Essential fatty acids and modern lifestyle: a reappraisal

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Controversy surrounds the effects of dietary fish oil supplementation on atherosclerosis. Three studies were undertaken, where Vervet monkeys were fed either a Western atherogenic diet (WAD) or a high carbohydrate diet (HCD). The first study indicated that enhanced atherosclerosis may be the result of an imbalance of fatty acids in plasma and tissue lipids as eicosapentaenoic acid (EPA; C20:5ω3) was increased with fish oil (FO) supplementation at the expense of arachidonic acid (AA; C20:4ω6). The second study investigated the effect of diet on the metabolism of EPA. Disappearance of EPA, after EPA loading, was delayed in Vervets on the WAD in comparison with those on the HCD. Results of this study indicate that diet is able to modulate EPA metabolism, and that the beneficial effects of a HCD on plasma lipoprotein concentrations can be augmented by EPA supplementation. The third study investigated the combined effect of a supplement that contained different ratios and dosages of gamma-linolenic acid (GLA; C18:3ω6) and EPA during ingestion of the WAD. Based on a favourable response to plasma lipoprotein cholesterol and a phosphatidylcholine fatty acid metabolism with increases in both EPA and dihomogamma-linolenic acid (DGLA; C20:3ω6), we conclude that a 4:1 ω6/ω3 fatty acid supplement at 200 mg/day would be the optimum supplement in our animal model. The long-term effects of this supplement on lipoprotein metabolism and atherosclerosis in the non-human primate model, is currently under investigation.

Key words: Essential fatty acids, atherosclerosis, African Green monkey

Introduction

The effects of fish oil (FO) supplementation on atherosclerosis vary, with some reporting regression[1,2], whilst others report no effect[3] or progression[4,5]. The effects on lipids in plasma and tissue are also inconsistent[1,5-12]. Unrealistically high doses of FO have been used in studies with primates[1,12]. These conflicting reports encouraged us to evaluate commercially available fish oils at a realistic dose. In the first study a supplement that supplied 2.5% of dietary energy was evaluated to determine its effect on established atherosclerosis in Vervet monkeys[13].

Thrombotic disorders are currently treated and prevented by utilising pharmacological concentrations of eicosapentaenoic acid (EPA; C20:5ω3)[14]. Controversy and disagreement about the beneficial effects of this essential fatty acid are legion. EPA has been shown to lower[12,15-18], elevate[19,20] or have no effect on cholesterol metabolism[13]. Supplementation of EPA to different diets may be the reason for the inconsistency of effects[6]. The purpose of the second study was therefore to investigate the metabolism of EPA (55% as free fatty acid) in Vervet monkeys as a supplement to a Western atherogenic diet (WAD) or a high carbohydrate diet (HCD).

Supplementation with ω3 fatty acids may enhance atherosclerosis[19], probably due to a lower ω6/ω3 fatty acid ratio. According to the modified essential fatty acid hypothesis of coronary heart disease (CHD), this could be caused by an inadequate or imbalanced supply of ω6 fatty acids[15]. Increased cholesterol concentration, especially low density lipoprotein cholesterol (LDL-C), which may occur with increased ω3 fatty acid intake, is not desirable. In contrast, ω6 essential fatty acids, especially gamma-linolenic acid (GLA; C18:3ω6) and dihomogamma-linolenic acid (DGLA; C20:3ω6), reduce LDL-C[21,22]. Supplementation of a WAD with ω3 essential fatty acids (EFA), without complementary supplementation with ω6 essential fatty acids, inhibits Δ6-desaturation of linoleic acid (LA; C18:2ω6) and thereby levels of their respective metabolites, DGLA and arachidonic acid (AA; C20:4ω6). In turn, prostaglandin synthesis may be related to atherosclerosis. Favourable levels of EPA and DGLA could prevent atherosclerosis and reduce mortality[14,21]. The third study was therefore designed to investigate the combined effect of a supplement that contained different ratios and dosages of GLA and EPA in Vervet monkeys on a WAD.

Methods

Experimental animals

The non-human primate is a well defined and acceptable model that can be used to study dietary treatments to improve blood lipid profiles. Availability and a well controlled environment[23] made it an excellent model for us to use. In the studies under discussion, African Green Vervet monkeys (*Cercophithecus aethiops*) were used as

This paper was presented at the International Workshop on 'Modern Lifestyles and Micronutrient Deficiency' in Nusa Dua, Bali on October 19-21, 1995.

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they were used successfully by us[24,25] and other workers[1,26] to determine the effect of modulation by diets on lipids, lipoproteins and atherosclerosis. Vervets are also phylogenetically related to humans[26] and when fed an atherogenic diet have lipoprotein concentrations, distributions and compositions similar to human beings that are at risk of having coronary heart disease (CHD)[14].

Experimental dietary treatment

Dietary manipulation was achieved in the studies under discussion by using either a WAD or a HCD. The WAD provided 40% and the HCD 20% energy from fat respectively. The dietary treatments have been described in previous work[19]. The diets used were formulated entirely from cooked foods normally consumed by people, with no extra synthetic cholesterol added and prepared as described[25,27]. The food was prepared by the same individual in the same kitchen, and the diets were analysed during the studies to monitor the consistency of the lipid composition. Fatty acid supplements were injected as required at multiple sites per food patty, and subsequently fed to the animals without delay, one patty each morning and afternoon[19].

Blood and tissue samples

Blood samples were collected into K_2-EDTA (1mg/mL blood) after an overnight fast, by femoral venipuncture without venous stasis, under ketamine-HCl anaesthesia (10mg/kg body weight i.m.) and atropine (0.05mg/kg). In the first study, monkeys were terminated and arteries collected as described[19].

Ethical approval

All three studies were approved by the Ethics Committee of the South African Medical Research Council.

Biochemical analyses

Lipoproteins

Lipoproteins were isolated by sequential ultracentrifugation at 10°C in a Beckman L8-80M ultracentrifuge with a 40.3 rotor. Plasma density was adjusted by addition of NaBr/NaCl solutions and LDL was isolated within a density range of 1.019-1.063g/mL[28]. High density lipoprotein (HDL) was isolated by precipitation of the apo B containing lipoproteins with heparin-manganese chloride[29].

Analytical methods

Total plasma cholesterol and high density lipoprotein cholesterol (HDL-C) were measured enzymatically (Boehringer Mannheim, CHOD-PAP, Cat. No. 237574). Cholesterol in the isolated lipoprotein fractions were determined by an enzymatic iodide method[30,31] as described[13]. Total protein was analysed by a modified Lowry procedure[32].

Tissue samples

Erythrocyte membranes (EMB) were prepared by haemolysing erythrocytes with different phosphate buffers[33,34].

Fatty acids

Lipids from studies 1 and 3 were extracted from plasma and EMB with chloroform/methanol (2:1; v/v), separated by thin layer chromatography and analysed for fatty acid composition of plasma triacylglycerol (TAG), cholesterol ester (CE) and phosphatidylcholine (PC), and EMB-PC by gas-liquid chromatography[35-37]. In study two, neutral lipids were extracted from plasma by the Dole method[38].

Study 1: *Effect of fish oil on atherosclerosis in Vervets fed Western atherogenic (WAD) and high carbohydrate (HCD) diets.*

Aim

To determine the effect of fish oil (FO) supplementation to a WAD and HCD on plasma lipoproteins, arterial lipids and fatty acids in Vervet monkeys.

Experimental design

This study was described previously[13]. Briefly the experimental design can be summarised as follows: The experimental Vervets were divided into four comparable groups, two groups were retained on the WAD, one of which was supplemented with fish oil (WAD/FO; n=9), whilst the other received a sunflower oil (WAD/SO; n=9) supplement. The remaining two groups were changed from the WAD to a HCD. One group was supplemented with the same FO supplement (HCD/FO; n=9) and the other group received the sunflower oil (HCD/SO; n= 10) supplement. Nine female Vervets that were never exposed to the WAD, served as a reference group. Blood was drawn prior to starting with the respective dietary regimens and oil supplementations, and again at 3, 8, 13 and 20 months. The Vervets were terminated after the experimental period of 20 months.

Statistical analysis

Statistical analysis was done as described[13] and as groups consisted of male and female Vervets, diet/sex interactions were tested and the results interpreted accordingly.

Results

Results were described previously[13] and can be summarised as follows:

Plasma lipoprotein and tissue cholesterol response

FO supplementation did not change the cholesterol concentrations of plasma, HDL and LDL significantly (Table 1). Vervets of the WAD/FO group had an increased (2.7 times; $p<0.001$) content of total cholesterol in their aorta intima compared to the WAD/SO group. The same trend was also evident after FO was supplemented to the HCD.

Fatty acid response

EPA was increased 7.5- and 6.5-fold respectively (both $p<0.001$) in plasma and aortic intima PC (Table 2). DGLA ($p<0.01$) AA ($p<0.001$) levels were reduced in the plasma PC after FO supplementation of the WAD, and similar effects were seen after supplementing the HCD with FO. In the aorta intima the AA was also reduced ($P<0.001$) on the WAD/FO. Docosahexaenoic acid (DHA; C22:6ω3) was also increased after the FO supplementation.

Table 1. The effect of fish oil on lipoprotein and arterial total cholesterol levels[13].

	WAD/SO n=9	WAD/FO n=9	HCD/SO n=10	HCD/FO n=9	HCD n=9
Plasma	332.5	344.9	145.7	144.3	180.7
(mg/dL)	(125.2)	(121.0)	(23.1)	(20.6)	(24.2)
LDL	300.9	265.9	49.5	49.7	86.9
(mg/dL)	(158.9)	134.2)	(21.2)	(13.6)	(26.6)
Intima	32.5	89.2**	44.2	83.7*	10.5
(µg/mg protein)	(26.6)	(78.3)	(70.9)	(125.2)	(4.9)

- WAD: Western atherogenic diet; SO: Sunflower oil; FO: Fish oil; HCD: High carbohydrate diet
- Significant difference between WAD/FO and WAD/SO or HCD/FO and HCD/SO: *p < 0.01; **p < 0.001

Table 2. The effect of fish oil on the fatty acid composition of plasma and intima phosphatidylcholine fatty acids[13].

	WAD/SO n=9	WAD/FO n=9	HCD/SO n=10	HCD/FO n=9	HCD n=9
Plasma					
C18:2ω6	25.6	18.1**	33.3	23.6[a]*	31.5
	(2.2)	(2.1)	(2.9)	(1.6)	(1.5)
C20:3ω6	1.5	1.2*	2.6	1.1[a]*	3.9
	(0.1)	(0.1)	(1.4)	(0.3)	(1.2)
C20:4ω6	12.1	9.4**	8.0	5.9**	8.8
	(1.0)	(0.5)	(1.0)	(0.7)	(0.8)
C20:5ω3	0.8	6.0**	0.4	5.3a*	0.3
	(0.1)	(0.7)	(0.1)	(1.1)	(0.1)
C22:6ω3	5.5	8.2**	3.0	7.6**	3.0
	(1.0)	(1.5)	(0.4)	(0.8)	(0.5)
Intima					
C18:2ω6	5.7	7.4*	6.3	7.0	5.1
	(0.8)	(1.3)	(1.0)	(1.7)	(0.8)
C20:3ω6	1.0	1.3	1.1	1.1	1.0
	(0.3)	(0.2)	(0.4)	(0.3)	(0.2)
C20:4ω6	19.6	15.0**	18.0	15.5	20.4
	(1.9)	(1.8)	(2.8)	(2.5)	(1.9)
C20:5ω3	0.2	1.3**	0.1	0.6[a]*	ND
	(0.1)	(0.4)	(0.1)	(0.1)	
C22:6ω3	3.1	3.7	2.1	3.0*	2.3
	(0.7)	(0.9)	(0.5)	(0.7)	(0.5)

- WAD: Western atherogenic diet; HCD: High carbohydrate diet; SO: Sunflower oil; FO: Fish oil; a: Males only; ND: Not detected.
- Significant difference between WAD/FO and WAD/SO or HCD/FO and HCD/SO: *p<0.01; **p<0.001

Correlations

In the plasma and aorta intima PC, EPA and AA respectively demonstrated the strongest negative and positive correlations with the intimal CE and FC contents (Table 3).

Table 3. Correlation coefficients (r) and p-values between the cholesterol ester (CE) and free cholesterol (FC) content of the aorta intima and plasma and intimal phosphatidylcholine (PC) fatty acids[13].

	Intima-CE		Intima-FC	
PC Fatty acid	r	p	r	p
Plasma				
C20:4ω6	-0.66	0.0029	-0.53	0.0245
C20:5ω3	0.75	0.0004	0.57	0.0126
Intimal				
C20:4ω6	-0.73	0.0007	-0.72	0.0005
C20:5ω3	0.78	0.0001	0.59	0.0095

Study 2: *The effect of diet on the metabolism of eicosapentaenoic acid.*

Aim

We studied the effect of diet on the metabolism of EPA in our Vervet monkey model receiving either a WAD or HCD.

Controversy surrounds the beneficial effects of EPA on lipoprotein metabolism because some researchers showed that EPA does lower plasma cholesterol concentrations[12,15-18], while others suggested a cholesterol elevating effect[19,20]. Although many factors could possibly explain these divergent results obtained with EPA, Harris[6] speculated that the type of diet which EPA is supplemented with, could possibly be the reason for these contradicting results.

Experimental design

Ten healthy female Vervet monkeys were randomly assigned to two groups, namely a HCD group and a WAD group. Experimental procedures were carried out in three phases.

Phase 1

During this phase of six weeks, animals received their respective WAD or HCD without any EPA supplementation. This phase was used to stabilise animals on their respective diets.

Phase 2

EPA (Callanish Pharmaceuticals, 50% free acid) was used as a supplement to the respective diets and were progressively increased every six weeks from 300mg/day to 2400 mg/day over a period of 24 weeks. The incorporation of EPA in plasma CE, plasma TAG, EMB-PC and in EMB phosphatidylethanolamine (PE) was investigated at the end of this phase.

Phase 3: Washout period

At the beginning of the third phase, EPA supplementation was withdrawn. Animals then continued on their respective diets for a further 12 weeks.

Blood samples

Blood was sampled from each animal at the end of Phase 2. During Phase 3, blood samples were collected after weeks 1, 2, 4, 6, 8 and 12.

Statistical analysis

Each animal served as its own control. The baseline value was taken before supplementation of EPA was started. This value was compared with the blood sample value obtained at maximal level of supplementation and at one, two, four, six, eight and 12 weeks after cessation of EPA supplementation. In order to minimise the possible effects of diet, differences in fatty acid intake and differences between individual animals on the amount of fatty acid accumulated, the following relationship was used: FAt/FAtot where FAt is equivalent to the percentage of EPA in plasma or EMB after a given time during the washout period and FAtot is equivalent to the percentage of EPA at the beginning of the washout period (end of supplementation period).

Results

In the WAD group the EPA content in the EMB-PC increased from 0.34%±0.18% to 11.32±3.06% (33-fold) during the supplementation period. In the HCD group the EPA content in the EMB-PC increased from 0.08±0.1% to 8.68±4.59 (109-fold) over the same period.

In the EMB-PE fraction, EPA content in the group consuming a WAD increased from 1.03±0.24% to 19.51±6.47% (19-fold) during the supplementation period. In the HCD group EPA content increased from 0.25±0.05% to 11.19±5.20% (45-fold) during the corresponding period.

EPA content in plasma

In plasma CE, EPA increased from 0.35±0.11% to 19.46±4.93% (56-fold) in the WAD group, while in the HCD group it increased from 0.05±0.04% to 20.04±10.94% (400-fold).

In the WAD group EPA content in plasma TAG increased from 0.16±0.13% to 19.31±7.98% (120-fold) during supplementation. In the HCD group EPA content increased from 0.03±0.03% to 18.02±11.78% (600-fold) during supplementation.

The disappearance role of EPA

EPA content in EMB-PC gradually decreased during the washout phase. The estimation of the half-life (t½) of EPA is given by the median of the t½ which is 34.3 and 22.8 days for the WAD and the HCD groups respectively (Table 4).

The t½ of EPA for the EMB-PE is estimated by the median of the individual median t½ which is 43.5 and 31.3 days for the WAD and HCD groups respectively (Table 4).

The t½ of EPA for plasma CE was found to be 23.5 days for the WAD and 14.1 days for the HCD (Table 4).

Different rates of disappearance of EPA from plasma TAG of the WAD and HCD animals were also observed. The WAD had a t½ of 17.4 days whereas the t½ for HCD was 9.4 days.

Table 4. Summary of the comparison of the estimated half-life (t½) (median of the individual median measurement in days) of eicosapentaenoic acid (EPA).

Compartment	WAD	HCD
EMB-PE	43.5	31.3
EMB-PC	34.3	22.6
Plasma CE	23.5	14.1
Plasma TAG	17.4	9.4

EMB: Erythrocyte membrane; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine; CE: Cholesterol ester; TAG: Triacylglycerol; WAD: Western atherogenic diet; HCD: High carbohydrate diet.

Plasma total cholesterol and HDL-C

In the EPA treated groups plasma total cholesterol levels increased by 17.1% in the WAD and decreased by 20.8% in the HCD. EPA supplementation reduced HDL-C by 36% in the WAD and 21.7% in the HCD.

Study 3: *Establishment of an optimal ω6/ω3 essential fatty acid ratio and dosage for the prevention of atherosclerosis in nonhuman primates fed a Western atherogenic diet.*

Aim

To determine the optimal ω6/ω3 fatty acid ratio and dosage of a fatty acid concentrate which will have the most favourable plasma lipid profile as well as the most favourable plasma and tissue fatty acid composition in a nonhuman primate model fed a WAD.

Experimental design

Twenty adult male Vervets were stabilised on a high carbohydrate diet for three months prior to experimental intervention at which time the diet was changed to a WAD for 20 weeks. Vervets were then randomly allocated to receive one of four possible fatty acid supplements for a period of 12 weeks according to their LDL-C response to the WAD. The major fatty acid compositions of the fatty acid concentrate mixture are given in Table 5. Group A (n=5) received 200mg/day of a fatty acid supplement with an ω6/ω3 fatty acid ratio of 9:1 and group B (n=5) received 800 mg/day of the same fatty acid supplement. Group C (n=5) received 200mg/day of a fatty acid supplement with an ω6/ω3 fatty acid ratio of 1:1 and Group D (n=5) received 800mg/day of the same fatty acid supplement. The supplementation period was followed by a 20 week washout period. The washout period was followed by a second supplementation period during which the Vervet groups received a different fatty acid supplement albeit at the same dosage. Group A (n=5) received 200mg/day of a fatty acid supplement with an ω6/ω3 fatty acid ratio of 4:1 and group B (n=5) received 800 mg/day of the same fatty acid supplement. Group C (n=5) received 200mg/day of a fatty acid supplement with an ω6/ω3 fatty acid ratio of 2:1 and Group D (n=5) received 800 mg/day of the same fatty acid supplement.

Blood and tissue samples

Blood samples were collected before and after each supplementation period and during the washout period.

Statistical Analysis

The analysis of variance model[39] used, assessed the effects of the experimental factors that included ratio of the ω6/ω3 fatty acid supplement (9:1, 4:1, 2:1 and 1:1), dosage of supplement (200 and 800mg/day), baseline measurements, as well as the interactions between these factors, so that the model could asses change that was observed in the experimental design. The change from baseline was accordingly investigated for significant effects of the different supplements.

Table 5. Major fatty acid compositions of the GLA (80) and EPA (50) concentrate mixtures.

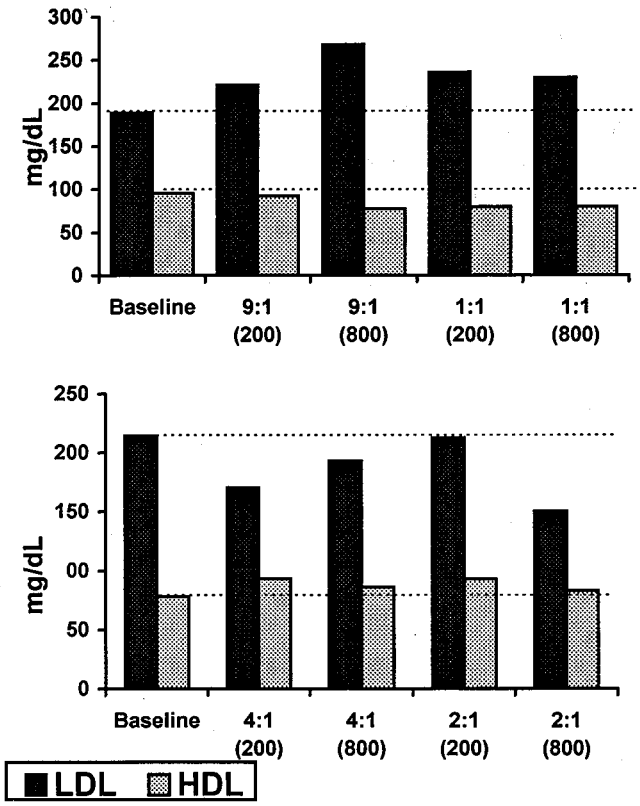
	Ratios			
Fatty acid	9:1	4:1	2:1	1:1
C18:2ω6	13.6	11.8	9.8	7.2
C18:3ω6	70.6	60.2	47.8	31.9
C20:5ω3	7.0	14.4	23.3	34.7
C22:6ω3	1.1	2.2	3.6	5.4
Σ ω6	84.4	72.3	58.1	40.0
Σ ω3	9.0	18.3	29.4	43.6
Σ ω6/Σ ω3	9.34	3.96	1.98	0.92

GLA: Gamma-linolenic acid; EPA: Eicosapentaenoic acid.

Results

Figure 1 illustrates the cholesterol response to the ω6/ω3 fatty acid supplement. A beneficial effect on cholesterol concentration was achieved only with the 4:1 ω6/ω3 fatty acid ratio at 200mg/day and with the 2:1 ω6/ω3 fatty acid ratio at 800mg/day. The 4:1 ω6/ω3 fatty acid supplement at 200mg/day lowered the LDL-C concentration with 19.8%, while the 2:1 ω6/ω3 fatty acid supplement at 800mg/day lowered the LDL-C concentration with 28.9%. The experimental factors that contributed significantly towards the LDL-C response, were the baseline value and interactions between the dose and ratio used. HDL-C concentrations were more increased with the 4:1 than the 2:1 ω6/ω3 fatty acid supplement (19% versus 6.1% respectively).

Figure 1. Cholesterol response to ω6/ω3 ratio and dosage.

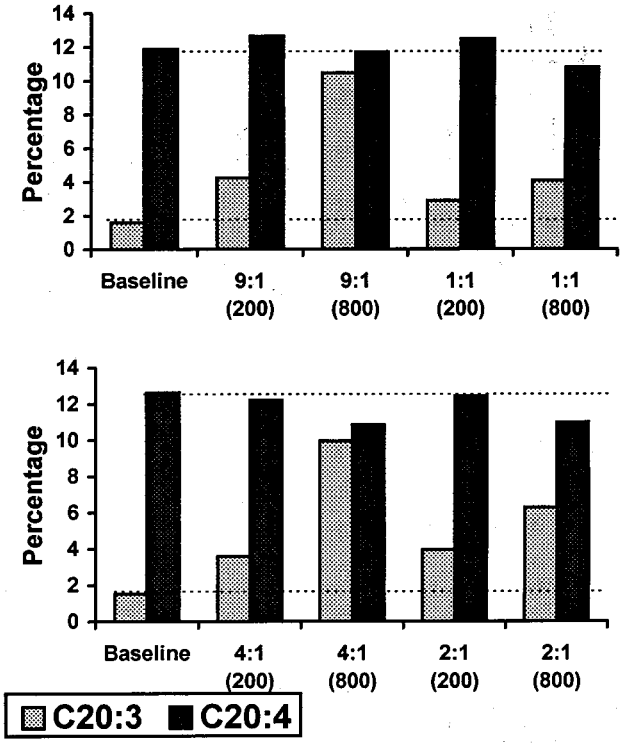


All the supplements at different ratios and dosages increased DGLA, EPA and DHA, with different responses (Figures 2 and 3). Based on the favourable plasma lipoprotein responses, the 4:1 ω6/ω3 fatty acid ratio at 200mg/day supplement was found to have a non-significant effect on plasma PC AA composition. EPA was only slightly increased by this supplement (Figure 3), favouring the accumulation of DGLA. The experimental factors that significantly contributed towards this accumulation, were the ratio, baseline value and interactions between the dose and ratio, and between baseline value and ratio.

Discussion

The most significant finding from Study 1 is that under the conditions of the study, neither plasma lipoprotein nor the arterial cholesterol concentrations improved as a result of supplementation of either the WAD or the HCD with FO. The lack of effect of dietary supplementation with FO on the plasma total cholesterol is in agreement with other studies that have compared FO with diets lacking a supplement, with monounsaturated fatty acids or with ω6 polyunsaturated fatty acid (PUFA) supplements[2-5]. In animal studies the effect of FO on LDL-C varied when diets were compared that lacked a supplement or with ω6 PUFA supplements and was either decreased, or had no effect or was increased within the same animal model[2,7,8]. Most animal studies compared the effect of FO with saturated fat diets that resulted in reduced LDL-C concentrations[9,10,40]. Our findings are consistent with studies in humans, especially normolipidaemic patients in which ω3 fatty acids had no effect on LDL-C[6].

Figure 2. Plasma phosphatidylcholine ω6 fatty acid response to ω6/ω3 ratio and dosage.

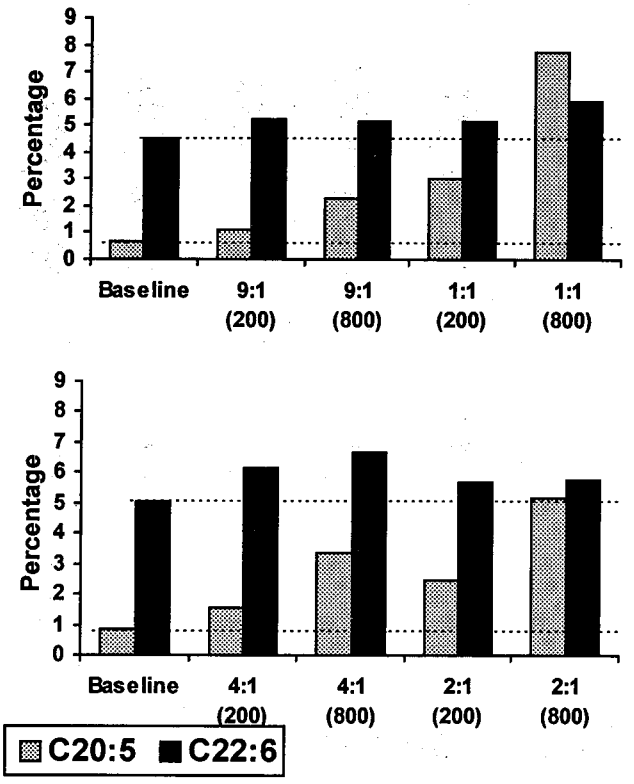


Regardless of the mechanism, the final assessment of FO must be at the arterial wall. The aorta intima total cholesterol concentration was almost three times higher on the WAD/FO and two times higher on the HCD/FO when compared to the respective SO supplemented groups[13]. Our results clearly disagree with reports that demonstrated no effect[11] of FO on the progressive accumulation of cholesterol in the aorta of rabbits, or decreases in the aorta of primates when compared to predominantly saturated fat diets[12]. On the contrary, not even platelet function was significantly inhibited by FO supplementation to either the WAD or the HCD[41]. None of the changes measured in terms of atherosclerosis after FO supplementation were favourable in the same individuals[20].

Study 1 strongly supported[13] the hypothesis[14] that a fatty acid imbalance of plasma and tissue lipids could be responsible for atherosclerosis, although others disagree with this hypothesis[44]. The increased EPA and decreased AA found in plasma and intimal PC after FO

supplementation, supports the results of other animal[43,44] and human studies[45,46]. The exact mechanism through which FO enhanced the accumulation of intimal cholesterol remains unclear from our results. The increased plasma and intimal EPA levels were nevertheless positively associated with intimal CE and FC contents[13]. This increase in EPA occurred at the expense of AA that was negatively associated with the intimal CE and FC contents[13]. The fatty acid results clearly indicated that the FO dose used, disturbed the fatty acid composition of plasma and arterial PC with dire consequences. This finding therefore supports the view that there may be an optimal anti-atherogenic ratio of ω6 and ω3 PUFAs in the diet[13].

Figure 3. Plasma phosphatidylcholine ω3 fatty acid response to ω6/ω3 ratio and dosage.



Results from the disappearance rate studies (Study 2) that more EPA was incorporated in tissues of animals consuming a WAD than those consuming the HCD, was probably due to the fact that EPA displaced saturated and/or other fatty acids. Furthermore, the disappearance rate of EPA from EMB and plasma was slower in the animals consuming the WAD than in those consuming the HCD, suggesting a slower rate of metabolism of EPA in animals consuming the WAD. This difference in disappearance rate observed between the two diets, however, appears to be unrelated to the level of incorporation of EPA into the tissues of animals on the respective diets. Differences in disappearance rates were not only observed between the two diets, but also between the EMB-PC and -PE fractions and between plasma CE and TAG. The disappearance of EPA from EMB-PE took nine days longer than from EMB-PC, and five to six days longer in plasma CE than in plasma TAG, independent of the type of diet.

The EMB-PC fraction is situated on the outside and -PE on the inside of the EMB[47]. Phospholipid renewal on the outside of the EMB can occur via direct exchange of the phospholipid between EMB and plasma lipoprotein, whereas phospholipids on the inside are renewed via acylation of lysophospholipids[48] and could therefore take longer to be renewed and metabolised. Present results strongly suggests that these acylation and metabolic processes were delayed by the WAD.

Differences between the disappearance of EPA from plasma CE and TAG probably relates to differences in their respective turnover and metabolic rates. The effect of the WAD diet on the slower disappearance of EPA from the CE and TAG relative to the HCD, is in line with the reported effect of the WAD on LDL metabolism in these animals consuming a WAD[28]. Results of Study 2 clearly showed that diet can modulate EPA metabolism and that the beneficial effects of a HCD on plasma lipoprotein levels can be augmented by EPA.

Although the LDL-C concentration was lowered more by the 2:1 ω6/ω3 fatty acid supplement at 800mg/day than the 4:1 ω6/ω3 fatty acid supplement at 200mg/day in Study 3, the HDL-C concentration was increased concomitantly more by the latter. The LDL-C lowering effect of the ω6/ω3 fatty acid supplements are promising indeed, as it has been shown that EPA can increase LDL-C levels in patients with coronary disease[49], hypercholesterolaemic men[50] and in hypertensive patients[51], although the consumption of cod liver oil was unable to affect HDL-C in male patients with myocardial infarction[52], probably as a result of its low EPA content[53]. HDL-C can, however, be increased by weight reduction[54], alcohol consumption[55] and a regular consumption of ω3 PUFA that reduces cholesterol transport to cells by LDL and increases efflux of cholesterol from cells by HDL[56]. The substantial HDL-C increase of 19% in this study by the 4:1 ω6/ω3 fatty acid supplement at 200mg/day indicates that a balanced ω6/ω3 fatty acid supplement is necessary as we were unable to change the HDL-C concentration in Vervets with established atherosclerosis on a fish oil supplement[13].

The EPA increase from baseline due to the 4:1 ω6/ω3 fatty acid supplement at 200mg/day was three times less than the EPA increase due to the 2:1 ω6/ω3 fatty acid supplement at 800mg/day. The 4:1 ω6/ω3 fatty acid supplement at 200mg/day reduced AA by 3%, while the 2:1 ω6/ω3 fatty acid supplement at 800mg/day reduced AA by 13%. The 2:1 ω6/ω3 fatty acid supplement at 800mg/day also increased DGLA nearly twice as much as the 4:1 ω6/ω3 fatty acid supplement at 200mg/day. As the ω6/ω3 PC fatty acid balance is easily disturbed by dietary imbalances[35,57,59], it seems that the ω6/ω3 fatty acid supplement that affects this delicate balance the least, would be the ω6/ω3 fatty acid supplement of choice.

Based on the favourable response to HDL-C and PC fatty acid metabolism, we therefore conclude that the 4:1 ω6/ω3 fatty acid supplement at 200 mg/day would be the optimum supplement to use in prospective intervention trials to test the hypothesis that ω3 fatty acids may regress atherosclerosis if the balance with ω6 fatty acids is not disturbed.

Conclusions

Supplementation of a WAD with ω3 fatty acids (FO; 32% ω3 fatty acids) did not regress atherosclerosis in Vervets on the WAD or prevent the progression of atherosclerosis on the HCD. The WAD delayed the metabolism of ω3 and ω6 (results not shown) fatty acids that could possibly explain the accumulation of lipids in arteries in Vervets on WAD studies as well as elevations of plasma lipoprotein. Recent experimental evidence in our model strongly suggest that we reappraise the role of ω3 and ω6 fatty acids in atherosclerosis. Results indicate that the impact of ω3 and ω6 fatty acids in the diet on atherosclerosis took place at very low levels, where not only amount but also the ratio appears to be critical.

Essential fatty acids and modern lifestyle: a reappraisal

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Asia Pacific Journal of Clinical Nutrition (1996) Volume 5, Number 3: 149-156

必需脂肪酸與現代生活方式：一個重新評價

摘要

有關膳食魚油補充對動脈粥樣硬化的效果各說不一。本文進行了三種連續的研究對這個問題進行評估。作者選用了 Vervet 猴子爲對象，分別喂以西方致動脈粥樣硬化膳食 (WAD) 或高碳水化合物膳食 (HCD)。第一個研究指出了動脈粥樣硬化增加，可能由於血漿脂肪酸和組織脂類（魚油的補充可使 20 碳 5 烯酸(EPA)增加而花生四烯酸消耗）不平衡所致。第二個研究探討了膳食對 EPA 代謝的作用。Vervet 猴子 EPA 負荷後 EPA 的消失在攝取 WAD 膳者較 HCD 膳者延緩。這個研究指出膳食能調節 EPA 代謝，同時攝取 HCD 膳對血漿脂蛋白水平的有益作用可由於 EPA 的補充而增加。第三個研究探討了攝取 WAD 膳時補充不同比例和數量 r-亞麻酸 (GLA : $C_{18}3W_6$) 和 EPA 的聯合作用。基於增加 EPA 和二高-r-亞麻酸 (DGLA : $C_{20}3W_6$) 對血漿脂蛋白膽固醇和卵磷脂脂肪酸代謝的有益反應，我們認爲每日補充 200 毫克 4:1 W_6/W_3 脂肪將是最適合我們的動物模型。長期補充對非人類靈長動物脂蛋白代謝和動脈粥樣硬化的作用，目前正在研究中。

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