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A controlled study on the effects of $n-3$ fatty acids on lipid and glucose metabolism in non-insulin-dependent diabetic patients

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Summary

Eight male non-insulin-dependent diabetic patients participated in a double-blind randomized cross-over study (2 weeks for each period) evaluating the effects of 10 g/day fish oil dietary supplementation on glucose and lipid metabolism. Fasting serum triglyceride concentrations were decreased by fish oil because of a reduction in VLDL (1.4 ± 0.2 vs. 1.9 ± 0.2 mmol/l, $P < 0.025$). LDL cholesterol concentration was instead increased (3.4 ± 0.3 vs. 2.8 ± 0.3 mmol/l, $P < 0.025$) and net changes in VLDL triglyceride and in LDL cholesterol were inversely correlated ($r = -0.86$, $P < 0.01$). Plasma free fatty acids concentrations and turnover rate ([^{3}H]palmitate method) were similar after fish oil and placebo. Fish oil supplement did not induce significant changes in fasting blood glucose (8.1 ± 1.1 vs. 8.5 ± 1.2 mmol/l) and average daily blood glucose (BG) (9.4 ± 3.2 vs. 9.3 ± 3.5 mmol/l). Glucose stimulated plasma insulin response during a hyperglycemic clamp was not significantly influenced by fish oil both in the early phase and during steady state. Insulin sensitivity (M/I index) was also unchanged. In conclusion, this study shows that a dietary supplement of fish oil decreases plasma triglyceride levels in non-insulin-dependent diabetic patients, an increased conversion rate of VLDL to LDL playing a role in this change. With this dosage of fish oil no relevant variations in glycemic control, insulin secretion and insulin sensitivity occurred.

Key words: Fish oil; $n-3$ fatty acids; Non-insulin-dependent diabetes mellitus; Hypertriglyceridemia; Glycemic control; Insulin secretion; Insulin sensitivity

Introduction

Fish consumption has been associated with a low incidence of cardiovascular disease [1–4]. This

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beneficial effect has been ascribed to the high $n-3$ fatty acid content of fish oil (eicosapentaenoic and docosahexaenoic acids) [5]. Diet supplement of fish oil has been shown to decrease plasma lipid levels [6–8], blood pressure [9,10] and platelet responsiveness [11,12]. Since all these risk factors for atherosclerosis are strongly operative in diabetic patients [13], $n-3$ fatty acids may be potentially useful for the management of these patients.

An improved glucose tolerance has been reported in hypertriglyceridemic patients after diet enrichment with $n-3$ fatty acids [10]. Furthermore, sardine oil is able to increase membrane fluidity in diabetic patients [14], with possible changes in the function of insulin receptors [15]. Moreover, eicosapentaenoic acid produces variations in prostaglandin I and leukotriene formation [16], which may affect insulin secretion [17].

While this study was undertaken the hypotriglyceridemic effect of fish oil has been reported also in type 2 (non-insulin-dependent) diabetic patients [18–20]. However, contrasting results concerning its effects on glucose metabolism have been shown. After dietary supplement of $n-3$ fatty acids in diabetic patients an increased metabolic clearance rate of glucose was observed [18], as well as an unchanged [19] or deteriorated glycemic control [19–21]. However, not all of these studies were conducted with a control group.

The purpose of the present study in type 2 (non-insulin-dependent) diabetic patients was to investigate in a double-blind crossover design the effects of a dietary supplement of fish oil on serum and lipoprotein lipid levels as well as on glycemic control. Particular emphasis was given to the evaluation of the effects of fish oil on insulin secretion, insulin sensitivity and turnover rate of free fatty acids (FFA).

Materials and methods

Subjects

Eight male patients with type 2 (non-insulin-dependent) diabetes mellitus [22] participated in a double-blind crossover study comparing the effects of a fish oil concentrate (Maxepa, Scherer, U.S.A.) versus placebo (olive oil) on lipid and glucose metabolism. Individual data regarding their clinical characteristics are given in Table 1. Patients were not suffering by liver, kidney or any other disease known to influence lipid and/or carbohydrate metabolism. Four of them were on glibenclamide, 3 on glibenclamide and metformin, and 1 patient was treated by diet only. Hypoglycemic therapy was continued throughout the study without dosage adjustments. Lipid-lowering treatment, if any, was stopped at least 4 weeks before the study. At the beginning of the study serum concentration of triglycerides was 2.5 ± 1.0 mmol/l and of cholesterol was 5.5 ± 1.4 mmol/l (mean $\pm$ SD). The study was approved by the Hospital Ethical Committee.

TABLE 1

INDIVIDUAL CLINICAL CHARACTERISTICS OF THE MALE NIDD PATIENTS PARTICIPATING IN THE STUDY

	Age (years)	Weight (kg)	BMI (kg/m^2)	Diabetes duration (years)	HbA_1 (%)
V.C.	57	63.1	23.6	14	9.3
L.P.	48	77.5	28.8	12	10.5
G.C.	55	68.5	23.2	2	7.4
D.S.	45	76.2	24.5	3	9.0
E.L.	47	63.8	20.8	10	8.8
A.M.	53	69.1	26.6	2	7.2
L.F.	46	82.0	31.2	9	8.0
V.C.	56	71.2	27.5	26	8.1
Mean $\pm$ SD	51 ± 5	71.4 ± 6.3	25.8 ± 3.2	9.8 ± 7.6	8.5 ± 1.0

Experimental design

Patients were admitted to the metabolic ward and given a weight maintaining diet which excluded fish (carbohydrates 53%, fat 31%, protein 16%, fiber 23 g, P/S ratio 1.2). Four days after hospitalization, subjects were randomly assigned to the diet supplements of 10 g per day of either fish oil or olive oil. Four patients started with fish oil and 4 with placebo. After 2 weeks they were crossed over to the alternate treatment which was continued for 2 weeks. The oil supplement was provided in 10 capsules per day (3 at breakfast, 4 at lunch, 3 at dinner). Ten capsules of Maxepa contained 1.8 g eicosapentaenoic acid and 1.2 g docosahexaenoic acid. No subjective reactions were reported during the treatment. On day 14 of

each supplementation period, blood samples for glucose, insulin and lipid analysis were drawn in the morning after fasting and before as well as 1, 2 and 3 h after a mixed standard meal was consumed at 1300 h (1000 kcal, carbohydrates 53%, fat 31%, protein 16%, fiber 14 g, P/S ratio 1.1). Plasma glucose concentration was also measured before and 2 h after dinner. On the following day, in the fasting condition, the measurement of free fatty acids (FFA) turnover rate and a hyperglycemic clamp followed by arginine stimulation were performed.

Procedures

Hyperglycemic clamp [23]: glucose was infused as an intravenous bolus (33 mg/kg body weight) and later at a variable rate in order to maintain the plasma glucose level around 11 mmol/l. This was achieved by measuring plasma glucose concentration of the arterialized venous blood at 5–10 min intervals by means of a Beckman Glucose Analyzer. The steady-state rate of glucose disposal (M value) was measured during the last 45 min of the clamp and corrected for the corresponding insulin concentrations (average of the measurements at 75, 90, 105 and 120 min of the clamp), yielding the M/I ratio. Because of technical problems, M/I values were calculated only from 7 patients.

120 min after the beginning of the clamp, 5 g arginine was i.v. infused in 30 sec and blood was sampled at 2, 4, 6, 10 and 15 min for glucose and insulin determination.

Basal FFA turnover rate [24]: [^{3}H]palmitate (Amersham International plc, England, 208 Ci/g), was used to trace plasma FFA turnover. In the fasting state, a priming dose of 30 μCi [^{3}H]palmitate was rapidly injected followed by a constant infusion of 1.3 μCi/min. After a 40-min equilibration period, 3 blood samples were taken at 5-min intervals for the determination of [^{3}H]FFA plasma specific activity. A steady state for FFA specific activity has been previously shown after a similar infusion time [25]. FFA turnover rate was calculated as the infusion rate divided by the specific activity and expressed as μmol FFA/min. No time trend was observed in this study in the samples obtained at 40, 45 and 50 min ($F = 0.02$ and $F = 0.33$ after Maxepa and placebo, respectively). Data on FFA turnover are available only for 6 patients.

Analytical methods

Plasma glucose concentration was measured by the glucose oxidase method using a Beckman Glucose Analyzer (Beckman Instrument Inc., U.S.A.). Serum insulin concentration was determined by radioimmunoassay [26]. Glycosylated hemoglobin was measured by affinity chromatography [27].

Lipoprotein separation was achieved by preparative ultracentrifugation [28], after addition to serum of EDTA and merthiolate to a final concentration of 0.05% and 0.01%, respectively. After ultracentrifugation at $d = 1.006$ kg/l for 40 h, dextran sulphate–manganese chloride was added to the infranatant, precipitating the apolipoprotein B containing low density lipoprotein (LDL) fraction. Cholesterol and triglyceride concentrations were measured by enzymatic methods [29,30] in the supernatant (very low density lipoproteins, VLDL) and in the infranatant (LDL + high density lipoproteins, HDL) before and after precipitation of the LDL fraction. Concentration in LDL fraction was then calculated (infranatant at $d =$ 1.006 kg/l before precipitation minus HDL).

Plasma FFA concentration was measured colorimetrically [31]. FFA radioactivity was measured after lipid extraction with chloroform/methanol and FFA were isolated from the lipid extract by thin-layer chromatography [32]. The coefficient of variation for the whole FFA extraction and isolation procedure was $3.3 \pm 0.5\%$.

Statistics

Data are expressed as mean $\pm$ SEM unless otherwise stated. Incremental areas, i.e. areas above the basal values, were calculated by a standard formula which utilized x and y coordinates of all vertices. Statistical analysis was performed according to standard methods [33]. Differences between the two treatments were evaluated by Wilcoxon's signed rank test. $P < 0.05$ is considered significant.

Results

Since the study design did not include a washout period between the two treatments, the in-

fluence of the treatment sequence was investigated to exclude a possible prolongation of the effects of fish oil. No differences in lipid and glucose responses were observed between the subjects who started with fish oil and those who started with the placebo (two way analysis of variance).

Body weight did not change throughout the study.

As shown in Table 2, fasting serum triglyceride concentration was lower after 2 weeks of fish oil supplement than after the placebo (1.94 ± 0.21 vs. 2.31 ± 0.21 mmol/l, $P < 0.05$). This difference was due to a lower triglyceride concentration in the VLDL fraction (1.41 ± 0.19 vs. 1.88 ± 0.21 mmol/l, $P < 0.025$), while no significant changes were present in the other fractions. Immediately before and at all time points after the test meal, serum triglyceride levels were lower during treatment with fish oil than with the placebo (Fig. 1). The incremental area for serum triglyceride was, however, not different after treatment with fish oil (2.21 ± 0.44 mmol/l per 3 h) or placebo (2.20 ± 0.32 mmol/l per 3 h).

Fasting concentrations of total cholesterol during fish oil or placebo treatment were similar in serum (4.76 ± 0.26 vs. 4.60 ± 0.26 mmol/l, n.s.) and HDL (0.59 ± 0.03 vs. 0.59 ± 0.03 mmol/l, n.s.) while changes in opposite directions were present in the VLDL and LDL fractions (Table 2). Fish oil, in fact, produced lower VLDL cholesterol concentrations than placebo (0.67 ± 0.08 vs. 0.88 ± 0.10 mmol/l, $P < 0.05$), but higher LDL concentrations (3.44 ± 0.34 vs. 2.84 ± 0.26 mmol/l, $P < 0.025$).

TABLE 2

FASTING SERUM AND LIPOPROTEIN LIPID CONCENTRATIONS AFTER 2 WEEKS DIETARY SUPPLEMENTATION WITH 10 g/DAY FISH OIL OR PLACEBO (OLIVE OIL) IN NIDD PATIENTS ($n = 8$)

Mean ± SEM. * $P < 0.05$, ** $P < 0.025$, versus "placebo".

	Placebo	Fish oil
Triglyceride (mmol/l)		
serum	2.31 ± 0.21	1.94 ± 0.21 *
VLDL	1.88 ± 0.21	1.41 ± 0.19 **
LDL	0.27 ± 0.03	0.29 ± 0.03
HDL	0.11 ± 0.01	0.10 ± 0.01
Cholesterol (mmol/l)		
serum	4.60 ± 0.26	4.76 ± 0.26
VLDL	0.88 ± 0.10	0.67 ± 0.08 *
LDL	2.84 ± 0.26	3.44 ± 0.34 **
HDL	0.59 ± 0.03	0.59 ± 0.03
Plasma FFA (μmol/l)	263 ± 32	274 ± 39

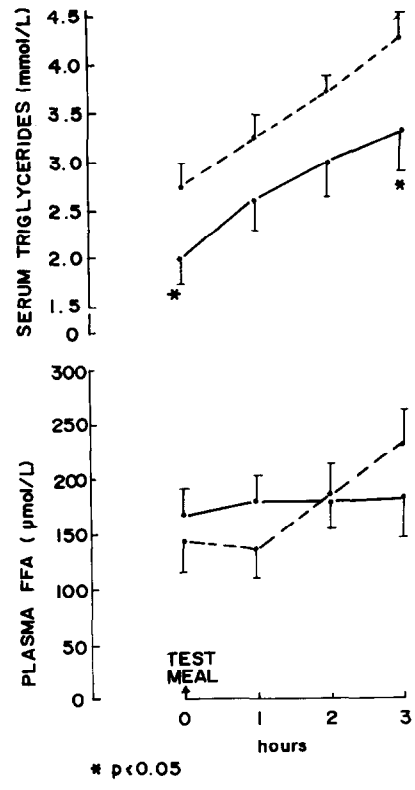

Fig. 1. Mean (± SEM) concentrations of serum triglyceride and plasma FFA during a standard mixed meal after 2-week dietary supplementation with 10 g/day fish oil (———) or placebo (olive oil) (— — —).

The net changes in VLDL triglyceride concentrations observed after the treatment with fish oil were inversely correlated with the net changes in LDL cholesterol concentrations ($r = -0.86$, $P < 0.01$) (Fig. 2).

Compared with placebo, fish oil supplement did not induce any change both in fasting and in postprandial plasma FFA concentrations (Table 2, Fig. 1). FFA turnover rate in the fasting state was similar during both treatments (248 ± 47 vs. 240 ± 33 μmol/min, n.s., fish oil and placebo, respectively) (Fig. 3).

Fasting plasma glucose concentration was not significantly affected by fish oil supplement (8.06 ± 1.11 mmol/l vs. placebo: 8.50 ± 1.22 mmol/l,

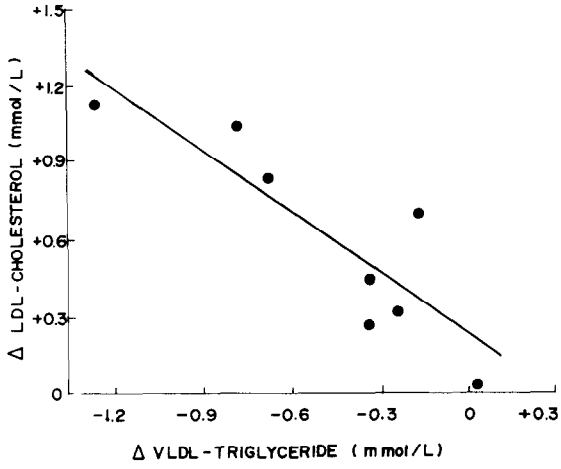

Fig. 2. Relationship between net changes of VLDL triglyceride and LDL cholesterol concentrations after fish oil dietary supplementation. Values represent concentrations after fish oil minus concentrations after placebo. The correlation coefficient (r) was -0.86 ($P < 0.01$).

n.s.). A tendency to a postprandial increase in plasma glucose concentrations was observed during the treatment with fish oil compared with the placebo (glucose incremental area = 7.3 ± 2.9 vs. 2.7 ± 1.9 mmol/l per 3 h, respectively, $P < 0.10$) (Fig. 4). However the average plasma glucose concentration during the day (7 measurements) was similar during fish oil and placebo supplements (9.44 ± 3.17 vs. 9.33 ± 3.50 mmol/l, respectively, n.s.).

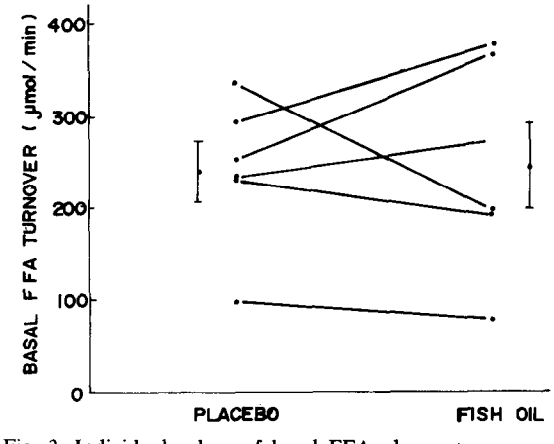

Fig. 3. Individual values of basal FFA plasma turnover rate measured by [^{3}H]palmitate infusion after 2-week dietary supplementation with 10 g/day fish oil or placebo (olive oil).

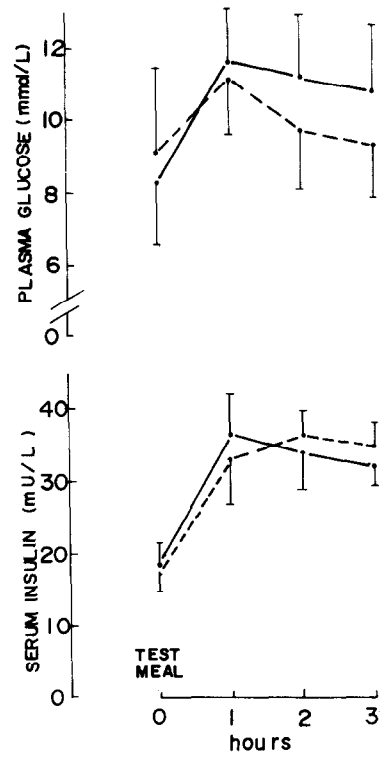

Fig. 4. Mean (±SEM) concentrations of plasma glucose and serum insulin during a standard mixed meal after 2-week dietary supplementation with 10 g/day fish oil (———) or placebo (olive oil) (— — —).

In the fasting state serum insulin levels were slightly lower during fish oil supplement (10 ± 1 mU/l) as compared with placebo (11 ± 1 mU/l, $P = 0.08$) (Table 3). Serum insulin profile after the standard meal was similar during administration of fish oil and placebo (Fig. 4); the insulin incremental area after the standard meal was 42 ± 8 and 44 ± 8 mU/l per 3 h, respectively, n.s. (Table 3).

The intravenous glucose load yielded a similar plasma glucose response during fish oil and placebo supplements (3-min peak = 19.3 ± 1.8 vs. 18.7 ± 1.2 mmol/l, respectively, n.s.). The first-phase insulin response to the intravenous glucose infusion was similarly low during both treatments (3-min insulin peak: 18 ± 4 vs. 21 ± 6 mU/l, n.s., and insulin incremental area: 35 ± 20 vs. 44 ± 27 mU/l per 7 min, n.s., respectively) (Table 3). Plasma glucose concentration during the last 45 min of the clamp was similar on fish oil (11.2 ± 0.4

TABLE 3

BASAL AND STIMULATED SERUM INSULIN LEVELS AFTER 2 WEEKS DIETARY SUPPLEMENTATION WITH 10 g/DAY FISH OIL OR PLACEBO (OLIVE OIL) IN NIDD PATIENTS ($n = 8$)

Mean ± SEM.

Serum insulin	Placebo	Fish oil
Fasting (mU/l)	11 ± 1	10 ± 1
Test meal		
0–3 h incremental area (mU/l per 3 h)	44 ± 8	42 ± 8
Hyperglycemic clamp		
3-min peak (mU/l)	21 ± 6	18 ± 4
0–7 min incremental area (mU/l per 7 min)	44 ± 27	35 ± 20
75–120 min average (mU/l)	18 ± 3	18 ± 4
Arginine test		
2-min peak (mU/l)	93 ± 17	87 ± 18
0–15 min incremental area (mU/l per 15 min)	431 ± 136	362 ± 85

mmol/l) and placebo (11.0 ± 0.4 mmol/l), with coefficients of variation of 4.6 ± 1.1 and 3.3 ± 0.7%, respectively. At this level of blood glucose there was no difference in the corresponding levels of serum insulin (17.7 ± 4.2 and 18.1 ± 3.4 mU/l, respectively) (Table 3).

The M/I ratio during the clamp was not different after supplements with fish oil (15.1 ± 2.7) or placebo (14.8 ± 1.5) (Fig. 5).

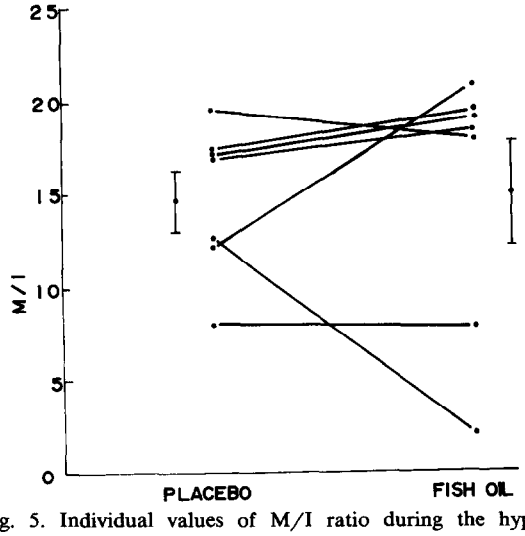

Fig. 5. Individual values of M/I ratio during the hyperglycemic clamps performed after 2-week dietary supplementation with 10 g/day fish oil or placebo (olive oil).

Arginine stimulation during the hyperglycemic clamp induced a rapid increase in serum insulin concentration to similar levels after both fish oil and placebo (2-min peak: 87 ± 18 and 93 ± 17 mU/l, respectively) (Table 3). The serum insulin incremental area after arginine infusion was not significantly different during administration of fish oil (362 ± 85 mU/l per 15 min) or of the placebo (431 ± 136 mU/l per 15 min).

Discussion

This study shows that under controlled clinical conditions dietary supplementation with $n-3$ fatty acids in non-insulin-dependent diabetic patients produces changes in the lipid and lipoprotein pattern which are similar to those observed in non diabetic individuals. In fact, after a dietary supplement of fish oil a decreased concentration of plasma triglyceride was found, due to a reduction of triglyceride in the VLDL fraction. In relation to cholesterol metabolism a significant decrease of cholesterol concentration in VLDL was observed after fish oil together with a significant increase in LDL. These changes question the usefulness of fish oil supplement in reducing the atherogenicity of the lipoprotein profile in type 2 (non-insulin-dependent) diabetic patients. Moreover, the hypothesis is supported that the protective effect of fish consumption on cardiovascular morbility and mortality [1–4] is more related to the effects on platelet function [11,12] than on the atherosclerotic plaque [34].

Inconsistent results on the effects of fish oil supplement on glucose metabolism in diabetic patients have been recently published [18–21]. More factors can explain this discordance. Most of these studies were not performed according to a controlled design and different methods were utilized to investigate glucose metabolism. Moreover, different fish oil doses were supplied, which often implied different cholesterol supplements. Our study did not show any effect of a daily supplement of 10 g fish oil on fasting blood glucose levels. In addition, glycemic control assessed as the average daily blood glucose concentration was not affected by the treatment with $n-3$ fatty acids. This is in agreement with the results shown by Schectman et al. [19] using a similar dosage of

fish oil; when instead, in the same study, the dose of omega-3 fatty acids was almost doubled, the fasting blood glucose concentration was significantly increased. A dose dependent effect should also be inferred from the finding of unchanged [21] and increased [20] fasting blood glucose levels after 9 g and 18 g of fish oil concentrate, respectively. In our study, the absence of any significant influence of $n-3$ fatty acids on blood glucose control in type 2 diabetic patients is in line with the lack of significant effects of fish oil on insulin secretion (insulinemia measured in the fasting state, after a standard meal, during a hyperglycemic clamp and after a maximal stimulus – arginine test during a hyperglycemic clamp) as well as on insulin sensitivity (M/I ratio during the hyperglycemic clamp).

In order to keep full control of drug compliance, food intake, physical activity and other possible confounding variables, our study was performed on an in-patient setting. This was only compatible with a short duration of the study. Therefore, although the present data show that fish oil, at the dosage employed in this study, has no clinically meaningful effect on glycemic control in type 2 (non-insulin-dependent) diabetic patients, we cannot exclude that it might exert some influence on glucose metabolism and/or insulin secretion if administered for a longer time. On the other hand, the duration of the study was confirmed to be enough to elicit clear lipid and lipoprotein changes and therefore to allow the evaluation of the underlying pathophysiologic mechanisms.

Insulin resistance and hypertriglyceridemia are commonly seen in patients with type 2 diabetes. A close relationship between these two conditions has been reported [35], suggestive of the hypothesis that hypertriglyceridemia is secondary to hyperinsulinemia and insulin resistance [36], possibly through the stimulation of hepatic VLDL triglyceride secretion [35,37]. Taking this possibility into account, the hypotriglyceridemic effect of $n-3$ fatty acids and the reported decreased incidence of diabetes in fish consuming populations, in spite of unchanged or even reduced insulin concentrations [38], could recognize a common mechanism of action, namely an improvement in insulin sensitivity. This hypothesis is not in line with our results. We found, in fact, reduced triglyceride levels but we did not observe any significant improvement in insulin sensitivity. Moreover, plasma glucose and FFA concentrations, which represent metabolic precursors of triglycerides, and plasma insulin levels which are believed to influence VLDL synthesis were not affected by fish oil. In addition, plasma FFA basal turnover rate was unchanged after the administration of $n-3$ fatty acids. According to the results of our study, other mechanisms may contribute to the hypotriglyceridemic effect of fish oil. A clear finding is that the LDL cholesterol concentration in all subjects increased after $n-3$ supplement and this increase was directly correlated to the reduction in VLDL triglyceride (Fig. 2). This suggests that an increased rate of conversion of VLDL to LDL had occurred. This increased conversion could be due to the presence of smaller VLDL particles, more slowly removed from the circulation than the large ones and therefore more easily transformed to LDL [39]. Smaller VLDL particles after fish oil concentrate dietary supplement have been reported [40]. These particles could be the result of either a hepatic secretion of VLDL particles depleted in triglycerides and/or changes in the lipolytic system operating after VLDL secretion.

In conclusion, this study shows that a dietary supplement of fish oil decreases plasma triglyceride levels in non-insulin-dependent diabetic patients. This is consistent with an increased conversion rate of VLDL to LDL. With a daily dosage of 10 g fish oil no relevant variations in glycemic control, insulin secretion and insulin sensitivity occurred. Taken together these observations do not support, at present, the use of fish oil supplement for the correction of metabolic abnormalities which are present in type 2 (non-insulin-dependent) diabetic patients.

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