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Dietary supplementation with n-3 fatty acids may impair glucose homeostasis in patients with non-insulin-dependent diabetes mellitus

B. VESSBY & M. BOBERG
From the Department of Geriatrics, Uppsala University, Uppsala, Sweden

Abstract. Vessby B, Boberg M (Department of Geriatrics, Uppsala University, Uppsala, Sweden). Dietary supplementation with n-3 fatty acids may impair glucose homeostasis in patients with non-insulin-dependent diabetes mellitus. *Journal of Internal Medicine* 1990; **228**: 165–171.

The effects on lipoprotein and glucose metabolism of addition of n-3 fatty acids were studied 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. After MaxEPA treatment, there was a marked increase in long-chain polyunsaturated fatty acids of the n-3 series in the plasma lipid esters and in the platelet phospholipids, while the n-6 fatty acid content decreased. The very low density lipoprotein (VLDL) triglyceride concentrations decreased significantly (by 22%) on MaxEPA treatment. However, these changes were not significantly different from those observed during the placebo period. The blood glucose concentration tended to increase during MaxEPA treatment, and to decrease during the placebo period, the changes under the two regimes being significantly different ($P < 0.01$). In addition, the rate constant for glucose disappearance (k value) for the intravenous insulin-tolerance test, which reflected the peripheral insulin sensitivity, tended to decrease during MaxEPA treatment and increase during administration of the placebo, there being a significant difference ($P < 0.03$) between the changes during the two treatments. The reason for the observed changes in blood glucose concentration and peripheral insulin sensitivity is still unclear.

Keywords: blood glucose, diabetes mellitus, insulin, lipoproteins, n-3 fatty acids, peripheral insulin sensitivity.

Introduction

Long-chain polyunsaturated fatty acids of the n-3 series will, when administered in high concentrations, reduce raised triglyceride concentrations in hypertriglyceridaemia [1]. The n-3 fatty acids from fish oil have been reported to prevent development of insulin resistance in rats that have been fed high-fat diets [2]. These results suggest that n-3 fatty acid supplements may be of value in the treatment of non-insulin-dependent diabetes mellitus, which is characterized by increased serum triglyceride levels and peripheral insulin insensitivity.

The aim of this study was to investigate the effects of adding commercially available n-3-fatty-acid concentrates to a conventional diabetic diet in non-insulin-dependent diabetic patients. The effects on serum lipoproteins, plasma fatty acid composition and glucose homeostasis were studied.

Abbreviations: ESA = eicosapentaenoic acid, DHA = docosahexaenoic acid, VLDL = very low density lipoprotein, LDL = low density lipoprotein, HDL = high density lipoprotein, apo B, A-I, A-II = apolipoproteins B, A-I, A-II.

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 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, active drug or placebo, were allocated to the patients in a random manner. 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. Serum lipoprotein lipids, serum apolipoproteins, the fatty acid composition of plasma lipid esters and platelet phospholipids, fasting blood glucose levels, serum insulin concentrations and HbA_{1c} values were determined at 0, 8 and 16 weeks. The intravenous insulin tolerance test and the intravenous glucagon stimulation test were performed at the same time. Serum lipid and high density lipoprotein (HDL) cholesterol levels, fasting blood glucose and HbA_{1c} concentrations, and urinary glucose excretion were also recorded after 4 and 12 weeks.

Patients

Patients with non-insulin-dependent diabetes, who had been receiving dietary treatment for at least 1 year, were included. All subjects were instructed to adhere to their ordinary diabetic diet throughout the study and to avoid major changes in their level of physical activity. The majority of the patients were on hypoglycaemic drugs in addition to dietary treatment. All medication was maintained unchanged throughout the study.

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 Uppsala University, Uppsala, Sweden.

Fourteen patients (11 men and three women, of age range 39–72 years) were included in the study. Five individuals were randomized to start with MaxEPA, and nine with the placebo (olive oil) preparation. The patients were given five 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 monounsaturated 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 of 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 antioxidant.

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 [3] and precipitation with a sodium phosphotungstate and magnesium chloride solution [4]. Triglyceride and cholesterol concentrations were determined in serum and in the isolated lipoprotein fractions by enzymatic methods using Boehringer–Mannheim (Munich, FRG) kits 126012 and 124087, modified for use in a Multistat III F/LS apparatus (Instrumentation Laboratories, Lexington, MA, USA).

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

The fatty acid composition of the plasma lipid esters and the platelet phospholipids was determined by gas liquid chromatography as described previously [6].

Blood and urinary glucose concentrations were determined by the glucose oxidase assay [7]. Serum insulin was measured using the Phadebas Insulin Test (Pharmacia, Uppsala, Sweden) [8], and haemoglobin A_{1c} was determined by high performance liquid chromatography (normal range 3.5–6.0%) [9].

An intravenous insulin tolerance test (IVITT) was performed by injection of crystalline insulin (0.1 U kg^{-1} body weight). The blood glucose concentrations were monitored at 5-min intervals, and the insulin sensitivity was expressed as the rate constant for glucose disappearance (k_{IVITT}) [10]. A glucagon stimulation test was carried out as described by Madsbad [11], using 0.5 mg of glucagon. Plasma C-peptide was measured as described by Heding [12].

Statistics

The variation of the variables was separated using an analysis of variance model with main factors for groups, patient (nested within group) and time. 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 effect could be demonstrated on the variables studied. Thus 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.

Results

Body weight remained constant throughout the study. Side-effects were only mild and transient, and they were mainly light gastrointestinal disturbances which could not be attributed to the addition of n-3 fatty acids. For one patient the study was interrupted during the placebo treatment period due to an acute myocardial infarction. The values obtained for this patient during the first 8 weeks (MaxEPA) were included in the calculations. All other 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 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.

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

During MaxEPA treatment, a pronounced increase in long-chain polyunsaturated fatty acids of the n-3 series (20:5, 22:5, 22:6) was observed in all plasma lipid esters and platelet phospholipids (Table 1), and there was a simultaneous decrease in n-6 fatty acids. The alterations in fatty acid composition differed significantly from those during the placebo period, when there were no major changes in the fatty acid pattern, except for a slight increase in the oleic acid content of all plasma lipid esters (Table 1). Furthermore, there was a slight increase in the oleic content and a decrease in the stearic acid content

Table 1. The adjusted means for the plasma and platelet phospholipid fatty acid composition in non-insulin-dependent diabetic patients on admission, and the estimated effects of MaxEPA and placebo treatment

	Plasma phospholipid fatty acid composition (%)			*P*-value for difference between treatment periods	Platelet phospholipid fatty acid composition (%)			*P*-value for difference between treatment periods
Variable	Baseline value on admission	M-A	P-A		Baseline value on admission	M-A	P-A	
16:0	33.2	+0.3	−0.3	0.08	25.7	+0.5	+0.1	0.48
16:1	0.9	−0.07	−0.1**	0.29	1.2	−0.3	+0.5	0.09
18:0	14.9	+0.4*	+0.2	0.20	18.5	−0.6	−1.2**	0.17
18:1	10.6	−0.7*	+0.4	0.001	16.3	+0.7	+0.9*	0.66
18:2 n-6	23.3	−4.7***	+0.2	0.0001	7.8	−0.9*	−0.4	0.18
18:3 n-6								
18:3 n-3	0.2	−0.03	+0.03	0.13				
20:3 n-6	3.1	−1.1***	−0.2	0.0001	1.6	−0.3***	−0.03	0.0002
20:4 n-6	7.3	−0.8**	−0.2	0.04	21.7	−3.0***	−0.5	0.0003
20:5 n-3	1.2	+4.0***	+0.1	0.0001	0.7	+3.2***	+0.5	0.0001
22:4 n-6					2.1	−1.2***	−0.1	0.0001
22:5 n-3	0.9	+0.6***	−0.1	0.0001	1.8	+1.0***	−0.1	0.0001
22:6 n-3	4.4	+2.1***	+0.01	0.0001	2.6	+0.7***	+0.01	0.001

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
M-A = change during treatment with MaxEPA.
P-A = change during treatment with placebo.

Table 2. The adjusted means for the lipoprotein and apolipoprotein concentrations on admission, and the estimated effects of MaxEPA and placebo treatment

Variable	Baseline value on admission	M-A	P-A	*P*-value for difference between treatment periods
VLDL triglyceride (mmol l^{-1})	2.68	−0.58** (−22%)	−0.42(*) (−16%)	0.45
VLDL cholesterol (mmol l^{-1})	1.05	−0.20 (−19%)	−0.14 (−13%)	0.59
LDL triglyceride (mmol l^{-1})	0.64	−0.03 (−5%)	−0.05 (−8%)	0.13
LDL cholesterol (mmol l^{-1})	4.08	−0.10 (−2%)	−0.24 (−6%)	0.36
HDL triglyceride (mmol l^{-1})	0.24	−0.01 (−4%)	0.02 (+8%)	0.12
HDL cholesterol (mmol l^{-1})	0.95	0.04 (+4%)	0.03 (+4%)	0.96
Serum triglyceride (mmol l^{-1})	3.58	−0.64** (−18%)	−0.40 (−11%)	0.28
Serum cholesterol (mmol l^{-1})	6.12	−0.31* (−5%)	−0.36* (−8%)	0.69
LDL cholesterol/HDL cholesterol	4.37	0.22 (−5%)	0.36* (−8%)	0.30
Serum cholesterol-HDL cholesterol / HDL cholesterol	5.71	0.55* (−10%)	−0.61* (−11%)	0.72
Apo A-I (g l^{-1})	0.99	−0.02 (−2%)	±0 (±0%)	0.68
Apo A-II (g l^{-1})	0.36	−0.03* (−8%)	−0.03(*) (−8%)	0.89
Apol B (g l^{-1})	1.02	−0.03 (−3%)	−0.03 (−3%)	0.88

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, (*) $P = 0.05$.
M-A = change during treatment with MaxEPA.
P-A = change during treatment with placebo.

of the platelet phospholipids after addition of olive oil, but these changes were not significantly different from those observed during the MaxEPA treatment period.

Effects of MaxEPA on serum lipoprotein lipids and apolipoproteins

During MaxEPA treatment there was a significant decrease in the concentration of serum triglycerides, corresponding to a reduction in the VLDL triglyceride levels (Table 2). There were no significant changes in the triglyceride concentrations of LDL or HDL. Although the reduction in VLDL triglyceride was more pronounced after MaxEPA treatment than during the placebo period, there was also a reduction in triglyceride levels, of borderline significance, during olive oil supplementation. The changes observed during MaxEPA treatment did not differ significantly from those following treatment with placebo capsules; this was true both for VLDL triglycerides and for the other lipid parameters.

There was a significant decrease in serum cholesterol concentration between admission to the study and treatment with MaxEPA. The LDL cholesterol/HDL cholesterol ratio was significantly reduced only during the placebo period, while the serum cholesterol-HDL cholesterol/HDL cholesterol ratio was significantly reduced during both treatment and placebo periods.

The serum apolipoprotein concentrations tended to decrease after MaxEPA treatment, although only in the case of apo A-II was the decrease significant. The apo A-II/HDL cholesterol ratio was significantly reduced during both MaxEPA and placebo periods, while the A-I/A-II ratio remained unchanged.

Table 3. The adjusted means for fasting blood glucose, HbA_{1c}, K_{IVITT}, and serum insulin and plasma C-peptide concentrations at the intravenous glucagon test on admission and the estimated effects of MaxEPA and placebo treatment

Variable	Baseline value on admission	M-A	P-A	*P*-value for difference between treatment periods
fB glucose (mmol l^{-1})	10.0	+0.62 (+0.6%)	−0.51 (−5%)	0.007**
HbA_{1c} (%)	8.1	+0.30 (+4%)	−0.15 (−2%)	0.18
K_{IVITT} (% min^{-1})	2.26	−0.33 (−15%)	−0.34 (+15%)	0.03*
Intravenous glucagon test				
S insulin 0′ (mU l^{-1})	14.6	−0.45 (−3%)	−0.87 (−6%)	0.86
S insulin 6′ (mU l^{-1})	41.4	+2.8 (+7%)	+1.4 (+3%)	0.70
pC peptide 0′ (nmol l^{-1})	0.86	+0.01 (+1%)	−0.04 (−5%)	0.47
pC peptide 6′ (nmol l^{-1})	1.40	+0.17 (+12%)	−0.00 (±0%)	0.13

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
M-A = change during treatment with MaxEPA.
P-A = change during treatment with placebo.

Effects of MaxEPA on glucose metabolism

Neither MaxEPA nor placebo treatment caused a significant change in mean blood glucose concentration compared to baseline levels (Table 3). However, there was a general trend towards increased values after MaxEPA treatment and reduced values after placebo treatment, and the changes during the two periods were significantly different ($P = 0.007$). Corresponding to these divergent effects on blood glucose levels, the mean HbA_{1c} concentration also increased after MaxEPA treatment and decreased slightly during placebo treatment, although the changes were not significantly different. In addition, the k value for the intravenous insulin-tolerance test, which reflected the peripheral insulin sensitivity, decreased after MaxEPA treatment and increased after administration of the placebo, and the changes during the two treatment periods were statistically different ($P = 0.03$).

Effects of MaxEPA on blood pressure

The mean blood pressure was 147/89 mmHg on admission, 143/83 mmHg after MaxEPA treatment and 144/86 mmHg after placebo treatment. The diastolic blood pressure after 8 weeks of treatment with n-3 fatty acids was significantly lower ($P = 0.01$) than on admission. However, the changes during the MaxEPA and placebo treatment periods were not significantly different ($P = 0.25$).

Discussion

The changes in fatty acid composition of the plasma lipid esters and the platelet phospholipids are very similar to those reported previously in non-diabetic subject [13]. The incorporation of the n-3 fatty acids, with a subsequent reduction in the content of fatty acids of the n-6 series and oleic acid, indicates the operation of highly specific metabolic pathways for incorporation of fatty acids into the various lipid esters in diabetic patients, too.

A decrease in serum triglyceride levels following addition of n-3-fatty acids has been reported in healthy [14] as well as hypertriglyceridaemic [1] subjects. The diabetic subjects in the present study were all hypertriglyceridaemic on admission. The reduction in VLDL triglyceride concentration by approximately 20% is comparable to the triglyceride changes observed during treatment with a similar dose of MaxEPA in previous studies [13]. The effect on serum cholesterol has been found to be variable [1, 13].

The degree of similarity between the changes in serum lipid levels caused by addition of 10 g of

MaxEPA and 10 g of olive oil, respectively, is rather remarkable. Several recent reports have indicated that substitution of olive oil for saturated fat will reduce the serum lipid levels effectively, and to an extent similar to that of polyunsaturated fatty acids of the n-6 series [15, 16]. Whether the addition of a small amount of olive oil *per se* could cause a reduction in lipid levels is at present unknown. Although a similar dose of olive oil administered in the same way caused no significant changes in the lipoprotein levels in hypertriglyceridaemic subjects [13], it may be asked whether this small amount of olive oil may also have metabolic effects. In future studies it may be wise to consider using a different placebo preparation.

In contrast to the more or less expected effects of MaxEPA on lipoprotein lipid concentrations, the effects on glucose metabolism were somewhat surprising. The changes in blood glucose concentration and peripheral insulin sensitivity, as measured by the intravenous insulin tolerance test, differed significantly during the two treatment periods, with a tendency toward increased blood glucose levels as well as a non-significant increase in the HbA_{1c} concentration after addition of n-3 fatty acids. To date, there have been relatively few studies on the effects of n-3 fatty acids in diabetic patients. However, as reviewed recently [17], some studies have shown that addition of n-3 fatty acids may increase blood glucose levels without a concomitant increase in insulin or C-peptide concentrations. In contrast to the present study, Popp-Snijders [18] reported an increased rate of metabolic clearance of glucose, which she interpreted to indicate an improved insulin sensitivity, during a constant glucose-insulin infusion. Glauber *et al.* [19] have reported an unchanged glucose clearance rate, using the euglycaemic clamp technique, at superphysiological insulin concentrations. Rivellese *et al.* [20] found no effect on peripheral glucose utilization as measured by the hyperglycaemic clamp technique.

The reasons for the observed changes in blood glucose concentration and insulin sensitivity in diabetic patients are still little understood. More controlled studies, over prolonged time periods, are required in order to evaluate the specific effects of n-3 fatty acids on glucose homeostasis in diabetes. Even if there is a tendency toward impaired glucose homeostasis, other effects on platelets, coagulation factors, blood pressure, prostaglandin metabolism and lipoprotein lipid concentrations may outweigh the risk of development of atherosclerotic cardiovascular disease. From a practical viewpoint, however, it appears to be important to monitor closely both glucose and lipid metabolism in diabetic patients who are being treated with n-3 fatty acids.

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Correspondence: Bengt Vessby, MD, Department of Geriatrics, PO Box 12042, S-750 12 Uppsala, Sweden.