Does vegetable oil attenuate the beneficial effects of fish oil in reducing risk factors for cardiovascular disease?[1–4]

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See corresponding editorial on page 188.

ABSTRACT Contradictory reports on the protective effect of fish consumption on cardiovascular disease (CVD) risk could be due to variations in the intake of n−3 and n−6 polyunsaturated fatty acids (PUFAs). Metabolic competition between n−3 and n−6 PUFAs suggests that n−6 PUFAs in vegetable oils could attenuate the efficacy of n−3 PUFAs in fish oil to favorably alter endpoints relevant to CVD risk. We determined the effects of varying dietary amounts of fish oil on lipid and thrombotic endpoints relevant to risk factors for CVD and whether these effects were attenuated by vegetable oils. Two randomized, double-blind, placebo-controlled, parallel studies were conducted in human subjects fed varying amounts of n−3 and n−6 PUFAs; n−3 PUFA intake was varied by using fish or placebo oil capsules, and n−6 PUFA intake was modified by incorporating varying amounts of safflower oil into the diet. Endpoints included changes in membrane fatty acid composition, blood lipids, and thrombotic profile. The results indicated that absolute amounts of fish oil, and not the relative amounts of fish and vegetable oil (ratios of n−3 to n−6 PUFAs), determined the magnitude of the reduction of arachidonic acid and increase in eicosapentaenoic acid in phospholipids of plasma and platelets. The suppression of plasma triacylglycerols by fish oil was not affected by varying amounts of dietary n−6 PUFAs. Fibrinogen concentrations decreased with 15 g but not with 9 g fish oil/d fed at the same ratio of n−3 to n−6 PUFAs. The efficacy of fish oil in favorably modifying certain risk factors for CVD was not attenuated by vegetable oil. *Am J Clin Nutr* 1997;66:89–96.

KEY WORDS Fish oil, ratio of n−3 to n−6 fatty acids, cardiovascular disease risk, polyunsaturated fatty acids, arachidonic acid, eicosapentaenoic acid, humans

INTRODUCTION

Results from epidemiologic studies about the relation between fish consumption and the risk of cardiovascular disease (CVD) have been contradictory. Kromhout et al (1) reported that fish consumption as low as 30 g/d once or twice a week over a 20-y period had a protective effect on mortality from cardiovascular disease. However, Ascherio et al (2) did not find a protective effect on CVD when fish intakes were increased from one to two servings a week to five to six servings a week in men who were initially considered to be free of CVD. Because the beneficial effect of fish consumption in reducing the risk of CVD in some populations is possibly due to the hypolipidemic and antithrombotic effects of the n−3 polyunsaturated fatty acids (PUFAs) that are abundant in marine oils (3–8), these contradictory findings could be due to variations in the actual intake of n−3 and n−6 PUFAs. It is well documented that metabolic competition exists between n−3 PUFAs and n−6 PUFAs, which predominate in vegetable oils (9–12). In rats, n−6 PUFAs suppress the incorporation of n−3 PUFAs into tissue lipids, and n−3 PUFAs inhibit the conversion of linoleic acid, the major n−6 PUFA in vegetable oils, to arachidonic acid, a precursor of proaggregatory eicosanoids (11, 12). This suggests that the relative amounts of dietary n−3 and n−6 PUFA, and thus the ratio of n−3 to n−6 PUFAs, may be as important as the absolute amount of n−3 PUFAs in lowering tissue concentrations of arachidonic acid and its metabolites.

We showed previously in rats that the ratio of n−3 to n−6 PUFAs in the diet, rather than the absolute amount of n−3 PUFA, is the determining factor in suppressing the amounts of arachidonic acid and its metabolites in tissue lipids (13). To determine whether humans respond similarly, we conducted two randomized, double-blind, placebo-controlled, parallel studies in healthy normal adults fed institutionally prepared diets supplemented with fatty acid capsules. In study 1, subjects were fed a fixed amount of fish oil in capsules, with varying amounts of n−6 PUFAs incorporated into the diet; thus, the ratios of ingested n−3 to n−6 PUFAs but not the absolute amount of n−3 PUFAs varied with each treatment group. In study 2, the n−6 PUFA content of the diet was held constant and fish oil intake, by capsule, was varied to obtain ratios of n−3 to n−6 PUFAs similar to those of study 1.

[1] From the Pennington Biomedical Research Center, Louisiana State University, Baton Rouge.

[2] The fish-oil and olive oil capsules and bulk safflower and olive oil ethyl esters were supplied under IND #41 886 from the Food and Drug Administration, NIH/NOAA Biomedical Test Material Program, NMFS, Southeast Fisheries Science Center, Charleston Laboratory, Charleston, SC.

[3] Supported by grants from the US Department of Agriculture (91-34115-6148, 93-37200-8961) and NIH (DK41868).

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Received May 14, 1996.

Accepted for publication January 9, 1997.

Results from these two studies were compared to determine whether there is a ratio or dose effect on lipid and thrombotic endpoints.

SUBJECTS AND METHODS

Selection of subjects

Newspaper advertisements were used to recruit 32 subjects (16 males and 16 females) for study 1 and 36 subjects (18 males and 18 females) for study 2 aged 18–49 y. Subjects were healthy and had a body mass index (BMI; in kg/m^2) between 19 and 27. The study protocol was approved by the Louisiana State University Institutional Review Board, and subjects gave written informed consent. All volunteers who entered the study were considered normal on physical examination and had normal results of blood pressure, complete blood count, chemistry 24 panel, and urine tests. Subjects were required to abstain from all medications, particularly nonsteroidal antiinflammatory agents and oral contraceptives, and alcohol for the duration of the study. As an incentive, subjects received a financial reward for completion of the study.

Study design

The experimental design for both studies is described in **Table 1**. All subjects consumed an institutionally prepared baseline diet supplemented with fatty acid capsules. Energy from fat, including the capsules, was 40% of total energy for all diets in both studies and protein and carbohydrates provided 15% and 45% of energy, respectively. All subjects consumed the baseline diet, supplemented with placebo-oil capsules (1 g olive oil/capsule) for a run-in period. After 4 wk (study 1) and 2 wk (study 2) of the run-in period, subjects were randomly assigned to control or treatment groups (Table 1).

In study 1, the minimum amount of n−6 PUFAs in the baseline diet was 8 g and the n−3 PUFA content was < 1.5 g. Subjects received 9 g n−3 PUFAs or placebo by capsule. To vary the ratio of n−3 to n−6 PUFAs, varying amounts of safflower (80% 18:2n−6) or olive (6.4% 18:2n−6) oil were incorporated into the diets (Table 1). In study 2, all subjects consumed the baseline diet, which contained 16 g n−6 PUFAs and < 1.5 g n−3 PUFAs. n−3 PUFA intakes, by capsule, were varied to obtain ratios of n−3 to n−6 PUFAs of 0, 0.3, and 0.8—similar to those of study 1. Placebo capsules were added to equalize fat and capsule intakes (Table 1).

Provision of the diet

The menus were planned and diets analyzed for nutrient composition by Moore's Extended Nutrient database (MENu; Pennington Biomedical Research Foundation, Baton Rouge, LA). All ingredients were weighed to 0.1 g on electronic balances. Mixed foods were prepared in batch quantities large enough for the entire study and were individually portioned, weighed, sealed, labeled, and frozen until used. A 3-d menu cycle that excluded fish and seafood was used for study 1 and a similar 4-d cycle was used for study 2. The subjects were provided with all foods for the duration of the study and were encouraged to consume all of the food provided. On weekdays, subjects were required to consume breakfast and dinner at the Pennington Biomedical Research Center. Lunch, snacks, and some weekend meals were packaged for consumption off-site.

The energy intake of the subjects was matched to their estimated energy requirements according to the Harris-Benedict equation (14). The diets were planned in 95.24-kJ increments (428.6–809.5 kJ). The PUFA and monounsaturated fat contents depended on the treatment and was the same for all subjects within a treatment (Table 1).

The energy from fat was maintained at 40% of total energy by the addition of saturated fats such as coconut oil. Adjustments in dietary energy were made as needed to maintain weight within 2 kg of initial weight. "Unit" foods (bread and cookies) containing 40% of energy from saturated fat were provided to adjust energy intake. Each "unit" was 23.8 kJ, so that some subjects consumed 1–3 units. Subjects were allowed unlimited decaffeinated, sugar-free, nonalcoholic beverages.

TABLE 1
Experimental design

	Fish oil[1]	Placebo olive oil[1]	Safflower oil[1]	Olive oil[1]	Dietary n−6 fatty acids	Ratio of n−3 to n−6	
						Calculated[2]	Analyzed[3]
				g/d			
Study 1							
Baseline (n = 32)	0	9	0	16	8	0	—
Control (n = 8)	0	9	0	16	8	0	0.04 ± 0.10
Treatment 1 (n = 8)	9	0	16	0	8	0.35	0.30 ± 0.04
Treatment 2 (n = 8)	9	0	8	8	8	0.48	0.44 ± 0.04
Treatment 3 (n = 8)	9	0	0	16	8	0.8	0.79 ± 0.08
Study 2							
Baseline (n = 36)	0	15	—	—	16	0	—
Control (n = 12)	0	15	—	—	16	0	0.01 ± 0.01
Treatment 1 (n = 12)	6	9	—	—	16	0.3	0.31 ± 0.05
Treatment 2 (n = 12)	15	0	—	—	16	0.8	0.73 ± 0.01

[1] Supplemental preparations obtained from the NIH/NOAA Biomedical Test Material Program: fish oil was fatty acid ethyl esters (in capsules) prepared from menhaden oil and contained 80% n−3 fatty acids, placebo olive oil was fatty acid ethyl esters (in capsules) prepared from virgin olive oil and contained 6.4% 18:2n−6 and 72.5% 18:1n−9, safflower oil was incorporated into the diets and contained 80% 18:2n−6, olive oil was incorporated into the diets and contained 6.4% 18:2n−6 and 72.5% 18:1n−9.

[2] Calculated by Moore's Extended Nutrient Database.

[3] Analyzed by GLC; n = 3–4.

They recorded any deviations from the diet and consumption of self-selected beverages in a daily diary.

Representative samples of all foods, beverages, and capsules for one subject for 1 d from each treatment were randomly selected and homogenized, and an aliquot was frozen for fatty acid composition analysis.

Compliance

Subject compliance with the study protocols was evaluated by a questionnaire administered during weekly meetings with the research nurse, assessment of the daily food diaries by the dietitian, measurement of body weight, and analysis of the fatty acid composition of plasma phospholipids.

Blood collection

Blood was drawn from the antecubital vein of each subject after an overnight fast by using a 19-gauge butterfly needle (Becton Dickinson, Franklin, NJ). Serum lipids and coagulation factors were determined by methods published previously (15–17). Whole blood was mixed with 10% of a 3.8% sodium citrate solution and used for the measurement of aggregation and the isolation of platelets. Blood was drawn before, in the middle of, and after treatment.

Fatty acid analysis

Total lipids were extracted from plasma, washed platelets, and diet homogenates by the method of Folch et al (18). Phospholipids were fractionated by thin-layer chromatography and the fatty acid composition of total phospholipids was determined by gas-liquid chromatography as described previously (13).

Platelet aggregation

Aggregation of citrated whole blood by collagen (1 mg/L; Chronolog Corp, Haverton, PA) was determined at 10 min by using a whole-blood aggregometer (model 560 VS; Chronolog Corp) as described elsewhere (19). Samples were centrifuged immediately at 1000 × g for 10 min at room temperature and plasma was stored at −70 °C for the analysis of thromboxane B_2 (TXB_2), the stable metabolite of the proaggregatory eicosanoid thromboxane A_2, by radioimmunoassay as described previously (13, 20).

Statistical analysis

The study was conducted with a randomized block design. The statistical analysis was designed to evaluate differences between treatment, time, and treatment-by-time interactions by using a repeated-measures model. To assess significance, a conservative Scheffé adjustment was used for all multiple comparisons between treatments to control overall Type 1 error to < 0.05. Means were compared at each time period (by using the Scheffé adjustment) to detect differences between treatments (21). There were no significant differences in variables among the control and treatment groups at pretreatment. The pretreatment value was the measurement made after the run-in and before the treatment period. For study 1 it was at 4 wk and for study 2 it was at 2 wk.

Pre- and posttreatment values were compared with contrasts by using a Bonferroni correction because the particular contrasts of interest had been selected before the collection of data. The Bonferroni correction controlled overall Type 1 error for all simultaneous multiple comparisons at < 0.05. Assumptions underlying this model were checked by standard residual analysis (22).

RESULTS

Subjects

Twenty-eight subjects completed study 1 and 34 subjects completed study 2. The four subjects from study 1 and the two subjects from study 2 who withdrew did so for personal reasons, but otherwise, the procedures were well tolerated. Body weight or BMI did not change significantly during either study (data not shown), indicating good subject adherence to the diets. The rapid drop in serum triacylglycerols with fish-oil treatment (**Figure 1**), which is a consistent observation in most human studies (23), and the rapid incorporation of eicosapentaenoic acid (20:5n−3), the major n−3 PUFA in fish oil, into plasma phospholipids of all fish-oil– fed subjects but not control subjects (**Figure 2**) also indicated excellent subject com-

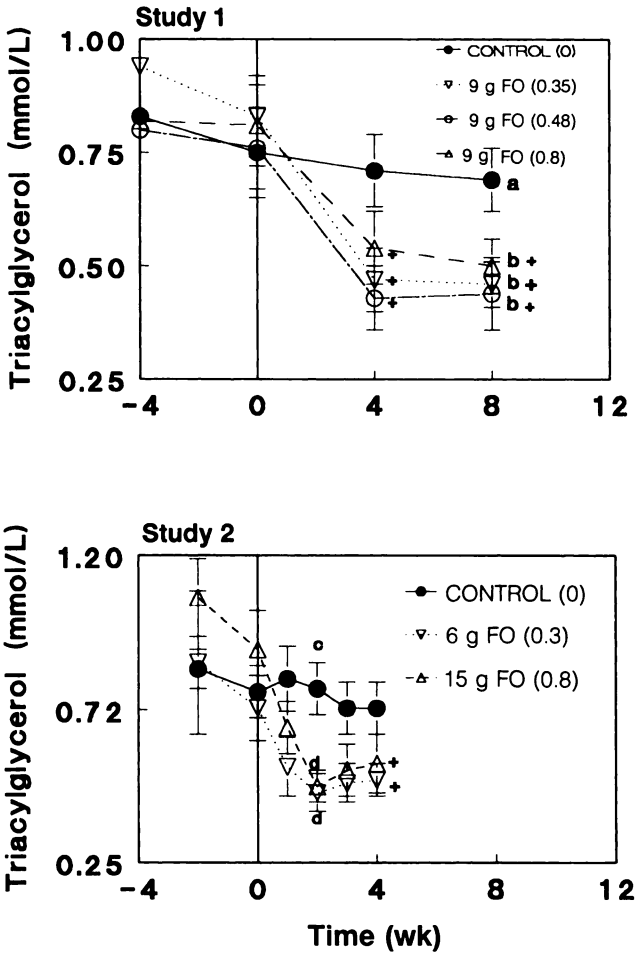

FIGURE 1. Plasma triacylglycerol concentrations. The number in parentheses in the key is the dietary ratio of n−3 to n−6 polyunsaturated fatty acids. Means with the same letter within the same time period are not significantly different. $^+$Significantly different from pretreatment values, $P < 0.05$ (repeated-measures ANOVA). To convert to mg/dL, divide by 0.0113. $\bar{x} \pm$ SEM; n = 6–8 (study 1), n = 11–12 (study 2). FO, fish oil

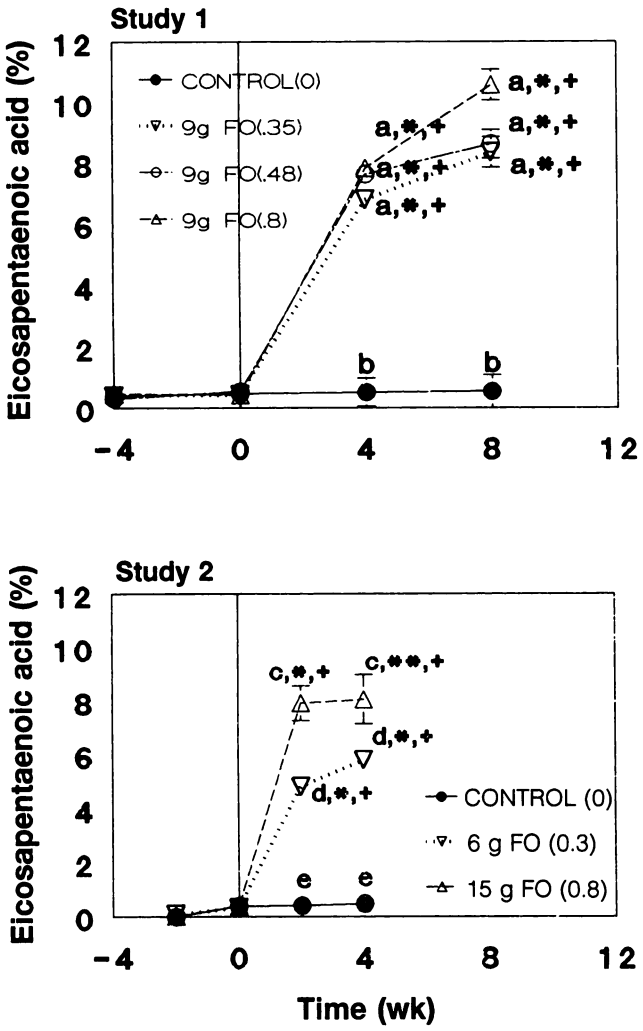

FIGURE 2. Plasma eicosapentaenoic acid concentrations (% by wt of total fatty acids) in plasma phospholipids. The number in parentheses in the key is the dietary ratio of n−3 to n−6 polyunsaturated fatty acids. Means with the same letter within the same time period are not significantly different. *Change from the pretreatment value is significantly different from the change in the control; **change from the pretreatment value is significantly different from the change in the group treated with 6 g fish oil. +Significantly different from the pretreatment value, $P < 0.05$. $\bar{x} \pm$ SEM; n = 6–8 (study 1), n = 11–12 (study 2). FO, fish oil.

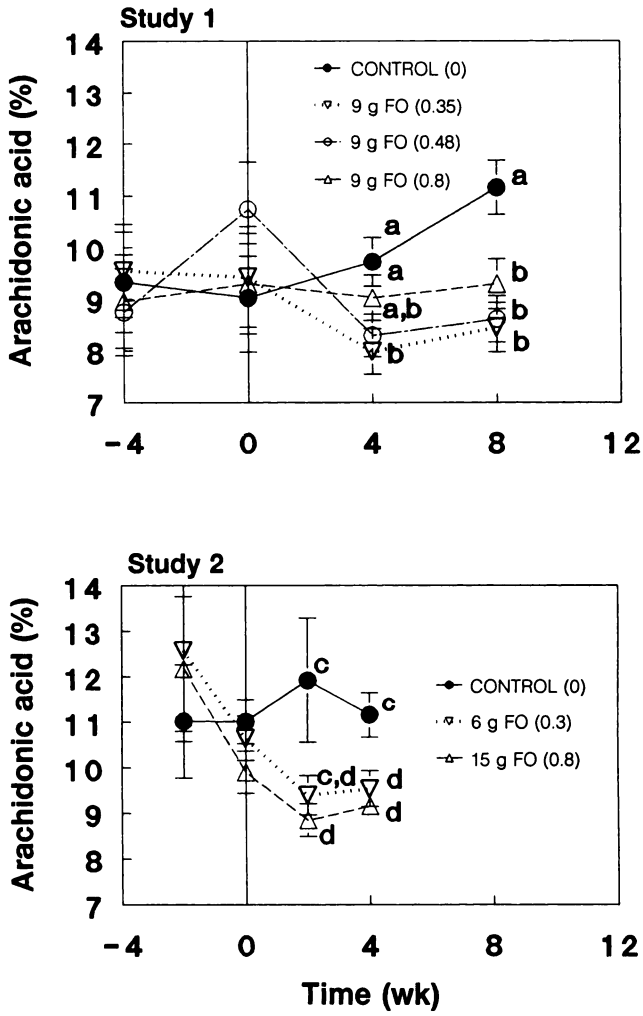

FIGURE 3. Arachidonic acid concentrations (% by wt of total fatty acids) in plasma phospholipids. The number in parentheses in the key is the dietary ratio of n−3 to n−6 polyunsaturated fatty acids. Means with the same letter within the same time period are not significantly different. $\bar{x} \pm$ SEM; n = 6–8 (study 1), n = 11–12 (study 2). FO, fish oil.

pliance with the study protocol. On the basis of the results from study 1 that changes in serum triacylglycerols and fatty acid composition of plasma phospholipids occurred within 4 wk of fish-oil treatment (Figures 1, 2 and **Figure 3**), the baseline feeding period was shortened to 2 wk and the dietary intervention periods were shortened to 4 wk in study 2.

Fatty acid composition of diets

Analysis of the fatty acid composition of the diets showed that the actual dietary ratios of n−3 to n−6 were in close agreement with calculated values (Table 1).

Serum triacylglycerols

Plasma concentrations of plasma triacylglycerols in all three fish-oil–treated groups were significantly lower ($P < 0.05$) than pretreatment values after 4 and 8 wk of the treatment in study 1 and were significantly lower than values for the control group at 8 wk (Figure 1). However, there was no significant difference among the three groups receiving fish oil. In study 2, plasma concentrations of triacylglycerols in both fish-oil–treated groups were significantly lower ($P < 0.05$) than concentrations of the control group after 2 wk, and significantly lower ($P < 0.05$) than pretreatment values after 4 wk of the treatment, but there was no difference between the two fish-oil–treated groups (Figure 1).

Eicosapentaenoic acid and arachidonic acid in plasma phospholipids

In study 1, concentrations of eicosapentaenoic acid in plasma phospholipids were significantly higher ($P < 0.05$) in all three fish-oil–treated groups than in the control group after 4 and 8 wk of the dietary treatment (Figure 2). However, there was no significant difference in eicosapentaenoic acid concentrations among the three groups receiving fish oil. In study 2, eicosapentaenoic acid concentrations showed a significant dose-de-

pendent increase ($P < 0.05$) with fish-oil treatment (Figure 2). In both studies, arachidonic acid concentrations, the major n−6 PUFA in membrane lipids, in plasma phospholipids were significantly lower ($P < 0.05$) in groups fed fish oil than in control groups (Figure 3). The arachidonic acid concentrations in subjects fed fish oil were not affected by varying the amount of dietary n−6 PUFA in study 1. However, in study 2 arachidonic acid concentrations in plasma phospholipids were significantly lower ($P < 0.05$) than control values in the group receiving 15 g fish oil but not in the group receiving 6 g fish oil after 2 wk of treatment. After 4 wk of treatment, arachidonic acid concentrations were significantly lower ($P < 0.05$) than control values in both fish-oil–treated groups; however, there was no significant difference between the two groups (Figure 3).

Eicosapentaenoic acid and arachidonic acid in platelet phospholipids

The data for concentrations of eicosapentaenoic acid and arachidonic acid in platelet phospholipids posttreatment (weeks 8 and 4 for studies 1 and 2, respectively) are presented in **Figures 4** and **5**. The pattern of dose-dependent, but not ratio-dependent, change in eicosapentaenoic acid concentrations (Figure 4) was similar to that of plasma phospholipids (Figure 2). There was no significant difference in the concentrations of arachidonic acid in platelet phospholipids among groups receiving the same amount of fish oil with varying ratios of n−3 to n−6 PUFAs in study 1 (Figure 5). The change from pretreatment concentrations of arachidonic acid in platelet phospholipids was significantly different ($P < 0.05$) in groups receiving fish oil compared with the control group. In study 2, compared with the control, the arachidonic acid concentration was significantly lower ($P < 0.05$) in the group receiving 15 g fish oil but not in the group receiving 6 g fish oil (Figure 5).

In both studies, the concentrations of arachidonic acid in platelet phospholipids of the control groups were significantly greater than pretreatment values (Figure 5). This increase may have been due to the possibility that arachidonic acid concentrations in the baseline diets (≈133 mg/d) were higher than those in the diets consumed by the subjects before the studies. There are no reliable estimates of the amount of arachidonic acid in the average American diet.

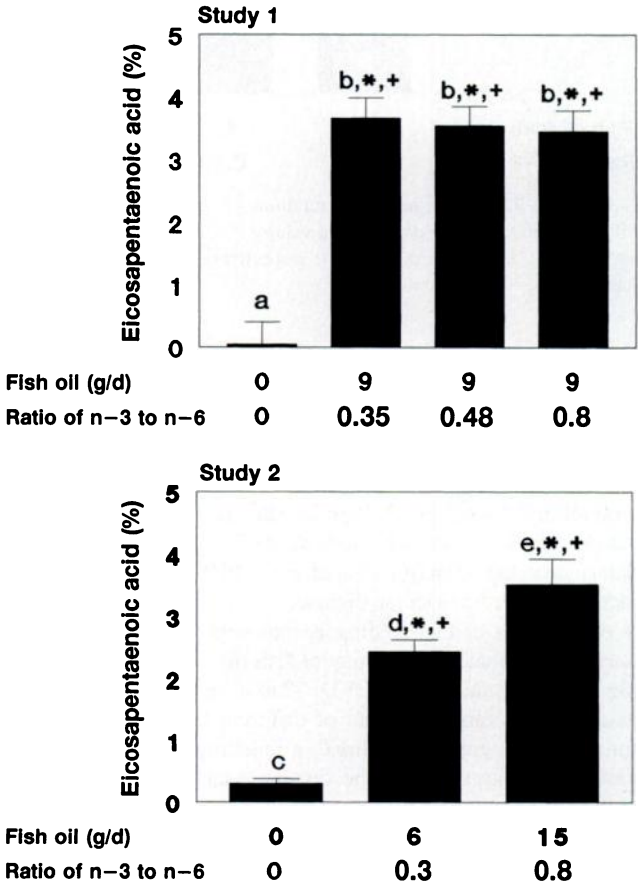

FIGURE 4. Posttreatment concentrations of eicosapentaenoic acid (% by wt of total fatty acids) in platelet phospholipids. Means with the same letter are not significantly different. *Change from pretreatment value is significantly different from the change in the control group, $P < 0.05$. +Significantly different from pretreatment values, $P < 0.05$. Values for study 1 and study 2 are at weeks 8 and 4, respectively. $\bar{x} \pm$ SEM; $n = 6$–8 (study 1), $n = 11$–12 (study 2).

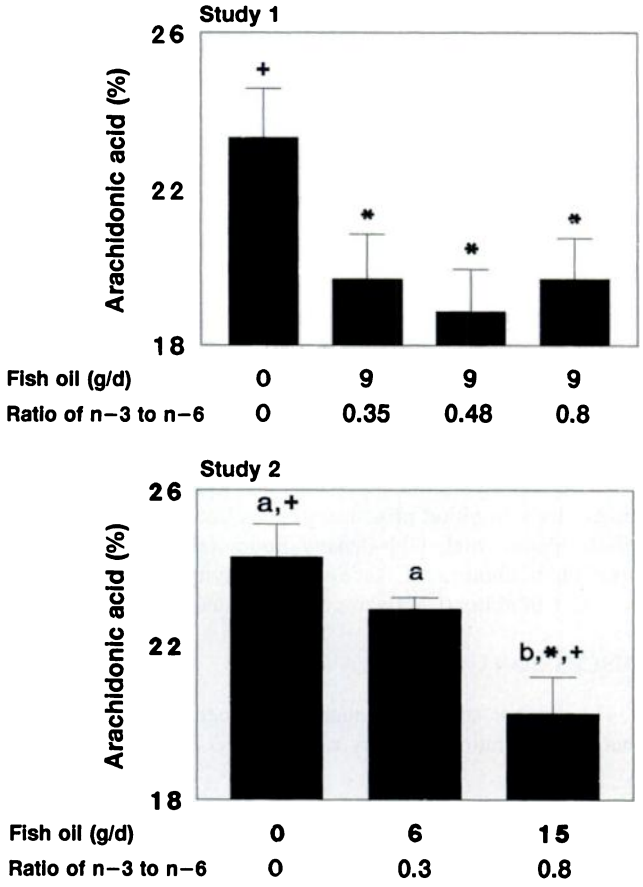

FIGURE 5. Posttreatment concentrations of arachidonic acid (% by wt of total fatty acids) in platelet phospholipids. Means with the same letter are not significantly different. *Change from the pretreatment value is significantly different from the change in the control group, $P < 0.05$. +Significantly different from pretreatment values, $P < 0.05$). Values for study 1 and study 2 are at weeks 8 and 4, respectively. $\bar{x} \pm$ SEM; $n = 6$–8 (study 1), $n = 11$–12 (study 2).

Thromboxane B_2 synthesis

Concentrations of TXB_2 in whole blood treated with collagen were not significantly affected by fish oil intake in study 1 (**Figure 6**). In study 2, the concentrations of TXB_2 in whole blood at week 4 were significantly lower ($P < 0.05$) than pretreatment concentrations in both fish-oil–fed groups, but there was no difference between the two fish-oil–fed groups (Figure 6).

Plasma fibrinogen

Plasma fibrinogen concentrations were unaffected by fish-oil treatment in study 1. In study 2, only the group receiving 15 g fish oil/d showed a significant reduction ($P < 0.05$), compared with pretreatment concentrations, in fibrinogen (**Figure 7**).

Other lipid and thrombotic endpoints

There was no significant change due to fish-oil treatment in either study in blood pressure; platelet counts; aggregation in whole blood; total, high-density lipoprotein, or low-density-lipoprotein cholesterol; factor VII activity; or plasminogen activator inhibitor-1 antigen concentrations (**Table 2**).

DISCUSSION

The present study challenges the generally accepted view that it is the ratio of dietary n−3 to n−6 PUFAs and not the absolute amount of n−3 PUFAs that determines the efficacy of dietary n−3 PUFAs in exerting their potential beneficial effects (3, 9, 10, 13). Results from study 1 that show no effects of the ratio of n−3 to n−6 PUFAs and those from study 2 that show an n−3 PUFA dose-related effect indicate that it is the amount of dietary n−3 PUFAs (in amounts used in these studies) rather than the ratio of n−3 to n−6 PUFAs that determines the effectiveness of n−3 PUFAs in altering risk factors for cardiovascular disease.

A corollary of this finding is that vegetable oil in the diet may not attenuate the efficacy of fish oil in favorably modifying some risk factors for CVD. This information is critical in assessing desirable amounts of different types of PUFAs for future dietary guidelines aimed at reducing the risk of cardiovascular diseases and in the dietary management of patients with CVD.

Plasma triacylglycerols were significantly reduced by fish oil intake in both of our studies. Schmidt et al (24) reported that 4 g/d was the minimum amount of n−3 PUFA required to suppress plasma triacylglycerols. Increasing dietary n−6 fatty acids did not attenuate the suppressive effect of fish oil (9 g/d) on plasma triacylglycerols in study 1, and there was no difference in plasma triacylglycerol concentrations between the group receiving 6 g fish oil/d and the group receiving 15 g/d in study 2. These results suggest that the amount of fish oil

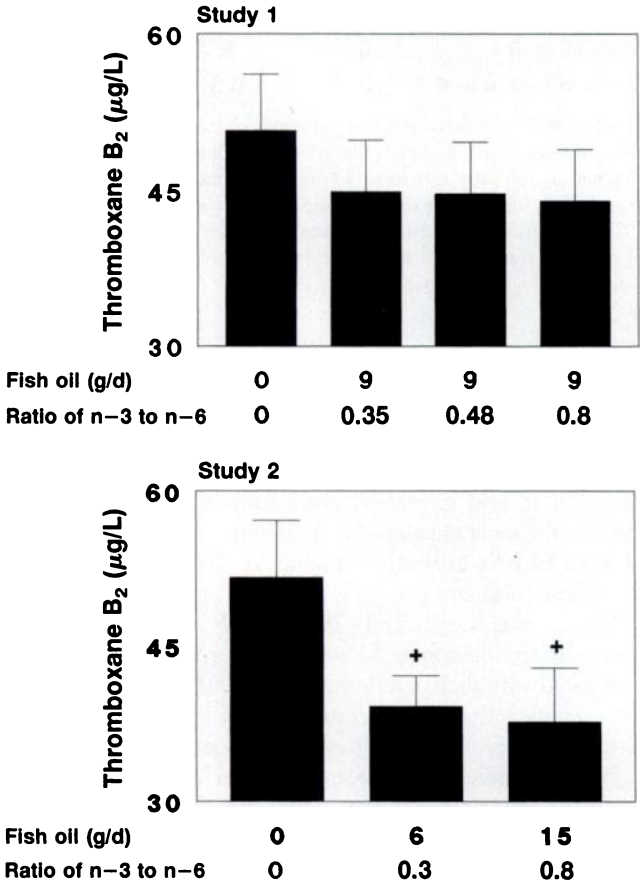

FIGURE 6. Posttreatment concentrations of thromboxane B_2 in whole blood aggregated with collagen. $^+$Significantly different from pretreatment values, $P < 0.05$. Values for study 1 and study 2 are at weeks 8 and 4, respectively. $\bar{x} \pm$ SEM; n = 6–8 (study 1), n = 11–12 (study 2).

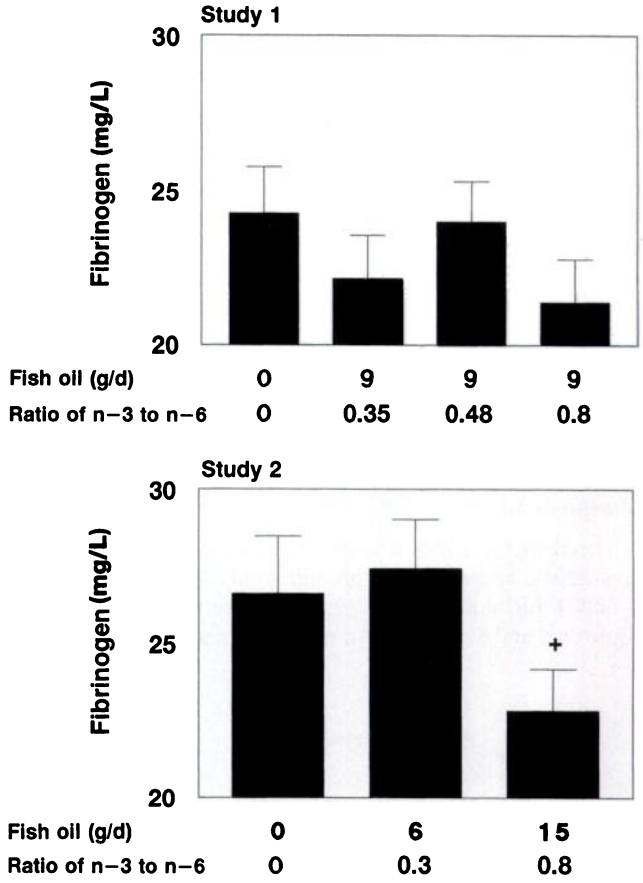

FIGURE 7. Posttreatment concentrations of plasma fibrinogen. $^+$Significantly different from pretreatment values, $P < 0.05$. Values for study 1 and study 2 are at weeks 8 and 4, respectively. $\bar{x} \pm$ SEM, n = 6–8 (study 1), n = 11–12 (study 2).

TABLE 2
Other lipid and thrombotic endpoints[1]

	Study 1				Study 2		
	Control (0)[2]	Treatment 1 (0.35)	Treatment 2 (0.48)	Treatment 3 (0.8)	Control (0)	Treatment 1 (0.3)	Treatment 2 (0.8)
Total cholesterol (mmol/L)[3]	4.58 ± 0.33	4.54 ± 0.29	4.72 ± 0.28	4.44 ± 0.30	4.14 ± 0.17	4.68 ± 0.29	4.30 ± 0.29
LDL cholesterol (mmol/L)	2.91 ± 0.27	3.15 ± 0.25	3.08 ± 0.23	3.08 ± 0.25	2.72 ± 0.13	3.38 ± 0.27	3.23 ± 0.24
HDL cholesterol (mmol/L)	1.30 ± 0.12	1.16 ± 0.11	1.08 ± 0.1	1.14 ± 0.11	1.08 ± 0.1	1.04 ± 0.06	0.82 ± 0.10
Factor VII (%)	93.33 ± 5.76	87.57 ± 5.34	82.75 ± 4.99	77.14 ± 5.34	87.64 ± 4.61	91.45 ± 6.60	96.5 ± 2.46
PAI (g/L)[4]	10.50 ± 3.76	7.86 ± 3.48	14.13 ± 3.25	16.00 ± 3.48	17.33 ± 2.07	23.19 ± 4.01	29.99 ± 5.76
Platelet count ($\times 10^9$/L)	228 ± 25	219 ± 18	172 ± 18	204 ± 19	181 ± 9	184 ± 13	173 ± 7
Whole blood aggregation (ohms)[5]	20.73 ± 1.35	19.71 ± 1.25	18.3 ± 1.17	20.29 ± 1.25	20.11 ± 1.08	18.73 ± 0.92	17.57 ± 1.16
Systolic blood pressure (mm Hg)	103 ± 3	107 ± 3	105 ± 3	109 ± 3	111 ± 2	115 ± 3	115 ± 3
Diastolic blood pressure (mm Hg)	70 ± 3	64 ± 2	68 ± 2	65 ± 2	70 ± 2	73 ± 2	75 ± 3

[1] $\bar{x}$ ± SEM.
[2] Values in parentheses indicate dietary ratios of n−3 to n−6 fatty acids.
[3] To convert mmol/L to mg/dL divide by 0.0259.
[4] Plasminogen activator inhibitor.
[5] Impedence in whole blood after aggregation with 1 mg collagen L for 10 min.

required for the maximum reduction of plasma triacylglycerols is < 6 g/d, and further increases in fish oil intake above this amount did not affect the magnitude of the reduction in plasma triacylglycerol concentrations. Furthermore, the suppressive effect of fish oil intake above this level on plasma triacylglycerol concentrations was not attenuated by increasing the amount of dietary n−6 PUFAs.

The results of the fatty acid analysis showed that the amount of arachidonic acid and the incorporation of eicosapentaenoic acid into plasma and platelet membrane phospholipids were dependent on the absolute amount of dietary n−3 PUFAs and not on the ratios of n−3 to n−6 PUFAs for the amounts of dietary n−3 and n−6 fatty acids used in our studies. These results are in contrast with the results from our previous animal studies, which showed that the ratio of n−3 to n−6 PUFAs and not the absolute amounts of dietary n−3 PUFAs was the important determinant in the efficacy of n−3 PUFAs to suppress tissue concentrations of arachidonic acid and its metabolites (13).

Other studies investigating the effect of dietary ratios of n−3 to n−6 PUFAs on lipid and thrombotic variables in animals varied the amounts of dietary n−3 fatty acids as well as the ratios of n−3 to n−6 PUFAs (25, 26). Thus, it is difficult to interpret the results from these animal studies. In human studies, Gronn et al (27) used a constant amount of dietary n−3 fatty acids with diets high or low in n−6 fatty acids. They reported that the incorporation of n−3 fatty acids into the phospholipids of platelets was inhibited in the group receiving higher amounts of n−6 fatty acids, suggesting that the ratio of n−3 to n−6 PUFAs is more important than the amount of dietary n−3 fatty acids. Their data do not agree with our findings. This may be because their subjects consumed self-selected diets after receiving instruction on how to increase or decrease their n−6 PUFA intake and, thus, their dietary PUFA intakes were not controlled.

In our studies, all meals and snacks consumed by the subjects for the entire feeding period, including the run-in period, were prepared by the metabolic kitchen, and the actual dietary ratios of n−3 to n−6 PUFAs were determined by analyzing the fatty acid composition of lipids in aliquots of the diets. Cleland et al (28) reported that incorporation of eicosapentaenoic acid into neutrophil phospholipids of human subjects consuming 2 g fish oil/d was lowered by increasing dietary n−6 PUFAs. Arachidonic acid concentrations were significantly reduced by fish oil intake but this reduction was not attenuated by increasing dietary n−6 PUFAs. Their subjects consumed self-selected diets and n−6 PUFA intakes were manipulated by supplying subjects with either a low- or high-linoleic acid spread and oil. However, actual intakes of dietary n−6 fatty acids were not measured, as they were in our study.

Aggregation of platelets in whole blood by collagen was unaffected by fish oil ingestion in either study (Table 2). Although several studies (29, 30) have shown a reduction in platelet aggregation in response to collagen in fish-oil–fed subjects, the review by Herold and Kinsella (6) showed that this response is variable. Synthesis of thromboxane A_2, as determined by TXB_2, in platelets of whole blood aggregated with collagen was not significantly affected by fish oil intake in study 1, possible because of the small sample size. In study 2, TXB_2 synthesis by platelets in whole blood was significantly lower than pretreatment values in both fish-oil–fed groups, but there was no difference between the two fish-oil–fed groups. The number of subjects per treatment was 12 in study 2, whereas it was 8 in study 1. A power analysis determined that for the variance in TXB_2 concentrations in the subjects in study 1 and the given sample size, a difference of 16% would be detected with 80% power. The difference observed was 10%. However, the sample size was determined to be sufficient to detect significant differences in plasma triacylglycerol and phospholipid PUFA concentrations with 80% power.

The effect of fish oil on clotting factors is controversial but several well-designed studies have reported reductions in fibrinogen in response to intakes as low as 1.3 g n−3 PUFAs/d (24). In study 2, only the group receiving 15 g fish oil/d showed

a significant reduction in fibrinogen concentrations. The fibrinogen concentrations were not reduced in the group receiving the diet with the same ratio of n−3 to n−6 PUFAs (0.8) achieved with 9 g fish oil/d in study 1. These results suggest that the reduction of fibrinogen concentrations depends on the amount of fish oil consumed and not onthe ratio of n−3 to n−6 PUFAs.

Our results, which show a lack of effect of the ratio of n−3 to n−6 PUFAs on the endpoints measured, have important implications in assessing desirable amounts of n−3 and n−6 fatty acids in human diets. The effective dose of fish oil varied with the type of endpoint measured. Results from our studies indicate that 6–15 g n−3 PUFAs/d were required to significantly alter some, but not all, lipid and thrombotic endpoints in 4 wk. To obtain these amounts by eating fish is unrealistic in the contemporary American diet. Lower amounts of dietary n−3 PUFAs may confer similar effects if consumed for a longer period of time, as suggested in the study by Kromhout et al (1). However, the study by Ascherio et al (2) indicated that ≈0.58 g n−3 PUFAs/d over 10 y did not have a beneficial effect on heart disease risk. Contradictory results reported from various clinical, epidemiologic, and prospective studies regarding the potential beneficial effects of fish or fish oil intake on cardiovascular disease may reflect variations in the actual intake of n−3 PUFAs and the time period over which they were consumed. Further studies are required to determine the minimum n−3 fatty acid intake necessary to exert favorable effects on endpoints relevant to cardiovascular disease, and whether this minimum amount is affected by the dietary ratio of n−3 to n−6 fatty acids.

Statistical analyses of the data for both studies were done by P Wozniak, R Machiavelli, and K Wilson.

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