Moderate Intake of n-3 Fatty Acids for 2 Months Has No Detrimental Effect on Glucose Metabolism and Could Ameliorate the Lipid Profile in Type 2 Diabetic Men

Results of a controlled study

JING LUO, MD, PHD
SALWA W. RIZKALLA, MD, PHD
HUBERT VIDAL, PHD
JEAN-MICHEL OPPERT, MD, PHD
CLAUDE COLAS, MD
ABDELGHANI BOUSSAIRI, PHD
MICHELE GUERRE-MILLO, PHD
ANNE-SOPHIE CHAPUIS, RN
ANNIE CHEVALIER
GEORGES DURAND, PHD
GÉRARD SLAMA, MD

OBJECTIVE — To evaluate the effect of a moderate dose of fish oil on glycemic control and in vivo insulin action in type 2 diabetic men with elevated plasma triacylglycerols and to determine the effect of the same treatment on gene expression of GLUT4, lipoprotein lipase (LPL), and hormone-sensitive lipase (HSL) in the abdominal adipose tissue.

RESEARCH DESIGN AND METHODS — A total of 12 type 2 diabetic men were randomly allocated to 2 months of 6 g daily of either fish oil or sunflower oil, separated by a 2-month washout interval, in a double-blind crossover design.

RESULTS — For glucose metabolism, 2 months of fish oil supplementation compared with sunflower oil led to similar fasting plasma insulin, glucose, and HbA_{1c}. Basal hepatic glucose production did not increase after fish oil. There was no difference in insulin suppression of hepatic glucose production nor in insulin stimulation of whole-body glucose disposal measured by the euglycemic-hyperinsulinemic clamp. Fish oil did not ameliorate the low mRNA level of GLUT4 in adipose tissue of these patients. For lipid profile, fish oil lowered plasma triacylglycerol more than sunflower oil ($P < 0.05$) and tended to increase the amount of mRNA of both LPL and HSL in adipose tissue.

CONCLUSIONS — A moderate dose of fish oil did not lead to deleterious effects on glycemic control or whole-body insulin sensitivity in type 2 diabetic men, with preserved triacylglycerol-lowering capacities.

In type 2 diabetic patients, the frequently observed high triacylglycerol concentrations have been shown to be a risk factor for coronary heart disease. Treatment of hypertriacylglycerolemia in these patients is based on restricted fat diet, optimal glycemic control, and weight loss. Pharmacological therapy is often required. Fish oil, rich in n-3 polyunsaturated fatty acids, has been proposed as an efficient treatment to lower plasma triacylglycerol concentrations in both diabetic and nondiabetic subjects. On the other hand, it is widely accepted that fish oil may have detrimental effect on blood glucose control of type 2 diabetic subjects, even though very few solid data support this hypothesis. Some uncontrolled studies have drawn attention to possible adverse consequences of fish oil therapy on fasting glycemia in type 2 diabetic patients (1–5). Placebo-controlled studies (6–11) have also been conducted but the results are still inconclusive: fasting glycemia was either deteriorated (6–8,11) or unchanged (9,10) after fish oil treatment. It should be noted that in these studies considerably higher doses of n-3 fatty acids (3–7.5 g/day) than that generally prescribed in France (1.8 g/day) (12) were used. Moreover, in most of these studies, dietary information was not available. Therefore, it is unclear whether the increase in fasting glycemia observed in some studies was related to fish oil intake itself or to changes in dietary habits of the patients.

In insulin-resistant rats, fish oil administration was shown to increase insulin action both in vivo (13) and in vitro (14). In type 2 diabetic patients, no significant effect of fish oil on insulin sensitivity was found in placebo-controlled studies (7, 10,15). The lack of positive results might, however, be explained by the long duration of insulin resistance in the type 2 diabetic patients in addition to relatively short-term experimental periods (2 or 3 weeks) (7,10), with the exception of the most recent study of Rivellese et al. (15) in which fish oil was given for 6 months.

The aim of the present study was to investigate the effects of a 2-month treatment with a moderate dose of fish oil on glycemic control, insulin sensitivity, and hepatic glucose production in hypertriacylglycerolemic type 2 diabetic men under careful dietary supervision. In addition, we also assessed the effects of fish oil treatment

From the Department of Diabetes (J.L., S.W.R., C.C., A.-S.C., A.C., G.S.), INSERM U341; the Departments of Biochemistry (A.B.) and Nutrition (J.-M.O.), Hôtel-Dieu, Paris; INSERM U449 (H.V.), Lyon; INSERM U177 (M.G.-M.), Paris; and Institut National de la Recherche Agronomique (INRA) (G.D.), Jouy-en-Josas, France.

Address correspondence and reprint requests to G. Slama, Department of Diabetes, Hôtel-Dieu, 1, place du Parvis Notre-Dame, 75181 Paris cedex 04, France.

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Abbreviations: HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; Lp(a), lipoprotein(a); PCR, polymerase chain reaction; RT, reverse transcription.

Table 1—*Clinical characteristics of the study subjects at the time of screening*

n	10
Age (years)	54 ± 3
Duration of diabetes (years)	6 ± 1
Body weight (kg)	84 ± 4
BMI (kg/m²)	28 ± 1
Fasting glycemia (mmol/l)	11.61 ± 0.08
Postprandial glycemia (mmol/l)	16.11 ± 0.13
HbA_{1c} (%) (normal values ≤5.6%)	8.4 ± 0.5
Plasma triaglycerols (mmol/l)	2.66 ± 0.24
Plasma cholesterol (mmol/l)	6.03 ± 0.46
Dietary fish intake (g/week)	441 ± 71
Alcohol intake (g/day)	8.7 ± 4.7
Sedentary/physically active*	7/3
Smokers/nonsmokers	2†/8

Data are means ± SEM or *n*. *The physical activities concerned were walking, bicycle, running, and tennis; †2 and 30 cigarettes/day, respectively.

on the expression of genes involved in glucose metabolism (GLUT4) and lipid storage (hormone-sensitive lipase [HSL] and lipoprotein lipase [LPL]) in abdominal subcutaneous adipose tissue.

RESEARCH DESIGN AND METHODS

Patients

A total of 12 type 2 diabetic men were recruited from patients attending the outpatient clinic of the Department of Diabetes. Patients were selected on the basis of having a fasting plasma glucose of 7.84–14.0 mmol/l, HbA_{1c} <10.5%, and plasma triacylglycerol of 1.72–4.6 mmol/l. This sample size was determined by fixing the probability of type 1 error at 0.05 and that of type 2 error at 0.10. Plasma triacylglycerol concentration was the parameter chosen for the calculation of sample size, with the expected difference between the variations during the two treatments being 40% and the expected SD being 0.80 mmol/l. The sample size of 12 was more than one-half the number obtained by calculation because of the crossover design. One patient was excluded 10 days after the beginning of the study because of a misunderstanding of the experimental design. Among the 11 other patients who completed the study, one subject stopped his oral antidiabetic therapy by error at the beginning of the study, which rendered his results inconsistent. This patient was excluded. The clinical and biological characteristics of these subjects are given in Table 1. Patients with abnormal renal, hepatic, and thyroid functions as determined by physical examination, blood cell count, and standard blood biochemical profile were excluded. Similarly, patients suffering from gastrointestinal disorders were not allowed to enter the study. One patient was under lipid-lowering treatment. He was asked to stop the treatment 2 months before the beginning of the study. Eight patients were taking oral antidiabetic agents (sulfonylurea and/or metformin) and two were receiving antidiabetic dietary regimen alone. None of the patients were or had been treated with insulin. Two patients were treated with beta-blockers, ACE inhibitors, and/or calcium antagonists for hypertension. All therapies except lipid-lowering treatment were continued unchanged throughout the study. The purpose, nature, and potential risks of the study were explained, and a written informed consent was obtained from each patient. The experimental protocol was approved by the ethics committee of Hôtel-Dieu Hospital.

The patients were randomly allocated to two periods of 2 months of daily 6 g of either fish oil (i.e., 1.8 g n-3 polyunsaturated fatty acids/day) or sunflower oil treatment with a double-blind random crossover design. The two treatment periods were separated by a 2-month washout interval. The fish oil and sunflower oil doses were supplied as two capsules three times a day (1 g identical gelatin capsules, each capsule contained 1 g oil, Pierre Fabre Médicament, Castre, France). The fish oil capsules contained 30% n-3 polyunsaturated fatty acids (18% eicosapentaenoic acid and 12% docosahexaenoic acid), 4% n-6 polyunsaturated fatty acids, 36% monounsaturated fatty acids, 30% saturated fatty acids, and 0.175% α-tocopheryl acetate. The sunflower oil capsules contained 65% n-6 polyunsaturated fatty acids, 0.2% n-3 polyunsaturated fatty acids, 24% monounsaturated fatty acids, 11% saturated fatty acids, and 0.056% α-tocopheryl acetate. The subjects were advised to take the oil capsules during meals with cold drinks and to avoid taking them with hot beverages, such as coffee. To assess compliance with the two treatments, fatty acid composition of plasma and erythrocyte membrane phospholipids was measured at the end of the two treatment periods.

Fasting blood samples were collected before and at the end of each treatment period for the determination of plasma glucose, HbA_{1c}, insulin, lipids, and lipoproteins. At the end of each treatment period, subjects were hospitalized from 0730 to 1700 after an overnight fast, to be submitted to an euglycemic-hyperinsulinemic clamp coupled with an isotopic measurement of glucose turnover. At 0730, before the clamp studies, a sample of abdominal subcutaneous adipose tissue was obtained by needle biopsy using a 14-gauge needle and a 30-ml syringe under local anesthesia with xylocaine 10% without adrenaline. The tissue obtained was rapidly frozen in liquid nitrogen and stored at −80°C.

Dietary follow-up

Before entering into the study, the subjects had been seen on a regular basis (at least every 6 months) at our department. All were well educated, especially concerning the type and quantity of foods they should consume. Each subject entered a run-in period of 2 months. Patients received individual counseling by a dietitian concerning dietary food intake. In addition, counseling sessions (in small groups) were conducted in the run-in period. During this period, we asked them to follow their usual diet more strictly. Patients were recommended to consume 55% of their caloric intake as carbohydrates, 15% as protein, and 30% as lipids. Dietary intake was prescribed individually according to data obtained from dietary questionnaires in order to maintain the initial caloric intake and nutrient proportions constant throughout the study. This was accomplished by providing a list to each individual of the recommended daily intake of commonly used foods and a substitution list to allow exchange within food groups. The subjects were not instructed to modify their fat intake in the diet to compensate the increased fat intake through fish oil and sunflower oil capsules. Before the beginning of each treatment period, dietary questionnaires were obtained again (baseline data, recall technique) in order to maintain the initial caloric intake and nutrient proportions constant throughout the study. To assess compliance with the dietary recommendations, patients were asked to keep a food diary to be completed the last 7 days of each treatment period. Household measuring cups or spoons and food pictures were used to quantify portion sizes of foods eaten. When each subject returned his records at the end of each 7-day period, the dietitian verified the contents of the records and clarified with the subject any ambiguous information. These records were analyzed using the computer program Profile Dossier V3 software (Audit

Conseil en Informatique Médicale, Bourges, France), whose dietary database is made up of 400 foods or groups of foods representative of the French diet. French food contents were obtained from Ciqual Repertory (16). There was ~10% of missing values. If a food described by a subject was not in the database, the ingredients of recipes or manufacturers' information were used to code according to Ciqual Repertory (16).

Nondietary follow-up

Patients were asked to keep a constant lifestyle throughout the study. Physical activity was assessed by recall questionnaires. The kind of activity and its frequency, as well as the mode and duration of transportation to and from work were questioned. Patients' physical activity remained constant during the study.

Euglycemic-hyperinsulinemic clamp studies

The studies of glucose dynamics were done during a short hospitalization at the end of each treatment period and consisted of a first step of 180 min of [6,6-$^2H_2$]glucose infusion followed by two steps of insulin infusion: a low dose insulin of 1 mU · kg^{-1} · min^{-1} (180 min) and a high dose of 6 mU · kg^{-1} · min^{-1} (120 min).

The euglycemic-hyperinsulinemic clamp study was performed as described previously (17). One catheter was placed in an antecubital vein for infusions of glucose, [6,6-$^2H_2$]glucose, and insulin. Another catheter was placed retrogradely into a contralateral wrist vein for blood sampling. Venous blood was arterialized by placing the hand in a heated box (70°C).

During insulin infusion (1 then 6 mU · kg^{-1} · min^{-1}), plasma glucose was allowed to decline to 5.5 mmol/l and was then clamped by adjusting the infusion rate of a 20% glucose solution according to plasma glucose concentration measured every 5 min. During the last 30 min of each step when a steady state was obtained, blood samples were collected at 10-min intervals to measure insulin concentrations.

Glucose turnover and hepatic glucose production

Isotopic measurement of glucose turnover was performed at the basal state and during the low-dose (1 mU · kg^{-1} · min^{-1}) insulin clamp. A primed continuous infusion of [6,6-$^2H_2$]glucose was given 180 min before the insulin infusion. The priming dose of [6,6-$^2H_2$]glucose was determined according to basal individual plasma glucose concentrations. The infusion rate of [6,6-$^2H_2$]glucose was maintained at 3 mg · kg^{-1} · h^{-1} during the basal state and elevated to 5 mg · kg^{-1} · h^{-1} during the low-dose (1 mU · kg^{-1} · min^{-1}) insulin clamp. To determine the [6,6-$^2H_2$]glucose enrichment, blood samples were withdrawn at the beginning of the isotope infusion and at 10-min intervals during the last 30 min of each step.

The calculation of glucose turnover was made on the basis of the assumption that the plasma glucose steady state was achieved: $R_a = i/E_p$, where i is the tracer infusion rate and E_p is the [6,6-$^2H_2$]glucose isotopic enrichment in the plasma.

In the basal state, the turnover of glucose equals the hepatic glucose production. During exogenous glucose infusion at a constant rate, R_a is equal to the sum of the endogenous production and the exogenous glucose infusion. Therefore, the residual hepatic glucose production rate is calculated by subtracting the known rate of exogenous glucose infusion from the calculated R_a. During the infusion of the high insulin dose of 6 mU insulin · kg^{-1} · min^{-1}, hepatic glucose production was supposed to be zero (18), so that the exogenous glucose infusion provides a measurement of the total body glucose disposal.

Gene expression of GLUT4, HSL, and LPL by reverse transcription-competitive polymerase chain reaction

Adipose tissue samples were pulverized in liquid nitrogen, and total RNA was prepared from the frozen powder by centrifugation over a cesium chloride cushion. Specific mRNAs for GLUT4, HSL, and LPL were quantified by reverse transcription followed by competitive polymerase chain reaction (RT-PCR) as described by Laville et al. (19). Briefly, for each mRNA, a specific first-strand cDNA synthesis was performed using thermostable reverse transcriptase and the specific antisense primer in 10 mmol/l Tris-HCl, pH 8.3, 90 mmol/l KCl, 1 mmol/l $MnCl_2$, and 0.2 mmol/l deoxynucleoside triphosphates. The reaction lasted 3 min at 60°C and 15 min at 70°C and was then stopped by heating at 99°C for 5 min. The product of the RT reaction was then subjected to PCR by using *Taq* polymerase and corresponding sense and antisense primers in 10 mmol/l Tris-HCl, pH 8.3, 100 mmol/l KCl, 0.75 mmol/l EGTA, 5% glycerol, and 0.2 mmol/l deoxynucleoside triphosphates. The reaction contained also defined concentrations of a multispecific competitor. The multispecific competitor was a 525-bp-long synthetic gene, the sequence of which corresponded to the juxtaposition of sense primer sequences for GLUT4, HSL, and LPL, followed by the juxtaposition of the complementary sequences of antisense primers in the same order. After 120 s at 94°C, the PCR mixtures were subjected to 40 cycles of PCR amplification with a cycle profile including denaturation for 40 s at 95°C, hybridation for 50 s at 55°C, and elongation for 50 s at 72°C. The amplification products of each PCR were separated in a 3% agarose gel stained with ethidium bromide and photographed. The band densities were evaluated from the negative film with a Vernon photometer-integrator. The logarithm of the density ratio of the competitor band to the target mRNA band was plotted versus the logarithm of the initial amount of competitor cDNA. The results were then normalized and presented by reference to the mRNA

Table 2—*Body weights and dietary intake at the end of 2 months of sunflower oil and fish oil treatments*

	Sunflower oil	Fish oil
Body weight (kg)	82 ± 4	82 ± 4
Energy (kcal/day)	2,228 ± 133	2,165 ± 188
Carbohydrates (g/day) (% of energy)	188 ± 12 (36 ± 1)	188 ± 19 (36 ± 2)
Protein (g/day) (% of energy)	98 ± 9 (18 ± 1)	92 ± 6 (18 ± 2)
Fish (g/week)	439 ± 80	383 ± 62
Fat (g/day) (% of energy)	100 ± 5 (44 ± 1)	99 ± 9 (44 ± 1)
Saturated fatty acids (% of fat intake)	45 ± 1	43 ± 1
Monounsaturated fatty acids (% of fat intake)	37 ± 2	37 ± 2
Polyunsaturated fatty acids (% of fat intake)	17 ± 2	20 ± 2
Fibers (g/day)	15 ± 2	15 ± 1
Cholesterol (mg/day)	388 ± 43	368 ± 28

Data are means ± SEM (n = 10). The diet composition does not include the contents of oil capsules.

Table 3—*Concentrations of n-3 fatty acids in phospholipids of plasma and of erythrocyte membranes at the end of 2 months of sunflower oil and fish oil treatments*

	Plasma phospholipids		Erythrocyte membranes	
	Sunflower oil	Fish oil	Sunflower oil	Fish oil
20:5 n-3 (%)	0.93 ± 0.11	2.49 ± 0.31†	1.03 ± 0.10	2.47 ± 0.26*
22:6 n-3 (%)	4.30 ± 0.28	6.02 ± 0.47†	5.29 ± 0.41	6.62 ± 0.50‡
n-6/n-3	5.59 ± 0.44	3.18 ± 0.28*	4.17 ± 0.31	2.73 ± 0.25†

Data are means ± SEM (n = 10). *$P < 0.0001$; †$P < 0.001$; ‡$P < 0.02$ vs. sunflower oil.

level of the constitutively expressed β2-μglobulin gene. This presentation had the advantage of erasing differences in total RNA quantities measured between samples.

Biological assays

Plasma glucose was measured by the glucose oxidase method. Plasma insulin was determined by a radioimmunoassay. The antiserum used in the test showed a cross-reactivity of 100% with human insulin and of 40% with proinsulin. VLDLs, LDLs, and HDLs were separated by sequential ultracentrifugation to measure their contents in triacylglycerols, total, free, and esterified cholesterol and phospholipids. Serum lipoproteins AI, AI:AII, and lipoprotein(a) [Lp(a)] particles and apolipoproteins AI and B were determined by an immunochemical assay. For the measurement of LP AI and LP AI:AII particles, the anti-apo AII antibody, incorporated in sufficient quantity, permits to block the family of LP AI:AII particles, when the LP AI particles go on to migrate and react with anti-apo AI, to give formation to an immunoprecipitation rocket with a height proportional to LP AI concentration. A single sample gives two rockets: the smallest and more stained one corresponds to apo AII containing particles. The highest and less stained corresponds to particles containing only the apo AI. Plasma [6,6-$^2H_2$]glucose isotopic enrichment was determined by capillary gas chromatography coupled with electron-ionization mass spectrometry. Quadruple samples were taken for the measurements of plasma glucose and insulin and for the determination of plasma [6,6-$^2H_2$]glucose isotopic enrichment during the clamp study. For the other measurements, single samples were used.

Statistical methods

The effects of fish oil and sunflower oil treatments were compared by a multiple analysis of variance followed by a post hoc test (LSD test). The main factors considered in the analysis were the following: treatment (with two levels: fish oil and sunflower oil), time (with two levels: baseline and 2-month treatment), and order (with two levels). Results of euglycemic clamp studies, hepatic glucose production, and the incorporation of n-3 in the plasma and the red blood cells' membranes were compared only at the end of the two treatment periods. Variables not normally distributed, such as plasma glucose, insulin and lipids, were subjected to a logarithmic transformation prior to statistical comparisons. All statistical analyses were performed using CSS statistical package (StatSoft, Tulsa, OK). Results are expressed as means ± SEM. A P value of <0.05 was considered significant.

RESULTS — Patients followed the two treatment periods without any difficulty. Fish oil and sunflower oil capsules were well tolerated, without any complaint or side effects. According to self-report, subjects' lifestyle was unchanged throughout the entire study.

There was no effect of the crossover design (starting with fish oil or sunflower oil) nor treatment-by-period interaction for any of the parameters studied.

Diets and body weight

Results of the 7-day dietary records are shown in Table 2. At the end of the two treatment periods, daily intakes of total energy, macronutrients, saturated and unsaturated fatty acids, and cholesterol were unchanged. Weekly fish intake was also unchanged. Concomitantly, body weight was comparable after fish oil and sunflower oil treatment (Table 2).

Compliance

The n-3 fatty acid composition of plasma and erythrocyte membrane phospholipids at the end of the two treatment periods are shown in Table 3. The contents of n-3 polyunsaturated fatty acids, both eicosapentaenoic and docosahexaenoic acids, were significantly higher after the fish oil period than after the sunflower oil period in both plasma and erythrocyte membrane phospholipids, reflecting good compliance with fish oil treatment.

Plasma glucose, insulin, lipids, and lipoproteins

Variations in fasting plasma glucose and HbA_{1c} in fish oil and sunflower oil treatment periods were similar (Table 4). Similarly, variations in fasting plasma insulin concentrations were comparable in the two treatment periods (Table 4).

Circulating lipid and lipoprotein concentrations after the two treatment periods are shown in Table 5. The decrease in plasma triacylglycerol concentrations was more important during fish oil treatment than during sunflower oil treatment ($P < 0.05$). Plasma Lp(a) concentrations were significantly lowered by fish oil treatment ($P < 0.02$) but not by sunflower oil treatment. The serum LDL and HDL cholesterols, free fatty acids, lipoproteins AI and AI:AII particles, apolipoproteins A and B were not significantly affected by either treatment.

Basal and insulin-mediated glucose metabolism (Table 6)

Basal plasma glucose and insulin concentrations were similar after 2 months of fish oil and sunflower oil treatments. Basal hepatic glucose production was comparable after fish oil and after sunflower oil treatments. During the two insulin infusion steps, plasma glucose was maintained at stable

Table 4—*Fasting plasma glucose, insulin, and HbA_{1c} concentrations at baseline and after 2 months of sunflower oil and fish oil treatments*

	Sunflower oil		Fish oil	
	Baseline	2 months	Baseline	2 months
Glucose (mmol/l)	11.50 ± 0.90	11.23 ± 1.20	10.86 ± 1.00	11.08 ± 1.00
Insulin (pmol/l)	91 ± 12	76 ± 10	84 ± 6	83 ± 7
HbA_{1c} (%)	8.6 ± 0.5	8.9 ± 0.6	8.8 ± 0.6	8.7 ± 0.5

Data are means ± SEM (n = 10).

Table 5—*Fasting circulating lipid and lipoprotein concentrations at baseline and after 2 months of sunflower oil and fish oil treatments*

	Sunflower oil		Fish oil	
	Baseline	2 months	Baseline	2 months
Triacylglycerols (mmol/l)	2.69 ± 0.33	2.21 ± 0.35	2.36 ± 0.34	1.73 ± 0.27*
Total cholesterol (mmol/l)	6.16 ± 0.46	5.72 ± 0.40	5.72 ± 0.31	5.69 ± 0.33
LDL cholesterol (mmol/l)	2.30 ± 0.34	2.49 ± 0.23	2.22 ± 0.20	2.59 ± 0.21
HDL cholesterol (mmol/l)	1.15 ± 0.12	1.03 ± 0.05	1.08 ± 0.04	1.10 ± 0.05
HDL_2 cholesterol (mmol/l)	0.25 ± 0.04	0.22 ± 0.02	0.26 ± 0.03	0.25 ± 0.02
HDL_3 cholesterol (mmol/l)	0.57 ± 0.07	0.64 ± 0.05	0.58 ± 0.06	0.65 ± 0.03
Phospholipids (mmol/l)	2.49 ± 0.11	2.26 ± 0.13	2.39 ± 0.14	2.29 ± 0.14
Lipoprotein(a) (g/l)	0.16 ± 0.04	0.16 ± 0.03	0.17 ± 0.04	0.14 ± 0.03†
Lipoprotein AI (g/l)	0.52 ± 0.03	0.47 ± 0.02	0.49 ± 0.03	0.49 ± 0.04
Lipoprotein AI:AII (g/l)	0.40 ± 0.02	0.38 ± 0.02	0.39 ± 0.02	0.38 ± 0.02
Apolipoprotein A1 (g/l)	1.54 ± 0.08	1.48 ± 0.08	1.48 ± 0.05	1.43 ± 0.07
Apolipoprotein B (g/l)	1.55 ± 0.16	1.50 ± 0.11	1.38 ± 0.08	1.43 ± 0.09
Free fatty acids (mmol/l)	—	0.86 ± 0.05	—	0.97 ± 0.14

Data are means ± SEM (n = 10).*$P < 0.05$; †$P < 0.02$ fish oil treatment vs. sunflower oil treatment, multiple analysis of variance followed by a post hoc LSD test.

euglycemic levels. Plasma insulin was raised and maintained at steady levels during the last 30 min of each hyperinsulinemic step, as expected. Neither plasma glucose nor plasma insulin differed significantly during the clamp after fish oil and that after sunflower oil treatments. The insulin suppression of hepatic glucose production was not significantly different at the end of the two treatment periods. Exogenous glucose infusion rate was not different after fish oil and sunflower oil treatments at any degree of hyperinsulinemia. The glucose disposal increased stepwise during the two insulin infusion steps. However, it was not significantly different after the two treatments, at any plasma insulin levels.

Gene expression of GLUT4, HSL, and LPL

Because of technical problems, abdominal subcutaneous adipose tissue mRNA (extracted from 0.2–0.8 g tissue) was successfully obtained after both fish oil and sunflower oil periods in five subjects only. Individual results of gene expression of GLUT4, HSL, and LPL are depicted in Fig. 1. GLUT4 mRNA levels were low in these NIDDM patients and did not seem to differ after the two treatments. LPL mRNA levels were higher after the fish oil period than after the sunflower oil period in all five subjects studied. Four of the five subjects had higher HSL mRNA levels after fish oil than after sunflower oil treatment, and the fifth one had the same levels after the two periods.

CONCLUSIONS — In the present study, 2 months of 6 g fish oil daily (1.8 g n-3 polyunsaturated fatty acids) supplementation to type 2 diabetic men induced a decrease in plasma triacylglycerol and did not deteriorate either fasting glycemia, HbA_{1c}, or basal hepatic glucose production. Daily energy and macronutrient intake as well as body weight remained stable during the 2-month fish oil and sunflower oil periods. Body weight was also maintained identical during the two periods. Thus, dietary or body weight changes could not be implicated in the observed results. The average daily fish intake of the subjects in this study was 60 g, which was consistent with the usual lifestyle of diabetic patients in our department. Epidemiological studies have shown that eating at least 30 g of fish/day results in a substantially reduced risk of coronary heart disease mortality in normoglycemic subjects. In a glucose-intolerant population, the possible protective effect of fish oil may be smaller than in normal subjects (20). The results of the present study, however, could not be attributed to the high habitual fish intake. This fish intake brought about 0.32 g n-3 fatty acids, whereas the capsules of fish oil contained 1.8 g n-3 fatty acids/day (6 g fish oil), which was sixfold higher. In addition, our patients were diabetic and not normoglycemic nor glucose intolerant as in most of the epidemiological studies. The fish oil dose used considerably changed n-3 fatty acid content in plasma and in erythrocyte membrane phospholipids compared with values of the same patients after the sunflower oil treatment. Another factor to be considered is the α-tocopheryl acetate contained in the fish oil and sunflower oil capsules. Tocopherol is known to be essential for the optimal functioning of cell membranes and can have significant effects on glucose homeostasis (21) and blood lipids (22). However, these studies used doses, between 100 and 500 mg of α-tocopheryl acetate daily, that are largely higher than the quantities contained in the daily fish oil (10.5 mg) and sunflower oil (3.9 mg) intake in the present study. Thus, neither habitual fish intake nor α-tocopheryl acetate content of the capsules could be implicated in the observed results after the fish oil treatment.

Table 6—*Plasma glucose and insulin concentrations, total body glucose disposal, exogenous glucose infusion, and hepatic glucose production at the end of 2 months of sunflower oil and fish oil treatments*

	Sunflower oil			Fish oil		
Insulin infusion rate (mU · kg^{-1} · min^{-1})	0	1	6	0	1	6
Glucose (mmol/l)	11.0 ± 1.10	5.86 ± 0.48	5.08 ± 0.15	10.84 ± 1.10	5.66 ± 0.53	5.59 ± 0.22
Insulin (pmol/l)	81 ± 5	370 ± 33	3,186 ± 284	75 ± 5	405 ± 46	2972 ± 370
Total body glucose disposal (mg · kg^{-1} · min^{-1})	2.20 ± 0.09	3.78 ± 0.53	8.75 ± 0.81	2.03 ± 0.11	3.28 ± 0.23	8.08 ± 0.88
Exogenous glucose infusion rate (mg · kg^{-1} · min^{-1})	0	2.69 ± 0.70	8.75 ± 0.85	0	2.66 ± 0.42	8.00 ± 0.93
Hepatic glucose production (mg · kg^{-1} · min^{-1})	2.20 ± 0.09	1.09 ± 0.33	—	2.03 ± 0.11	0.62 ± 0.33	—

Data are means ± SEM (n = 10).

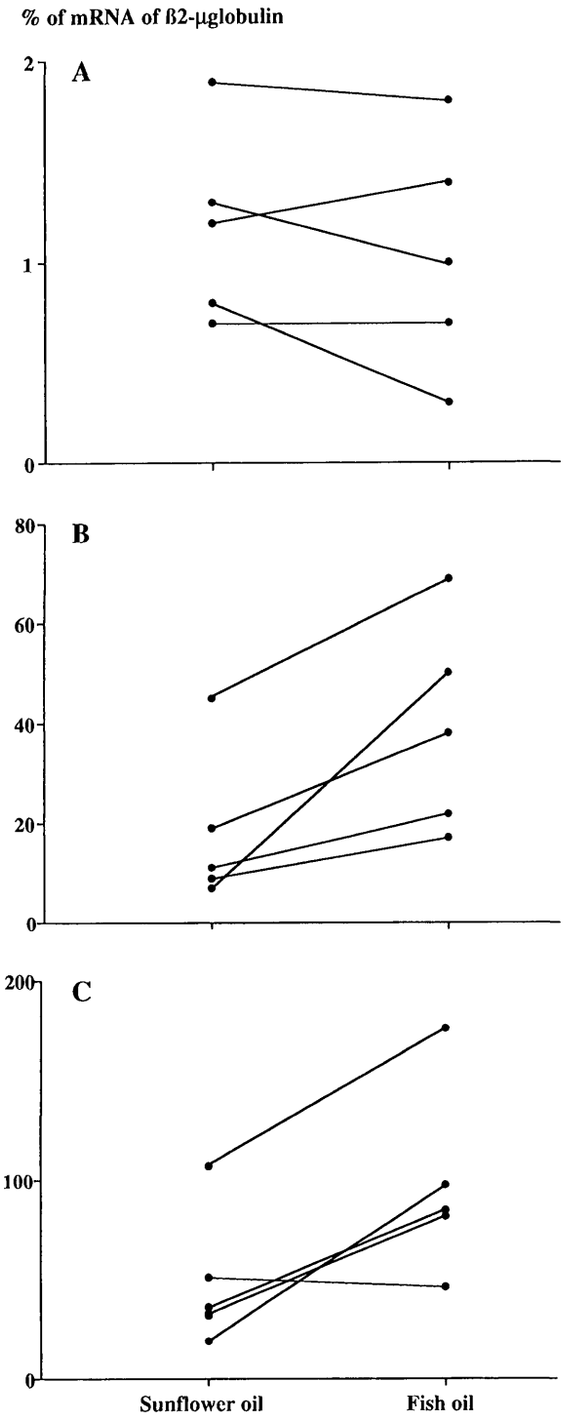

Figure 1—*Effects of 2 months of fish oil and placebo treatments on GLUT4 (A), LPL (B), and HSL mRNA levels (C) (expressed as % of mRNA of β2-µglobulin) in abdominal subcutaneous adipose tissue of five patients.*

In diabetes, fish oil supplementation was found to have potential deleterious effects that prevent its recommendation. Fasting plasma glucose and HbA_{1c} were found to be deteriorated when using doses of n-3 fatty acids above 3 g/day (1–5). In these studies, subjects were withdrawn from their habitual hypoglycemic therapy before giving fish oil treatment (3) (there was no placebo-controlled group) or dietary follow-up was missed. It was, therefore, difficult to conclude whether the increase in fasting glycemia was due to fish oil intake, changes in dietary regimen, or absence of habitual therapy. Although a considerable number of placebo-controlled studies have been undertaken to evaluate the effects of fish oil on glycemic control (6–11), the results were contradictory and the problem is still unsettled. In these studies, investigators used at least twice the dose of 1.8 g n-3 fatty acids/day (6 g fish oil) used in the present study, provided by fish oil preparations (6–10) or by high dietary fish intake in a low-fat regimen (11). Even with these high doses of fish oil, Puhakainen et al. (9) and Annuzzi et al. (10) did not find any change in glycemic control. These results, however, were observed after short-term treatment and in the absence of a washout period. It was questionable if these results are also true after longer periods and with a sufficient washout period between fish oil and placebo treatments. As far as we know, the present study is the first to demonstrate that by using a moderate dose of fish oil for 2 months in a crossover design and after a sufficient washout period, accompanied by controlled dietary regimen, no deterioration of fasting glycemia or HbA_{1c} could be found in type 2 diabetic men. In a double-blind, placebo-controlled design with a parallel group sequence, Rivellese et al. (15) did not find any deterioration in blood glucose control after 6 months of a moderate dose of fish oil in type 2 diabetic patients. The same results with a small dose of fish oil were found in a total of 935 hyperlipidemic or glucose-intolerant patients of both sexes in 63 Italian clinical centers (23).

The present study demonstrated unchanged hepatic glucose production after a long-term fish oil treatment. Similarly, Borkman et al. (7) did not find any change in hepatic glucose production using a double-blind crossover design. The duration of the latter study, however, was shorter (3 weeks) than ours (2 months). Puhakainen et al. (9) in another controlled study for 6 weeks found an increase in glycerol gluconeogenesis that was not immediately translated by an increase in hepatic glucose production despite the large dose of n-3 fatty acids used (12 g/day). On the other hand, Glauber et al. (3) found an elevated hepatic glucose production after 1 month of a dose of 5.5 g/day of n-3 fatty acids in subjects withdrawn from their habitual hypoglycemic therapy. Increasing the dose of fish oil treatment and/or withdrawal of hypoglycemic therapy might lead to the increased hepatic glucose production found in some studies.

Insulin-stimulated glucose disposal assessed by the euglycemic clamp was not modified by 2 months of fish oil supplementation, compared with sunflower oil. In a randomized study with a controlled crossover design, Borkman et al. (7), in spite of the absence of a washout interval between the two 3-week experimental periods, did not find any change in insulin sensitivity in diabetic patients. Similarly, Annuzzi et al. (10), even with a high dose of 10 g/day of fish oil for 2 weeks in a controlled double-blind random study, did not find any change in glycemic control, insulin secretion, or insulin sensitivity. The same group by using a moderate dose for 6 months also did not find any change in insulin sensitivity (15). However, in the double- or single-blind placebo-controlled studies of Schectman et al. (6) and Hendra et al. (8), insulin sensitivity was not assessed.

The results of the present study suggest also that a moderate dose of n-3 polyunsaturated fatty acids to type 2 diabetic men had no effect on adipose tissue GLUT4 gene expression, which was already low in this type of patient. In NIDDM, impairment of insulin action at the cellular level (muscle and adipose tissue) was found to be, in part, due to decreased glucose transport activity (24–27), which is in turn associated with pretranslational suppression of the expression of the adipocyte GLUT4 transporter isoform, expressed exclusively in insulin-sensitive tissues (27). In insulin-resistant rats, a recent study from our laboratory showed that fish oil–induced amelioration of insulin sensitivity in adipocytes was associated with an increase in GLUT4 gene expression and protein content in adipocytes but not in muscle (28). The discrepancy between the increase in GLUT4 in adipocytes by fish oil in insulin-resistant rats but not in type 2 diabetic patients could be due to the difference in the duration of insulin resistance. In rats, it is represented by some weeks, whereas in humans it is some years. Furthermore, recently, Kozka et al. (29) showed that the large human adipocytes are much less responsive to variations in glucose transport activity than are rat adipocytes.

In the literature, possible deleterious effects of fish oil on plasma lipid profile were found in type 2 diabetes. A dose of 7.5 g fish oil/day led to increased total and LDL cholesterol (6,30). In our study, there was an increase of cholesterol in both LDL and HDL fractions to the same extent so that the LDL/HDL ratio was comparable after the two treatment periods.

The dose of 1.8 g n-3 fatty acids/day was sufficient to lower plasma triacylglycerol concentrations, which was consistent with previous findings in type 2 diabetic patients (2,3,6–9,15). Rivellese et al. (15) found that after a 6-month fish oil treatment, this is attributed in particular to a decrease in VLDL fraction. This decrease in triacylglycerol may be due to reduction of hepatic triacylglycerol production (31). Patients with type 2 diabetes are subjected to increased risk of hypertriacylglycerolemia for two reasons. First, hyperinsulinemia may favor hepatic triacylglycerol production. Second, the increase in plasma free fatty acids usually present in type 2 diabetes may provide more substrate for triacylglycerol production (32). However, no modification in either insulin or free fatty acid levels was found after fish oil treatment compared with that after sunflower oil treatment in our study. However, it was shown (15) that in this type of patient, 6 months of fish oil treatment is needed to decrease free fatty acids. Other mechanisms could be implicated in the decreased plasma triacylglycerol concentrations by fish oil. It has been demonstrated in rats that the fish oil–induced low rates of hepatic lipogenesis is associated with high rates of fatty acid oxidation (31,33). Moreover, it has been demonstrated that VLDL particles produced under n-3 fatty acid treatment are small (34) and more efficiently degraded than those found after treatment with other fatty acids (35).

Another important aspect of the metabolic role of fish oil is its effect on lipid accumulation. Previous studies in our laboratory (14) and in others (36) showed that in rats, a diet rich in (n-3) polyunsaturated fatty acids, compared with a control diet rich in saturated or n-6 polyunsaturated fatty acids, produced less fat without changing body weight. Therefore, we decided to study lipid storage–related enzymes such as the LPL, which is the key enzyme for plasma triacylglycerol removal and the HSL, which is the key enzyme for adipose tissue lipolysis. Our results suggest an increase in mRNA levels of LPL in the adipose tissue after the fish oil treatment period, which is consistent with the increase in LPL activity reported previously (4,37,38). The increase in LPL gene expression might contribute to the fish oil–induced decrease in plasma triacylglycerol concentrations. However, the hypothesis could not be excluded that the increase in LPL gene expression might simply be the consequence of low plasma triacylglycerol concentrations. Concerning the fish oil–induced increase in mRNA levels of HSL, there is no relevant information in the literature. This enzyme catalyzes the first and second steps in the breakdown of triglycerides, releasing fatty acids (3). Plasma free fatty acid concentrations have been found to be unchanged after fish oil treatment in the present study and in others (3,9,31). Therefore, the change of HSL gene expression had no detectable physiological consequences on tissue lipolysis in this study. However, we could not ignore that the expression of this enzyme in adipose tissue is regulated by insulin levels (39) and that decreased insulin levels negatively regulate the levels of HSL mRNA. In the present study, changes in fasting plasma insulin levels could not be detected. Indeed, the findings related to gene expression in this study were successfully obtained in only five of the ten subjects studied. Greatest care must be taken in the interpretation of these findings.

In conclusion, contrary to what has been believed for a couple of years, a moderate dose of n-3 polyunsaturated fatty acids did not deteriorate glycemic control or basal hepatic glucose production in type 2 diabetic men, with preserved triacylglycerol-lowering capacities.

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