Effect of omega-3 fatty acids on cardiovascular risk factors in patients with type 2 diabetes mellitus and hypertriglyceridemia: an open study

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Abstract. – *Background and Objectives:* Epidemiological and interventional studies suggest that a high dietary intake of n-3 polyunsaturated fatty acids may confer a protective effect against atherosclerotic disease and reduce serum triglycerides levels. The aim of our study was to investigate the effectivity on triglyceride levels and inflammatory markers of a concentrated of n-3 fatty acids in patients with type 2 diabetes mellitus and hypertriglyceridaemia.

Subjects: A total of 30 patients (16 males and 14 females) with diabetes mellitus type 2 and hypertriglyceridemia (>200 mg/dl) were included in the study. Patients received two capsules of eicosapentaenoic 465 mg and docosahexanoic 375 mg daily for 12 weeks.

Results: Triglycerides levels and non HDL-cholesterol decreased (326 ± 113.5 vs. 216.4 ± 57 mg/dl; $p<0.05$) and (103.87 ± 44 vs. 89.6 ± 14 mg/dl; $p<0.05$), respectively. HDL-cholesterol levels increased (39.6 ± 10.7 vs. 46.4 ± 8.7 mg/dl; $p<0.05$). C reactive protein decreased (5.98 ± 3.9 vs. 3.9 ± 1.6 mg/dl; $p<0.05$) and TNF-alpha levels decreased (16.24 ± 5.5 vs. 13.3 ± 5.8 pg/dl; $p<0.05$), without significant changes in IL-6 levels.

In conclusion, an n-3 polyunsaturated intervention improved lipid profile and inflammatory markers in patients with diabetes mellitus type 2 and hypertriglyceridaemia.

Key Words:

Inflammatory markers, Hypertriglyceridaemia, n-3 fatty acids, Type 2 diabetes mellitus.

tients[1]. Several potential mechanisms have been suggested to contribute to this phenomenon, including an enhanced atherogenic potential of low density lipoprotein (LDL) in the hypertriglyceridemic subjects[2].

Management of hypertriglyceridaemia should focus initially on non-pharmacological modalities of treatment, such as diet, exercise, weight control, reduced alcohol intake and strict glycaemic control[3].

If the measures prove unsuccessful, severely elevated triglycerides levels should be treated with lipid-lowering drugs in order to prevent pancreatitis and/or cardiovascular risk. Such drugs include fibric acid derivatives and nicotinic acid and its analogues. These compounds both decrease triglycerides and increase HDL cholesterol. Some of these compounds have shown modest reductions in fatal and non-fatal myocardial infarctions, but for neither could a decrease demonstrated[4]. Epidemiological and interventional studies suggest that a high dietary intake of n-3 polyunsaturated fatty acids may confer a protective effect against atherosclerotic disease and reduce serum triglycerides levels[5].

The aim of our study was to investigate the effectivity on triglyceride levels and inflammatory markers of a concentrated of n-3 fatty acids in patients with type 2 diabetes mellitus and hypertriglyceridaemia.

Introduction

Subjects with moderate hypertriglyderidaemia are considered to be at increased risk for coronary heart disease (CHD), especially diabetic pa-

Material and Methods

Study Participants

A total of 30 patients (16 males and 14 females) with diabetes mellitus type 2 and hyper-

triglyceridemia (>200 mg/dl) were included in an open study. The following variables were specifically recorded: age, years of diabetes duration, pharmacological treatment to diabetes (dose and drug), and blood pressures, weight, height and body mass index (BMI). These patients were followed in a Diabetes and Nutrition Clinic.

Naïve patients were included if they had fasting serum triglycerides levels higher than 200 mg/dl. Concomitant medication remained unchanged throughout the trial. Patients were excluded if they had serum alanine amino transferase (ALT) >3 timers upper normal level, serum creatinine >1.5 mg/dl. Patients with poorly controlled diabetes mellitus (HbA1c >8.5%), asthma, alcohol abuse, coronary heart disease were also excluded from the study. The study was approved by the local Ethical Committee.

Study Design

The study consisted of a treatment period of 12 weeks preceded by a 9-week run-in during which the patients received dietary advice both orally and in written form by the physicians performing the study, and dietary assessesment throughout the study. After this period patients received two capsules of eicosapentaenoic 465 mg and docosahexanoic 375 mg daily for 12 weeks. Treatment to diabetes mellitus was maintained during the protocol.

At each visit (basal and week 12) blood samples were drawn after 12 hour fast and 24 hours abstinence from alcohol, and lipid profiles were measured.

Laboratory Parameters

Before and after 3 months of the treatment a biochemical determination was performed: glucose, insulin, HOMA, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, IL-6, TNF alpha and C reactive protein.

Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y., USA), while HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL cholesterol was calculated using Friedewald formula

Haemoglobin A1c levels were measured by using high-pressure liquid chromatography. Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, CA, USA).

Interleukin 6 and TNF-alpha were measured by ELISA (R&D systems, Inc., MN, USA) with a sensitivity of 0.7 pg/ml and 0.5 pg/ml, respectively. Normal values of IL6 was (1.12-12.5 pg/ml) and TNF-alpha (0.5-15.6 pg/ml).

C reactive protein CRP (c-reactive protein) was measured by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany), analytical sensivity 0.5 mg/dl. Insulin was measured by enzymatic colorimetry (Insulin, WAKO Pure-Chemical Industries, Osaka, Japan) and the homeostasis model assessment for insulin sensitivity (HOMA) was calculated using these values[6].

Blood pressure was measured twice after a 10 minute rests with a random zero mercury sphygmomanometer, and averaged. Body weight was measured to an accuracy of 0.1 kg and body mass index computed as body weight/(height^2).

Statistical Analysis

We hypothesized that intervention with n3 fatty acids will reduce triglyceride levels by 30%. To achieve this at $p<0.05$ and 1-beta=0.80, we needed good paired data from 30 subjects. The results were expressed as mean ± SD deviation. The distribution of variables was analyzed with Kolmogorov-Smirnov test. Quantitative variables with normal distribution were analyzed with a two-tailed, paired Student's-t test. Non-parametric variables were analyzed with the Friedman and Wilcoxon tests. Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. A p-value under 0.05 was considered statistically significant.

Results

Thirty patients gave informed consent and were enrolled in the study. The mean age was 54.7±12.2 years, with diabetes mellitus duration of 8.7±8.3 years. Baseline characteristics (epidemiological data and chronic complications) of patients were presented in Table I.

Table II shows changes from baseline in metabolic parameters. Weight, body mass index, blood pressure, total cholesterol, LDL-cholesterol, insulin, HOMA did not change. Serum triglycerides and non HDL-cholesterol levels decreased (326±113.5 vs 216.4±57 mg/dl; $p<0.05$) (-33.7%) and (103.87±44 vs.

Table I. Clinical and epidemiological characteristics of study population.

Characteritics	Values
Age (years)	54.7 ± 12.2
Sex (male/female)	16/14
Diabetic duration (years)	8.7 ± 8.4
BMI (kg/m^2)	31.2 ± 5.6
HbA1c	7.4%
Smoking habit	53.3%
Insulin treatment	40%
Oral hypoglycemic treatment	60%
Retinopathy	33.3%
Nephropathy	13.3%
Neuropathy	6.6%

BMI: body mass index.

89.6±14 mg/dl; *p*<0.05) (-13.47%), respectively. Serum HDL-cholesterol levels increased (39.6±10.7 vs. 46.4±8.7 mg/dl; *p*<0.05) (+14.6%). Before the treatment, a 100% of the patients had HDL levels <50 mg/dl and triglyceride levels >200 mg/dl. After treatment, a 26.1% of the patients had HDL levels >50 mg/dl and a 34.1% of the patients had triglyceride levels <200 mg/dl.

Plasma C reactive protein decreased (5.98±3.9 vs. 3.9±1.6 mg/dl; *p*<0.05) (-34.8%) and serum TNF-alpha levels decreased (16.24±5.5 vs. 13.3±5.8 pg/dl; *p*<0.05) (-18.1%), without significant changes in IL-6 serum levels. No side effects were detected during the treatment. No patient dropped out of the study.

Discussion

We showed that an n-3 polyunsaturated intervention improved lipid profile and inflammatory markers in patients with diabetes mellitus type 2 and hypertriglyceridemia.

N3 fatty acids are considered to be well tolerated drug in the treatment of hypertriglyceridaemia, as our data shown. Certain populations have a high dietary intake of n-3 fatty acids and a low incidence of cardiovascular disease[7]. Fish are a rich source of n-3 fatty acids. These fish oils have been shown to improve endothelial function *in vitro* in patients with hypercholesteroaemia, and to reduce cardiovascular risk through that mechanism[8]. The effects of n-3 fatty acids on HDL cholesterol and its subfractions are unclear. However, one of the hypothesis is that the HDL- cholesterol subfractions change in composition and absolute size upon n-3 fatty acid treatment: the level of HDL2 tends to rise compared with the level of HDL3. Some studies have demonstrated that an increase in HDL2/HDL3 ratio may reduce cardiovascular risk[9]. Omega-3 fatty acids lower triglycerides by reducing the hepatic secretion of VLDL cholesterol[10]. Omega 3 fatty acids supplementation in patients with hypertriglyceridaemia may decrease triglycerides while increase LDL cholesterol[11]. It has been proposed that n-3 fatty acids increase LDL cholesterol by promoting production of triglycerides poor VLDL cholesterol and accelerating conversion of VLDL cholesterol to LDL cholesterol. However, this mechanism re-

Table II. Metabolic parameters.

Factors	Initial	3 months
Weight (kg)	81.4 ± 10.7	80.9 ± 10.5
BMI (kg/m^2)	31.2 ± 5.6	30.8 ± 5.2
Basal glucose (mg/dl)	148.3 ± 67	147.5 ± 61.6
Insulin (mU.I/l)	21.3 ± 16.3	18.9 ± 7.1
HOMA	7.4 ± 9.3	6.1 ± 3.5
Total cholesterol (mg/dl)	218.3 ± 61	215.3 ± 48.1
LDL-cholesterol (mg/dl)	95.4 ± 24	102 ± 20
HDL-cholesterol (mg/dl)	39.6 ± 10	46.5 ± 8.7*
Non-HDL-cholesterol (mg/dl)	103.87 ± 44	89.6 ± 14
Triglyceride (mg/dl)	326.3 ± 183	216.4 ± 48*
Systolic BP (mmHg)	139 ± 13	137 ± 14
Diastolic BP (mmHg)	81 ± 8.9	81 ± 7.2

*(*p*<0.05) BP: blood pressure. HDL: high density lipoprotein. LDL: low density lipoprotein. HOMA: homeostasis model assessment.

mains unclear and our study has shown a neutral effect on LDL levels such as other studies[12].

The NCEP ATP III guidelines identify non-HDL cholesterol as a treatment target for cardiovascular risk reduction in individuals with significant triglyceride levels (>200 mg/dl)[13]. The results of NEPTUNE II survey indicated that inadequate treatment of hypertriglyceridaemia is common; only 27% of patients with triglyceride levels >200 mg/dl and cardiovascular disease with diet and/or drug therapy achieved cholesterol goals[14]. In previous studies in patients with hypertriglyceridaemia who were receiving stable doses of statin therapy, the addition of n-3 fatty acids to statin for 24 weeks resulted in further reductions in triglyceride (28.9%) and VLDL cholesterol (21.1%)[12,15]. The Gruppo Italiano per lo Studio della Streptochinasi nell'Infarto miocardico (GISI) Prevenzione Trial, in which 25% of 11300 post-myocardial infarctation patients were randomised to either n-3 fatty acids or controls in addition to the usual care, showed a statistically significant 20% reduction in total mortality and a 45% decrease in sudden death[16]. Perhaps, other mechanisms could be implied in these beneficial results. First, n-3 fatty acids improved electrical remodelling and arrhythmia induction[17]. Second, n-3 fatty acids could modify indices of heart rate variability. However, Hammad et al[18] have failed to show any effect on heart rate variability with n-3 fatty therapy. Third, n-3 fatty acid could improve peripheral markers of coagulation, endothelial function or inflammation. Thies et al suggest that n-3 fatty acids might act to stabilize advanced atherosclerotic plaques, through their antinflammatory effects[19]. Only a few studies have investigated the effects of n-3 fatty acids on plasma IL-6 and TNF-alpha levels in humans, but the results are inconsistent[20-23]. However, our present study shows a significant influence of n-3 fatty acids supplements on plasma C reactive protein and TNF alpha. This is despite medium baseline inflammatory markers in our high risk patients (diabetic patients). Perhaps, the use of n-3 fatty acids may be a new primary approach to treat hypertriglyceridaemia and inflammatory status in diabetic patients. Recently, Kabir et al[24] have demonstrated this hypothesis with beneficial effects linked to morphologic and inflammatory changes in adipose tissue.

A limitation of our study is the lack to comparation with placebo and the short duration of treatment (12 weeks). Longer trials may better characterize the long-term efficacy and tolerability of n 3 fatty acids as primary treatment in this type of patients. However, the decrease of the inflammatory state of patients with diabetes mellitus type 2 is a relevant data to taking account in our daily clinical practice. PUFAs prevent insulin resistance by increasing membrane fluidity and GLUT4 transport[25].

In conclusion, our data detected an improvement in lipid profile and antinflammatory markers in naïve patients with diabetes mellitus type 2 and hypertriglyceridaemia, with a good tolerability.

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