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Heterogeneous responsiveness of normolipemic women to n-3 long chain fatty acid supplementation. Changes in serum lipids and apoproteins

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The effect of 10 day-low dosage of n-3 long chain fatty acids (390 mg/day of EPA and 252 mg/day of DHA) on lipid and apolipoprotein (Apo) concentrations has been studied in nine normolipidaemic women aged 28.9 ± 4.2 years. n-3 fatty acid supplementation did not significantly decrease total cholesterol and triglyceride levels but markedly decreased the Apo A1 and Apo B concentrations (12.7 %, $p < 0.01$ and 23.1 %, $p < 0.001$, respectively), while the Apo A1/Apo B ratio significantly increased (14.8 %, $p < 0.02$). In contrast to the individual variations found for triglycerides and cholesterol, Apo changes indicate a fairly homogeneous response to the fish oil supplement. In seven women Apo A1 decreased (>10%), whereas Apo B decreased (> 10 %) in all of them. The Apo A1/Apo B ratio increased (> 10 %) in five of these nine women. Changes in Apo A-1 and Apo B did not significantly correlate with changes in serum lipids. These findings suggest that short-term supplementation with low amount of n-3 long chain fatty acids, EPA and DHA, influences the serum Apo content more than the lipid levels in normolipidaemic women.

Key words: Apoproteins A1 and B, Cholesterol, PUFA, n-3, Triglycerides, Women.

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Abbreviations

APO, apolipoprotein(s); DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL, high density lipoproteins; ø HDL-C, high density lipoprotein cholesterol; LDL, low density lipoproteins; LDL-C, low density lipoprotein cholesterol; mRNA, messenger ribonucleic acid; MUFA PUFA, mono and polyunsaturated fatty acids; SFA, saturated fatty acids; VLDL, very low density lipoproteins.

Clinical and epidemiological studies have led to the hypothesis that n-3 polyunsaturated fatty acids (PUFA), eicosapentaenoic (EPA, C20:5 n-3), and docosahexaenoic acid (DHA, C22:6 n-3), occurring in fatty fish and fish oil may be useful for ameliorating atherosclerosis (3-5, 9, 15, 18). However, most studies have investigated the effect of rather large amounts of EPA+DHA (10, 16). Thus, a high dietary supply of n-3 PUFA by increasing fish intake is considered to be the most effective dietary intervention in reducing hypertriglyceridemia (11, 21, 26). In contrast, the effectiveness of fish in reducing plasma cholesterol is more controversial (11, 12, 21, 29). However, there is limited information on the influence of n-3 fatty acids on apolipoprotein (Apo) concentration (1, 10, 25, 32). The effect of n-3 preparations on blood lipids and Apo of subjects with normal or even low serum lipid levels have been scarcely reported.

This study, therefore, was designed to investigate the influence of a standardized fish oil preparation containing moderate level of n-3 PUFA on the concentration of serum lipids, and Apo in normolipidaemic women.

Materials and Methods

The study was carried out in 9 women, aged 28.9 ± 4.2 years (range, 24 to 40 years) weighing 58.5 ± 10.6 kg (range, 51.0 to 75.4 kg), with a body mass index of 23.0 ± 2.6 kg/m^2 (range, 19.0 to 27.3 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 traning, 1 to 3-h/week), were matched by sociocultural level (University students) and with similar food 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 ingest 6 capsules per day (2 at breakfast, 2 at lunch, and to 2 at dinner) for 10 days.

Each capsule contained 500 mg fat, 0.34 mg vitamin E, 4.2 mg cholesterol, 60 mg carbohydrates, 115 mg proteins, 5.2 kcal (21.8 kJ) being its energy content. A total daily supplementation of 870 mg of n-3 fatty acids (390 mg EPA and 252 mg DHA) was given to each volunteer.

Women were blood sampled once, at the start of the experiment after fasting for 12 to 15-h, and then 28 days later. Fish-oil supplementation started on day 17 of the study and ended 2 days before the second blood venipuncture. This experimental design eliminates bias due to lipid variation during the hormonal cycle and enabled us to measure the n-3 fatty acid influence on serum lipids and Apo in women, avoiding the confusing effects of the menstrual cycle (35). The experimental design also eliminates nutritional changes as the women maintained their habitual diets throughout the experiment.

Several capsules were randomly selected and opened their oil being homogeneously mixed and saponified with 0.5 N sodium hydroxide in methanol, and then it was methylated following the IUPAC method (14). The fatty acid methyl esters were analyzed by gas chromatography. Details of the method and the calculations used have previously been published (28).

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. Cholesterol in sera was measured using the enzymatic cholesterol esterase-cholesterol oxidase method (2) of Boehringer Mannheim, Germany. Triglycerides were tested according to the enzymatic glycerol-phosphate-oxidase method (33) of Boehriger Mannheim. Apo A1 and B were determined by kinetic immuno-

turbidimetry (27) following the indications and control of Behring Institute.

Lipids internal quality control was carried out according to the laboratory manual of the Lipid Research Clinics Program. Apo 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, Apo A1 and Apo B were 3.5 %, 3.7 %, 5.0 % and 4.5 %, respectively.

A statistical study of the data was carried out using the paired Student *t* test. Pearson product-moment correlations between different lipids and Apos were also studied (8).

Table I. *Major fatty acid composition of the n-3 supplement used in the study.*
Values ar percentages of total fatty acids (mean ± SD) of three determinations.

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
EPA	14.53 ± 0.16
DHA	8.90 ± 0.07
Total SFA	29.62 ± 0.25
Total MUFA	25.60 ± 0.10
Total PUFA	39.21 ± 0.20
Total n-3 PUFA	32.06 ± 0.20
PUFA/SFA	1.32 ± 0.02

Results

The major fatty acid composition of the fish oil supplement is given in table I. This preparation is similar to some sardine oil-concentrates, as the EPA plus DHA concentration accounts for approximately 25 % of total fatty acids.

The low supplement-dosage of n-3 fatty acids used during a period of 10 days induced a non significant decrease in total cholesterol and triglycerides (table II). Nevertheless, a marked decrease in serum Apo concentrations was found.

Contrariwise to what occurs with the apolipoproteins, there is no homogeneity in the individual response of the triglycerides and of cholesterol to the fish oil supplementation. Thus, only three of the nine displayed a substantial reduction of triglycerides (> 10 %), whereas one suffered a triglyceride increase (> 10 %). Total serum cholesterol decreased (> 10 %) in only two women. However, in seven women Apo A1 levels fell (> 10 %), whereas Apo B decreased (> 10 %) in all of them. Furthermore, the Apo A1/Apo B ratio increased in all nine women studied.

Table II. *Effects of the n-3 supplement on lipids and apoproteins A1 and B in women.*
Values are mean ± SD of nine women.

	Initial	Final
Triglycerides[1] (mg/dl)	66.61 ± 24.01	60.61 ± 21.87
Total cholesterol[2] (mg/dl)	198.84 ± 30.31	185.77 ± 34.75
Apo A1 (g/l)	1.50 ± 0.20	1.31 ± 0.15*
Apo B (g/l)	1.04 ± 0.25	0.80 ± 0.23***
Apo A1/Apo B	1.55 ± 0.52	1.78 ± 0.62**

[1] To transform into mmol/l divide by 89.

[2] To transform into mmol/l divide by 38.7.

* $p < 0.01$; ** $p < 0.02$; *** $p < 0.001$.

Five of these nine women exhibited a marked increase (> 10 %) of this ratio.

Changes in serum lipid concentrations do not significantly correlated with those found in Apo A1 and Apo B.

Discussion

This study shows that fish oil supplement induced minor changes in serum lipids. In contrast, the Apo profile was markedly affected.

Present results on serum cholesterol are in agreement with those described by several authors (1, 6, 11, 12, 16, 19-21). However, the absence of any significant change in triglyceride concentrations is rather difficult to explain, as n-3 PUFA are well known to decrease serum triglyceride levels (6, 10-12, 19-23, 32). Current results may be related to the low EPA + DHA dosage studied, since according to HARRIS (11), supplementary EPA + DHA lowers triglyceride levels in a dose-dependent manner. SCHMIDT *et al.* (30) reported that 4 g/day was the minimum amount of n-3 PUFA required to suppress plasma triglycerides.

Lipid-change tendencies were fairly different or even opposite in some women independently of their basal lipids concentrations. The heterogeneity in the response to such n-3 dosage in the current study is difficult to explain (bias of age, physical activity, sociocultural influence, diet, and sex were eliminated. ORDOVAS *et al.* (24) have reviewed the current knowledge regarding the gene-diet interaction in relation to plasma lipid response to dietary intervention. Several candidate loci have been examined in humans: Apo A1, Apo AIV, Apo B and Apo E. The mechanisms responsible are still unknown, but these authors suggest that mutations at these loci have been found to be associated with responsiveness to diminution of plasma lipids due to dietary fat and cholesterol restriction.

Relatively few investigators have examined the effects of fish oils on plasma Apo levels (1, 20, 22, 26, 31, 32, 34). In contrast to the current results. ÅGREN *et al.* (1) did not find any significant changes in total Apo B or Apo A1 levels after a moderate fish oil supplementation. Apo B levels decreased in trials reporting a drop in LDL-C (22, 26). However, in 42 hypertense 60 year old subjects, SUZUKAWA *et al.* (31) observed that LDL-C concentrations rose with fish oil but without a concomitant increase in plasma Apo B. Apo A1 levels were reported to decrease in healthy volunteers (20) but did not change in type IIb and V patients given doses of fish oil (26).

Fish oil + EPA diminished the production of Apo B in human and rat (5, 22, 23) and may reduce the daily flux of transport of VLDL-Apo B. Herring and lamprey oils suppress the expression of mRNA for Apo A1 and B whereas safflower oil enhances the expression of Apo B, suggesting that n-3 PUFA may inhibit hepatic and intestinal synthesis of Apo A1 and B (17).

In summary, a low daily dosage of n-3 fatty acids markedly decreased the serum Apo A1 and B concentrations of normolipidemic women but was unable to change their serum lipids.

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sensibilidad a la suplementación con ácidos grasos n-3 de cadena larga en mujeres normolipémicas. Cambios en lípidos y apoproteínas. J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (4), 349-354, 1997.

En nueve mujeres normolipémicas de 28,9 ± 4,2 años de edad se estudia el efecto de la utilización durante 10 días de una pequeña dosis de ácidos grasos n-3 de cadena larga (390 mg/día de EPA y 252 mg/día de DHA) sobre los niveles de lípidos y apoproteínas (Apo) del suero. Tal suplemento no modifica significativamente los niveles de colesterol y triglicéridos, pero disminuye de forma marcada la concentración de Apo A1 y Apo B (12,7 %, $p < 0,01$ y 23,1 %, $p < 0,001$, respectivamente). En contraste con las variaciones dispares intraindividuales encontradas para el colesterol y los triglicéridos, los cambios son bastante mas homogéneos respecto a las Apo. En 7 de las 9 mujeres disminuye la Apo A1 (> 10 %), mientras que la Apo B desciende (> 10 %) en todas ellas. El cociente Apo A1/Apo B se eleva (> 10 %) en 5 mujeres. Los cambios en la concentración sérica de los lípidos no se correlacionan significativamente con las modificaciones en las Apo. Estos resultados señalan que la suplementación con pequeñas dosis de EPA y DHA durante 10 días afecta en mayor medida a los niveles en suero de las Apo que a los de los lípidos.

Palabras clave: Apoproteínas A1 y B, Colesterol, Mujeres, PUFA n-3, Triglicéridos.

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