membrane phospholipids, there were strong positive correlations between the peak ACh response and DHA in: phosphatidylcholine ($r=0.71$, $P<0.0001$); phosphatidylserine ($r=0.54$, $P=0.01$) and phosphatidylethanolamine ($r=0.43$, $P=0.04$). Additionally, although no significant changes were observed in the SNP response after tuna oil supplementation, there were significant correlations between the peak SNP response and; plasma DHA ($r=0.53$, $P=0.005$) and DHA in phosphatidylserine ($r=0.60$, $P=0.003$).

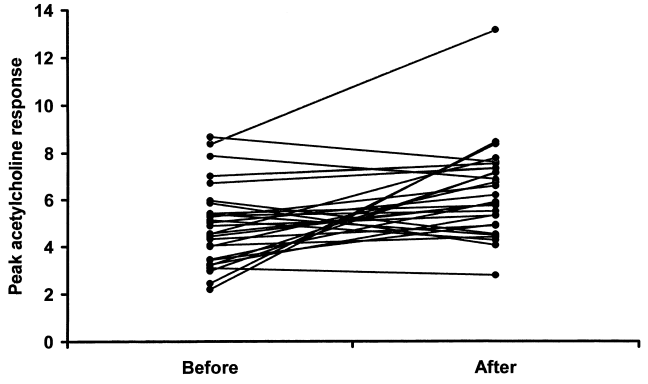

Fig. 1. Changes in peak acetylcholine response (expressed as the ratio of the peak response over baseline) before and after tuna oil supplementation in individual subjects ($n=28$).

4. Discussion

In this study, we have investigated the effects of increasing (dietary achievable) levels of n-3 and n-6 fatty acids on endothelial function in the microvasculature of healthy subjects over a 7–8 month period. Eight months duration was selected as earlier, higher dose n-3 PUFA studies [16,17] had shown maximal effects at 4 months and it was considered that lower doses may require 7–8 months. The reason for giving oil supplements in this study rather than encouraging dietary change were as follows: a standard equal amount of each fatty acid could be consumed by all subjects and thereby reduce the wide variation in dietary intake which would otherwise occur. Furthermore, compliance was likely to be greater than by encouraging active dietary change, which would also need continuous careful monitoring by a dietician. Nevertheless, the supplements given reflect an achievable dietary change and the estimated percentage dietary increases in the fatty acids derived from the supplements were achieved in all groups [18]. These supplements were daily for 8 months as peppermint flavoured 20% oil–water emulsions to avoid the extra caloric intake from gelatine capsules, reduce the chance of weight gain, and increase compliance. A mixed group of n-3/n-6 PUFA-rich oils was selected because n-3 fatty acids interfere with n-6 metabolism and vice versa. High doses of one can produce deficits of the other [19].

In the tuna-supplemented group a significant decrease in dihomo γ-linolenic acid ($C_{20:3}$) was observed, which was not reflected in the tuna/EPO-supplemented group. The

significant reduction in $C_{20:3}$ is probably due to competition between the n-3 and n-6 fatty acids for position on the phospholipids. In the tuna/EPO-supplemented group the lack of reduction in $C_{20:3}$ suggests that a balance might have been reached between the n-3 and n-6 fatty acids. Docosahexaenoic acid ($C_{22:6}$) was significantly reduced in placebo (Scottish diet), oleate and soya supplemented groups, suggesting inhibition of n-3 fatty acid desaturation by the n-6 fatty acids in these supplements.

The results of this study show that 8 months dietary addition of an equivalent to eating fish three times a week produced a significant improvement in the peripheral endothelial function, tested by application of ACh to the forearm skin microvessels using iontophoresis. This non-invasive test in peripheral vessels is being used increasingly to examine endothelial function [20–23]. Although the tests have been performed in the skin microvessels, we believe that the measurements made here are representative of the microvasculature elsewhere and therefore are a reflection of generalised cardiovascular integrity. It has been shown that healthy young men at increased risk of developing hypertension have impaired skin microvascular responses [24]. We have shown that the dilator responses to ACh in the forearm skin correlates significantly with other markers of systemic endothelial function, such as plasma nitric oxide (positive correlation) and plasma endothelin-1 (negative correlation) [14], providing support for the notion that our measurements in the forearm microvasculature are representative of generalized endothelial function. Others have shown that skin microvascular responses to iontophoresis of ACh correlate well with peak oxygen consumption during exercise [23], suggesting that microvascular responses of skin reflect those of resistance vessels in working muscles and therefore reflect generalised microvascular impairment. The relevance of our measurements as indicators of changes in cardiovascular disease status is also shown in a previous study of ours in which we saw significant augmentation of skin microvascular responses after cholesterol-lowering in patients with peripheral vascular disease [25]. Recently, it has been shown that forearm skin microvascular responses to upper arm occlusion correlate significantly with cardiovascular risk in healthy female subjects [26].

This technique has not been compared with the non-invasive test of arterial endothelial function using flow-mediated vasodilation. However, in a recent study the latter test did not correlate with other techniques for measuring endothelial function, indicating that endothelial function probably differs between conduit and resistance vessels [27].

Endothelium-independent vasodilation (SNP responses) following supplementation with placebo oil showed a trend to worsening. We believe this is a true effect of the placebo supplementation. Although the statistical trend for a significant decrease in the placebo group was only seen for endothelium-independent responses, Table 3 shows that there was also a lesser trend for a decrease in endothelium-dependent responses in this group. We have also seen a significant increase in the levels of thrombomodulin (a marker of endothelial damage) and a significant increase in collagen-induced platelet aggregation after supplementation in the placebo group (unpublished observations). This suggests that the placebo oil provided a less healthy fatty acid mix than that obtained by these volunteers from their usual diet. This might have occurred because a higher proportion of the subjects in the study had non-manual (71%) than manual (29%) occupations, and the former tend to eat more healthily than the population in general [28].

The mechanisms by which tuna oil supplementation significantly improved endothelium-dependent responses are not clear but could possibly be due to the increased levels of n-3 PUFA. In support of this there were strong positive correlations between endothelium-dependent vasodilation and DHA in erythrocyte phospholipids in this group of subjects. The effects of these fatty acids could be related to enhanced production of vasodilator substances such as nitric oxide [29] and PGI_3 from EPA, and to reduced production of vasoconstrictors such as thromboxane A_2 due to the low activity of the EPA derived thromboxane A_3 [30]. That nitric oxide might be involved is supported by our finding of a positive association between nitric oxide mediated vasodilatation and DHA levels. The n-3 fatty acids are also known to reduce plasma triacylglycerol concentrations [2], high levels of which have been linked to impaired endothelium-dependent vasodilation, but not endothelium-independent vasodilation responses. The combination of reduced triacylglycerol concentration and altered eicosanoid balance could explain why there was a significant increase in endothelium-dependent vasodilation but not in endothelium-independent vasodilation.

It is unlikely that the augmentation in ACh response seen in the tuna-supplemented group was due to the slightly lower pre-supplementation response compared with the other groups. This group contained two subjects with relatively small responses to ACh, and exclusion of these subjects from the statistical analysis still resulted in significant augmentation in the ACh response. An increase in ACh responses was observed in 71% of subjects.

An intriguing finding was that in spite of producing increased plasma levels of n-3 fatty acids in the EPO/tuna supplemented group, to levels similar to those in the tuna alone group, we did not find any augmentation in endothelium-dependent vasodilatation. One possibility is that in the tuna/EPO supplemented group there is a production of both vasoconstrictor thromboxane A_2, derived from arachidonic acid, in addition to production of vasodilator prostaglandin I_2 [31]. In this group there were no significant changes in DHLA levels. While the changes in DHLA

alone might not be sufficient to affect blood flow per se, as shown by no significant change in the EPO-supplemented alone group where DHLA levels increased, the lack of change in the tuna/EPO supplemented group might be sufficient to counteract the vasodilator effects. This can also be seen in the tuna-supplemented group where the increases in EPA and DHA levels were accompanied by a significant fall in the DHLA (with less thromboxane A_2 formed perhaps) producing an overall net vasodilator effect. A further reason for the differences in observed blood flow changes in the tuna and EPO/tuna supplemented groups is that, in contrast to the tuna-supplemented group, we found no significant correlations in the EPO/tuna supplemented group between DHA levels and ACh responses and it might also be that the lack of this relationship is responsible in some way. Leeson et al. [12] recently showed that the positive association between flow mediated dilatation and n-3 fatty acids was largely accounted for by DHA.

The endothelium-dependent and independent vasodilation was not significantly improved after oleic acid (n-9) rich sunflower oil in this study. This is consistent with other studies [32]. In one study [33], it was shown that high oleic and linoleic acid meals impaired flow-mediated dilatation in comparison with a low fat meal. Oleic acid has also been shown to inhibit endothelium-dependent responses to ACh [34]. In hyperlipidaemic males, however, monounsaturated fatty acid rich and low fat diets are both associated with improved endothelial function as compared with a high saturated fat diet. It seems that oleate might improve endothelial function in disease states rather than in healthy individuals. Dietary PUFAs may also reduce leucocyte aggregation and monocyte adhesion to endothelial cells [35,36].

An optimum study design might have been a dietary exchange rather than a dietary supplementation, but in practice, this would have been difficult to achieve. In the present study, the level of supplementation can be equated to feasible dietary change. As such, these results suggest that a relatively simple dietary change to eating oil-rich fish 2–3 times a week, or easy and cheap supplementation could produce a beneficial change in endothelial function in healthy individuals. The increasing availability of n-3-supplemented food items such as bread, fat spread, eggs, etc. may also facilitate improved n-3 PUFA status. It would be expected that producing such dietary change in the wider population might have significant clinical benefits, and perhaps to an even greater extent in people with risk factors for vascular disease, e.g., smoking, obesity, hypertension and hyperlipidaemia.

In conclusion, the current study showed a significant improvement in endothelium-dependent vasodilation after tuna oil (long-chain n-3 PUFAs) supplementation. This work adds to the growing literature that supports increasing dietary long-chain n-3 fatty acids as a safe approach to improving vascular health, and potentially reducing risk of coronary heart disease.

Acknowledgements

The Ministry of High Education, Libya, funded K.E. The authors gratefully acknowledge the Ministry of Agriculture Fisheries and Food UK (MAFF) contract AN0233 for financial support and TENOVUS who supplied the laser Doppler imager. Thanks to Steven Fenton for subject recruitment and data management, and to Rosemary Price for considerable help with dietary assessment and analysis. We are extremely grateful to the study volunteers for their time and dedication to the project.

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