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Supplementation with n-3 fatty acids reduces triglycerides but increases PAI-1 in non-insulin-dependent diabetes mellitus

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Abstract. The effects of dietary supplementation with n-3 fatty acids on lipid and glucose metabolism and on fibrinolysis were evaluated in 14 non-insulin-dependent diabetic patients who were given 10 g of MaxEPA (3 g n-3 fatty acids) or placebo (olive oil) per day in a randomized double-blind cross-over study during two consecutive 8-week periods. The serum triglyceride (TG) concentrations decreased by 27% ($P < 0{\cdot}01$) after addition of MaxEPA with a reduction of VLDL TG by 36% ($P < 0{\cdot}05$) while LDL cholesterol increased by 6% ($P = 0{\cdot}05$). The fasting blood sugar and HbA_{1c} concentrations increased significantly after addition of MaxEPA but the changes were not significantly different from those during the placebo period. The highest glucose concentrations at fasting and after an i.v. glucose injection were seen after MaxEPA while the serum insulin concentrations were unchanged. The peripheral insulin sensitivity, as measured by a euglycaemic, hyperinsulinaemic clamp technique, did not change during the study. The mean plasminogen inhibitor-1 (PAI-1) activity of the patients was elevated compared with healthy controls. In spite of the reduction of the triglyceride concentrations and unchanged insulin levels, there was a significant increase of the activity of PAI-1 (+21%, $P < 0{\cdot}01$) after MaxEPA suggesting a possible impairment of the fibrinolytic capacity. In many situations there seems to be a reduction of PAI-1 when the triglycerides are lowered. In the diabetic patients given n-3 fatty acids this was not the case.

Introduction

Long chain, polyunsaturated n-3 fatty acids will, when given in high concentrations, reduce triglyceride concentrations in hypertriglyceridaemia [1]. Similar effects have been recorded also in patients with non-insulin-dependent diabetes mellitus (NIDDM) with elevated triglyceride levels [2–5]. However, at the same time there have been reports of impaired blood glucose control and the place of the n-3 fatty acids in the treatment of patients with NIDDM is at present equivocal. More controlled studies are needed to evaluate the effects, and mechanisms behind the metabolic changes, of n-3 fatty acids in diabetes.

The present study was undertaken to further investigate the effects of n-3 fatty acids on the lipoprotein composition and glucose homeostasis, especially with regard to the peripheral insulin sensitivity, in patients with unsatisfactorily controlled NIDDM. Also, the effects of n-3 fatty acids on the fibrinolysis were evaluated.

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Patients and methods

Design of the study

Patients were treated with 10 g MaxEPA (Seven Seas Health Care Ltd, Hull, UK), a natural triglyceride concentrate of selected marine oils containing high concentrations of n-3 fatty acids, in a double-blind cross-over trial using olive oil as placebo. The patients were given 5 capsules containing 5 g of MaxEPA or the equivalent amount of olive oil twice daily. The fatty acid content of MaxEPA was declared to be approximately 50% saturated and mono-unsaturated fatty acids, 18% eicosapentaenoic acid, 20:5 n-3 (EPA), and 12% docosahexaenoic acid, 22:6 n-3 (DHA), corresponding closely to the results of analyses performed in our laboratory. A daily dose of 10 g MaxEPA thus provided 1·8 g of EPA and 1·2 g of DHA. Vitamin E (1 mg) was added to each capsule as an anti-oxidant. The dose of the oil supplement corresponded to the maximal daily dose recommended by the manufacturer.

The duration of the trial was 16 weeks, with cross-over after 8 weeks. The initial treatments with active drug or placebo were allocated to the patients in a random manner. Five individuals were randomized to start with MaxEPA and 9 with the placebo (olive oil) preparation. No lipid lowering drugs were used.

The patients were examined before and at 4-week intervals during the study. Body weight, blood pressure and possible side-effects were recorded at every visit. All sampling was done after a 12-h overnight fast. All laboratory analyses were performed at 0, 8 and 16 weeks. In addition, serum lipid, high density lipoprotein cholesterol levels, fasting blood glucose and HbA_{1c} concentrations, and urinary glucose excretion were recorded also after 4 and 12 weeks.

Patients

Fourteen patients (12 men and 2 women, mean age 65, range 55–75 years) with non-insulin-dependent diabetes, who had been receiving dietary treatment [6] for at least one year, were included. The average body weight was 77·9 kg (range 60·5–100·9), corresponding to BMI 26·2 kg m^{-2} (22·7–34·5) which remained unchanged during the study periods. All subjects were instructed to adhere to their ordinary diet throughout the study and to avoid major changes in their level of physical activity. In addition to dietary treatment the majority of the patients were on hypoglycaemic drugs which were kept unchanged throughout the study. Ten patients took sulfonyl urea, another two patients in combination with metformin, one metformin only, while one patient was on dietary treatment only without any hypoglycemic drugs. All patients were in a stable cardiovascular state. One patient had suffered a myocardial infarction 3 years before entering the study with a remaining mild angina pectoris. Six patients were on antihypertensive treatment. All medication such as selective beta-adrenegic-blocking drugs, diuretics or glyceryl trinitrate was also maintained unchanged. All patients had normal renal, thyroid and liver function.

All subjects had given their informed consent prior to participating in the study, and the protocol was approved by the Ethical Committee of the Medical Faculty of the University of Uppsala, Uppsala, Sweden.

Laboratory analyses

Lipoprotein lipid concentrations were determined in serum after an overnight fast. Very low density (VLDL), low density (LDL) and high density (HDL) lipoproteins were isolated using a combination of preparative ultracentrifugation [7] and precipitation with a sodium phosphotungstate and magnesium chloride solution [8]. Triglyceride and cholesterol concentrations were determined in serum and in the isolated lipoprotein fractions by enzymatic methods using Boehringer-Mannheim (Mannheim, Germany) kits, modified for use in a Multistat III, F/LS apparatus (Instrumentation Laboratories, Lexington, MA, USA). The coefficients of variation in the assays were 2·7 and 1·7% respectively. The concentrations of plasma free fatty acids were determined by an enzymatic colorimetric method using a commercially available kit (Waho Chemicals GmbH, Neuss, Germany) applied for use in the Multistat III centrifugal analyzer.

The concentrations of serum apolipoproteins (apo) A-I and B were determined by turbidimetry in the Multistate III F/LS apparatus using monospecific polyclonal antibodies against apo A-I and B, respectively. The coefficients of variation were 2·5 and 2·4%, respectively. The samples were preincubated with triglyceride lipase prior to the assay as suggested by DaCol and Kostner [9].

The fatty acid composition of the plasma lipid esters and the platelet phospholipids was determined by gas liquid chromatography as described previously [10].

Blood and urinary glucose concentrations were determined by the glucose oxidase assay [11]. Serum insulin was measured using the Phadebas Insulin Test (Pharmacia, Uppsala, Sweden) [12] and haemoglobin A_{1c} was determined by high performance liquid chromatography (normal range 3·5–6·0%) [13].

An intravenous glucose tolerance test was performed as earlier described [14]. The insulin sensitivity was evaluated by an euglycaemic hyperinsulinaemic clamp technique according to De Fronzo [15] as earlier described [16]. The insulin sensitivity is expressed as the insulin sensitivity index (M/I_{60-120}) which is a measure of the tissue sensitivity to insulin expressed per unit of insulin achieved by dividing the mean glucose uptake (M) by the mean insulin concentration during the last 60 min of the clamp study. A glucagon stimulation test was carried out as described by Madsbad *et al.* [17] with 0·5 mg of glucagon. Plasma C-peptide was measured according to Heding [18]. Fibrinogen was assayed by an immunological turbidimetric method using the Multistat III centrifugal analyzer. Reference interval was 2·4–4·3 g l^{-1}. Factor VII_C (biological activity) was assayed by means of titration with plasma using a one-stage clotting assay with $FVII_C$-deficient plasma as test-base (reference interval 80–120%). Tissue plasminogen activator inhibitor (PAI-1) activity in plasma was measured with Spectrolyse/pL kits from Biopool Sweden using polylysine as a stimulator [19]. The reference range was < 15 U ml^{-1}.

Statistics

The variation of the variables was separated using an analysis of variance model with main factors for groups, patient and time periods. The interaction between group and time has been included in the model. Hypotheses of interest were tested by contrasts in the combination of factors. No significant carry-over or order effect could be demonstrated on the variables studied, unless indicated below. The combined data for the two treatment periods (MaxEPA and placebo) are presented together, irrespective of the order of treatment. As least-square means (adjusted mean values) constitute the basis of the tests and estimates in the analysis, the results are presented in that form. We are grateful to Dr Lars Berglund who performed the statistical analyses.

Results

All patients completed the study according to schedule. Adherence to the study protocol was monitored by capsule counting and by determination of the fatty acid composition in the plasma lipid esters and platelets (see below). For all patients there were significant changes in the fatty acid pattern in plasma

Table 1. Effects of n-3 fatty acids on serum lipid and apolipoprotein (apo) composition in NIDDM patients ($n = 14$)

	Admission	MaxEPA	Placebo	M-A	P-A	M-A compared with P-A
S-chol mmol l^{-1}	6·13	6·14	5·90	±0%	−4%	NS
S-TG mmol l^{-1}	2·62	1·90	2·87	−27%†	+10%	$P<0·001$
S-apo B g l^{-1}	1·26	1·26	1·17	±0%	−7%*	$P=0·01$
S-apo A-I g l^{-1}	1·10	1·03	1·13	−6%	+3%	$P<0·05$
VLDL TG mmol l^{-1}	1·83	1·18	2·06	−36%*	+13%	$P<0·01$
LDL chol mmol l^{-1}	4·45	4·72	4·13	+6%(*)	−7%	$P<0·001$
HDL-chol mmol l^{-1}	0·88	0·95	0·91	+8%	+3%	NS
LDL chol/HDL chol	5·16	5·14	4·64	±0%	−10%*	$P=0·01$

TG, Triglycerides; Chol, Cholesterol; M-A, Change during MaxEPA compared with admission; P-A, Change during placebo compared with admission; * $P<0·05$; † $P<0·01$; ‡ $P<0·001$; (*), $P=0·05$; NS, not significant.

Table 2. Effects of n-3 fatty acids on glucose homeostasis in NIDDM patients ($n = 14$)

	Admission	MaxEPA	Placebo	M-A	P-A	M-A compared with P-A
fB glucose mmol l^{-1}	9·2	10·7	10·5	+16%*	+13%*	NS
HbA_{1C}%	7·32	7·74	7·66	+6%*	+5%	NS
fP insulin mU l^{-1}	8·2	9·1	9·2	0·9	1·0	NS
fP C-peptide nmol l^{-1}	0·65	0·70	0·71	0·06	0·07	NS
K_{IVGTT}% min^{-1}	0·47	0·44	0·46	−6%	−2%	NS
$M/I_{60\ 120}$	5·46	4·71	4·87	−14%	−11%	NS

K_{IVGTT}, Fractional removal rate (K-value) at the intravenous glucose tolerance test; $M/I_{60\ 120}$, Insulin sensitivity index (mg glucose × kg body weight^{-1} × min^{-1} × mU^{-1} insulin multiplied by 100); For other abbreviations see Table 1.

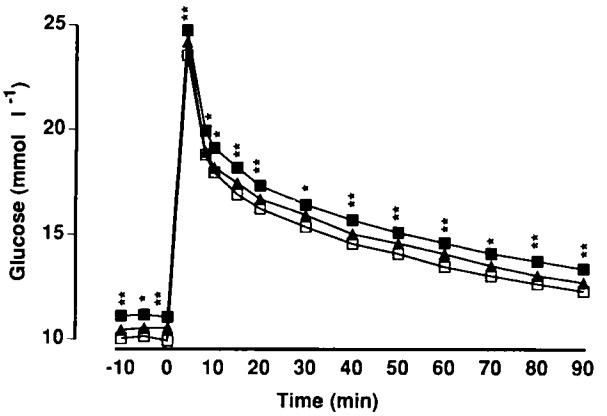

Figure 1. Glucose concentrations at fasting and after intravenous blood glucose injection on admission (open squares), after MaxEPA (filled squares) and after placebo (filled triangles).
* $P<0·05$; ** $P<0·01$, after MaxEPA compared with on admission.

and in platelets, with an increase in n-3 fatty acid content when on MaxEPA treatment. These observations indicate that the patients were taking the capsules as prescribed. Side-effects were only mild and transient, and could not be attributed to the addition of n-3 fatty acids.

Effects of MaxEPA on serum lipoprotein lipids and apolipoproteins

During treatment with MaxEPA there was a significant reduction of the serum trigylceride concentration by 27% ($P<0·01$) while the serum cholesterol concentration remained unchanged (Table 1). This corresponded to a reduction of the VLDL triglycerides by 36% ($P<0·05$). The change during the MaxEPA treatment period was significantly different from that during treatment with placebo. There was an increase of LDL cholesterol by 6% ($P=0·05$) in contrast to a slight mean reduction on the placebo treatment period by 7% (NS). The changes during the two periods were highly significantly different ($P<0·001$). The HDL concentration did not change during the treatment. The 'atherogenic index', the ratio between LDL cholesterol and HDL cholesterol, remained unchanged during treatment with MaxEPA but decreased during the placebo period by 10%. Also the changes of the serum apolipoprotein B and A-I concentrations were significantly different during the two treatment periods (Table 1).

Effects of MaxEPA on glucose homeostasis

The mean fasting blood glucose concentrations were increased at the end of both treatment periods and the

Table 3. Effects of n-3 fatty acids on PAI-1, factor VII_C, fibrinogen and free fatty acid concentrations in NIDDM patients ($n = 14$)

	Admission	MaxEPA	Placebo	M-A	P-A	M-A compared with P-A
PAI-1 U l^{-1}	18·7	22·6	17·7	+21%†	−5%	$P<0·01$
Factor $VII_{C\%}$	113	114	110	+1%	+3%	NS
Fibrinogen g l^{-1}	3·10	2·85	3·08	−8%	−1%	NS
FFA mmol l^{-1}	0·59	0·57	0·60	−3%	+2%	NS

For abbreviations see Table 1.

Table 4. Effects of dietary n-3 fatty acids on plasma phospholipid fatty acid composition (%) in NIDDM ($n = 14$)

	Admission	MaxEPA	Placebo	M-A	P-A	M-A compared with P-A
18:1 n-9	11·5	10·3	12·2	−10%‡	+6%†	$P<0·001$
18:2 n-6	22·6	19·0	22·7	−16%‡	±0	$P<0·001$
20:3 n-6	2·8	1·6	2·8	−43%‡	±0	$P<0·001$
20:4 n-6	8·2	6·8	7·4	−17%‡	−10%†	$P<0·05$
20:5 n-3	1·6	5·3	1·5	+231%‡	−6%	$P<0·001$
22:5 n-3	0·9	1·3	0·09	+44%‡	±0	$P<0·001$
22:6 n-3	4·9	6·8	4·7	+39%‡	−4%	$P<0·001$

For abbreviations see Table 1.
(The contents of fatty acids are expressed as relative percent of 13 identified fatty acids with chain lengths from 16 to 22 carbon atoms).

changes during MaxEPA and placebo were not significantly different (Table 2). Also the HbA_{1C} concentration increased significantly during MaxEPA. A similar, not significant, tendency during the placebo period was probably due to a carry-over effect from the MaxEPA period as indicated by the statistical analysis. When the comparison was restricted to the first treatment period the changes between MaxEPA and placebo were significantly different ($P<0·05$) with an increased HbA_{1C} concentration after MaxEPA by 9% ($P=0·01$), while the HbA_{1C} concentrations remained unchanged in the group starting with placebo (−2%, NS). The fasting plasma insulin and C-peptide concentrations were the same after the two periods. The fractional removal rate at the intravenous glucose-tolerance test was identical after MaxEPA and placebo, as was the peripheral insulin sensitivity (insulin sensitivity index) measured by the euglycaemic clamp technique (Table 2).

When the blood glucose concentrations at fasting and after intravenous glucose injection were compared (Fig. 1) the blood glucose concentrations were at all time points significantly higher after MaxEPA than on admission. The blood glucose concentrations after the placebo period were intermediate both at fasting and after glucose injection and significantly lower than after MaxEPA at 8 min ($P<0·05$) and at 10 min ($P=0·05$) after glucose injection. The serum insulin concentrations measured on admission and after Max-EPA and placebo, respectively, were similar in fasting, as well as after the intravenous glucose infusion (data not shown). Thus, in spite of the differences in blood glucose concentrations, there were no differences between serum insulin concentrations at any time point neither before, nor after glucose injection.

Effects of MaxEPA on free fatty acids, fibrinogen, factor VII_C and PAI-1

While there were no changes of free fatty acids, fibrinogen and factor VII_C (Table 3) there was a clearly significant increase ($P<0·01$) of the PAI-1 -concentration after Max-EPA-treatment by 21% while the values remained unchanged after the placebo period. The mean PAI-1 activity was already above the reference range for healthy subjects (<15 U l^{-1}) before the study and showed a further increase after addition of n-3 fatty acids. The changes during the MaxEPA and placebo periods were significantly different ($P<0·01$).

Effects of MaxEPA on fatty acid composition of plasma lipid esters and platelet phospholipids

A pronounced increase in long-chain polyunsaturated fatty acids of the n-3 series (20:5, 22:5, 22:6), was, as expected, observed in all plasma lipid esters and platelet phospholipids during MaxEPA treatment. There was a simultaneous decrease in n-6 fatty acids. The most significant changes of the plasma phospholipid fatty acid composition are shown in Table 4.

Discussion

The effects of supplementation with n-3 fatty acids in NIDDM are still controversial. While the VLDL lipoprotein concentrations usually decrease, the LDL cholesterol is unchanged or moderately increased. The addition of the n-3 fatty acids, especially when given in high doses, tend to impair the blood glucose control while the serum insulin levels are unchanged or reduced [20]. However, there are few randomized and controlled studies published on the use of n-3 fatty acids in diabetic patients.

Also in the present study the VLDL triglyceride concentrations were reduced but LDL cholesterol increased and the atherogenic index (LDL cholesterol/HDL cholesterol) remained unchanged. The lipoprotein changes are similar to those in previous reports [2–5,20].

Whether the addition of a small amount of olive oil *per se* could cause a reduction in lipid levels is at present unknown. In an earlier study [5] the same dose of olive oil caused a similar effect including a slight but significant reduction of the LDL/HDL ratio. Thus, in future studies it may be wiser to consider using a different placebo preparation.

The tendency to increased blood glucose concentrations before and after intravenous glucose is also in line with earlier findings [2,4,5,21,22]. It is obvious that the n-3 fatty acids, at least when given in higher doses during a limited period of time, may impair the blood glucose control in patients with non-insulin-dependent diabetes mellitus. The mechanisms behind these changes are still unexplained. The present investigation, as well as other studies [2,4], indicate that this may be due to an impaired pancreatic insulin response to glucose. It may also be associated with an elevated basal hepatic glucose production due to increased availability of gluconeogenic precursors or increased hepatic resistance to fasting levels of insulin as suggested by Glauber and colleagues [21]. An increased metabolic clearance rate of glucose after addition of n-3 fatty acids has been suggested [23]. However, in accordance with the results of this study, other investigators have also been unable to show any change of the peripheral glucose elimination [21,24]. A common finding in all studies is that, in spite of the tendency to increased blood glucose concentrations, the serum insulin concentrations remain unchanged or reduced, also after glucose injection, compatible with the suggested reduced pancreatic insulin response to glucose.

A new finding in the present study was an increased activity of the plasminogen activator inhibitor PAI-1 suggesting an impaired fibrinolytic capacity after n-3 fatty acids. Similar results have been reported in non-diabetic normo-lipidemic [25,26] and hyperlipidemic [27] subjects given n-3 fatty acids. In accordance with an earlier report in diabetic patients [28] the PAI-1 activity was high at baseline in our study and increased further after addition of MaxEPA. An increased PAI-1 activity was demonstrated to be a risk factor for recurrent myocardial infarction in patients who had suffered from myocardial infarction [29]. An impairment of the fibrinolytic capacity in diabetics, who as a group have a high risk for coronary heart disease, may thus possibly further increase this risk.

It has been shown that the reduction of VLDL lipoprotein concentrations by n-3 fatty acids is probably due to a decreased production of triglyceride rich lipoproteins rather than to an increased removal [1]. Enhanced insulin production, elevated free fatty acid levels and poor glycaemic control have all been linked to an elevated VLDL-production in NIDDM [30]. During the treatment with n-3 fatty acids, reduced VLDL-levels were achieved in spite of increased plasma glucose concentrations and unchanged free fatty acids and insulin concentrations indicating that other mechanisms may contribute to the reduced lipoprotein VLDL levels.

Also the reduction of the fibrinolytic capacity, in spite of the reduced serum triglyceride levels, is interesting. A suggested coupling between reduction of serum triglycerides and improvement of fibrinolytic capacity [31] was not present in our study.

It is too early to definitely conclude anything about the benefits or draw-backs of supplementation with n-3 fatty acids in NIDDM patients. The effects of n-3 fatty acids may be related to the dose. Evidence is lacking regarding long-term effects of treatment with low doses. Possibly, negative effects on glucose homeostasis and lipid metabolism may be considered at high doses only, while other beneficial effects, e.g. on platelet reactivity, may be retained also at lower doses. The effect on fibrinolysis may possibly be regarded as a physiological response to the increased bleeding time seen after administration of n-3 fatty acids.

Subjects with NIDDM are advised to increase their intake of polyunsaturated fatty acids, including a higher intake of fatty fish. However, general use of different fish-oil preparations with high concentrations of n-3 fatty acids in NIDDM patients should be discouraged before further studies have been performed.

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