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ORIGINAL CONTRIBUTION

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Small supplements of N-3 fatty acids change serum low density lipoprotein composition by decreasing phospholipid and apolipoprotein B concentrations in young adult women

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Summary In order to investigate the effect of a short-term application of marine n-3 polyunsaturated fatty acids on the composition of serum very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL), nine women aged 29 ± 4.2 years, following a diet with a SFA/MUFA/PUFA profile of 2.4/3/1, received supplements of six capsules daily, each capsule containing 0.137 g of n-3 fatty acids (14.5 % eicosapentaenoic acid (EPA) and 8.9 % docosahexaenoic acid (DHA)) for 10 d. Food consumption, assessed during two 10-days periods indicates that percentage contribution of SFA, MUFA, and PUFA to the daily energy intake did not change through the fish-oil supplementation period, but the daily consumption of n-3 fatty acids increased 2.3 times. N-3 fatty supplementation increased EPA and DHA percentages in serum phospholipids, but failed to decrease ($p > 0.05$) the cholesterol and triglyceride concentration in serum LDL and HDL, although it did so in VLDL. In contrast, the lipoprotein-phospholipid and lipoprotein-protein concentrations were markedly affected, mainly in LDL and HDL (at least $p < 0.01$). HDL and VLDL compositions were not affected but the total mass (lipid + protein in mg/dl) concentration of these lipoproteins significantly decreased ($p < 0.05$), suggesting a lower number of these particles in circulating blood after the n-3 treatment. The LDL-cholesterol/LDL-apolipoprotein B ratio increased ($p < 0.01$) reflecting a probable increase in LDL size. Following fish oil supplementation, LDL particles contained a significantly lower amount of phospholipids, which also suggests changes in the surface/core ratio of the average LDL. Changes in serum lipoprotein lipids did not significantly correlate with any dietary change other than the n-3 fatty acid increase. The results indicate that a 10-day application of a small supplement of n-3 change the LDL composition leading to less atherogenic LDL particles with lower phospholipid and apolipoprotein (Apo) B concentrations.

Key words Diet – lipoprotein composition – n-3 fatty acids – phospholipids – women

Introduction

It is well known that fish oils rich in n-3 polyunsaturated fatty acids (PUFA) are able to lower serum triglyceride levels in humans (14, 21, 22, 25, 31, 34), but their effect on size and composition of low and high density lipoproteins (LDL and HDL, respectively) has been less reported. Several factors are involved in the variability of the human response to dietary fish oil supplementations: the type of patients being studied (normo or hyperlipidaemics) and their dietary habits, the n-3 PUFA dosage, the n-3 PUFA/n-6 PUFA ratio, and the study duration (21–23, 31). Moreover, trials using fish oil supplements have shown a hypotriglyceridaemic effect, especially in hypertriglyceridaemic patients, and a reduction in low density lipoprotein cholesterol (LDL-C) levels when saturated fat intake is partially replaced by fish oil (20). Nevertheless, the information on the influence of n-3 fatty acids on apolipoprotein (Apo) concentrations and lipoprotein composition, above all in normo or even hypolipidaemic healthy subjects is limited (2, 42). On the other hand, oleic and linolenic acids have been shown to increase, whereas linoleic acid is known to decrease, the uptake of eicosapentaenoic acid (EPA) by the cell (38). Thus, the metabolic effect of n-3 fatty acids may be modulated by the presence of other dietary fatty acids, such as oleic acid (36).

Two week-study is considered a bare minimum for achieving dietary perturbation on plasma lipids (Grande, personal communication). However, recent data of our research group on women supplemented with n-3 fatty acids show that changes on plasma Apo are present after only 10 days of dietary treatment.

Therefore, we investigated the influence of a short-term application of a fish-oil preparation on the serum lipoprotein composition of young adult women consuming a high level of fat and monounsaturated fatty acids (MUFA), and a relatively low level of n-6 PUFA.

Materials and methods

Subjects and experimental design

The study was carried out in 9 women, aged 29 ± 4.2 years, 58.5 ± 10.6 kg, with a body mass index of 23.0 ± 2.6 kg/m^2. None of them were taking oral contraceptives or other drugs affecting lipoprotein metabolism. All of them performed a similar physical activity (aerobic training, 1 to 3 h/week), were matched by sociocultural level (university graduate students), and had similar eating habits. The study was performed in accordance with the Helsinki Declaration of 1964 (as amended in 1983 and 1989). All the women voluntarily consented to take 6 capsules per day (2 at breakfast, 2 at lunch, and the remaining 2 at dinner) of a fish oil supplement for 10 days. Each capsule (Pulse®, Grifols, S.A. Parets del Vallès, Barcelona, Spain) contained 500 mg of fat, 0.34 mg of vitamin E, 4.2 mg of cholesterol, 60 mg of carbohydrates, 115 mg of proteins, and an energy content of 5.2 kcal (21.8 kJ). Each volunteer consumed a daily dosage of 870 mg of total n-3 fatty acids (390 mg EPA and 252 mg DHA).

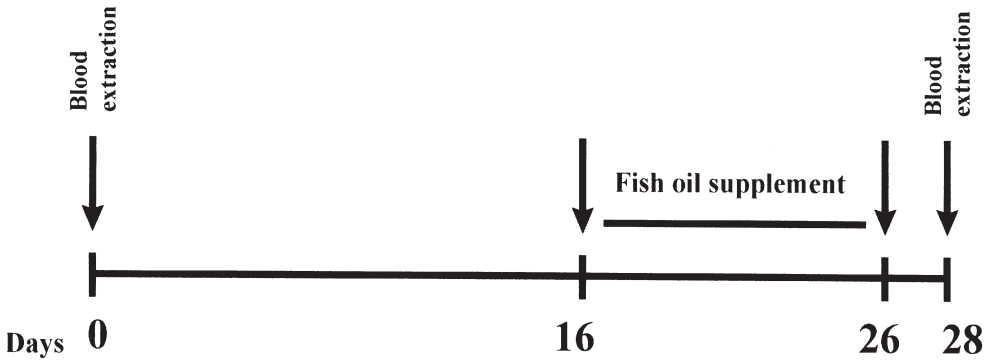
Women were blood sampled after fasting for 12–15 h once at the start of the experiment and then 28 days later. Fish oil supplement started on day 17 of the study and ended 2 days before the second venipuncture was performed (Fig. 1). This experimental design eliminated bias due to lipid variation during the hormonal cycle. This made it possible to quantify the n-3 fatty acid influence on serum lipids, lipoproteins and Apo in women, avoiding the confusing effects of the menstrual cycle. The experimental design also eliminated nutritional change influences because the women ate their normal diets throughout the experiment. Any possible significant dietary change which occured was evaluated by performing a survey of two 10-day periods, the first of which corresponded to the period before the basal blood extraction (basal period) and the other to the fish oil supplementation period. The diet was recorded and studied throughout out the two 10-day periods. These 10-day periods provide acceptable estimates of macronutrients, e.g., fat and energy (5) and of cholesterol (7). Energy and nutrient intakes per person and day were calculated using a computer program compiled from tables for the raw food weights (29). The possible change in the atherogenic potential of the diets was calculated according to the Zilversmit (44) and Connor et al. (13) equations.

Fatty acids and lipoprotein analyses

Several capsules were randomly selected and opened and the oil they contained was homogeneously mixed and saponified with 0.5N of sodium hydroxide in methanol, then methylated following the IUPAC method (24). The fatty acid methyl esters were analyzed by gas chromatography. Details of the method and calculations used have previously been published (35).

Blood was allowed to clot for 1 h at room temperature and the serum was then separated by centrifugation at 1 200 *g* and 20 °C for 20 min. Lipoproteins (VLDL, LDL, and HDL) were isolated by 21 h density gradient ultracentrifugation at 40 000 rpm (272 000 *g*) and 8 °C (40). Cholesterol in sera and lipoproteins was measured using the enzymatic cholesterol esterase-cholesterol oxidase method (3) proposed by Boehringer Mannheim, Germany. Triglycerides were tested according to the enzymatic glycerol-phosphate-oxidase method (41). Phospholipids were measured according to the enzymatic method of Boehringer Mannheim (39). Apo A1 and B were determined by kinetic immunoturbidimetry (33) following the indications and control of the Behring Institute.

Fig. 1 Experimental design



Lipids internal quality control was carried out according to the laboratory manual of the Lipid Research Clinics Program. Apo A1 and B were standardized against the IUIS-NHLBI-CDS 1883 control assayed in the International Collaborative Study Centers for Disease Control for Apolipoproteins Standardization. Variation coefficients for cholesterol, triglycerides, phospholipids, Apo A1, and Apo B were 3.5, 3.7, 3.2, 5.0, and 4.5 %, respectively.

Although it was not possible to determine the fatty acid profile of serum phospholipids of all the volunteers, serum lipids were extracted from some of the samples according to the methods of Folch et al. (18): phospholipids from the lipid extract were isolated by thin layer chromatography and saponified with 0.5N sodium hydroxide in methanol, and then methylated following the IUPAC method (24). The fatty acid mathyl esters were analyzed by gas chromatography as indicated above.

Statistic analyses

Data were statistically studied using the paired Student-*t* test. Pearson product-moment correlations between different lipoprotein compounds and between intraindividual dietary variations and intraindividual lipid and lipoprotein changes were also studied (16).

Results

Dietary data related to the basal and experimental periods are presented in Table 1. Due to the macronutrient composition and fatty acid formulation of capsules and their low energy content, the daily energy contribution of protein, carbohydrates, fat, saturated fatty acids (SFA), MUFA, and n-6 PUFA of the diet was not modified during the fish oil supplementation period. Only n-3 PUFA and the n-3 PUFA/n-6 PUFA ratio changed in a relevant and significant manner, because the fish oil supplement contributed more n-3 fatty acids than the food itself did. No significant or relevant other dietary changes occured during the experimental period in relation to the basal period. The major fatty acid composition of the fish oil supplement is given in Table 2.

Table 3 shows that the low supplement-dosage of n-3 fatty acids used during a period of 10 d induced a non-significant decrease in serum total cholesterol and LDL-C, while the drop (7.81 %) in HDL-cholesterol (HDL-C) was at the borderline for statistical significance.

Table 1 Daily intake of energy and some nutrients during the basal and fish oil supplement periods

	Basal period	Supplement period*
Energy, MJ	8.2 ± 2.6	7.9 ± 3.1
Protein, g	89.3 ± 29.0	90.6 ± 52.7
Carbohydrates, g	192.6 ± 68.0	188.2 ± 100.6
Fat, g	96.1 ± 30.6	90.5 ± 32.5
Alcohol, g	1.2 ± 2.6	2.1 ± 4.1
Fiber, g	13.0 ± 4.4	12.5 ± 5.1
SFA, g	32.3 ± 15.0	31.2 ± 10.5
MUFA, g	39.4 ± 11.6	37.8 ± 12.6
Total PUFA, g	13.2 ± 1.9	12.5 ± 4.1
N-6 PUFA, g	12.6 ± 2.1	11.0 ± 4.3
N-3 PUFA, g	0.6 ± 0.3	1.5 ± 0.2[a]
N-3 PUFA/n-6 PUFA	0.05 ± 0.03	0.15 ± 0.07[b]
Cholesterol, mg	389.8 ± 70.8	363.1 ± 89.4
Vitamin E, mg	8.0 ± 3.4	7.8 ± 2.3
Energy contribution		
Protein, %	18.3 ± 3.1	19.1 ± 4.9
Carbohydrates, %	37.0 ± 6.2	37.2 ± 6.2
Fat, %	44.3 ± 3.6	42.9 ± 9.6
SFA, %	14.9 ± 2.7	14.8 ± 2.9
MUFA, %	18.2 ± 1.2	17.9 ± 4.2
Total PUFA, %	6.1 ± 2.4	6.6 ± 1.4
N-6 PUFA, %	5.8 ± 2.3	5.2 ± 1.2
N-3 PUFA, %	0.3 ± 0.2	0.7 ± 0.4[b]
CSFI	26.7 ± 4.6	26.0 ± 4.5
CI	23.0 ± 4.0	22.5 ± 4.2

Mean values ± SD of the 10-d period which bear superscripts [a] and [b] are significantly different ($p < 0.001$ and $p < 0.05$ respectively). (Paired Student *t*-test). *Diet plus fish oil supplement. SFA, saturated fatty acids. MUFA, monounsaturated fatty acids. PUFA, polyunsaturated fatty acids. CSFI, cholesterol-saturated fat index/1000 kcal of Connor et al. (13). CI, Cholesterol index/1000 kcal of Zilversmit (44)

Table 2 Major fatty acid composition of n-3 supplement

Myristic acid	6.97 ± 0.11
Palmitic acid	16.17 ± 0.14
Palmitoleic acid	8.66 ± 0.07
Oleic acid	13.06 ± 0.08
Linolenic acid	8.78 ± 0.06
Eicosapentaenoic acid	14.53 ± 0.16
Docosahexaenoic acid	8.90 ± 0.07
Total saturated	29.62 ± 0.25
Total monounsaturated	25.60 ± 0.10
Total polyunsaturated	39.21 ± 0.20
Total n-3 polyunsaturated	32.06 ± 0.20
Polyunsaturated/saturated ratio	1.32 ± 0.02

Results (% of total fatty acids) are mean ± SD of three determinations

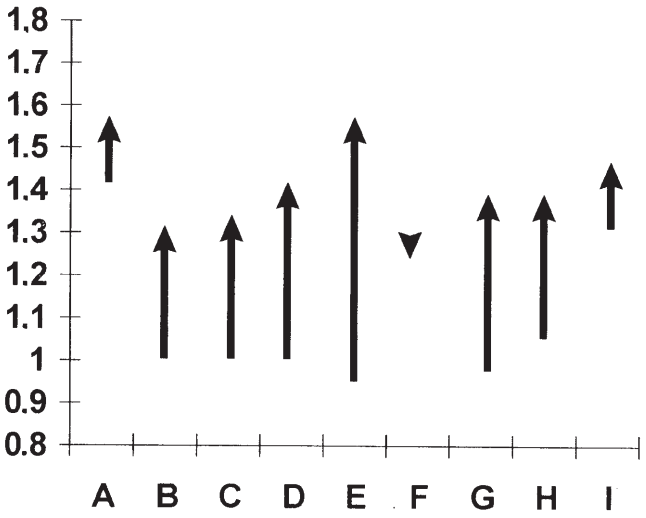


Fig. 2 LDL cholesterol/apoprotein B ratio changes produced by the fish oil supplementation in the 9 subjects. Up-arrows indicate increase, whereas down-arrow means decrease in the ratio LDL cholesterol/Apoprotein B

Very low density lipoprotein cholesterol (VLDL-C) decreased significantly ($p < 0.05$). Serum, LDL- and HDL-triglycerides were not significantly affected but VLDL-triglyceride values decreased significantly ($p < 0.05$). A significant reduction in serum phospholipid ($p < 0.001$), VLDL-phospholipid ($p < 0.05$), LDL-phospholipid ($p < 0.001$), and HDL-phospholipid ($p < 0.05$) values was shown. Total protein in VLDL ($p < 0.05$), LDL ($p < 0.001$), and HDL ($p < 0.01$) decreased after the n-3 supplementation. HDL-Apo A1 fell by 13.28 % ($p < 0.01$).

Table 3 Effects of n-3 supplementation on serum lipids, lipoproteins, and apolipoproteins (Apo) in young adult women

	Basal	After supplementation	Change (%)	P
Serum				
Cholesterol, mmol/l	5.14 ± 0.78	4.80 ± 0.90	-6.57	NS
Phospholipids, mmol/l	3.50 ± 0.36	2.81 ± 0.33	-19.71	<0.001
Triglycerides, mmol/l	0.75 ± 0.78	0.68 ± 0.25	-9.01	NS
VLDL				
Cholesterol, mmol/l	0.34 ± 0.10	0.28 ± 0.14	-18.56	<0.05
Phospholipids, mmol/l	0.15 ± 0.05	0.11 ± 0.04	-23.05	<0.05
Triglycerides, mmol/l	0.43 ± 0.18	0.39 ± 0.18	-10.91	<0.05
Apo B, mg/dl	2.45 ± 0.78	1.57 ± 0.83	-32.69	<0.05
Total protein, mg/dl	6.24 ± 2.31	4.20 ± 1.56	-35.92	<0.05
LDL				
Cholesterol, mmol/l	3.03 ± 0.70	2.87 ± 0.83	-5.30	NS
Phospholipids, mmol/l	1.22 ± 0.27	0.67 ± 0.33	-45.40	<0.001
Triglycerides, mmol/l	0.25 ± 0.11	0.21 ± 0.09	-12.67	NS
Apo B, mg/dl	101.75 ± 22.67	78.73 ± 21.84	-22.62	<0.001
Total protein, mg/dl	103.84 ± 24.94	81.00 ± 23.76	-22.00	<0.001
LDL-cholesterol/LDL-ApoB[a]	1.14 ± 0.15	1.39 ± 0.20	+21.23	<0.01
HDL				
Cholesterol, mmol/l	1.80 ± 0.31	1.66 ± 0.32	-7.81	BL (0.08)
Phospholipids, mmol/l	1.89 ± 0.28	1.63 ± 0.21	-14.13	<0.01
Triglycerides, mmol/l	0.07 ± 0.03	0.09 ± 0.03	+24.23	NS
Apo A1, mg/dl	145.3 ± 19.8	126.0 ± 15.2	-13.28	<0.01
Total protein, mg/dl	224.22 ± 33.34	200.69 ± 34.26	-10.49	<0.01

Results are means ± SD of nine women. NS: Not significant. BL: At the borderline for statistical significance. To transform cholesterol, phospholipids, and triglycerides in mg/dl multiply by 38.7, 75, and 89, respectively. [a] Calculated from data in mg/dl

Table 4 Percentage contribution of lipids and protein to the lipoprotein mass

	Basal	After supplement	P
VLDL			
Total mass, mg/dl	69.9 ± 15.5	55.7 ± 10.1	<0.05
Cholesterol	19.71 ± 3.27	18.47 ± 5.42	NS
Phospholipids	16.15 ± 2.91	15.10 ± 2.52	NS
Triglycerides	56.11 ± 5.96	59.56 ± 3.03	NS
Protein	8.03 ± 3.77	6.87 ± 2.42	NS
LDL			
Total mass, mg/dl	334.4 ± 80.1	259.0 ± 63.1	<0.05
Cholesterol	35.05 ± 3.99	43.27 ± 2.75	<0.001
Phospholipids	27.57 ± 3.71	18.43 ± 3.80	<0.01
Triglycerides	6.38 ± 2.39	6.91 ± 2.06	NS
Protein	31.01 ± 2.09	31.61 ± 2.52	NS
HDL			
Total mass, mg/dl	442.2 ± 30.3	395.7 ± 28.5	<0.05
Cholesterol	13.75 ± 1.28	16.22 ± 1.36	NS
Phospholipids	32.14 ± 1.16	31.05 ± 2.42	NS
Triglycerides	1.41 ± 0.59	1.98 ± 0.73	NS
Protein	50.70 ± 2.09	50.75 ± 3.28	NS

Data are means ± SD; NS: Not significant

Apo B decreased significantly in VLDL as well as LDL ($p < 0.05$, and $p < 0.001$, respectively). The LDL-C/LDL-Apo B ratio increased significantly by 21.23 % ($p < 0.01$).

The VLDL mass (lipids + proteins), LDL mass, and HDL mass decreased significantly (all, $p < 0.05$). Percentage contributions of lipids and protein to the total lipoprotein mass were unaffected in VLDL and HDL, but cholesterol increased and phospholipids decreased in the average LDL particle (Table 4).

A detailed study of the data shows that the LDL-C/LDL-Apo B ratio rose considerably (> 25 %) in 5 of the 9 women studied, while 8 of the 9 presented an increased LDL-C/Apo B ratio (> 10 %) (Fig. 2).

Table 5 sets forth the serum phospholipid fatty acid profile. A tendency of the EPA and DHA percentages to increase was found.

Table 5 Effect of n-3 fatty acid supplementation on selected fatty acids in serum phospholipids (% total fatty acids)

	Basal period	After supplementation
Oleic acid	18.3	17.2
Linoleic acid	13.2	11.4
Arachidonic acid	9.1	8.4
Eicosapentaenoic acid	0.5	1.4
Docosahexaenoic acid	2.1	5.0

Data are the mean of three samples

Discussion

This study shows that the use for 10 d of a 3 ml fish oil supplement in healthy young adult women induced minor changes in serum lipoprotein cholesterol and triglyceride concentrations. In contrast, the lipoprotein phospholipid and protein concentrations were markedly affected, above all in LDL and HDL.

According to the fat intake data from both dietary periods studied, the women presented a Mediterranean dietary profile with a high percentage of MUFA. The PUFA/SFA ratio was quite low (< 0.4). Cholesterol consumption was above the recommended 300 mg/day. Data from different Spanish populations (18, 30) agree with the findings of the present study.

Results of n-3 supplement on serum lipoproptein cholesterol are similar than those described by several authors (2, 15, 20, 22, 25) who have indicated that the intake of moderate amounts of n-3 fatty acids have minor influence on LDL-C and HDL-C concentrations. Present results on VLDL triglycerides agree with several author results (22, 25, 31) and although small were significant. Current results must be a consequence of both the previous low fasting triglyceride concentration in the young female subject studied, and the low-dosage n-3 PUFA supplement used. Lower fasting and postprandial concentrations of triglyceride together with the higher concentration of HDL-C are notable features of the female lipid profile (42). Doses of < 5 g fish oil/day (1 g EPA) have been shown to have negligible effects on triglycerides and plasma lipoproteins (2, 15, 37).

Different studies have shown that EPA and DHA do not necessarily affect lipoprotein metabolism in a similar way (2, 12). Childs et al. (12) compared oils with different EPA to DHA ratios and indicate that only DHA-rich oils can decrease LDL-C and are better at maintaining HDL concentrations than EPA-rich oils. Moreover, it has been shown that while the correlation of the EPA concentration with HDL-C is positive, that of the DHA concentration with HDL-C is negative, and only EPA is inversely correlated with the decrease in triglycerides (6). As we have commented, the two major fatty acids in the fish oil supplement used in the current study were EPA and DHA. The balance between EPA and DHA might be related to the lack of changes observed in serum and lipoprotein cholesterol and triglyceride concentrations in the current study.

Our results regarding the influence of fish oil supplementation on the fatty acid profile of serum phospholipids agree with the information previously documented in man (1, 2, 32), where EPA and DHA percentages tended to increase with respect to the basal levels, while that of other fatty acids, such as arachidonic acid, decreased. Nevertheless, samples of serum phospholipids analyzed showed a relatively high percentage of DHA, suggesting some influence on the n-3 fatty acids consumed by the high and low energy contribution of oleic and linoleic acids, respectively. Oleic, linoleic, and linolenic acids have been shown to influence the metabolic effect of n-3 fatty acids (38).

On the other hand, references about the changes induced by n-3 PUFA in the absolute amount of serum phospholipids in humans are very scarce. All of the women in the current study displayed a drop in serum phospholipids, and this reduction was relevant (> 10 %) in 7 of them.

Present results shows a market effect of the n-3 supplement on lipoprotein-Apo A1 and Apo B levels. Herring and lamprey oils suppress the expression of messenger ribonucleic acid (mRNA) for Apo A1 and Apo B whereas safflower oil enhances the expression of Apo B, suggesting that n-3 PUFA may inhibit hepatic and intestinal synthesis of Apo A-I and Apo B (26). Apo A1 levels were reported to decrease in healthy volunteers but did not change in type IIb and V patients given doses of fish oil (32).

In the present study, a significant decrease of the VLDL lipid and protein contents was observed. Due to the fact that the contribution (percentage) of protein and the different lipids on the average VLDL particle was unaffected, it can be assumed that the fish oil supplement induced a decrease in the number of circulating VLDL particles. This reduction is known to be related to the effect of n-3 fatty acids because fish oil + EPA diminishes the production of Apo B in humans and rats (14, 34) and may reduce the daily flow of VLDL-Apo B (14).

LDL particle composition studies and epidemiological observations have shown that small, dense LDL is characterized by a high protein content and a low number of cholesterol esters (27). Apo B-rich LDL particles have been associated with coronary artery disease, even in subjects with normal lipid levels (4, 10).

As each LDL particle contains only one Apo B molecule, it can be calculated that women will present 22 % fewer average LDL particles after taking the n-3 PUFA supplement than before the treatment. The number of cholesterol molecules per Apo B particle in the average LDL of these women can be determined considering a MW of 550 kd for Apo B. The women in our study presented about 2 290 cholesterol molecules per Apo B particle after the n-3 treatment, while the same women presented about 1 870 before treatment. These average LDL particles contain more cholesterol and fewer phospholipids; thus, it can be confirmed that the n-3 supplement used induces the presence of large LDL particles. In fact, extrapolating the LDL-C/LDL-Apo B ratio to the ratios found by Campos et al. (11), the average LDL particles during the basal period would be $LDL_{4–5}$, whereas after n-3 fatty acid supplementation the average LDL particles would be $LDL_{2–3}$. Small dense LDL particles are more susceptible to oxidative damage than larger LDL; thus, the theoretical increase in LDL size after n-3 fatty-acid supplementation might be expected to reduce atherogenic risk (19).

In contrast to our results, it has been also found that the amount of total lipids per mg of LDL protein was significantly lower in LDL in the subjects given fish oil (19). These authors did not find any significant change in the ratio between surface-to-core components of the LDL particles; however, the percentage of phospholipids in the LDL particle composition tended to decrease (19). Differences observed with respect to our study data must be attributed to differences in experimental conditions (length, dosage, and subjects) of both studies.

It is not easy to understand the relevance and the metabolic events involved in the LDL phospholipid decrease, but this reduction might be a consequence of the low VLDL phospholipid levels after the treatment, as well as of the possible increase in the transfer of surface material from VLDL to HDL to guarantee adequate levels of HDL in the women during the n-3 fatty acid supplementation period.

Metabolic studies of Apo in HDL in human volunteers have revealed that variations in HDL levels are partially due to differences in fractional catabolic rates. Large HDL particles with increased HDL-cholesterol/HDL-Apo A1 plus Apo AII ratios exhibit decreased fractional catabolic rates (8, 9). These results suggest that HDL composition might be important in controlling HDL levels and in turn, in processes that influence the development of atherosclerosis. The fish oil treatment did not alter the percent contribution of lipids and protein in the average

HDL particle. From current data it can also be calculated that the cholesterol/Apo A1 molar ratio did not change, and owing to the significant decrease in the HDL total mass observed, it can be deduced that the number of average HDL particles decreased.

The small and non-significant changes in the intake of oleic, linoleic, and linolenic acids between both periods of the current study were not found to correlate significantly with variations in lipids, lipoproteins or Apo. However, the greater the intraindividual differences in SFA intake between the basal and the supplementation period, the greater the decrease in LDL-Apo B (r: 0.6804; $p < 0.001$) and the lower the reduction observed in HDL-Apo A1 (r: -0.5024; $p < 0.05$).

In short, a low daily dosage of n-3 fatty acids for 10 d markedly decreased the serum and lipoprotein phospholipid levels, the HDL-Apo A1, the Apo B concentration in VLDL and LDL, and the LDL-cholesterol/LDL-Apo B ratio in young adult women but was unable to decrease in a significant manner the cholesterol and triglyceride concentrations in LDL and HDL. A decrease in the total number of VLDL, LDL, and HDL particles can be suggested.

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References

1. Anttolainen M, Valsta LM, Alfthan G, Kleemola P, Salminen I, Tamminen M (1996) Effect of extreme fish consumption on dietary and plasma antioxidant levels and fatty acid composition. Eur J Clin Nutr 50:741–746
2. Agren JJ, Hänninen O, Julkunen A, Fogelholm L, Vidgren H, Schwab U, Pynnönen O, Uusitupa M (1996) Fish diet, fish oil and docosahexaenoic acid rich oil lower fasting and postprandial plasma lipid levels. Eur J Clin Nutr 50:765–771
3. Allain CC, Poon LS, Chan SCG, Richmond W, Fu PC (1974) Enzymatic determination of total serum cholesterol. Clin Chem 20:470–475
4. Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM (1988) Low-density lipoprotein subclass patterns and risk of myocardial infarction. J Am Med Assoc 260:1917–1921
5. Balogn M, Kahn HA, Medalie JH (1971) Random repeat 24-hour dietary recalls. Am J Clin Nutr 24:304–310
6. Bønnaa KJ, Bjerve KS, Nørdoy A (1992) Docosahexaenoic and eicosapentaenoic acids in plasma phospholipids are divergently associated with high density lipoproteins in humans. Arterioscler Thromb 12:675–681
7. Brekke MJ, Brekke ML, Quiter ES, Peters JR, Mullis RM, Hunninghake DB (1992) Appropriate days for measuring intake of dietary fat and cholesterol. Ann Nutr Metab 36:318–327
8. Brinton EA, Eisenberg S, Breslow JL (1989) Elevated high density lipoprotein cholesterol level correlated with decreased apolipoprotein A-I and Apo A-II fractional catabolic rate in women. J Clin Invest 84:262–269
9. Brinton EA, Eisenberg S, Breslow JL (1991) Increased Apo A-I and Apo A-II fractional catabolic rate in patients with low high density lipoprotein cholesterol levels with or without hypertriglyceridemia. J Clin Invest 87: 536–544
10. Campos H, Genest Jr. JJ, Blijlevens E, McNamara JR, Jenner JL, Ordovas JM, Wilson PWF, Schaefer EF (1992) Low density lipoprotein particle size and coronary artery disease. Arterioscler Thromb 12:187–195
11. Campos H, Blijlevens E, McNamara JR, Ordovas JM, Posner BM, Wilson PWF, Castelli WP, Schaefer EF (1992) LDL particle distribution: Results from the Framingham Offspring study. Arterioscler Thromb 12:1410–1419
12. Childs MT, King IB, Knopp RH (1990) Divergent lipoprotein responses to fish oils with various ratios of eicosapentaenoic acid and docosahexaenoic acid. Am J Clin Nutr 52:632–639
13. Connor SL, Gustafson JR, Artaud-Wild SB, Flavell DP, Classick-Kohn CJ, Hatcher LF, Connor WE (1986) The cholesterol/saturated-fat index: An indication of hypercholesterolaemic and atherogenic potential of food. Lancet 1:1229–1232
14. Connor WE (1997) Do the n-3 fatty acids from fish prevent deaths from cardiovascular disease? Am J Clin Nutr 66:188–189
15. Davidson DM, Gold KV (1988) Cardiovascular effects of n-3 fatty acids. N Engl J Med 319:580 (Letter)
16. Domenech JM (ed) (1982) In: Bioestadistica. Métodos estadisticos para investigadores. 4th ed. Herder: Barcelona
17. EPCUM (1993) Estudio de prevalencia y prevención primaria de las enfermedades cardiovasculares en Madrid. Presentación. Hospital Universitario de San Carlos. Madrid. Area de Sanidad y Consumo del Ayuntamiento de Madrid. Madrid
18. Folch J, Lees M, Sloane-Stanley (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509
19. Graaf J de, Hak-Lemmers HLM, Hectors MPC, Demackers PNM, Hendriks JCM, Stalenhoef AFH (1991) Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healthy subjects. Arterioscler Thromb 11:298–306
20. Harris WS (1989) Fish oil and plasma lipid and lipoprotein metabolism in humans: A critical review. J Lipid Res 30:785–807
21. Harris WS (1996) n-3 fatty acids and lipoproteins: Comparison of results from human and animal studies. Lipids 31:243–252
22. Harris WS (1997) n-3 fatty acids and serum lipoproteins: Human studies. Am J Clin Nutr 65 (suppl):645S–1654S
23. Hwang DH, Chanmugam PS, Ryan DH, Boudrau MD, Windhauser MM, Tulley RT, Brooks ER, Bray GA (1997) Does vegetable oil attenuate the beneficial effects of fish oil in reducing risk factors for cardiovascular disease? Am J Clin Nutr 66:89–96
24. IUPAC (1987) In: Standard methods for the analysis of oils, fats and derivatives. Paquot C, Hatfenne A (prepared by). 7th revised and enlarged edition. Blackwell Scientific Publications: Oxford, pp 123–129
25. Kinsella JE, Lokesh B, Stone RA (1990) Dietary n-3 polyunsaturated fatty acids and amelioration of cardio-

vascular disease: Possible mechanisms. Am J Clin Nutr 52:1–28
26. Matsumoto A, Aburatani H, Mizuta Y, Kaga A, Itakura H (1987) Dietary n-3 fatty acids (fish oils) suppress the expression of apolipoprotein B and A1 genes in rats. Atherosclerosis 7:506 (Abstract)
27. McNamara JR, Campos H, Ordovas JM, Peterson J, Wilson PWF, Schaefer EJ (1987) Effect of gender, age, and lipid status on low density lipoprotein subfraction distribution. Results of the Framingham Offspring study. Arteriosclerosis 7:483–490
28. Moreiras-Tuni O, Carbajal A, Perea del Pino IM (eds) (1990) Evolución de los hábitos alimentarios en España. Ministerio de Sanidad y Consumo. Secretaría General Técnica. Artes Gráfícas Iberoamericanas S.A., Madrid
29. Moreiras O, Carbajal A, Cabrera L (eds) (1992) La composición de los alimentos. EUDEMA S.A., Madrid, pp 1–91
30. Nestel PJ, Wong S, Topping DL (1986) Dietary long chain polyenoic fatty acids: Suppression of triglyceride formation in rat liver. In: Simopoulos AP, Kifer RR, Martin RE (eds) Health effects of Polyunsaturated Fatty Acids in Seafoods. Academic Press, New York, pp 211–218
31. Nestel PJ (1990) Effects of n-3 fatty acid on lipid and lipoprotein metabolism. Annu Rev Nutr 10:149–167
32. Phillipson BE, Rothrock DR, Connor WE, Harris WS, Illingworth DR (1985) Reduction of plasma lipids, lipoproteins and apoproteins by dietary fish oils in patients with hypertriglyceridemia. N Engl J Med 312:1210–1216
33. Rifai N, King ME (1986) Immunoturbidimetric assays of apolipoproteins A, A-1, A-2 and B in serum. Clin Chem 32:957–961
34. Sánchez-Muniz FJ (1987) Prevención con dieta para una vida longeva. Relevancia del consumo de pescado. Rev Clin Esp 180:43–47
35. Sánchez-Muniz FJ, Viejo JM, Medina R (1992) Deep-frying of sardines in different culinary fats. Changes in the fatty acid composition of sardines and frying fats. J Agric Food Chem 40:2252–2256
36. Sánchez-Muniz FJ, Bastida S, Quintas E, Merinero MC, Rodríguez-Gil S (1997) Heterogeneous responsiveness of normolipemic women to n-3 long chain fatty acid supplementation. Changes in serum lipids and apoproteins. J Physiol Biochem 53:349–354
37. Schmidt EB, Kristensen SD, De Caterina R, Illingworth DR (1993) The effects of n-3 fatty acids on plasma lipids and lipoproteins and the other cardiovascular risk factors in patients with hyperlipidemia. Atherosclerosis 103: 107–121
38. Simopoulos AP, Kifer RR, Martin RE, Barlow SM (1991) Preface. World Rev Nutr Diet 66:XVI–XIX
39. Takayama MN, Itoh S, Nagasaki T, Tinimizu I (1977) A new enzymatic method for determination of serum choline containing phospholipids. Clin Chem Acta 79:93–98
40. Terpstra AHM, Woodward CJM, Sánchez-Muniz FJ (1981) Improved techniques for the separation of serum lipoproteins by density gradient ultracentrifugation: Visualization by prestaining and rapid separation of serum lipoproteins from small volumes of serum. Anal Biochem 111:149–157
41. Wahlefeld AW (1974) In: Methoden der enzimatischen Analyse. 3 ed., vol II, Verlag Chemie, Weinheim, p 1878
42. Williams CM (1997) Cardiovascular risk factors in women. Proc Nutr Soc 56:383–391
43. Zampelas A, Peel AS, Gould BJ, Wright J, Williams CM (1994) Polyunsaturated fatty acids of the n-6 and n-3 series: Effects on postprandial lipid and apolipoprotein levels in healthy men. Eur J Clin Nutr 48:842–848
44. Zilversmit DB (1979) Cholesterol index of foods. J Am Dietet Assoc 74: 562–565