55

PLASMA LIPIDS ON THREE LEVELS OF FISH OIL INTAKE IN HEALTHY HUMAN SUBJECTS

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ABSTRACT

Fourteen healthy females and four males consuming typical Western diets were restricted from fish for one month and then fed a daily fish oil supplement providing approximately 1, 3 and 6 g w3 fatty acids during three 4-week periods. A 3-week washout period separated each treatment period. Plasma fatty acids, triglycerides, lipoproteins and bleeding times were determined prior to and at the end of each treatment period. The total w3 fatty acids in plasma, including eicosapentaenoic acid, increased significantly with each increasing dose at the expense of total w6 and w9 fatty acids. Plasma very low-density lipoprotein cholesterol and plasma triglyceride levels were significantly reduced only at the 6 g dose. Bleeding times tended to increase but the changes were not significant. It appears that in the short-term, 3 to 6 g of total w3 fatty acids as fish oil, supplemented to the typical Western diets of healthy subjects, can alter plasma fatty acids and lipids.

INTRODUCTION

Epidemiological studies with Greenland Eskimos have revealed an unusually low incidence of coronary heart disease which seemed to correlate with an abundant consumption of seafood (1,2). Intervention studies in humans have since demonstrated that consumption of fatty fish and fish oil rich in the omega-3 (w3) fatty acids, eicosapentaenoic acid (EPA, 20:5w3) and docosahexaenoic acid (DHA, 22:6w3) can alter plasma fatty acids, triglyceride (TG), lipoprotein concentrations and bleeding time (3).

It has been proposed that the effect on serum lipids and on platelet aggregation that follows the consumption of fatty fish and fish oils may contribute to the lower incidence of coronary heart disease seen in the Greenland Eskimos (2).

The question of minimum effective dose of fish or fish oils has received little attention. The study by Kromhout (4) in the Netherlands led to the suggestion that a low habitual fish intake, equivalent to one or two fish dishes

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a week, might be of value in the prevention of coronary heart disease. In that study the group with the highest fish intake consumed an average of 67 g per day (supplying an estimated 0.4 g EPA/day). We found a few studies in the literature where multiple doses of fish oils or fish oil concentrate were compared. In healthy subjects, daily doses of w3 fatty acids ranging from 1.4 g to 8.2 g for 4 weeks produced a shift in serum lipids favoring w3 fatty acids with all dosages (5). Only at the 8.2 g dose were serum TG and very low-density lipoprotein (VLDL) levels decreased. In 11 hypertriglyceridemics patients (6) plasma TG were reduced with 15 ml fish oil (5 g w3 fatty acids) fed daily for 6 weeks. Plasma TG in five healthy men were lowered with 10 g fish oil fed daily for 3 weeks (supplying approximately 3.28 g w3 fatty acids/day), but not by 5 g fish oil (7).

Our study sought to expand the examination of the effect of increasing doses of fish oil on plasma lipids and bleeding times by administering low doses to healthy subjects consuming their typical Western diets.

MATERIALS AND METHODS

The study design and all procedures used were approved by an independent human research review committee. Fourteen females and four males, all healthy and normolipidemic (with the exception of one female whose baseline serum TG was 334 mg/dl) were recruited from the corporate office pool of approximately 600 employees. Informed consent was obtained. A description of the subjects is given in Table I.

Table I. **Description of Subjects**

	Mean ± STD	Range
Age (years)	37 ±11	30 - 54
Body Weights (Kg)	58 ±14	53 - 103
Percent Suggested Body Weight	110 ±20	89 - 159
Baseline Plasma Lipids:		
Total Cholesterol (mg/dl)	180.3 ±28.2	144 - 235.5
Triglyceride (mg/dl)	75.5 ±71.0	27 - 343

Suggested body weights were calculated as follows: For men, the sum of 48.08 Kg for every 152.4 cm of height ±1.07 Kg/cm above or below 152.4 cm of height, respectively, was calculated. For women, the sum of 45.36 Kg for every 152.4 cm of height ±0.89 Kg/cm above or below 152.4 cm of height, respectively, was calculated.

All volunteers consumed typical Western diets. Volunteers normally consuming vegetarian or fish meals were excluded from participation as were those taking blood lipid-altering medications. Subjects were asked to refrain from consuming fish or fish oils, and to maintain typical diet, exercise, smoking, and

medication habits for the duration of the study. Investigators met with subjects individually approximately every two weeks to record body weight and review compliance with the diet, exercise and lifestyle regimen.

The subjects maintained their typical self-selected diets with the restriction of fish and fish oil for a one month period of stabilization. Fish oil capsules (Table II) were then administered daily at doses of 3, 9, and 18 capsules (Shaklee EPA, Shaklee Corporation, San Francisco, CA) during three 4-week periods.

Table II. **Fatty Acid Composition of Fish Oil Capsules**

Dose	EPA (g)	DHA (g)	Total w3 Fatty Acids (g)
3 capsules	0.556	0.265	0.996
9 capsules	1.667	0.796	2.989
18 capsules	3.335	1.591	5.979

Fish oil capsules contained 51.1% w3 fatty acids. In addition to EPA (20:5w3) and DHA (22:6w3), smaller amounts of 18:3w3, 18:4w3, 20:4w3, and 22:5w3 were present and contributed to the total w3 fatty acid content shown in Table II. The cholesterol content of each capsule was 6.5 mg, consequently the three doses contributed 19.5, 58.5, and 117 mg cholesterol/day respectively.

Each treatment period was separated by a three week washout period. To minimize the potentially variable carry-over effects on blood parameters resulting from the ingestion of fish oil, capsules were administered in the order of increasing dose. The study design did not offer an opportunity to assess possible carry-over effects.

Two 12-hour overnight fasting venous blood specimens were drawn within two days of each other at the beginning of each treatment period, and one each at the end of week 3 and 4 of each treatment period. The beginning values and ending values at weeks 3 and 4 were then averaged, respectively. A single bleeding time (Simplate II, General Diagnostics, Warner-Lambert Co., Morris Plains, NJ) was obtained prior to and at the end of each treatment period. Blood samples were collected in tubes containing 0.1% ethylene diamine tetra-acetic acid anticoagulant. Plasma was immediately separated by centrifugation, refrigerated overnight, and then shipped in ice for analysis to the Oregon Health Sciences University, Portland, OR. Total cholesterol and TG levels were measured with an AutoAnalyzer II (Technicon Instruments, Terrytown, NY). The cholesterol and TG contents of VLDL, low-density lipoprotein (LDL), and HDL were determined by Lipid + Research Clinic procedures (8). Lipids were extracted from plasma by the procedure of Bligh and Dyer (9). Lipid classes were separated by thin-layer chromatography (10), and fatty acid methyl esters were analyzed by capillary column gas-liquid chromatography as previously described (11).

A one-way repeat measures ANOVA model (12) was applied to the actual and rank transformed data. When ANOVA tested significant ($p < 0.05$), the Duncan's Multiple Range test was applied to assess significance between the

dose effects. Only those cases where dose effects were monotonically increasing or decreasing with dose (p 0.05), with both actual and rank transformed data, were accepted as proof that a dose effect was taking place. In order to protect against the confounding of time and increasing dose, the pre-treatment baseline values for each subject were subtracted from the subsequent treatment effects. Furthermore, this pre-treatment baseline value was subtracted for each subject from the subsequent baseline values of each washout period for that subject, and tested for differences using the above statistical model at alpha = 0.05 level of significance. Any significant differences between these baseline values cast doubt on the claim of significant changes with increasing dose and these cases are noted.

RESULTS

There was a small increase in body weight (mean ± SEM) over the course of the study (from 68 ±3 Kg to 69 ±4 Kg), but it was not statistically significant.

The plasma fatty acids that showed a monotonic response to increasing dose of fish oil are presented in Table III. The percent of total w3 fatty acids in the total plasma fatty acid pool increased significantly with each increasing dose at the expense of the percent of total w6 and total w9 fatty acids. Of the w3 fatty acids, the percent of EPA (20:5w3), DHA (22:6w3), and 22:5w3 were significantly increased. The percentage of w6 fatty acids, 20:3w6 and 22:4w6, were significantly decreased. Fatty acids 18:1w9 and 24:1w9 were decreased in the w9 family. The significance of the effects of dose on DHA and 18:1w9 should be interpreted with caution because baselines were found to be significantly different.

The concentrations of linoleic (18:2w6), linolenic (18:2w3) and arachidonic acid (AA, 20:4w6) in the plasma fatty acid pool did not change significantly (data not shown). However, the ratio of EPA to AA in the total plasma fatty acid pool increased significantly (p 0.001) with increasing dose from 0.064 at pre-treatment baseline to 0.154, 0.445, and 0.673 after 1, 3, and 6 g w3 fatty acids, respectively.

Bleeding times (means ± SEM) tended to increase slightly over the course of the study (from 5.11 ±0.30 min to 6.19 ±0.41 min) but the changes were not significant.

Plasma VLDL and TG were significantly reduced at the 6 g w3 fatty acid dose (Table IV). Plasma HDL and the ratio of HDL/LDL and HDL/VLDL were significantly increased at the 6 g w3 fatty acid dose. The significance of the HDL increase must be interpreted with caution because baseline values were also significantly different. The levels of total cholesterol and low-density lipoprotein cholesterol did not change significantly (data not shown).

DISCUSSION

This study demonstrated that healthy free-living humans consuming typical "Western" diets can alter blood lipids and lipoproteins with low doses of fish oil supplements. Consistent with past studies (3), the percent of EPA and total w3 fatty acids in the plasma pool increased with increasing dietary levels. Three g w3 fatty acids yielded a significantly greater increase than 1 g, while 6 g yielded a greater increase than did 3 g. Furthermore, the ratio of total plasma

Table III. **Monotonically Changing Plasma Fatty Acids** (% of Fatty Acids in Total Plasma Fatty Acid Pool)

		Daily Dosage of W3 Fatty Acids			P-value[1]	P-value[2]	P-value[3]
Fatty Acid	Baseline	1g	3g	6g	Baseline	Raw	Ranked
18:1w9	21.194 ±0.7288	19.389 ±1.1777 (-1.806 ±1.1420)[a]	19.361 ±0.5918 (-1.833 ±0.5952)[a]	17.112 ±0.6110 (-4.147 ±0.5240)[b]	0.0049	0.0073	0.0001
18:4w3	0.039 ±0.0216	0.000 ±0.0000 (-0.039 ±0.0216)[a]	0.006 ±0.0056 (-0.033 ±0.0229)[a]	0.024 ±0.0106 (-0.018 ±0.0274)[b]	NS[4]	0.0300	0.0033
20:3w6	1.217 ±9.9827	1.028 ±0.0963 (-0.189 ±0.1353)[a]	1.083 ±0.0682 (-0.133 ±0.1439)[a]	0.612 ±0.1475 (-0.594 ±0.1889)[b]	NS	0.0044	0.0004
20:5w3	0.400 ±0.0754	1.006 ±0.0962 (0.606 ±0.1366)[a]	3.300 ±0.2789 (2.900 ±0.2531)[b]	4.618 ±0.5236 (4.224 ±0.5076)[c]	NS	0.0001	0.0001
22:4w6	0.133 ±0.0268	0.100 ±0.0229 (-0.033 ±0.0396)[a]	0.111 ±0.0212 (-0.022 ±0.0298)[a]	0.018 ±0.0128 (-0.118 ±0.0246)[b]	NS	0.0086	0.0028
20:1w9	0.039 ±0.0183	0.056 ±0.0185 (0.017 ±0.0259)[a]	0.083 ±0.0246 (0.044 ±0.0336)[a]	0.000 ±0.0000 (-0.041 ±0.0193)[b]	NS	0.0057	0.0007
22:5w3	0.283 ±0.0487	0.406 ±0.0539 (0.122 ±0.0756)[a]	1.033 ±0.0577 (0.750 ±0.0829)[b]	1.206 ±0.2136 (0.924 ±0.2126)[b]	0.0233	0.0002	0.0001
22:6w3	1.011 ±0.1022	1.489 ±0.0847 (0.478 ±0.1153)[a]	3.094 ±0.1440 (2.083 ±0.1994)[b]	3.141 ±0.2933 (2.153 ±0.3346)[b]	0.0002	0.0001	0.0001
Total w9	21.688 ±0.8370	21.067 ±0.5758 (-0.676 ±0.7174)[a]	19.639 ±0.5194 (-2.106 ±0.5736)[b]	17.647 ±0.6351 (-4.269 ±0.5605)[c]	NS	0.0001	0.0001
Total w6	43.659 ±1.2518	42.950 ±0.8741 (-0.718 ±0.8362)[a]	44.917 ±0.8126 (1.265 ±1.0435)[a]	37.529 ±1.4225 (-6.588 ±1.3897)[b]	NS	0.0001	0.0001
Total w3	2.235 ±0.1900	3.417 ±0.1727 (1.241 ±0.2180)[a]	7.028 ±0.5038 (4.871 ±0.6066)[b]	9.153 ±1.0518 (6.969 ±1.1719)[c]	NS	0.0001	0.0001

Data are means ± standard error of means.
Numbers in parenthesis are changes from baseline; differing superscripts indicate significant differences in treatment effects.
[1]P-values are from F-test comparing baselines for each treatment.
[2]P-values are from F-test comparing change from initial baseline for each treatment.
[3]P-values are from F-test comparing ranked change from initial baseline for each treatment.
[4]NS refers to "not significant".

Table IV. **Monotonically Changing Plasma Lipids** (mg/dl)

Lipid	Baseline	Daily Dosage of W3 Fatty Acids 1g	3g	6g	P-value[1] Baseline	P-value[2] Raw	P-value[3] Ranked
VLDL	14.389 ±3.0914	13.583 ±1.9701 (-0.806 ±1.6119)[a]	12.944 ±2.3277 (-1.444 ±1.1997)[a]	11.806 ±2.0497 (-2.583 ±1.3911)[b]	NS[4]	0.0272	0.0080
HDL	52.000 ±2.3996	52.056 ±2.6715 (0.056 ±1.3576)[a]	53.861 ±2.8793 (1.861 ±1.7610)[a]	59.028 ±3.2634 (7.028 ±1.7875)[b]	0.0132	0.0001	0.0001
Triglycerides	75.528 ±17.231	68.250 ±9.8146 (-7.278 ±9.7472)[a]	64.361 ±11.661 (-11.167 ±7.3879)[a]	58.556 ±10.245 (-16.972 ±8.1891)[b]	NS	0.0158	0.0054
HDL/LDL Ratio:	0.509 ±0.0343	0.452 ±0.0377 (-0.057 ±0.0202)[a]	0.442 ±0.0418 (-0.067 ±0.0395)[a]	0.554 ±0.0464 (0.045 ±0.0251)[b]	NS	0.0033	0.0011
HDL/VLDL Ratio:	5.440 ±0.6902	5.299 ±0.7243 (-0.141 ±0.4196)[a]	6.071 ±0.7940 (0.631 ±0.4599)[a]	7.308 ±0.9540 (1.868 ±0.6026)[b]	NS	0.0005	0.0049

Data are means ± standard error of means.
Numbers in parenthesis are changes from baseline; differing superscripts indicate significant differences in treatment effects.
[1]P-values are from F-test comparing baselines for each treatment.
[2]P-values are from F-test comparing change from initial baseline for each treatment.
[3]P-values are from F-test comparing ranked change from initial baseline for each treatment.
[4]NS refers to "not significant".

lipid EPA/AA increased with increasing dose. Dyerberg (13) proposed that high levels of plasma lipid EPA and low levels of AA could lead to an antithrombotic stage in which an active antiplatelet aggregating substance and an inactive platelet aggregating substance are formed and possibly protect against thrombosis. Despite the observed shift in the EPA/AA ratio, no significant effects on bleeding times were noted. Thorngren et al. (14) observed significant changes in platelet fatty acid composition within one week in volunteers fed 150 g of fatty fish per day, but bleeding times did not increase until 6 weeks after initiation of the fish diet. It is possible that bleeding times would also have responded to dose had the treatment period been extended beyond 4 weeks. In light of Kromhout's (4) findings that small amounts of fish appeared to have a protective effect against mortality from coronary heart disease, it would seem advisable to study the possible longer-term effects of low-dose fish oil consumption.

The significant decreases in plasma TG and VLDL are consistent with many studies which have administered fish oils or fish oil concentrates (3). The minimum effective dose for altering plasma TG or lipoproteins in this short-term study appeared to be the highest dosage fed, 6 g w3 fatty acids per day. This minimum daily dose is in the range found effective in healthy subjects (3.3 to 6.6 g w3 fatty acids per day 7 , and 8.2 g w3 fatty acids 5), and in hypertriglyceridemics (5 g w3 fatty acids 6).

In summary, a 3 g/day dose of w3 fatty acids from fish for 4 weeks was able to significantly increase the percent of EPA, total w3 fatty acids, and the ratio of EPA/AA in the plasma fatty acid pool as compared to a 1 g dosage. A minimum daily dose of 6 g w3 fatty acids was required to significantly alter plasma VLDL and TG. Bleeding times were not changed significantly. The long-term effects of low levels of fish oils in the diet deserves further attention.

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