Effects of omega-3 fatty acid supplements on serum lipids, apolipoproteins and malondialdehyde in type 2 diabetes patients

F. Shidfar,[1] A. Keshavarz,[2] S. Hosseyni,[3] A. Ameri[4] and S. Yarahmadi[5]

تأثير المكملات المحتوية على الأحماض الدهنية أوميغا 3 على شحميات المصل، وصميم البروتين الشحمي ومالون ثنائي الألدهيد في السكريين من النمط الثاني

فرزاد شيدفر، علي كشاورز، شريعه حسيني، أحمد عامري، شهين ياراحمدي

الخلاصة: هدفت هذه الدراسة إلى اختبار ما إذا كان يمكن تحسين التحكُّم في مستوى شحميات الدم وسكر الدم في المرضى السكريين من خلال المكملات الغذائية المحتوية على الأحماض الدهنية من النمط أوميغا 3. ولقد أجريت تجربة مزدوجة التعمية مشهَّدة بالغفل، على 50 من السكريِّين من النمط الثاني، تم اختيارهم بصورة عشوائية، وأُعطوا جرعة يومية مقدارها 2 غرام من الأحماض الدهنية أوميغا 3 المنقاة أو من الغفل لمدة عشرة أسابيع. ولقد انخفضت نسبة معدل الغليسريدات الثلاثية على الريق بصورة يعتد بها لدى من أُعطوا المكملات المحتوية على الأحماض الدهنية أوميغا 3، مقارنةً بمن أُعطوا الغُفْل (P = 0.01). كما كان هناك انخفاض يعتد به إحصائياً في ApoB-100 ومالون ثنائي الألدهيد مقارنةً بالمستوى القاعدي وبالمجموعة الشاهدة. و لم يكن للأحماض الدهنية أوميغا 3 أي تأثير يُعتدُّ به إحصائياً على مستويات الشحميات أو الصميم A-1، أو الغلوكوز أو الأنسولين أو الهيموغلوبين الغلوكوزي وHbA1c.

ABSTRACT In order to test whether hyperlipidaemia and glycaemic control can be improved among diabetes patients by dietary supplementation with purified omega-3 fatty acids, we carried out a double-blind, placebo-controlled trial on 50 type 2 diabetes patients randomized to 2 g/day purified omega-3 fatty acids or placebo for 10 weeks. Fasting triglycerides decreased significantly with supplementation relative to placebo (***P*** = 0.01). There was a significant decrease in ApoB-100 and malondialdehyde compared to baseline values and compared to the control group. Omega-3 fatty acids had no significant effect on serum lipid levels, ApoA-I, glucose, insulin and HbA1c.

Effets de l'apport en acides gras oméga 3 sur les lipides sériques, les apolipoprotéines et le malondialdéhyde chez les diabétiques de type 2

RÉSUMÉ Dans cette étude, nous avions pour objectif de vérifier si l'hyperlipidémie et le contrôle de la glycémie pouvaient être améliorés chez les diabétiques par une supplémentation alimentaire à base d'acides gras oméga 3 purifiés. Nous avons réalisé un essai en double aveugle contre placebo sur 50 patients atteints de diabète de type 2 randomisés pour recevoir 2 g/jour d'acides gras oméga 3 purifiés ou un placebo pendant 10 semaines. Les triglycérides à jeun ont diminué de manière significative avec la supplémentation, par rapport au placebo (p = 0,01). On a observé une réduction importante de l'apoB-100 et du malondialdéhyde par rapport à la valeur de référence et au groupe témoin. Les acides gras oméga 3 n'avaient pas d'effet significatif sur les niveaux de lipides sériques, d'apoA-I, de glucose, d'insuline et de $HbA1_c$.

[1]Department of Nutrition; [4]Department of Health, School of Health, Iran University of Medical Sciences, Tehran, Islamic Republic of Iran (Correspondence to F. Shidfar: farzadshidfar@yahoo.com).
[2]Department of Nutrition, School of Health; [5]Department of Endocrinology, School of Medicine, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran.
[3]Department of Chemistry, Azad Islamic University, Sari, Islamic Republic of Iran.
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Introduction

The prevalence of type 2 diabetes has increased greatly in the past few years, and an even greater increase is foreseen in the next few years [*1*]. For this reason, the disease is considered to be one of the epidemics of the new century.

Cardiovascular disease (CVD) accounts for up to 80% of mortality in persons with type 2 diabetes, with the age-adjusted relative risk of death due to CVD being 2–4-fold higher than in the general population [*2,3*]. Patients with type 2 diabetes are predisposed to increased platelet aggregation [*4,5*], impaired fibrinolysis [*6,7*], endothelial dysfunction [*8,9*], increased platelet activation [*10,11*], hyperlipidaemia and insulin resistance [*1–3*]. This increases their risk of atherothrombosis, an important process in the determination of morbidity and mortality from coronary heart disease (CHD).

There is considerable evidence for a protective effect of dietary omega-3 fatty acids in the prevention of heart disease [*12–15*], especially in a high-risk population [*16,17*]. Omega-3 fatty acids improve dyslipidaemia [*2,18–20*], vascular [*2,8,11*] and platelet function [*2,11,20*], and heart rate [*19,21*]. They lower blood pressure in hypertensive patients [*18,20*] and can reduce the risk of sudden cardiac death [*20,22*]. In addition, insulin sensitivity has been positively correlated with concentration of omega-3 fatty acids in skeletal muscle [*23*]. Therefore, an increased intake may be of particular benefit to type 2 diabetes patients [*2,11,13*].

Some reports have raised the possibility of an adverse effect of high doses of omega-3 fatty acids on glycaemic control in type 2 diabetes patients [*20,24–26*]. The results of other studies indicated that omega-3 fatty acids do not seem to have any effect on glycaemic control [*22,23*], at least in humans [*19,27,28*], or even mentioned favourable effects on glycaemic control [*13,22,29*].

The importance of this relates to evidence that the level of glycaemia in type 2 diabetes patients predicts all-cause and CVD mortality [*30*], and that intensive blood glucose control decreases the risk of microvascular complication [*31*]. Consequently, some experts urge caution in the use of omega-3 fatty acids in type 2 diabetes patients [*20,24–26*] whereas others encourage such use, albeit at doses < 3 g/day [*13,32,33*].

On the other hand, a concern remains with respect to the potential for increased lipid peroxidation following omega-3 fatty acid intake [*2,21,34*]. To date, the *in vivo* data are inconclusive, owing primarily to limitations in the methodologies employed. Some previous data do not support the literature suggesting adverse effects of omega-3 fatty acids on lipid peroxidation [*2,21,35*].

In view of the increasing use of omega-3 fatty acids in the diet as food additives or as therapeutic substances, it is important to determine the extent of any effects, and possible benefits have to be weighed against the potential for impairment of glycaemic control and lipid peroxidation in type 2 diabetes patients.

The aim of this study was to determine the effects of purified omega-3 fatty acids on serum lipoproteins, apolipoprotein B-100 (Apo B-100), apolipoprotein A-I (Apo A-I), malondialdehyde (MDA) (as index for lipid peroxidation), and glycaemic control in patients with type 2 diabetes.

Methods

Participants

We recruited non-smoking, treated hypertensive, type 2 diabetes patients (28 men and 28 women) aged 33–75 years from the endocrine clinic of Shariati Hospital,

Tehran, Islamic Republic of Iran. The study was carried out over the period October 2004–February 2005; during this time there were a total of 56 patients who met the selection criteria.

Patients had been diagnosed with diabetes within the previous 6 years. All had been on antihypertensive therapy for a minimum of 3 months and showed evidence of diabetes (fasting glucose ≥ 126 mg/dL or 2-hour postprandial glucose ≥ 200 mg/dL before diagnosis and treatment) [*9*]. Patients were included if they were taking oral hypoglycaemic agents but not insulin. All patients had HbA1c < 9%, body mass index < 30 kg/m^2, clinic systolic blood pressure > 115 mmHg and < 180 mmHg, and diastolic blood pressure < 110 mmHg, serum total cholesterol and triacylglycerol ≥ 200 mg/dL. None ate > 2 fish meals per week nor were they regular consumers of fish oil supplements [*11,18*]. Patients were excluded if they had a recent (within previous 3 months) or past history of symptomatic heart disease; myocardial infarction; angina pectoris or stroke; surgery; liver, renal (plasma creatinine > 1.62 mg/dL) or thyroid disease; or used nonsteroidal anti-inflammatory drugs, estrogen, progesterone or antioxidant vitamins. Patients on lipid-lowering drugs and aspirin were included, but were asked not to change the dose [*9,11,18*].

The ethics committee of Tehran University of Medical Sciences approved the study and all participants gave written informed consent.

Study design

This study was done as a double-blind, placebo-controlled trial of parallel design. During a 3-week baseline period, participants continued their usual diet. They were stratified by sex, serum total cholesterol (TC) and triacylglycerol (TG), and randomly assigned to 2 g/day purified omega-3 fatty acids or placebo (control) capsules (supplied as 1-g capsules) (Super EPA 2000, Advanced Nutritional Technologies, United States of America) for 10 weeks. The purified omega-3 fatty acids capsules contained only 520 mg eicosapentaenoic acid and 480 mg docosahexaenoic acid. The placebo capsules contained 300 mg saturated fatty acids, 100 mg monounsaturated fatty acids, and 600 mg linoleic acid [*21,36*].

All patients were asked to maintain their usual diet and physical activity level and not to alter their lifestyle during the intervention. Dietary intake was monitored by the same dietitian throughout the study and participants were asked to complete a 24-hour diet recall questionnaire at the beginning and after 3, 6 and 10 weeks and a lifestyle questionnaire (e.g. physical activity, income) at baseline and at the end of the 10 weeks intervention. The patients were followed up by telephone each week; patients who had no phone were instructed to return to the clinic every other week. Participants were required to provide venous blood samples after fasting overnight for 12–14 hours on day 0 and at the end of the intervention (10th week).

Laboratory analyses

Fasting serum lipoproteins, Apo B-100, Apo A-I, MDA, glucose and insulin were measured twice at baseline and twice at the end of intervention. All samples were collected while the patient rested in a supine position for 10 minutes and all analyses were carried out within 12 hours of collection. Samples were stored at –3 °C in the interim. There was no preservative in the glucose samples. Serum glucose was measured with a Cobas MIRA analyser (Roche Diagnostic, Basel, Switzerland) by enzymatic method (MAN Co., Tehran). Serum insulin was measured by radioimmunoassay with an automated immunoassay analyser (Tosoh Corporation, Tokyo). HbA1c was measured by colorimetric method (MAN Co.).

Serum lipoproteins, Apo B-100 and Apo A-I were assayed with a Cobras MIRA analyser (Roche Diagnostics). TC and TG levels were measured enzymatically with the triacylglycerol GPO-PAP-cholesterol CHOD-PP kit (MAN Co.). Serum high-density lipoprotein cholesterol (HDL-C) was determined enzymatically using the CHOD-PAP kit after precipitation of the chylomicrons, very low density lipoprotein cholesterol and low density lipoprotein cholesterol (LDL-C) with phosphotungstic acid and Mg^{+2}. Serum LDL-C was determined enzymatically using the CHOD-PAP kit after precipitation of LDL with heparin and sodium citrates, from the following formula: LDL-C = TC – cholesterol in supernatant.

Serum Apo A-I and Apo B-100 were measured immunoturbidometrically (Pars Azmun Co., Tehran,). Serum MDA was measured colorimetrically using thiobarbiruric acid (TBA) reagent (Daiichi Pure Chemical Co. Ltd, Tokyo) dissolved in 2M sodium sulfate by heating with 1,1,3,3-tetraethoxy-propane (Tokyo Kasei Co. Ltd, Tokyo) as a standard solution [*37*].

The intra-assay coefficients of variation for these assays (n = 10) were 1.4%, 1.4%, 1.3%, 1.4%, 2.18%, 3.6%, 1.9%, 1.9% and 2.3% for TC, HDL-C , LDL-C, TG, ApoB-100, ApoA-I, MDA, glucose and insulin respectively and the inter-assay coefficients of variation (n = 10) were 0.9%. 1.1%, 0.95%, 1.84%, 2.35%, 2.1%, 2.1%, 2.2% and 2.2% respectively. Accuracy (sensitivity and specificity) was assessed for the analytes (e.g. sensitivity for Apo A-I and Apo B-100 were 0.2 and 0.3 mg/dL respectively). We used a Randox serum control as a quality control material [*18*].

Statistical analyses

All data are expressed as mean (standard deviation). The level of significance chosen was $P < 0.05$. The normal distribution of the variable was checked by Kolmogorov–Smirnov test. In order to test whether the differences between the mean values of the items studied in both groups were significant, the Student t-test was used. Differences in the same hyperlipidaemic patient before and after 10 weeks of intervention were evaluated by paired t-tests; diet records were analysed using *Food processor II* software [*38*]. For comparison of means in different intervals of 24-hour recall questionnaires, analysis of variance was used. For qualitative variables (e.g. education, occupation, income), chi-squared test was used. Data were analysed using *SPSS*, version 10.

Results

Only 50 of the 56 randomly assigned patients completed the study. Baseline characteristics of the patients confirmed that they were well matched for the inclusion criteria (Table 1). There were no significant differences between the groups in type and number of antihypertensive or oral hypoglycaemic medications. Evidence of adherence to the diets came from analysis of diet records and capsule count.

There were no significant differences between the groups in total energy intake, nutrient intake or body weight at baseline, and no significant changes took place during the intervention.

Relative to placebo, omega-3 fatty acids had no significant effects on fasting glucose, insulin or HbA1c levels (Table 2).

There were no significant differences in fasting serum lipoproteins, glucose, insulin, HbA1c, apoproteins and MDA at baseline between groups (Table 2). There were no significant changes in lipoproteins with placebo supplementation. In the omega-3 fatty acid group, fasting TG decreased significantly by 31.2 % (P = 0.01) relative to the placebo group; there was also a significant decrease at the end of the study compared

Table 1 **Baseline characteristics of patients in test and control groups**

Characteristic	Control group (*n* = 25)		Omega-3 fatty acid group (*n* = 5)	
	Mean	SD	Mean	SD
Age (years)	54.1	11.1	53.4	11.7
Weight (kg)	89.1	2.3	88.7	2.0
BMI (kg/m^2)	29.0	0.7	28.4	0.5
Waist-to-hip ratio	0.93	0.01	0.94	0.01
Male/female ratio	12/13	12/13		

SD = standard deviation.
BMI = body mass index.

to in the test group ($P = 0.002$) (Table 2). There was a significant decrease in Apo B-100 and MDA after intervention compared to baseline value ($P = 0.02$ for both) and also compared to the control group ($P = 0.03$ for both) (Table 2). At the end of the study, TG/HDL-C had significantly decreased compared to baseline levels ($P = 0.04$ for both) and also compared to the control group ($P = 0.04$) (Table 2).

Discussion

Our finding of no adverse effect of omega-3 fatty acids on glycaemic control is supported by reports of a non-significant change in fasting glucose after consumption of omega-3 fatty acids or fish oils [*1,6,19,27*]. The other reports found a significant increase in HbA1c and fasting glucose in type 2 diabetes patients after a fish diet [*38*] or omega-3 fatty acids [*6,18,20,24–26,33*]. Fish oil may worsen glycaemic control by diverting substrates from lipogenesis to gluconeogenesis in the process of inhibiting hepatic synthesis [*6,38*].

The disparate findings concerning effects on glycaemic control in type 2 diabetes patients may be related to the dose of omega-3 fatty acids (it appears > 3 g omega-3 fatty acids/day has an adverse effect) [*7,18*]; differences in oral diabetic medication; degrees of obesity and insulin resistance; the presence of other conditions such as hypertension that may also affect insulin sensitivity; and lack of diet control during intervention [*1,6,19,28*]. There was no difference in fasting insulin between groups after the intervention. This concurs with other controlled studies in type 2 diabetes patients [*1,6,7,19*], however, in hyperlipidaemic patients with hyperinsulinaemia, 3.4 g fish oil/day for 6 months caused a significant decrease in fasting insulin [*20*]. The increase in fasting glucose in other studies may be due to hepatic glucose output, which is highly correlated with the degree of fasting hyperglycaemia [*11*].

The TG-lowering effect of omega-3 fatty acids is well established [*2,18–20*] and may be related to an increase in hepatic glucose output [*18*]. An increase in peroxisome proliferator-activated receptor α with supplementation leads to increased hepatic uptake and oxidation of free fatty acids as well as increased fatty acid oxidation in skeletal muscle. The consequent decrease in free fatty acid availability would lead to decreased TG synthesis. It is conceivable that TG might influence the binding of insulin to its receptor or interfere with early post-binding steps [*18,33*]. Moreover, higher serum TG leads to a resistance to the antilipolytic effect of insulin. Therefore, a reduction in serum TG levels might improve insulin sensitivity [*1,19,28*]. We did not, however, measure insulin sensitivity, and this is one of the limiting factors of this study.

The effects of omega-3 fatty acids on lipoproteins are contradictory, these findings may be explained in part by variations in the amount of omega-3 fatty acids consumed, the manner in which they are presented (fish, fish oils or purified oils), and the lipoprotein phenotype of the patients [*21,33*]. Previous

Table 2 **Blood analysis for fasting patients in test and control groups at baseline and post-intervention**

Serum component	Control group (*n* = 25)		Omega-3 fatty acid group (*n* = 5)	
	Mean	SD	Mean	SD
Glucose (mg/dL)				
Baseline	146.1	27.7	148.2	30.2
Post-intervention	142.3	30.1	151.8	30.9
Insulin (pmol/L)				
Baseline	111.6	57.2	115.9	64.0
Post-intervention	119.7	84.5	119.7	84.5
HbA1c (%)				
Baseline	7.1	0.1	7.4	0.2
Post-intervention	7.0	0.1	7.3	0.3
ApoB-100 (mg/dL)				
Baseline	157.8	21.1	151.3	19.5
Post-intervention	152.1	13.2	134.7[a,b]	23.2
ApoA-I (mg/dL)				
Baseline	174.0	23.7	175.5	49.1
Post-intervention	167.0	21.4	176.1	40.2
MDA (nmol/mL)				
Baseline	3.3	1.1	3.1	1.4
Post-intervention	3.5	0.9	1.9[c,d]	0.7
Total cholesterol (mg/dL)				
Baseline	249.6	45.3	244.3	30.9
Post-intervention	237.7	34.6	234.2	38.1
HDL-C (mg/dL)				
Baseline	38.1	8.3	38.9	8.7
Post-intervention	37.8	9.1	37.1	10.1
LDL-C (mg/dL)				
Baseline	168.3	37.2	160.6	41.2
Post-intervention	170.1	41.2	158.1	44.0
LDL-C/HDL-C				
Baseline	4.4	1.1	4.1	2.3
Post-intervention	4.5	2.1	4.2	1.9
TG/HDL-C				
Baseline	8.0	2.1	7.6	2.9
Post-intervention	8.5	2.9	5.5e[e,f]	1.8
TG (mg/dL)				
Baseline	306.4	39.1	299.2	28.1
Post-intervention	322.0	36.5	205.1[b,g]	23.5

SD = standard deviation.

[a]***Significantly different from control group at end of study (t-test of difference scores):*** P ***= 0.03.***

[b]***Significantly different from baseline of supplemented group (paired t-test):*** P ***= 0.02.***

[c]***Significantly different from control group at end of study (t-test of difference scores):*** P ***= 0.03.***

[d]***Significantly different from baseline of supplemented group (paired t-test):*** P ***= 0.02.***

[e]***Significantly different from control group at end of study (t-test of difference scores):*** P ***= 0.04.***

[f]***Significantly different from baseline of supplemented group (paired t-test):*** P ***= 0.04.***

[g]***Significantly different from control group at end of study (t-test of difference scores):*** P ***= 0.01.***

studies showed that the TG-lowering effect of omega-3 fatty acids is greater in those with higher initial TG concentration and higher consumption of omega-3 fatty acids [*38*]. In a placebo-controlled study, 4 g omega-3 fatty acids reduced TG by 42% in comparison with 31.2% in our study [*33*]. We observed no significant effect of omega-3 fatty acids on TC. In contrast, Grimsgaard et al. [*39*] reported an increase in TC with omega-3 fatty acids. The difference may have been related to differences in the baseline serum lipoprotein concentration of the type 2 diabetes patients.

The significant decrease of Apo B-100 at the end of study indicates a decrease in the number of LDL and VLDL particles. This might be expected to contribute to a reduction in atherogenic risk in people with type 2 diabetes [*15,32,38*]. Both TG and HDL-C are major determinants of LDL particle size [*20,29,38*] and the TG/HDL-C ratio is a stronger predictor of myocardial infarction than LDL-C/HDL-C in type 2 diabetes patients [*6,19,20,25*]. TG/HDL-C was significantly lower at the end of study compared to placebo ($P < 0.05$). This is associated with decreased risk of coronary heart disease and preventing the transition from atherosclerosis to atherothrombosis [*19,20,24*].

Omega-3 fatty acid supplementation leads to a significantly lower level of MDA compared to the placebo group, which had good clinically relevant aspects. Potential mechanisms for the decrease in MDA may relate to the assembly of omega-3 fatty acids in membrane lipids and lipoproteins making the double bonds less available for free radical attack, inhibition of the pro-oxidant enzyme phospholipase A2 and stimulation of anti-oxidant enzymes [*2,21,37*]. In this regard, omega-3 fatty acids upregulate gene expression of antioxidant enzymes and down regulate genes associated with production of reactive oxygen species [*35,37*]. Consequently, the data relating to the effects of omega-3 fatty acids on MDA *in vivo* are contradictory [*34*]. These inconsistencies could be related to differences in the population studied, the duration of the study, the antioxidant content of the supplement or background diet. The most plausible explanation, however, is differences in the methodologies employed to assess lipid peroxidation [*2,21*]. Sample size in these studies was rather small, so extrapolating the conclusions to the general population may not be valid in all cases.

Our findings suggest that 2 g/day purified omega-3 fatty acids offer substantial advantages on prevention of CVD in type 2 diabetes patients. In view of their overall favourable effects on CVD reduction, there appears to be no reason why omega-3 fatty acids should not be given to type 2 diabetes patients and these finding suggest that regular fish consumption should be considered as part of a healthy diet for diabetes management.

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