Effects of a Small Quantity of ω-3 Fatty Acids on Cardiovascular Risk Factors in NIDDM

A randomized, prospective, double-blind, controlled study

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OBJECTIVE — To study the effects of a low dose of ω-3 fatty acids on platelet function and other cardiovascular risk factors in patients with non-insulin-dependent diabetes mellitus (NIDDM).

RESEARCH DESIGN AND METHODS — We performed a randomized, prospective, double-blind, controlled study of a low dose of ω-3 fatty acids (2.5 g/day) in 20 ambulatory subjects with NIDDM. Subjects ingested five 1-g fish oil capsules each containing 0.5 g ω-3 fatty acids or five 1-g safflower oil capsules per day for 6 weeks followed by a 6-week washout period.

RESULTS — Nine subjects completed the study in each group. Both groups exhibited moderate control of glucose levels; modest elevations in baseline total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglyceride (TG) levels; and normal blood pressure values. In the fish oil group, plasma ω-3 fatty acid levels increased significantly. Fish oil significantly reduced the slope of the dose-response curves for collagen-induced platelet aggregation to one-third the value observed with safflower oil. No difference was observed in collagen-induced production of thromboxane A_2 (TXA_2, measured as the stable derivative TXB_2), or in adenosine-5′-diphosphate- (ADP) induced platelet aggregation or TXA_2 generation. Patients with high initial collagen-induced platelet TXA_2 production showed a significantly larger drop after fish oil than safflower oil. Fish oil significantly reduced TG levels by 44 mg/dl and decreased upright systolic blood pressure (sBP) by 8 mmHg compared with safflower oil. Fish oil caused a significant but small increase in HbA_{1c} (0.56%) and total cholesterol (20 mg/dl) but had no effect on fasting glucose, high-density lipoprotein cholesterol, or LDL-cholesterol levels.

CONCLUSIONS — Small doses of fish oil inhibit platelet aggregation and TXA_2 production, reduce upright sBP and TG levels, and have only a small effect on glucose and cholesterol levels in patients with moderately controlled NIDDM. Small quantities of ω-3 fatty acids or dietary fish are safe and potentially beneficial in NIDDM patients.

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NIDDM, non-insulin-dependent diabetes mellitus; IDDM, insulin-dependent diabetes mellitus; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; BP, blood pressure; sBP, systolic blood pressure; dBP, diastolic blood pressure; TXA_2, thromboxane A_2; TXA_3, thromboxane A_3; CVD, cardiovascular disease; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ADP, adenosine-5′-diphosphate; RIA, radioimmunoassay; BMI, body mass index.

Omega-3 fatty acids, either as dietary fish or as an extract, modify multiple factors involved in the pathogenesis of atherosclerotic cardiovascular disease (CVD) such as platelet aggregation, plasma lipoprotein metabolism, and blood pressure (BP) in the nondiabetic population (1–3). The active ω-3 fatty acids are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Because some of these factors may contribute to the development of diabetic microvascular disease, ω-3 fatty acids could conceivably prevent the development of diabetic microvascular disease.

The initial enthusiasm for the use of ω-3 fatty acids in diabetes was modified by reports of potentially deleterious effects including increased levels of plasma glucose, GHb, plasma total cholesterol and low-density lipoprotein (LDL) cholesterol, and serum apolipoprotein B (4). These adverse effects were achieved with large, possibly excessive, doses of ω-3 fatty acids, in the range of 4–10 g/day (4). The magnitude of these adverse effects has been small, typically 10–36% (4). Although some studies have explored the effects of low doses of ω-3 fatty acids in patients with non-insulin-dependent diabetes mellitus (NIDDM), these studies examined a limited number of end points, often were not vigorously controlled, or did not use suitable placebo substances (4,5).

Because diabetes is a heterogeneous group of diseases, the effects of ω-3 fatty acids may be different in patients with insulin-dependent diabetes mellitus (IDDM), NIDDM, and other types of diabetes (4,5).

In this study, we performed a randomized, prospective, double-blind, controlled trial of the effects of a small dose of ω-3 fatty acids compared with a placebo containing safflower oil on risk factors for CVD in patients with NIDDM, a disease with increased platelet activation (6).

RESEARCH DESIGN AND METHODS— We studied 20 patients with NIDDM. Patients 21–65 years of age were included in this study if their NIDDM was in moderate control with no increase or decrease in body weight in the 2 months preceding randomization, if the HbA_{1c} level was <9.5% (normal 3.8–6.4%) and if the hemoglobin was at least 13 g/dl in men or 12 g/dl in women. Patients were accepted if they had background diabetic retinopathy but were excluded if they had proliferative retinopathy or a history of intraocular hemorrhage. Patients were also excluded if they had any bleeding disorder or bleeding tendency or if they had taken any aspirin, aspirin-containing compound, or another nonsteroidal or steroidal anti-inflammatory agent in the 2 weeks before entry into the study. Patients were given a detailed and comprehensive list of aspirin-containing compounds for use before and during the study. A dietary history was obtained by a nutritionist (E.W.) before randomization with special emphasis on the intake of fish. Patients who were already on a high fish intake (i.e., >1 g ω-3 fatty acids per day) were to be excluded from randomization, but no one required exclusion on this basis. The dietary history included methods of food preparation, responses to a food frequency questionnaire, and representative meal patterns. The diet was monitored throughout the study and at the end of the study by the same dietitian (E.W.) to ensure that it remained unchanged.

Study design

The study was performed using the ambulatory patient facilities of the Massachusetts General Hospital General Clinical Research Center. Twenty patients were randomized to receive either the fish oil capsules or a placebo containing safflower oil. Randomization was performed by the hospital pharmacy from a randomization list in a double-blind fashion. Eighteen patients completed the study, and 9 were in each group. One subject was excluded from the study because of noncompliance and one withdrew because of the development of small bowel obstruction caused by previously unsuspected metastatic carcinoma of the colon.

The fish body oil capsules (Super EPA^r) and placebo capsules were provided by Pharmacaps (Elizabeth, NJ). Each 1-g capsule of Super EPA^r contained ~31% EPA, 21% DHA, 11% eicosenic acid (20:1 ω-11), 9% oleic acid, 4% docosapentaenoic acid (22:5 ω-3), 3% stearic acid, 3% linoleic acid, and smaller percentages of other fatty acids. The safflower oil capsules contained 1 g of safflower oil rather than fish oil and were indistinguishable from the fish oil capsules. Safflower oil contains ~78% linoleic acid, 12% oleic acid, 7% palmitic acid, and 2% stearic acid. Written informed consent was obtained from each participant. The study was approved by the Massachusetts General Hospital Subcommittee on Human Studies.

Two sets of baseline observations were obtained on successive days before ingestion of fish oil or placebo capsules. Further observations were made at 4 and 6 weeks, after which the fish oil or placebo supplements were discontinued and the patients continued their diabetic diets as before. Additional observations were made 6 weeks after cessation of the supplements. At each time of observation (baseline observations, after 4 and 6 weeks of fish oil or placebo and 6 weeks after cessation of capsules), the following measurements and samples were obtained: weight; supine and upright BP; fasting blood samples for determination of platelet aggregation and platelet production of thromboxane B_2 (TXB_2), serum TXB_2, plasma fatty acid composition, plasma glucose and lipids (cholesterol, LDL-cholesterol, HDL-cholesterol, and triglyceride [TG]), and serum creatinine; and a 24-h urine specimen for measurement of creatinine clearance. Diastolic blood pressure (dBP) was measured at the fading of the Korotkoff sounds. Each urine specimen was brought to the General Clinical Research Center upon completion, at which time the weight and BP were measured and the fasting blood samples were obtained. All observations were made and blood samples obtained after an overnight fast before breakfast and before ingestion of the fish oil or safflower oil supplements. Each subject was also seen at the end of the second week and the end of the ninth week of the study to enhance compliance and to measure the weight and BP.

Analytical methods

The plasma glucose, the serum and urine creatinine, and the plasma total cholesterol, HDL-cholesterol, and TG levels were measured by standard methods in the clinical laboratory of the Massachusetts General Hospital (7). The LDL-cholesterol concentration was estimated by using the equation of Friedewald, Levy, and Frederickson (8). The total plasma fatty acid composition, platelet aggregation, platelet TXB_2 generation, and serum TXB_2 levels were determined in the Core Laboratory of the General Clinical Research Center.

The total plasma fatty acid composition was determined after extraction with a modified Folch procedure (9) and esterification of the fatty acids in the lipid extracts by an acid methanolysis procedure. The methyl esters were analyzed by gas chromatography using appropriate standards on a Hewlett-Packard gas chromatograph model no. 5890 with an auto-injector model no. 7673 using Chemstation software for integration.

Platelet aggregation was determined using the method of Born (10) in a Chronolog Model 550 VS Dual Channel Platelet Aggregometer with a model 707 Dual Pen Recorder. Platelet aggregation was determined in response to graded doses of collagen and adenosine-5′-diphosphate (ADP). Aggregation was induced by the addition of collagen (Hormon-Chemie, Munich) or ADP (Bio/Data, Hatboro, PA) to a cuvette containing 0.45 ml of platelet-rich plasma in the aggregation profiler at 37°C with

Table 1—*Baseline characteristics of study population*

	Safflower oil	Fish oil	Statistical significance
Age (years)	58.1 ± 3.6	56.0 ± 2.3	NS
Duration of diabetes (years)	8.7 ± 2.7	7.8 ± 2.3	NS
Height (cm)	171.6 ± 2.2	173.8 ± 2.6	NS
Weight (kg)	82.1 ± 5.1	81.9 ± 3.6	NS
Ideal body weight (%)	119.2 ± 5.1	115.7 ± 3.3	NS
BMI (kg/m^2)	27.9 ± 1.2	27.0 ± 0.8	NS
Fasting blood glucose (mg/dl)	168.3 ± 16.9	192.7 ± 15.7	NS
HbA_{1c} (%)	7.4 ± 0.4	7.6 ± 0.3	NS
Serum creatinine (mg/dl)	1.0 ± 0.1	1.0 ± 0.1	NS
Cholesterol			
Total	220.7 ± 15.0	201.7 ± 10.1	NS
HDL	34.6 ± 2.4	39.9 ± 3.1	NS
LDL	144.7 ± 12.9	131.9 ± 8.6	NS
TGs	207.2 ± 26.1	148.6 ± 33.9	NS
BP (supine)			
sBP	137.6 ± 9.6	125.6 ± 6.1	NS
dBP	78.0 ± 2.6	78.4 ± 4.7	NS
BP (upright)			
sBP	134.2 ± 7.6	128.9 ± 6.3	NS
dBP	83.8 ± 4.4	85.6 ± 5.3	NS

Data are means ± SE.

constant stirring. Concentrations of collagen were 1, 2, 4, 6, 8, 10, and 20 μg/ml. Concentrations of ADP were 10^{-5}, 2×10^{-5}, 4×10^{-5}, 6×10^{-5}, 8×10^{-5}, 10^{-4}, and 2×10^{-4} M. Plasma for determination of platelet production of TXB_2 was obtained by a modification of the method of Halushka et al. (11). A 50-μl aliquot of an ice-cold solution containing 0.20 mM EDTA and 0.1 mg/ml indomethacin in Krebs-Ringer phosphate buffer was added to each cuvette at 4 min to stop the reaction. Each cuvette was decanted, and the plasma was centrifuged in a Microfuge (Eppendorf) for 10 min. The supernatant was assayed for TXB_2 by radioimmunoassay (RIA) (12). Serum TXB_2 generation was determined after incubation of whole blood for 1 h at 37°C (13). Serum was separated by centrifugation, stored at −20°C, and assayed for TXB_2 by RIA. Thromboxane A_3 (TXA_3) is formed in small amounts in serum from clotted whole blood of normal volunteer subjects on a diet rich in ω-3 fatty acids (14). The measurement of platelet TXB_2 generation by RIA using the antibody employed in our study corresponds well to measurements using gas chromatography/mass spectrometry in volunteer subjects on this diet (14).

Statistical analysis

The analysis of the effect of treatment on platelet aggregation or TXB_2 generation was conducted as follows. For each blood sample, the level of aggregation or TXB_2 generation was plotted against the log-transformed agonist values. Next, a line was fitted to these points using linear regression. The slope of this line was used as the response variable in subsequent analyses.

The data on age, known duration of NIDDM, height, weight, percentage of ideal body weight, body mass index (BMI), metabolic characteristics, BP, dietary composition, plasma fatty acid composition, platelet aggregation, platelet TXB_2 generation, and serum TXB_2 generation were all analyzed in the same way. The observations on the two baseline days were averaged and considered as a baseline. Data on day 28 and day 42 were averaged and considered as an estimate of the treatment effect. Day 42 was also used alone as an end-of-treatment estimator. Values on day 84 were used to indicate values after washout except for BP and body weight, for which the values for day 63 and day 84 were averaged and used to estimate values after washout. When one of the values to be averaged was absent, the other was used to supply the whole value. The averaging of baseline, treatment, and washout values was performed to maximize the power of this small study and was planned before the analysis. Baseline observations for the two groups were compared using a Student's *t* test. An analysis of covariance was used to test for treatment, end-of-treatment, and washout effect, in each case with baseline value as a covariate. That is, the differences caused by treatment are net differences after the effect of differences at baseline are taken into account. A test for interaction was also performed. This tested whether the relationship between the outcome measurement and the baseline measurement was different for subjects receiving fish oil than it was for subjects receiving safflower oil. Data are presented as means ± SE.

RESULTS

Study population

The age, known duration of NIDDM, height, weight, percentage of ideal body weight, BMI, metabolic characteristics, and BP readings of the two groups of subjects are presented in Table 1. No significant difference was found in any of the variables measured upon entry into the study including the creatinine clearance (not shown). The dietary composition of the subjects' intakes in the two study populations is displayed in Table 2. The protein intake in grams per day was significantly higher in the safflower

Table 2—*Dietary composition*

	Safflower oil	Fish oil	Statistical significance
Calories (kcal/day)	1974 ± 98	1691 ± 157	NS
Protein (g/day)	116 ± 10	88 ± 8	$P = 0.050$
Protein (% of total calories)	23.2 ± 1.4	21.4 ± 1.9	NS
Fat (g/day)	68.3 ± 6.2	57.7 ± 6.9	NS
Fat (% of total calories)	31.3 ± 2.8	30.8 ± 2.9	NS
Carbohydrate (g/day)	223.6 ± 21.6	206.7 ± 29.2	NS
Carbohydrate (% of total calories)	44.6 ± 3.5	47.7 ± 4.0	NS
Cholesterol (mg/day)	239.3 ± 67.5	191.0 ± 14.0	NS
Polyunsaturated fatty acids (g/day)	14.1 ± 1.7	12.7 ± 1.7	NS
Saturated fat (g/day)	19.9 ± 2.3	15.9 ± 6.3	NS
P/S ratio	0.76 ± 0.10	0.90 ± 0.12	NS
EPA (g/day)	0.08 ± 0.05	0.06 ± 0.02	NS
DHA (g/day)	0.12 ± 0.05	0.10 ± 0.03	NS

Data are means ± SE.

oil group than in the fish oil group ($P = 0.050$). No significant difference was observed between the two groups in the protein intake as a percentage of total calories or in any other measurement of dietary composition shown in Table 2. No subject experienced gastrointestinal or other side effects in either group. Thus, the subjects and the investigators remained blinded throughout the study and the analysis of the data.

In the fish oil group, 4 subjects were treated with diet alone, 1 subject with diet and glyburide, and 4 subjects with diet and insulin. In the safflower oil group, 4 subjects were treated with diet alone, 2 with a diet and glyburide, and 3 with a diet and insulin. In the fish oil group, 3 subjects were on medication for hypertension; in the safflower oil group, 2 subjects received such treatment. There was no change in the treatment regimen for NIDDM or hypertension in any subject during the course of the study. In the fish oil group, 5 subjects had no known complications of diabetes, 2 had background retinopathy and/or peripheral neuropathy, and 2 had coronary artery disease. In the safflower oil group, 6 had no known complications, 1 had background retinopathy and/or peripheral neuropathy, and 2 had coronary artery disease. In the fish oil group, 7 subjects were white and 2 were black. In the safflower oil group, 8 were white and 1 was of Asian-Indian origin.

Incorporation of ω-3 fatty acids into plasma fatty acids

The plasma fatty acid composition of the participants in the safflower oil group and the fish oil group is depicted in Table 3. In the fish oil group, the plasma-EPA level was significantly higher at 4 and 6 weeks of treatment ($P = 0.040$) and at the end of treatment ($P = 0.020$) than at baseline, and the plasma-DHA level was not significantly higher at 4 and 6 weeks of treatment ($P = 0.125$) but was significantly higher at the end of treatment ($P = 0.041$) than at baseline. Both EPA and DHA values had returned to baseline at the end of the washout period. In the fish oil group, the linoleic acid (18:2 ω-6) level was significantly lower at the end of the washout period than at baseline ($P = 0.039$). In the fish oil group, the plasma level of docosapentaenoic acid (22:5 ω-3), the product of EPA, was not significantly higher at 4 and 6 weeks of treatment ($P = 0.137$) but was significantly higher at the end of treatment ($P = 0.00075$) than at baseline. In the fish oil group, the plasma

Table 3—*Plasma fatty acid composition*

	Safflower oil				Fish oil			
Fatty acid	Baseline	Treatment	End of treatment	Washout	Baseline	Treatment	End of treatment	Washout
18:2ω6	19.71 ± 1.38	20.59 ± 1.02	21.26 ± 1.40	21.44 ± 1.16	20.91 ± 0.98	19.67 ± 1.38	20.08 ± 1.32	19.07 ± 1.27
20:4ω6(AA)	11.22 ± 0.99	10.94 ± 0.58	11.21 ± 0.61	11.50 ± 0.59	13.45 ± 0.97	10.76 ± 0.57	11.12 ± 0.62	11.99 ± 0.75
20:5ω3(EPA)	0.94 ± 0.30	1.02 ± 0.28	0.84 ± 0.23	1.13 ± 0.47	0.60 ± 0.10	2.14 ± 0.48	2.15 ± 0.51	0.73 ± 0.17
22:5ω3	0.98 ± 0.11	0.96 ± 0.09	0.88 ± 0.07	1.04 ± 0.13	1.14 ± 0.07	1.31 ± 0.14	1.41 ± 0.09	1.13 ± 0.16
22:6ω3(DHA)	3.69 ± 0.29	3.78 ± 0.46	3.78 ± 0.41	4.12 ± 0.64	3.52 ± 0.20	4.35 ± 0.44	4.48 ± 0.43	3.46 ± 0.37

Data are presented as the means ± SE percentage of total fatty acids.

Samples were obtained after an overnight fast before breakfast and before ingestion of safflower oil or fish oil capsules. Baseline values represent the mean values for the two sets of observations before treatment. Treatment values represent the mean values for the two sets of values obtained after 4 and 6 weeks of treatment. Values at the end of treatment are those obtained after 6 weeks of treatment. Washout values were obtained at end of washout period, 6 weeks after cessation of dietary supplementation.

level of arachidonic acid was not significantly lower at 4 and 6 weeks of treatment ($P = 0.14$) or at the end of treatment ($P = 0.08$) than at baseline. All other comparisons with baseline in Table 3 were not statistically significant. We also measured the levels of 16:0, 18:0, 18:1, 18:3ω6, 18:3ω3, 18:4ω3, and 20:3ω6 (not shown), for which there were no significant differences from baseline values.

Effects of ω-3 fatty acids on platelet aggregation, platelet TXB_2 generation, and serum TXB_2 concentrations

No significant difference was detected at baseline in collagen- or ADP-induced platelet aggregation or TXB_2 generation between the fish oil and safflower oil groups.

We compared the slopes of each subject's dose-response curves for collagen- or ADP-induced platelet aggregation or TXB_2 generation after 6 weeks of fish oil or safflower oil supplementation. The subjects who received fish oil exhibited a significant loss of collagen-induced platelet aggregation compared with those who received safflower oil. Fish oil reduced the slope for collagen-induced platelet aggregation to one-third the value observed with safflower oil ($P = 0.035$) (one-sided test), indicating decreased platelet aggregation in response to fish oil supplementation. The slopes were 13.3 ± 3.7 per unit change in log agonist value for the safflower oil group and 4.5 ± 2.7 for the fish oil group. The individual slopes in the safflower oil group were −1.3, 0.7, 1.3, 10.1, 18.4, 19.8, 19.9, 22.1, and 29.1. The individual slopes in the fish oil group were −3.4, −1.6, −1.2, −1.0, 1.9, 3.8, 6.9, 16.1, and 19.3. No difference was found in the collagen-induced generation of TXB_2 or in the ADP-induced platelet aggregation or TXB_2 generation between those who received fish oil and those who received safflower oil.

The difference between the slopes of the collagen-induced platelet aggregation curves at 6 weeks of supplementation and at baseline was significantly greater in the fish oil group than the safflower oil group ($P = 0.022$), but no such difference was observed for collagen-induced TXB_2 generation.

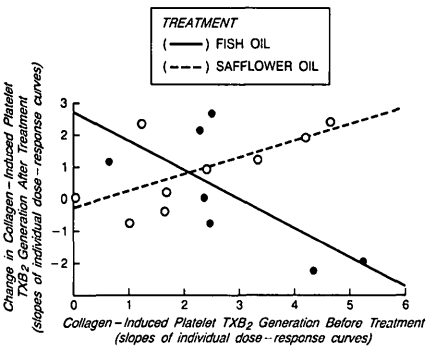

Figure 1—*The relationship between the changes from baseline in response to treatment in the slopes of the individual dose-response curves for collagen-induced platelet TXB_2 generation (ordinate) as a function of the extent of platelet TXB_2 generation at baseline (abscissa) is depicted for the subjects who received fish oil (●) and those who received safflower oil (○). Each symbol depicts the slope before treatment (abscissa) and the change after 6 weeks of treatment (ordinate). The slopes were determined as described in* METHODS.

We also compared the differences between the slopes of collagen-induced platelet aggregation or TXB_2 generation in response to dietary supplementation and the slopes at baseline as a function of the slope of aggregation or TXB_2 generation, respectively, at baseline. Patients with high initial TXB_2 generation showed a significantly larger drop from baseline after fish oil than after safflower oil when the mean values at 4 and 6 weeks of treatment were used by a test for interaction ($P = 0.038$) (Fig. 1). This difference was still present at the end of the washout period ($P = 0.031$).

No significant difference was found in serum TXB_2 concentrations at baseline (179 ± 28 ng/ml in the fish oil group and 148 ± 21 ng/ml in the safflower oil group). Fish oil had no effect on the serum TXB_2 concentration when the average of values at 4 and 6 weeks of treatment, the values at 6 weeks alone, or the values at the end of the washout period were compared with baseline.

Effects of ω-3 fatty acids on glucose, HbA_{1c} and lipid levels, and body weight

When the HbA_{1c} values at 4 and 6 weeks of treatment were compared with the corresponding values for safflower oil, fish oil produced a small but significant increase in HbA_{1c} of 0.56% ($P = 0.009$) (Fig. 2). After 6 weeks of treatment, fish oil produced a small but significant increase in HbA_{1c} of 0.72% compared with safflower oil ($P = 0.006$). At the end of the washout period, HbA_{1c} was not significantly different in the two study groups.

When the total cholesterol values at 4 and 6 weeks of treatment were compared with the corresponding values for safflower oil, the effect of fish oil on the total cholesterol, an increase of 8 mg/dl, was not significant ($P = 0.129$) (Fig. 2). After 6 weeks of treatment, fish oil produced a small but significant increase in the total cholesterol level of 20 mg/dl ($P = 0.030$). At the end of the washout period, the total cholesterol level was not significantly different in the two study groups.

When the mean of the TG values at 4 and 6 weeks of treatment was compared with the corresponding value for the safflower oil, fish oil produced a significant decrease of 44 mg/dl ($P = 0.027$) (Fig. 2). After 6 weeks of treatment, fish oil produced a decrease in the plasma TG level of 43 mg/dl compared with safflower oil ($P = 0.077$). At the end of the washout period, the plasma-TG level was not significantly different in the two study groups.

Fish oil had no effect on fasting glucose, HDL- or LDL-cholesterol levels, or body weight, when the average of the values at 4 and 6 weeks of treatment, the values at 6 weeks alone, or the values at

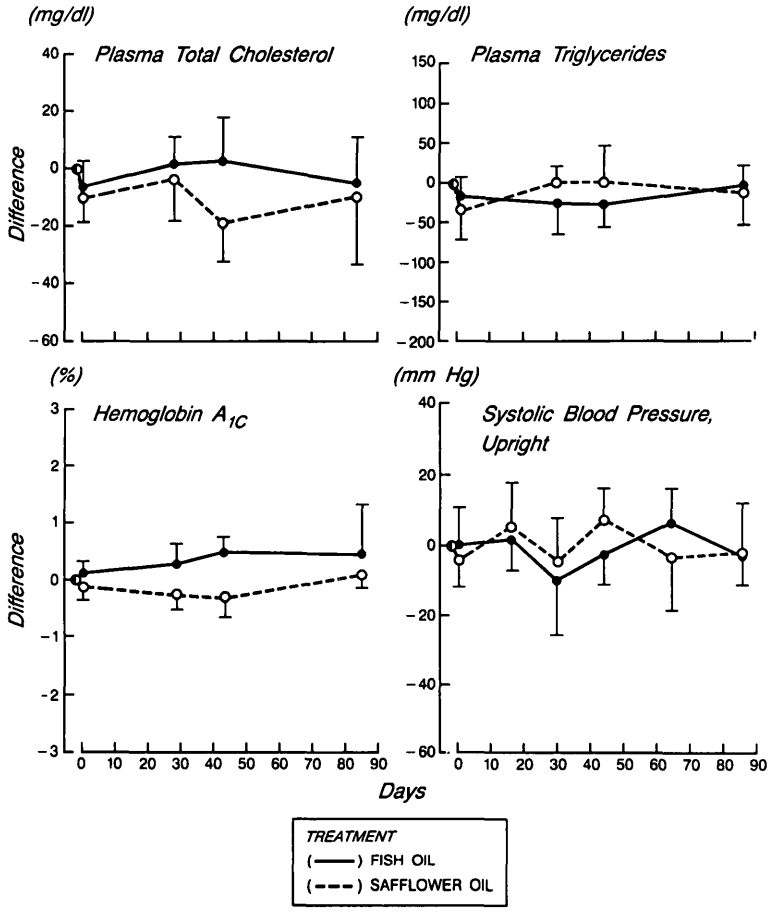

Figure 2—*The effect of fish oil (●) versus safflower oil (○) on plasma total cholesterol, plasma TGs, HbA_{1c}, and upright sBP. The data are expressed as differences from the observations on the first baseline day. For statistical analysis, the observations on the two baseline days were averaged and considered as a baseline; the average values are presented in Table 1.*

the end of the washout period were compared with the corresponding values for safflower oil.

Effect of ω-3 fatty acids on BP

When the values for upright systolic blood pressure (sBP) at 4 and 6 weeks of treatment were compared with the corresponding values for safflower oil, fish oil produced a significant fall of 8 mmHg ($P = 0.043$) (Fig. 2). After 6 weeks of treatment, fish oil produced a fall in the upright sBP of 10 mmHg compared with the corresponding values for safflower oil ($P = 0.092$). At the end of the washout period, the value for upright sBP was not significantly different in the two study groups.

Fish oil had no effect on sBP in the supine position or on dBP in the supine or upright position when the values at 6 weeks alone, the average of the values at 4 and 6 weeks of treatment, or the values at the end of the washout period were compared with the corresponding values for safflower oil.

CONCLUSIONS— We conducted a randomized, prospective, double-blind, controlled study of the effects of a small dose of fish oil (5 g/day, containing 2.5 g of ω-3 fatty acids) in comparison with the same quantity of safflower oil on cardiovascular risk factors in patients with moderately controlled NIDDM. Although we administered a small dose of ω-3 fatty acids, we observed a significant increase in the plasma levels of EPA and DHA during treatment and a return of these values to baseline levels at the end of a 6-week washout period. Our study demonstrates a beneficial effect of a small dose of ω-3 fatty acids on collagen-induced platelet aggregation and generation of TXA_2 (measured as the stable derivative TXB_2), plasma TG levels, and upright sBP in NIDDM patients who were in moderate metabolic control. This was achieved with only a small increase in HbA_{1c} of 0.56%, corresponding to an increase of ~18 mg/dl in plasma glucose (~10.7%) and a small increase in total cholesterol of 20 mg/dl (~9.9%), but no significant effect on fasting glucose or LDL- or HDL-cholesterol levels.

These findings support the view that a single dietary intervention can have a beneficial effect on multiple cardiovascular risk factors in NIDDM patients, as in the nondiabetic population (1–3), without a deleterious effect on glucose control or circulating lipid levels. In our study, the dietary and pharmacological treatment of NIDDM was held constant for the duration of the study to prevent a confounding effect on the variables we measured. The observed effects of fish oil on HbA_{1c} and total cholesterol levels were so small that they would probably not be recognized routinely in clinical practice. If they were appreciated, they probably could be prevented by conventional treatment for diabetes or hypercholesterolemia. Thus, it may be possible to achieve the beneficial effects of fish oil without an adverse effect on glucose or cholesterol levels (4).

Previous studies have suggested that ω-3 fatty acids (and, by extension, fish rich in ω-3 fatty acids) have potentially deleterious effects on circulating glucose, GHb, total cholesterol, LDL-cholesterol, and apolipoprotein B levels (4,5). That is, these studies suggested that a dietary intervention that improves numerous cardiovascular risk factors in the nondiabetic population might be unsafe for the diabetic population. How-

ever, the magnitude of these adverse effects was small, typically 10–36%, and was achieved with large doses of ω-3 fatty acids (4–10 g/day). These doses correspond to quantities of fish rich in ω-3 fatty acids that a person could not reasonably be expected to eat on a regular basis, i.e., 0.33–0.83 kg/day (0.73–1.83 lbs), assuming a content of 1.2 g of ω-3 fatty acids/100 g of fish (4). These doses are also large in terms of the benefits that have been ascribed to fish consumption vis-a-vis death from coronary heart disease (3,15,16). Moreover, many of the effects of ω-3 fatty acids, such as their effects on plasma TG and glucose levels, are related to dose in diabetic patients (4). An intake of 2.7–4 g/day in NIDDM patients had a transient effect or no effect on fasting plasma glucose and GHb levels (17–20). These considerations suggested to us that the doses of ω-3 fatty acids used in most previous studies of diabetic patients were excessive and that beneficial effects might be achieved and adverse effects averted at lower doses.

We used a small dose of fish oil, 5 g/day, containing a low dose of ω-3 fatty acids, 2.5 g/day, of which 1.5 g was EPA and 1.0 g was DHA. This corresponds to 0.21 kg (0.46 lb) of fish that is rich in ω-3 fatty acids, a quantity that might be consumed on a regular basis. Even at this low dose, we observed a decrease in collagen-induced platelet aggregation and TXA_2 generation, plasma TG levels, and upright sBP. Not surprisingly, the effect of fish oil on collagen-induced TXA_2 production was only evident in those with high baseline platelet TXA_2 generation. The decrease in upright sBP of 8 mmHg (6.2%) occurred in patients with normal or well-treated BP. A beneficial effect of 2.7 g/day of ω-3 fatty acids on sBP and dBP compared with baseline in NIDDM patients was observed in a previous study, which lacked a control group or a washout period (17). The detection of an effect of 2.5 g/day of ω-3 fatty acids on BP in NIDDM patients is noteworthy in view of the failure of doses as low as 3 g/day of ω-3 fatty acids to reduce BP in men with mild essential hypertension (21). Similarly, the decline in the plasma TG level of 44 mg/dL (29.6%) is noteworthy because it occurred in patients with normal or near-normal baseline values and is nevertheless of large magnitude. Our study does not provide any information about the effects of fish oil on BP and TG levels in NIDDM patients with hypertension or hypertriglyceridemia.

Our ability to detect small differences from normal or near-normal baseline values may reflect our rigorous study design and our use of safflower oil as the placebo. In a previous study, in which a small dose of ω-3 fatty acids (3 g/day) was compared with olive oil in NIDDM patients, fish oil produced an improvement in hypertriglyceridemia, a transient rise in plasma glucose, and a slight rise in LDL cholesterol, but no effect on BP, total cholesterol, or HDL cholesterol (19). Interestingly, fish oil produced a significant fall from baseline in collagen-induced TXB_2 generation within the treatment group and a decrease in spontaneous platelet aggregation but no effect on ADP- or epinephrine-stimulated TXB_2 generation, agonist-induced platelet aggregation, or other measures of platelet function (19). In that study, fish oil was compared with olive oil, which may by itself exert beneficial effects on circulating glucose and lipid levels and platelet function in nondiabetic subjects (22–25) and in NIDDM patients (26), possibly obscuring the beneficial effects of fish oils.

We compared fish oil, which contains polyunsaturated ω-3 fatty acids, to safflower oil, which contains polyunsaturated ω-6 fatty acids. Consequently, the effects of fish oil we observed can be attributed to the structure of the polyunsaturated fatty acids and not to the quantity of saturated or unsaturated fatty acids. The fish oil capsules contained not only 50% ω-3 fatty acids but a variety of other fatty acids (as indicated under METHODS), as in most if not all studies of these fish extracts. Although we cannot exclude an effect of these other constituents, an effect of the small quantities of the other fatty acids is unlikely; the quantity of oleic acid was comparable in the fish oil and safflower oil capsules.

Although the rigorous design of our study and the choice of safflower oil for comparison with fish oil minimize the effect of uncontrolled variables and facilitate the interpretation of our data, they also tend to minimize the potential impact of fish oils or dietary fish in the diet of the patient with NIDDM. In the community, individuals do not usually choose fish oils (or fish) versus safflower oil. Rather, they often choose fish (or poultry) as an alternative to red meat, which is rich in saturated fats. Our study demonstrates that a small dose of fish oil is safe and potentially beneficial in NIDDM patients. It also suggests that dietary fish rich in ω-3 fatty acids is a safe and a potentially beneficial alternative to red meat in patients with well-controlled NIDDM.

In our study, fish oil had a dramatic effect on collagen-induced platelet aggregation and TXA_2 generation but had no effect on ADP-induced platelet aggregation or TXA_2 generation. Collagen and ADP stimulate platelet function by different mechanisms. Collagen initiates the first phase of platelet aggregation, an effect that is mediated by a specific receptor on the surface of the platelet. ADP stimulates the second phase of platelet aggregation. Our findings suggest that ω-3 fatty acids act at the platelet surface, possibly by modifying the interaction between collagen fibrils and the membrane receptor that initiates platelet aggregation.

Similarly, agonist-induced platelet TXA_2 generation and serum TXA_2 production measure different aspects of platelet function. Agonist-induced platelet aggregation or TXA_2 generation measure graded degrees of platelet function in response to graded doses of an agonist. In contrast, when serum TXA_2 production is determined, platelet aggrega-

tion is allowed to go to completion. Our findings indicate that ω-3 fatty acids produce a partial rather than a complete inhibition of platelet function.

The findings of our study do not necessarily apply to IDDM patients, in whom fish oil has little or no deleterious effect on glycemic control or GHb levels, for example (5). Finally, although our study demonstrates a beneficial effect of a low dose of fish oil on several risk factors for CVD in NIDDM patients, we did not measure the effects of fish oil on cardiovascular morbidity or mortality. This will require prolonged studies of large populations.

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