Long-term Treatment of N-3 PUFAS on Plasma Lipoprotein Levels and Fatty Acid Composition of Total Serum and Erythrocyte Lipids in Hypertriglyceridemic Patients

Supanee Putadechakum, DSc*, Vichai Tanphaichitr, MD, PhD, FACP, FRACP**
Preeya Leelahagul, DSc*, Varapat Pakpeankitvatana, DSc***,
Thitima Surapisitchart, BS*, Surat Komindr, MD**

** Department of Research Center, Ramathibodi Hospital, Mahidol University*
*** Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University*
**** Department of food chemistry, Faculty of Pharmacy, Mahidol University*

The low incidence of coronary heart disease in Greenland Eskimos and Japanese fishermen who have high consumption of fish and seafood has called the attention in the role of eicosapentaenoic (EPA = 20:5n-3) and docosahexaenoic (DHA = 22:6n-3) acids, the major n-3 polyunsaturated fatty acids (PUFAs) in fish oils, in reducing cardiovascular risk. N-3 PUFAs may reduce serum lipids by promoting fatty acid oxidation and decrease VLDL producing from the liver, depress tissue arachidonate synthesis and alter the composition and tissue PUFAs.

Plasma lipoprotein and fatty acid composition of total serum and erythrocyte lipids in 9 hypertriglyceridemic patients consisting of 4 males and 5 females aged 39-72 yr who attended the Nutrition Clinic, Ramathibodi Hospital were investigated. The study period consisted of 4 wks of dietary advice only followed by 48 wks of dietary advice with a daily intake of 6 g of fish oil capsules (FOC). Six grams of FOC provided 1080 mg of 20:5n-3 and 720 mg of 22:6n-3.Their plasma triglyceride (TG) levels at wk 0, 12, 24, 36, 48 were 356.7, 230.1, 209.7, 192.9, 227.4 mg/dL and M-particle (equivalent to very low density lipoprotein, VLDL) were 484.4, 354.8, 383.1, 349.7, 453.2 mg/dL respectively that decreased significantly, whereas their plasma low density lipoprotein cholesterol (LDL-C) levels at the same periods were 139.4, 164.9, 171.0, 157.1, 158.3 mg/dL that increased significantly. Serum and erythrocyte 20:5n-3 and 22:6n-3 in these subjects were significantly higher than those at wk 0 throughout the study. These findings indicate the bioavailability of 20:5n-3 and 22:6n-3 in TG lowering effect of FOC. Fatty acids from fish oil have a remarkable effect on the synthesis and clearance of TG-rich lipoproteins, especially VLDL and chylomicrons. Though daily treatment with 6 g of FOC has a striking effect in increasing plasma LDL-C levels.

Keywords: *Fish oil capsules, Plasma lipoprotein, EPA, DHA, PUFAs, Hypertriglyceridemic patients*

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The low incidence of coronary heart disease in Greenland Eskimos and Japanese fishermen who have a high consumption of fish and seafood has called the attention in the role of eicosapentaenoic (EPA), docosahexaenoic (DHA) acids, and n-3 polyunsaturated fatty acids (PUFAs) in fish oils, in reducing cardiovascular risk[1]. In the multiple risk factor intervention trial cardiovascular mortality was noted to be inversely proportional to the intake of n-3 fatty acids over the 10.5 years of follow up[2]. A protective role in the secondary prevention of coronary heart disease (CHD) was also been seen in the Diet And Reinfarction Trial (DART)[3], in which a 29% decrease over 2 years in overall mortality in men who ate fatty fish twice a week, with no decrease in the rate of non-fatal myocardial infarction. In two large-scale observational studies, the Health Professionals

Correspondence to : Putadechakum S, Department of Research Center, Ramathibodi Hospital, Mahidol University, Bangkok, 10400, Thailand.

Study[4] and the US Physicians' Health Study[5] a similar pattern was noted. The Lyon Diet Heart Study[6] and the Indian trial by Singh et al[7] also strongly suggested a protective effect of n-3 PUFAs against CHD. These results are supported by the findings of GISSI Trial, which showed that treatment with n-3 PUFAs but not with vitamin E significantly lowered the risk of death, non-fatal myocardial infarction and stroke[8]. The epidemiological studies have associated n-3 PUFAs with decreased cardiac death due to its potential to beneficially modulate rhythm disorder and improve overall survival rates. N-3 PUFAs may reduce serum lipids by promoting fatty acid oxidation and decreasing VLDL produced from the liver, depress tissue arachidonate synthesis, alter the composition and tissue PUFAs, improve vascular tone and modify cell to cell interaction by altering eicosanoid balance. The present study was carried out to prove that a daily intake of fish oil can lower the VLDL.

Material and Method

Patients

Nine hypertriglyceridemic patients consisting of 4 males and 5 females aged 39-72 yr attending the Nutrition Clinic, Ramathibodi Hospital were enrolled into a duration of 52 weeks' study. Their mean (± SEM) of age, height, body weight are shown in Table 1. All of them were apparently healthy and were not receiving any medical treatment.

Experimental design

The study consisted of 4 wks of dietary control period followed by 48 wks of dietary control plus daily treatment with 6 g of fish oil capsule (FOC). Throughout the study all of the patients were instructed to consume diets with energy distribution of 15% protein, 30% fat and 55% carbohydrate-calories. They were advised to restrict their cholesterol intake (less than 300 mg/d) and to stop alcoholic consumption. During the FOC treatment period each patient consumed 3 capsules of FOC (1 g/capsule) after breakfast and dinner (6 g/day). Each capsule of FOC contains 180 mg of eicosapentaenoic acid (EPA) and 120 mg of docosahexaenoic acid (DHA). Thus, each patient obtained 1080 mg EPA and 720 mg DHA per day for 48 wks.

Anthropometric measurement

Body weight, height, triceps skinfold thickness (TST), mid upper arm circumference (MUAC), waist and hip circumferences in each patient were measured by using standard techniques[9-11] at the entry of the study (wk 0). Except height the remaining anthropometric parameters were also determined at wks 12, 24, 36 and 48. Mid-upper arm muscle circumference (UAMC) was calculated from the following formula[9]; UAMC = MUAC - 3.1416 TST. Standard values of TST, MUAC, UAMC were taken from the standard source[9]. MUAC, TST and UAMC in each subject was expressed as absolute values of these anthropometric parameters at the same periods.

Body mass index (BMI)[12] was calculated from weight in kg divided by height in (meters)^2. Waist and hip circumferences were measured and waist-over-hip circumference ratio (WHR) was computed[11]. The body fat (BF) of each patient was measured, using infrared light absorption analysis with the Body Composition Analyzer Futrex-5000 A (Futrex Inc. Gaithersburg, MD, USA)[13]. Body fat mass (BFM) was calculated from (body weight in kg x BF as% BW) ÷ 100 whereas fat-free mass (FFM) was calculated from body weight minus BFM in kg at the aforesaid period.

Blood collection

Venous blood was obtained from each patient after a 12-14 hr fast at wks 0, 12, 24, 36, 48. Whole blood and plasma were appropriately prepared for hematological study by H-1 hematology analyzer and biochemical determination by SMA-12 at the General Clinical Chemistry Laboratory, Ramathibodi Hospital at wk 0 and 48.

Plasma total cholesterol (TC)[14], triglyceride (TG)[15], and high density lipoprotein-cholesterol (HDL-C)[14,16,17] were measured by enzymatic colorimetric method. Low density lipoprotein-cholesterol (LDL-C) was calculated by Friedewald formula[18]: LDL-C = TC - (TG ÷ 5) - HDL-C. Serum L-, M-, S-particles reflecting chylomycron, very low density lipoprotein (VLDL) and LDL, respectively, by the micronephelometric method[19]. All of the aforementioned parameters were determined at wks 0, 12, 24, 36, 48.

Serum and erythrocyte fatty acids composition were determined by GLC method at wk 0, 12, 24,36, 48. The fatty acids were derived from the area under each peak of the chromatogram and expressed as percentages of total fatty acids.

Statistical analysis

Conventional statistical methods were used for the calculation of means and standard error of means (SEM) of various parameters. The results were tested for statistical significance among the groups by using

the Anova with repeated measurement. Statistical significance was considered at $p < 0.05$.

Results

Table 1 revealed the characteristics of the study cases.

Table 2 revealed the anthropometric parameters of the study cases in 48 weeks.

Table 3 revealed the plasma lipoprotein levels of the study cases in 48 weeks.

Table 4 and 5 revealed the serum and RBC 20:5 n-3 and 22:6 n-3 levels while receiving daily treatment with 6 g of FOC.

Discussion

The present study showed serum L-, M- and

Table 1. Initial characteristics of 9 hypertriglyceridemic patients on daily treatment with 6 g of FOC

No.	Sex	Age	Height	Weight	BMI	WHR	BP	Alcohol drinking
		yr	m	kg	Kg/m^2		mmHg	
1	M	43	1.67	81.2	29.1	1.00	115/75	-
2	M	52	1.80	77.8	24.0	0.90	125/85	-
3	M	39	1.61	63.3	24.4	0.90	130/90	-
4	M	43	1.68	80.5	28.5	0.96	130/80	+
5	F	56	1.50	52.0	23.1	1.00	150/90	+
6	F	49	1.50	55.0	24.4	0.88	105/75	+
7	F	64	1.44	51.2	24.7	0.97	135/75	-
8	F	72	1.50	52.8	23.5	1.02	145/90	-
9	F	49	1.50	67.2	29.9	0.91	135/90	-
Mean±SEM		51.89±3.55	1.58±0.04	64.56±4.22	25.73±0.88	0.95±0.02		

Table 2. Anthropometric parameters in 9 hypertriglyceridemic patients on daily treatment with 6 g of FOC

Parameter	Mean ± SEM				
	wk 0	wk 12	wk 24	wk 36	wk 48
BW (kg)	64.4 ± 4.3	64.4 ± 4.2	64.3 ± 4.4	64.3 ± 4.2	64.7 ± 4.1
BMI (kg/m^2)	25.7 ± 0.9	25.7 ± 0.9	25.6 ± 0.9	25.6 ± 0.9	25.8 ± 0.9
WHR	0.95 ± 0.02	0.94 ± 0.02	0.95 ± 0.01	0.96 ± 0.02	0.95 ± 0.01
BFM (kg)	22.4 ± 1.0	22.6 ± 1.0	23.2 ± 1.2	22.9 ± 1.2	23.3 ± 1.0
BF (%BW)	35.9 ± 1.9	36.1 ± 1.8	36.7 ± 1.6	36.3 ± 1.9	36.8 ± 1.8
FFM (kg)	41.7 ± 3.9	41.5 ± 3.7	41.0 ± 3.6	41.4 ± 3.7	41.4 ± 3.7
MUAC (cm)	29.7 ± 1.0	29.7 ± 1.0	30.2 ± 1.2	30.3 ± 1.0	30.5 ± 1.1
TST (mm)	18.5 ± 2.8	19.5 ± 3.3	20.5 ± 3.1[a]	20.8 ± 3.1[a]	20.4 ± 2.7[a]
UAMC (cm)	23.9 ± 1.2	23.6 ± 1.3	23.7 ± 1.6	23.8 ± 1.3	24.0 ± 1.2

[a] Significant difference from wk 0: $p < 0.05$

Table 3. Plasma lipoprotein levels in 9 hypertriglyceridemic patients on daily treatment with 6 g of FOC

Plasmalipoprotein	Mean ± SEM				
	wk 0	wk 12	wk 24	wk 36	wk 48
			mg/dL		
TC	238.2 ± 21.6	241.7 ± 18.9	245.9 ± 20.8	228.6 ± 17.8	239.0 ± 20.1
LDL-C	139.4 ± 17.0	164.9 ± 18.9	171.0 ± 20.8[a]	157.1 ± 16.6	158.3 ± 17.8
HDL-C	29.7 ± 1.5	30.9 ± 1.5	34.0 ± 1.5	33.2 ± 3.5	32.8 ± 2.7
TG	356.7 ± 38.1	230.1 ± 23.9[a]	209.7 ± 36.3[a]	192.9 ± 19.5[a]	227.4 ± 33.6[a]
L particle level	25.2 ± 5.4	14.7 ± 2.0	15.5 ± 4.7[a]	12.1 ± 2.1[a]	17.3 ± 4.4
M particle level	484.4 ± 66.6	354.8 ± 53.1[a]	383.1 ± 55.9	349.7 ± 51.8[a]	453.2 ± 58.9[b]
S particle level	394.8 ± 41.0	434.2 ± 40.2	438.0 ± 40.3	390.7 ± 41.8	404.2 ± 35.8

[a] Significant difference from wk 0: $p < 0.05$, [b] Significant difference from wk 12: $p < 0.05$

Table 4. N-3 and n-6 fatty acid composition of total serum lipids in 9 hypertriglyceridemic patients on daily treatment with 6 g of FOC

Wk	Mean ± SEM						
	18:3n-3	20:5n-3	22:5n-3	22:6n-3	18:2n-6	20:3n-6	20:4n-6
	% of total fatty acids						
0	1.9±0.1	0.4±0.1	0.8±0.2	3.6±0.3	21.0±1.4	1.1±0.1	5.8±0.4
12	1.8±0.2	0.8±0.2[a]	1.5±0.2[a]	5.4±0.4[a]	20.5±0.9	1.2±0.1	5.1±0.5
24	1.6±0.2	1.0±0.2[a]	2.0±0.3[a]	6.7±0.4[a,b]	19.4±1.0	1.1±0.1	4.6±0.3
36	1.6±0.1	1.1±0.1[a]	2.1±0.4[a]	6.9±0.4[a,b]	20.3±0.8	1.1±0.1	4.7±0.5
48	1.9±0.1	1.1±0.1[a]	2.6±0.3[a,b]	7.3±0.3[a,b]	22.1±1.1	1.1±0.1	4.8±0.3

[a] Significant difference from wk 0: $p < 0.05$, [b] Significant difference from wk 12: $p < 0.05$

Table 5. N-3 and n-6 fatty acid composition of total erythrocyte lipids in 9 hypertriglyceridemic patients on daily treatment with 6 g of FOC

Wk	Mean ± SEM						
	18:3n-3	20:5n-3	22:5n-3	22:6n-3	18:2n-6	20:3n-6	20:4n-6
	% of total fatty acids						
0	1.5.±0.1	2.9±0.3	2.7±0.2	7.5±0.2	8.7±0.2	1.6±0.2	14.4±0.2
12	1.4±0.1	3.3±0.3	3.0±0.1	8.4±0.3[a]	8.5±0.2	1.6±0.2	13.4±0.3
24	1.5±0.1	3.7±0.3[a]	3.4±0.1[a]	9.0±0.4[a]	8.2±0.2	1.6±0.2	13.3±0.3
36	1.4±0.2	3.7±0.4[a]	3.9±0.2[a,b]	9.7±0.6[a]	8.7±0.3	1.6±0.1	13.5±0.2
48	1.5±0.1	3.9±0.4[a]	3.9±0.2[a,b]	9.6±0.5[a]	8.5±0.2	1.7±0.2	13.7±0.3

[a] Significant difference from wk 0: $p < 0.05$, [b] Significant difference from wk 12: $p < 0.05$

S-particle levels at wk 0 were higher than normal limits (Table 3). These findings are consistent with their high plasma TG and LDL-C levels at wk 0. The elevated serum L-, M-particles may reflect that their high plasma TG levels were derived from their chylomicrons and VLDL. During FOC treatment, their serum L-, M-particles levels were lower than the initial levels. These findings are consistent with the current knowledge that fatty acids from fish oil and fish have a remarkable effect on the synthesis and clearance of TG-rich lipoproteins, especially VLDL and chylomicrons[20,21].

The bioavailabilities of EPA and DHA in the present study were evidenced by significant increases in their serum and RBC 20:5n-3 and 22:6n-3 levels while receiving daily treatment with 6 g of FOC (Table 4 and 5). The significant increases in their serum and RBC 22:5n-3 levels while receiving FOC indicate the competency of elongase activity in converting 20:5n-3 to 22:5n-3**.** The mean net increase in their serum 20:5n-3 levels at wks 12, 24, 36, 48 from that at wk 0 were 100, 150, 175 and 175% respectively; the mean net increase in their serum 22:5n-3 levels at the aforesaid periods were 88, 150, 163 and 225% respectively; the corresponding figures for their serum 22:6n-3 were 50, 86, 92 and 103%. The corresponding figures for their RBC 20:5n-3 levels were 14, 28, 28 and 34%; 11, 26, 44 and 44% for their RBC 22:5n-3 levels; and 12, 20, 29 and 28% for their RBC 22:6n-3 levels respectively.

High dietary intakes of 20:5n-3 and 22:6n-3, most usually from fish and fish oils, increase the amounts of 20:5n-3 and 22:6n-3 in plasma and tissue phospholipids and also reduce 20:4n-6. The decrease in 20:4n-6 is explained by inhibition of the $\Delta 6$ and $\Delta 5$ desaturases, reducing synthesis of 20:4n-6 from 18:2n-6, and by competition between 20:4n-6 and 20:5n-3 from acylation into phospholipids.

Conclusion

The present study has demonstrated the sustainable TG-lowering effect of FOC treatment on hypertriglyceridemic patients. These findings are consistent with the current knowledge that fatty acids from fish oil have a remarkable effect on the synthesis and clearance of TG-rich lipoproteins, especially VLDL and chylomicrons. Though daily treatment with 6 g of FOC has a striking effect in increasing plasma LDL-C levels. Anthropometric and body composition determinations revealed no significant changes in BMI, BFM, FFM and WHR throughout the study. These findings indicate that overall and abdominal obesity

are not the confounding factors in their plasma lipid changes during FOC treatment. The bioavailabilities of EPA and DHA were evidenced by significant increases in serum and RBC 20:5n-3 and 22:6n-3 levels while receiving daily treatment with 6 g of FOC that indicate the competency of elongase activity in converting 20:5n-3 to 22:5n-3.

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ผลการรับประทานน้ำมันปลาต่อภาวะไขมันในผู้ป่วยที่มีไขมันไตรกลีเซอไรด์ในเลือดสูง

สุภาณี พุทธเดชาคุ้ม, วิชัย ตันไพจิตร, ปรียา ลีฬหกุล, วราภัสร์ พากเพียรกิจวัฒนา, ฐิติมา สุรพิศิษฐชาติ, สุรัตน์ โคมินทร์

การรักษาผู้ป่วยไตรกลีเซอไรด์สูงในเลือด (พลาสมาไตรกลีเซอไรด์ (TG) > 200 มิลลิกรัม/เดซิลิตร) ด้วยแคปซูลไขมันปลา (FOC) จำนวน 9 คน (ชาย 4 คน หญิง 5 คน) ที่มารักษาที่คลินิกโภชนวิทยา ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี การรักษาเริ่มด้วยระยะควบคุมอาหารเพียงอย่างเดียวเป็นเวลา 4 สัปดาห์ และเพิ่มการรักษาด้วย FOC วันละ 6 กรัม เป็นเวลา 48 สัปดาห์ ใน 1 แคปซูลน้ำมันปลาประกอบด้วย eicosapentaenoic acid (EPA) 180 มิลลิกรัม และ docosahexaenoic acid (DHA) 120 มิลลิกรัม ปริมาณของ EPA และ DHA ในซีรัมและเม็ดเลือดแดงของผู้ป่วยเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติตลอดการศึกษา การลดลงของพลาสมา TG พบได้ตลอดการศึกษา (ร้อยละ 29-46) พลาสมา LDL-C ในผู้ป่วยเหล่านี้เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติในสัปดาห์ที่ 4 และ 24 (ร้อยละ 6-32) การศึกษายังพบว่าผู้ป่วยมีระดับของอนุภาค L และ M ในซีรัมลดลง ซึ่งตรงกับความรู้ในปัจจุบันที่ EPA และ DHA ลดการสังเคราะห์และเพิ่มการกำจัดไลโปโปรตีนที่อุดมด้วย TG ได้แก่ chylomicron และ VLDL สรุปผลการวิจัยนี้พบว่ากรดไขมันจากน้ำมันปลามีผลต่อการสังเคราะห์ และสลายตัวของไลโปโปรตีนที่อุดมด้วยไตรกลีเซอไรด์ โดยเฉพาะอย่างยิ่งคืออนุภาคไคโลไมครอนและวีแอลดีแอล แม้ว่าจะทำให้เพิ่มระดับของพลาสมาแอลดีแอลโคเลสเตอรอลก็ตาม