# Long-Term Effects of Fish Oil on Insulin Resistance and Plasma Lipoproteins in NIDDM Patients With Hypertriglyceridemia

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**OBJECTIVE** — The aim of this study was to evaluate the long-term (6-month) effects of moderate fish oil supplementation on insulin sensitivity and plasma lipoproteins in NIDDM patients with hypertriglyceridemia.

**RESEARCH DESIGN AND METHODS** — The study has been performed according to a randomized double-blind placebo-controlled design with a parallel group sequence. After a washout period of 4 weeks and a run-in period of 3 weeks, 16 NIDDM patients with hypertriglyceridemia (triglyceride [TG], 2.25–5.65 mmol/l) were randomly assigned to either fish oil (2.7 g/day eicosapentaenoic plus docosahexaenoic acid for 2 months, then 1.7 g/day for 4 more months) ($n = 8$) or placebo ($n = 8$). Diet and hypoglycemic drugs remained unchanged throughout the whole experiment. At baseline and after 6 months, insulin sensitivity was measured by euglycemic hyperinsulinemic clamp (insulin infused, 2.0 mIU · kg$^{-1}$ body wt · min$^{-1}$). At the same time, blood glucose control, fasting and postprandial serum insulin and nonesterified fatty acid (NEFA) concentrations, and fasting plasma lipoprotein concentrations were evaluated.

**RESULTS** — In the group treated with fish oil compared with the baseline, there was: *1*) a significant reduction in both plasma TG (2.92 ± 0.23 vs. 3.85 ± 0.32 [mean ± SE] mmol/l, $P < 0.01$) and VLDL-TG (2.35 ± 0.24 vs. 4.25 ± 0.66 mmol/l, $P < 0.01$), without significant changes in blood glucose control; 2) a significant reduction in fasting NEFA concentrations (572 ± 100 vs. 825 ± 131 μmol/l, $P < 0.01$); and 3) a significant enrichment in long-chain ω-3 fatty acids of erythrocyte membrane phospholipids. In the placebo group, there were no changes in any of the variables analyzed. The insulin-mediated glucose uptake was unchanged in both groups (fish oil, 4.04 ± 0.82 mg · kg$^{-1}$ · min$^{-1}$ at baseline and 3.96 ± 0.50 mg · kg$^{-1}$ · min$^{-1}$ at 6 months; placebo, 3.51 ± 0.62 mg · kg$^{-1}$ · min$^{-1}$ at baseline and 4.09 ± 0.49 mg · kg$^{-1}$ · min$^{-1}$ at 6 months).

**CONCLUSIONS** — In NIDDM patients with hypertriglyceridemia, moderate amounts of fish oil induce a long-term significant reduction in plasma triglycerides, VLDL triglycerides, and NEFA and a significant enrichment in the erythrocyte phospholipid content of long-chain ω-3 fatty acids, without deteriorating blood glucose control. However, this amount of ω-3 fatty acids was unable to improve insulin sensitivity in this group of patients.

Insulin resistance is one of the major metabolic abnormalities present in patients with NIDDM (1,2). This abnormality can be even more marked in NIDDM patients with hypertriglyceridemia. Moreover, insulin resistance and hypertriglyceridemia seem to be pathogenetically linked (3,4). Therefore, it is likely that changes induced in insulin resistance are followed by changes in triglyceride levels and vice versa.

Among other factors, diet seems to have important influence on the development and modulation of insulin resistance (5–8). In particular, studies in rats have shown that fish oil, rich in long-chain ω-3 fatty acids, has beneficial effects on insulin resistance, since it completely prevents the development of insulin resistance induced by a diet rich in fat (9). One of the possible explanations for this effect seems to be the incorporation of long-chain ω-3 fatty acids in the phospholipids of skeletal muscles. In fact, the insulin resistance of rats is inversely and significantly correlated with the long-chain ω-3 fatty acid content of their skeletal muscles (10). Moreover, studies in vitro support the hypothesis that changes in the fatty acid composition of skeletal muscles can affect insulin action either nonspecifically through modifications in membrane fluidity or more directly through changes in the diacylglycerol second-messenger function (11–13).

Unfortunately, the few intervention studies in humans have not completely confirmed the results that were obtained on insulin resistance in rats (14–16), although it is known that fish intake delays the development of diabetes in glucose-intolerant individuals (17) and that the incidence of diabetes is rare in Greenland, a country with a high intake of ω-3 fatty acids in the diet (18). There could be several possible explanations for the divergent effects of fish oil on insulin resistance in humans and rats, but if the hypothesis that fish oil acts on insulin resistance through changes in the composition of cell membranes is true, the main reason could be the short duration of the studies performed in humans (2–3 weeks). In fact, it takes several months for changes in cell membrane composition to occur, and no real long-term study on this aspect has been performed so far.

Therefore, the first aim of this study was to evaluate the long-term (6-month) effects of moderate fish oil supplementation

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EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; M value, rate of glucose utilization; NEFA, nonesterified fatty acid; TG, triglyceride.

on insulin sensitivity and the phospholipid fatty acid composition of erythrocytes with a randomized double-blind placebo-controlled study. Erythrocytes were chosen in view of their easy accessibility. However, cell membranes in all body tissues derive their fatty acid constituents from the plasma pool that, in turn, is influenced by the dietary fatty acid composition (19).

Moreover, since the long-term effects of fish oil on other important parameters, such as lipid metabolism, blood glucose control, and serum insulin concentrations, are not known in the long run, a second aim of our study was to evaluate also the effects of prolonged fish oil intake on these variables.

We chose to evaluate these effects on NIDDM patients with hypertriglyceridemia because this group of patients is characterized by marked insulin resistance and high levels of plasma triglycerides and, thus, could gain greater benefits from a clinical point of view from a possible positive action of fish oil.

## RESEARCH DESIGN AND METHODS

### Patients

Sixteen patients with NIDDM (World Health Org. criteria) (20) and type IIB or IV hyperlipoproteinemia (Friederikson's classification) (21) participated in the study, which was part of a multicenter trial on the effects of fish oil on plasma lipids and blood glucose control. All patients were regularly seen in the diabetic clinic of our Institute. Selection criteria for the study were: *1*) NIDDM for at least 2 years and in almost stable metabolic control during the 3 months before the study, with either diet alone or diet plus sulfonylureas; *2*) no significant changes in body weight during the 3 months before enrollment; *3*) fasting levels of plasma triglycerides between 2.25 and 5.65 mmol/l, with plasma cholesterol levels ≤7.75 mmol/l in the absence of any hypolipidemic drug for at least 4 weeks; *4*) age between 40 and 75 years; and *5*) only women in the postmenopausal phase.

Patients with moderate arterial hypertension that was well controlled by calcium antagonists or ACE-inhibitors could be enrolled.

Patients with proliferative retinopathy, vitreal hemorrhage, hepatic or renal failure, bleeding disorders, or who used any drug capable of influencing hemostatic variables were excluded from the study.

### Study design

The study was performed according to a randomized double-blind placebo-controlled design with a parallel group sequence.

After a washout period of 4 weeks, during which all hypolipidemic drugs were withdrawn and patients were stabilized on their own isoenergetic diet and previous hypoglycemic treatment (only sulfonylureas were admitted), there was a run-in period of 3 weeks during which patients received three capsules per day of placebo. At the end of the run-in period, patients were randomized to receive either fish oil or placebo capsules containing olive oil. Eight patients followed the fish oil treatment and eight received placebo. All patients completed the study, which lasted 6 months, during which they were seen each month on an outpatients basis. During the first 2 months, the patients took three capsules of fish oil or placebo per day; thereafter, the dosage was two capsules per day. Both fish oil and placebo capsules were provided by Pharmacia, Farmitalia Carlo Erba, Milan, Italy. Each capsule of fish oil contained 0.3 g eicosapentaenoic acid (EPA) and 0.5 g docosahexaenoic acid (DHA), and the remaining 20% was a mixture of different fatty acids. Therefore, the daily amount of ω-3 fatty acids was 2.5 g (0.96 g EPA, 1.59 g DHA) during the first 2 months and 1.7 g (0.64 g EPA, 1.06 g DHA) during the last 4 months. Each placebo capsule contained 1 g olive oil.

Throughout the experiment, patients followed their own isoenergetic diet composed of 50% carbohydrate, 30% fat (8% saturated, 6% polyunsaturated, and 16% monounsaturated), and 20% protein, with about 30 g/day dietary fiber. No attempt was made to change the patients' diets, particularly their usual fish consumption, which was on average 1 portion of fish per week at both the beginning and the end of the study. Compliance with the diet, which was checked by a semiquantitative dietary questionnaire at the beginning and the end of the study, was acceptable, and there were no indications of changes in the patients' dietary habits.

Hypoglycemic drugs, as well as antihypertensive treatment for the hypertensive patients enrolled, were maintained unchanged throughout the experiment.

Written informed consent was given by each participant; the study was approved by the ethical committee of the Federico II University.

At the end of the run-in and the two treatment periods, the following measurements were performed:

- Plasma lipids: blood samples after a 12-h fast were taken for determination of cholesterol and triglyceride on three separate occasions. On one of these occasions, samples for lipoprotein separation (VLDL, LDL, and HDL) were also taken.
- Blood glucose control: fasting plasma glucose and $HbA_{1c}$ were measured. Plasma glucose was also evaluated at 1, 2, and 3 h after lunch.
- Serum insulin and nonesterified fatty acids (NEFA) at fasting and 1, 2, and 3 h after lunch.
- Phospholipid fatty acid composition of erythrocyte membranes.
- Peripheral insulin sensitivity.

### Euglycemic hyperinsulinemic clamp

Peripheral insulin sensitivity was evaluated by a 2-h euglycemic hyperinsulinemic clamp, as previously described (22). Briefly, after a 10–12 h overnight fast, a polyethylene cannula was inserted into a cubital vein to perform the infusions. A second cannula was placed retrogradely into a forearm vein of the contralateral arm for blood sampling; the forearm was kept warm in a heated box (60°C) to ensure arterialization of venous blood. Then, regular human insulin was administered intravenously at a rate of 2 mIU · $kg^{-1}$ body wt · $min^{-1}$. A variable amount of a 20% glucose solution was also infused to maintain blood glucose concentration at approximately 5.5 mmol/l. The glucose infusion rate was adjusted according to plasma glucose levels, which were measured at 5-min intervals on a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA). Arterialized blood samples for insulin measurements were taken in the basal state and every 20 min during the clamp. The steady-state rate of glucose disposal (M value) was measured during the last 60 min of the clamp.

### Analytical methods

Plasma glucose concentrations were measured by the glucose-hexokinase method (Roche, Basle, Switzerland). $HbA_{1c}$ was measured by high-pressure liquid chromatography (23): normal values in our laboratory were <6.7%. Serum insulin levels were measured by radioimmunoassay (Techno Genetics, Milan, Italy) (24).

Plasma NEFA concentrations were measured by a standard enzymatic colorimetric method (Wako Chemicals, GmbH, Germany) (25). Total cholesterol and triglycerides were assayed in serum, and isolated lipoproteins on a COBAS-MIRA autoanalyzer (Roche, Basle) by enzymatic colorimetric methods using commercially available kits (Boehringer-Mannheim, Mannheim, Germany) (26,27). Quality control of lipid analysis was regularly ensured in our laboratory by the World Health Organization Prague Reference Center (28). Lipoprotein separation was achieved by preparative ultracentrifugation combined with a precipitation method (phosphotungstic acid plus magnesium chloride), after the addition of EDTA and merthiolate to serum for a final concentration of 0.05 and 0.01%, respectively. VLDL were isolated at a density of 1.006 g/ml (29). HDL were obtained by precipitation on serum (30). LDL were calculated by the difference between bottom fraction d 1.006 and HDL (31).

The fatty acid composition of erythrocyte phospholipids was determined after the extraction of lipids with a mixture of chloroform and methanol (2:1 vol/vol), containing 0.01% BHT as antioxidant, according to the Folch method (32), and transmethylation with 2% $H_2SO_4$ in methanol for 18 h at 65–70°C (33). The methyl fatty acids were then separated and quantitated on a gas chromatograph 5890 series II (Hewlett-Packard) fitted with a capillary column (Omegawax 320, Supelco Inc., Bellefonte, PA). Fatty acids were identified by comparing retention times with those of authentic standard mixture. Each sample was run in triplicate, and the samples of each subject (at baseline and 6 months) were determined in the same run. In our laboratory, the percentage recovery for all the procedure was 90%, and the within-day coefficient of variation is ≤3% for fatty acids representing >5% of total fatty acids, ≤5% for fatty acids representing 1–5%, and ≤10% for fatty acids present in amounts <1%.

### Statistical analysis

Data are expressed as mean ± SE, unless otherwise indicated. Fasting plasma triglycerides and cholesterol represent the mean of three samples. This average was planned to try to minimize the great variability in triglycerides levels. Postprandial values are the mean of the three measurements performed after lunch (1st, 2nd, and 3rd h). Variables that were not uniformly distributed were log transformed before statistical analysis. Statistical analysis was performed according to standard methods (34) using the Statistical Package for Social Sciences (SPSS/PC) software.

Baseline data were compared using the Student's *t* test for unpaired data. The effect of treatment was analyzed comparing the results within each group at baseline and at 6 months with the Student's *t* test for paired data. Moreover, comparisons between the two treatments were performed using the Student's *t* test for unpaired data on the differences between data at 6 months and at baseline. Relationships between changes in insulin sensitivity and cell membrane fatty acid composition were analyzed by the Pearson's correlation. $P < 0.05$ was considered statistically significant. The one-tailed *P* value was used only for plasma and lipoprotein triglycerides in consideration of the known hypotriglyceridemic effects of ω-3 fatty acids.

The sample size (8 patients for each arm of the study) was chosen to be able to detect a treatment effect on insulin sensitivity of about 30%.

**Table 1—*Clinical characteristics of the participants***

| | Fish oil | Placebo |
|---|---|---|
| *n* | 8 | 8 |
| Age (years) | 56 ± 3 | 57 ± 2 |
| Sex (F/M) | 5/3 | 4/4 |
| BMI (kg/m²) | 26 ± 1 | 30 ± 2 |
| Diabetes duration (years) | 8 ± 3 | 6 ± 1 |
| Fasting plasma glucose (mmol/l) | 10.2 ± 1.2 | 9.2 ± 0.6 |
| $HbA_{1c}$ (%) | 7.3 ± 0.4 | 6.9 ± 0.6 |
| Hypoglycemic therapy | | |
| Diet | 2 | 2 |
| Diet + sulfonylureas | 6 | 6 |
| Plasma cholesterol (mmol/l) | 6.26 ± 0.57 | 5.76 ± 0.29 |
| Plasma triglycerides (mmol/l) | 3.85 ± 0.32 | 3.29 ± 0.38 |
| HDL cholesterol (mmol/l) | 0.88 ± 0.04 | 0.93 ± 0.07 |

Data are *n* or means ± SE.

**RESULTS** — The main clinical characteristics of the two groups of patients at baseline are shown in Table 1. There were no significant differences between the two groups, the group on fish oil presenting higher plasma triglycerides and blood glucose levels and the one on placebo tending to be more obese. For both the fish oil and the placebo group, two patients were treated only by diet and six by diet plus glibenclamide. One patient in the fish oil group and three on placebo were on stable antihypertensive treatment (ACE-inhibitors or calcium antagonists), and did not change their treatment throughout the whole experiment. In both groups, there was no difference in body weight between baseline and the end of treatment (fish oil, 69 ± 5 vs. 70 ± 5 kg; placebo, 74 ± 3 vs. 74 ± 4 kg).

### Lipids and lipoproteins

Fish oil induced a significant decrease in serum triglycerides (2.92 ± 0.23 vs. 3.85 ± 0.32 mmol/l, $P < 0.01$) at six months and no significant change in serum cholesterol (5.71 ± 0.26 vs. 6.27 ± 0.57 mmol/l, NS). The decrease in serum triglycerides was mainly due to a significant and consistent (45%) reduction of VLDL-triglycerides (2.35 ± 0.24 vs. 4.25 ± 0.66 mmol/l) without changes in the triglyceride content of either LDL (0.37 ± 0.03 vs. 0.39 ± 0.07 mmol/l, NS) or HDL (0.19 ± 0.01 vs. 0.25 ± 0.05 mmol/l, NS) (Table 2).

With respect to lipoprotein cholesterol levels, fish oil produced opposite effects on VLDL and LDL cholesterol values. In fact, there was a significant reduction in VLDL (1.47 ± 0.13 vs. 2.77 ± 0.76 mmol/l, $P < 0.05$) and a significant increase in LDL (3.29 ± 0.49 vs. 2.88 ± 0.20 mmol/l, $P < 0.01$). HDL cholesterol did not change, and the ratio between LDL and HDL cholesterol tended to increase, although the difference was not significant (Table 2). As expected, placebo did not induce any effect on serum and lipoprotein lipid concentrations. When the changes obtained with fish oil were compared with those obtained with placebo,

**Table 2**—*Plasma lipoprotein triglyceride and cholesterol concentrations before and after 6 months on fish oil and placebo in hypertriglyceridemic NIDDM patients*

| | Fish oil ($n$ = 8) | | | Placebo ($n$ = 8) | | |
|---|---|---|---|---|---|---|
| | Baseline | 6 months | Δ | Baseline | 6 months | Δ |
| Triglycerides | | | | | | |
| VLDL (mmol/l) | 4.25 ± 0.66 | 2.35 ± 0.24* | −1.90 ± 0.54† | 2.76 ± 0.40 | 2.97 ± 0.77 | 0.21 ± 0.98 |
| LDL (mmol/l) | 0.39 ± 0.07 | 0.37 ± 0.03 | −0.02 ± 0.04 | 0.38 ± 0.04 | 0.41 ± 0.05 | 0.04 ± 0.03 |
| HDL (mmol/l) | 0.25 ± 0.05 | 0.19 ± 0.01 | −0.06 ± 0.04 | 0.28 ± 0.04 | 0.25 ± 0.04 | −0.03 ± 0.05 |
| Cholesterol | | | | | | |
| VLDL (mmol/l) | 2.77 ± 0.76 | 1.47 ± 0.13‡ | −1.30 ± 0.06 | 1.59 ± 0.20 | 1.99 ± 0.64 | 0.40 ± 0.7 |
| LDL (mmol/l) | 2.88 ± 0.20 | 3.29 ± 0.49* | 0.63 ± 0.11 | 3.38 ± 0.20 | 3.30 ± 0.38 | −0.08 ± 0.29 |
| HDL (mmol/l) | 0.88 ± 0.04 | 0.89 ± 0.05 | 0.01 ± 0.04 | 0.93 ± 0.07 | 0.95 ± 0.10 | 0.02 ± 0.06 |
| LDL/HDL | 3.31 ± 0.30 | 4.06 ± 0.30 | 0.74 ± 0.20 | 3.72 ± 0.21 | 3.87 ± 0.41 | 0.15 ± 0.22 |

Data are means ± SE. *$P < 0.01$; ‡$P < 0.05$ vs. baseline; †$P < 0.05$ vs. Δ placebo. Δ is the difference between 6-month and baseline values.

VLDL triglycerides showed a statistically significant difference (−1.9 ± 0.54 mmol/l after fish oil vs. 0.21 ± 0.98 mmol/l after placebo, $P < 0.05$) (Table 2).

The effects of fish oil and placebo on NEFA concentrations are shown in Fig. 1. There was a decrease in NEFA both in the fasting state and postprandially with fish oil, but the decrease was statistically significant only in the fasting state (572 ± 100 vs. 825 ± 131 μmol/l, $P < 0.01$). There was no significant change with placebo. Moreover, the changes induced by fish oil on fasting NEFA concentrations were significantly different from those observed in the placebo group (−252 ± 79 vs. 55 ± 86 μmol/l, $P < 0.05$).

## Blood glucose control and serum insulin concentrations

The effects of treatment on blood glucose control and serum insulin concentrations are shown in Table 3. In both the fish oil and the placebo group, there was no significant change in blood glucose control, which was evaluated either as fasting and postprandial plasma glucose levels or as $HbA_{1c}$, although there was a tendency for $HbA_{1c}$ to increase in both groups (1.0% for the fish oil and 0.7% for the placebo group). Moreover, no difference was observed when the two groups were compared for 6-month–baseline changes. Regarding serum insulin concentrations, fasting insulin concentrations increased significantly in the fish oil group; however, no difference was found when the 6-month–baseline changes were compared with that observed in the placebo group (fish oil group, 31 ± 9 pmol/l; placebo group, 15 ± 22 pmol/l) (Table 3). Postprandial plasma insulin levels did not change significantly in either the fish oil or the placebo group. Accordingly, there was no significant difference between the changes induced by fish oil and placebo (Table 3).

## Phospholipid fatty acid composition of erythrocyte membranes

The phospholipid fatty acid composition of erythrocytes at baseline and at the end of the fish-oil and placebo periods is shown in Table 4. Fatty acids are expressed as a percentage of the total fatty acids identified. Minor peaks (<1% of total fatty acids) are not reported with the exception of long-chain ω-3 fatty acids. As expected, all long-chain ω-3 fatty acids (C20:5, C22:5, C22:6) increased significantly in the fish oil group. In the same group, there was a statistically significant decrease in the long-chain ω-6 fatty acids (C20:3, C20:4). Saturated and monounsaturated fatty acids did not change after either fish oil or placebo.

## Insulin sensitivity

Peripheral insulin-stimulated glucose disposal was not affected by treatment. Glucose utilization during the euglycemic clamp (Fig. 2) was, in fact, the same at baseline and after 6 months in both the fish oil (M, 4.04 ± 0.82 vs. 3.96 ± 0.50 mg · kg$^{-1}$ · min$^{-1}$, NS; Δ, −0.08 ± 0.56 mg · kg$^{-1}$ · min$^{-1}$, 95% CI −1.42 to 1.25) and the placebo group (M, 3.51 ± 0.62 vs. 4.09 ± 0.49 mg · kg$^{-1}$ · min$^{-1}$, NS; Δ, 0.57 ± 0.68 mg · kg$^{-1}$ · min$^{-1}$, 95% CI −1.04 to 2.20).

Blood glucose concentrations during the clamp were 5.31 ± 0.99 mmol/l at baseline and 5.35 ± 0.05 mmol/l at 6

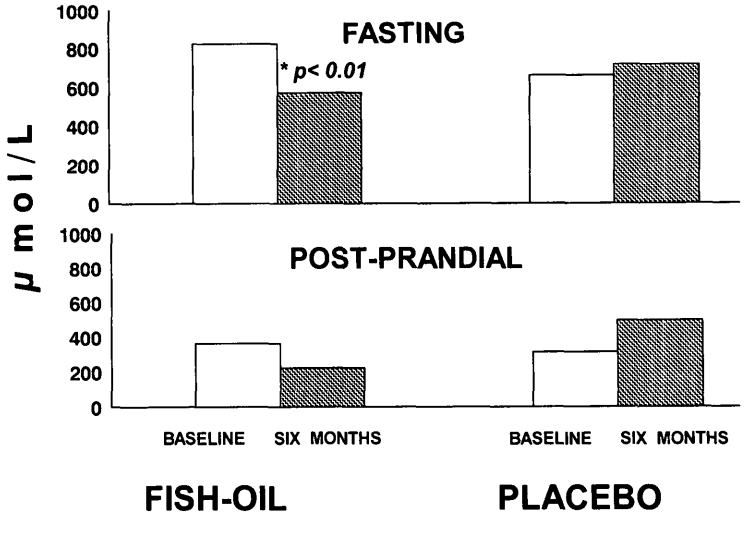


**Figure 1**—*Fasting and postprandial plasma NEFA concentrations before and after 6 months on fish oil or placebo in NIDDM patients with hypertriglyceridemia.*

**Table 3—*Blood glucose control and serum insulin concentrations before and after 6 months on fish oil or placebo in hypertriglyceridemic NIDDM patients***

| | Fish oil ($n$ = 8) | | | Placebo ($n$ = 8) | | |
|---|---|---|---|---|---|---|
| | Baseline | 6 months | Δ | Baseline | 6 months | Δ |
| Fasting plasma glucose (mmol/l) | 10.2 ± 1.2 | 10.9 ± 0.5 | 0.7 ± 1.0 | 9.2 ± 0.6 | 10.3 ± 1.0 | 1.2 ± 1.0 |
| Plasma glucose (mmol/l)* | 12.9 ± 1.8 | 12.1 ± 1.7 | −0.8 ± 2.0 | 11.5 ± 1.2 | 12.9 ± 1.6 | 1.5 ± 1.8 |
| $HbA_{1c}$ (%) | 7.3 ± 0.4 | 8.3 ± 0.5 | 1.0 ± 0.6 | 6.9 ± 0.5 | 7.7 ± 0.5 | 0.8 ± 0.7 |
| Fasting plasma insulin (pmol/l) | 75 ± 9 | 105.6 ± 15.6† | 31 ± 9 | 121 ± 18.4 | 135 ± 14 | 15 ± 22 |
| Plasma insulin (pmol/l)* | 244 ± 38 | 258 ± 29 | 14 ± 31 | 323 ± 68 | 243 ± 16 | −80 ± 61 |

Data are means ± SE. *Mean of three measurements 1, 2, and 3 h after lunch; †$P < 0.01$ vs. baseline; Δ is the difference between 6-month and baseline values

months for the fish oil group and 5.48 ± 0.11 mmol/l at baseline and 5.40 ± 0.11 mmol/l at 6 months for the placebo group. The time to reach euglycemia during insulin infusion was similar in the fish oil (40.2 ± 12.3 min at baseline, 37.2 ± 9.5 min at 6 months) and the placebo group (50 ± 6.2 min at baseline, 40.6 ± 12.5 min at 6 months). The steady-state insulin levels achieved during the euglycemic clamp studies were also similar (fish oil group, 738 ± 78 pmol/l at baseline and 696 ± 78 pmol/l at 6 months; placebo group, 720 ± 42 pmol/l at baseline and 708 ± 48 pmol/l at 6 months).

Correlation analysis between changes in insulin sensitivity and cell membrane ω-3 fatty acid composition did not show any significant relationship ($r = -0.23$, NS for the fish oil group).

**CONCLUSIONS** — The most important result of this study is that moderate amounts of fish oil, taken for a long period of time (6 months), do not improve insulin resistance in humans—at least in NIDDM patients with hypertriglyceridemia—as occurs in rats. In fact, in our study, there was no variation in peripheral insulin-stimulated glucose disposal after long-term treatment with fish oil (2.7 g/day for 2 months and 1.5 g/day for the other 4 months). On the other hand, this kind of treatment was able to change the composition of cell membranes significantly. In fact, the phospholipids of erythrocyte membranes, which were taken in our study as an index of membrane composition of the whole body and, therefore, also of skeletal muscles (19), showed a significant enrichment in EPA (by more than twofold) and in DHA (31%) after 6 months of treatment.

Therefore, the hypothesis that fish oil improves insulin sensitivity and that the improvement in peripheral insulin action is reached through changes in the composition of membrane phospholipids (10) does not seem to apply to humans, at least to this group of patients affected by NIDDM, hypertriglyceridemia, and marked insulin resistance. We chose to study this particular type of patient because they are the ones who could draw greater benefits from fish oil treatment from a clinical point of view, although, at the same time, the insulin resistance of these patients could be so strong and of such long duration as to have become unresponsive to any treatment.

Two more points should be taken into account in discussing our data: *1*) the dosage of fish oil utilized in our study, and 2) the kind of diet followed by our patients. Regarding the dosage, we used moderate amounts of fish oil, which might account for the lack of effect on insulin resistance in our study. On the other hand, considering the possible negative effects of higher amounts of fish oil on glucose control and insulin secretion reported in NIDDM patients by other authors (35,36), only moderate amounts could be used in clinical practice.

Another aspect to be considered involves the diet followed by our patients, which was low in fat, especially saturated fats. In fact, the beneficial effects of fish oil on insulin resistance that have been reported in rats seem to be more relevant with a fat-rich diet. A high-fat diet, especially a high saturated-fat diet, is not suitable for NIDDM patients with hypertriglyceridemia, and the results obtainable with this kind of diet would, therefore, not be clinically relevant in this type of patients.

After taking into account the possible differences discussed above, it remains that the increased incorporation of EPA and DHA in the phospholipids of erythrocyte membranes in humans is not associated with an improvement of peripheral insulin action. This may be attributable to the fact that ω-3 fatty acids in humans might not be specifically incorporated into phosphatidylinositol, which is considered to have a major role in the insulin signal-trans-

**Table 4—*Phospholipid fatty acid composition of erythrocyte membranes before and after 6 months on fish oil or placebo in hypertriglyceridemic NIDDM patients***

| | Fish oil ($n$ = 8) | | Placebo ($n$ = 8) | |
|---|---|---|---|---|
| | Baseline | 6 months | Baseline | 6 months |
| Saturated | | | | |
| C16:0 | 24.2 ± 0.7 | 24.3 ± 0.4 | 23.7 ± 0.6 | 23.5 ± 0.6 |
| C18:0 | 12.8 ± 0.4 | 12.5 ± 0.3 | 12.9 ± 0.2 | 12.6 ± 0.3 |
| Monounsaturated | | | | |
| C18:1 | 14.2 ± 0.7 | 14.4 ± 0.5 | 13.9 ± 0.5 | 14.0 ± 0.6 |
| Polyunsaturated ω-6 | | | | |
| C18:2 | 10.1 ± 0.5 | 10.9 ± 0.8 | 10.6 ± 0.4 | 12.6 ± 0.9* |
| C20:3 | 1.6 ± 0.1 | 1.4 ± 0.1† | 1.7 ± 0.4 | 1.8 ± 0.5 |
| C20:4 | 12.2 ± 0.3 | 9.9 ± 0.3‡ | 11.7 ± 0.3 | 11.8 ± 0.4 |
| Polyunsaturated ω-3 | | | | |
| C20:5 | 0.7 ± 0.2 | 2.2 ± 0.3‡ | 0.7 ± 0.1 | 0.6 ± 0.1 |
| C22:5 | 1.9 ± 0.1 | 2.6 ± 0.1‡ | 1.9 ± 0.2 | 1.7 ± 0.1 |
| C22:6 | 5.1 ± 0.3 | 6.7 ± 0.2‡ | 5.0 ± 0.4 | 4.7 ± 0.3 |

Data are means ± SE. Fatty acids are expressed as a percentage of total fatty acids; some minor peaks of fatty acids are not shown. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ vs. baseline.

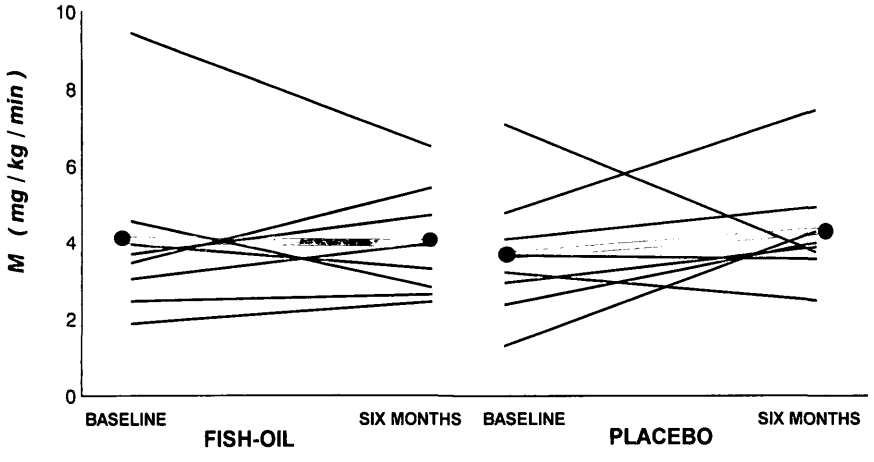


**Figure 2**—*Individual and average insulin-stimulated peripheral glucose disposal rate before and after 6 months on fish oil or placebo in NIDDM patients with hypertriglyceridemia.*

duction process (37,38). Therefore, if the above assumption is correct, the content of ω-3 long-chain fatty acids in human cell membranes should not have the same impact in the regulation of insulin action as in rats. Moreover, in our study, the increase in long-chain ω-3 fatty acids of erythrocytes was accompanied by a significant decrease in the long-chain ω-6 fatty acids, with no change in the total amount of polyunsaturated fats. In healthy and insulin-resistant individuals, indexes of insulin sensitivity are significantly related to the total amount of phospholipid long-chain polyunsaturated fatty acids (ω-3 plus ω-6) in skeletal muscles and not specifically to the content of long-chain ω-3 (39). Therefore, it is also possible that the insulin resistance of our patients was not significantly affected by fish oil treatment because the total amount of long-chain polyunsaturated fatty acids did not change with this treatment.

The results of our study on lipid metabolism confirm, for the first time on a long-term basis, results already demonstrated in short-term studies (14,35,36). In fact, with fish oil, there was a significant and clinically relevant reduction of triglycerides, attributable in particular to a decrease in the VLDL fraction (of 45% for VLDL triglycerides and 47% for VLDL cholesterol). This reduction was accompanied by a significant increase in LDL cholesterol (14%), which could be a consequence of the inhibitory effect of fish oil on LDL receptors (40). The opposite effects of fish oil on VLDL and LDL levels may explain, at least in part, why not all the studies are concordant in showing a positive effect of high fish-oil intake in reducing cardiovascular risk (41,42).

The reduction of triglycerides was not associated with any change in peripheral insulin-mediated glucose disposal. This result, reported also in some studies with fibrates (43), suggests that hypertriglyceridemia is a consequence—rather than a cause—of insulin resistance in NIDDM patients. Another possible alternative explanation is that the reduction in plasma triglyceride levels after fish oil, although relevant from a clinical point of view, was not enough to have significant effects on insulin action.

In our study, fish oil treatment also induced a significant reduction of fasting plasma NEFA concentrations, suggesting a possible antilipolytic effect. This may well contribute to the reduction of hepatic VLDL synthesis, which is generally reported to be the main mechanism of the hypotriglyceridemic action of fish oil (44). Very few studies with fish oil have analyzed NEFA concentrations, and no effect has been reported so far in short-term studies (14,15); therefore, the reduction that we found may represent a long-term effect of fish oil supplementation.

Regarding triglycerides, the decrease in NEFA was not associated with any improvement in insulin resistance. This, of course, does not exclude the importance of the Randle cycle (45), but suggests its partial contribution to the insulin resistance of NIDDM patients and the fact that it does not play a major role in nonobese patients.

Even if it was not a primary end-point of our study, we looked also at the possible negative effects of fish oil on blood glucose control and serum insulin concentrations. After 6 months of fish oil supplementation, no real and clinically relevant negative effects on either blood glucose control or serum insulin concentrations were found. Very contrasting data, especially for blood glucose control in NIDDM patients, are reported in the literature. However, from a careful examination of all the data, it becomes clear that all the negative effects on blood glucose control and serum insulin concentrations occur at a dosage of fish oil >4 g/day (46). In this respect, our study confirms that a moderate supplementation of fish oil is also safe for NIDDM patients with long-term administration.

In conclusion, this study confirms that moderate amounts of fish oil, taken for long periods of time, have a beneficial triglyceride-lowering action in NIDDM patients with hypertriglyceridemia and are safe for blood glucose control. Moreover, the long-term use of fish oil reduces NEFA concentrations. At the same time, this is the first study showing that a long-term supplementation of moderate amounts of fish oil has no significant effect on the marked insulin resistance of NIDDM patients with hypertriglyceridemia. However, this result does not rule out the possible effect of fish oil on the modulation of insulin action in people with normal or mildly abnormal insulin sensitivity.

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