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Original Article

Effects of Omega-3 Fatty Acids Supplement on Antioxidant Enzymes Activity in Type 2 Diabetic Patients

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Abstract

Background: Diabetes is a major cause of death. Oxidative stress mainly caused by hyperglycemia is the primary reason of related complications. Omega-3 fatty acids are prescribed in diabetes but the effect on antioxidant defense is controversial. This study investigated effects of omega-3 supplementation on antioxidant enzymes activity in type 2 diabetic patients.

Methods: A randomized, placebo controlled, double blind clinical trial was performed on 90 type2 diabetic patients. The treatment group took, daily, three capsules of omega-3 for two mo, which totally provided 2714mg omega-3 (EPA=1548 mg, DHA=828 mg and 338 mg of other omega=3 fatty acids). Placebo contained 2100 mg sunflower oil (12% SFA, 65% linoleic acid, 23% MUFA), which is the main oil used in the study population. Food intakes, anthropometric and demographic characteristics, and therapeutic regimen data were recorded before and after the intervention. Fasting blood samples were taken before and after the intervention to measure super oxide dismutase, glutathione peroxidase, glutathione reductase, catalase and total antioxidant capacity in erythrocytes.

Results: A total of 81 subjects completed the study. Two study groups were similar as regards duration of diabetes, age and the enzymes at baseline. Energy and macro- and micronutrients intakes, weight and hypoglycemic agent consumption were similar in the two groups at baseline and did not change. Supplementation had no effect on antioxidant enzyme status. Glycated hemoglobin showed a significant reduction by supplementation.

Conclusion: Daily supplementation of 2714 mg mega-3 for two mo results in a significant reduction in HbA1c level in type2 diabetic patients with no effects on antioxidant enzymes activity.

Keywords: Type 2 diabetes, Omega-3 supplement, Antioxidant enzymes

Introduction

Chronic diseases account for two-third of deaths in the world (1). The major chronic diseases are cardiovascular diseases, cancer, chronic respiratory disease, and diabetes (2). Diabetes mellitus is known as one of the major causes of death (3), and could lead to cardiovascular diseases and cancer (4). Estimations suggest that 285 million people were affected with diabetes in 2010 which will increase to 439 million in 2030 (5).

Diabetes is associated with increased oxidative damage mainly due to hyperglycemia (3), which could be the main contributors to cardiovascular diseases, cancer and diabetes itself (4). Antioxidant defense includes enzymatic and non-enzymatic path ways. There are quite a number of

non-enzymatic antioxidants, namely vitamins (A), ascorbic acid, tocopherol, enzyme cofactors (Q10), nitrogen compounds (uric acid), and peptides (glutathione) (6). Common enzymatic strategies are super oxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (7). Oxidative enzymes activity is changed in diabetes, which could be a target for antioxidant therapy (8).

Long chain omega-3 fatty acids may reduce mortality in diabetic patient's trough reduction of blood triglycerides, platelet aggregability and improving endothelial functions (9). As omega-3 PUFAs decrease blood triglyceride and fatty acids (10, 11), increasing some oxidant cleavage pathways, they maybe act as antioxidants (12). Whether omega-3 PUFAs can reduce the oxidative damage is not clearly understood; moreover, some studies believe that they will increase pro-oxidative conditions (13).

This research project studied the effect of omega-3 supplementation on antioxidant enzymes including SOD, GPx, GR, catalase, and total antioxidant capacity (TAC) in erythrocytes of diabetic patients.

Materials and Methods

A randomized, placebo controlled, double blind clinical trial was performed on 90 type2 diabetic patients. Subjects were members of the Iranian Diabetic Association diagnosed as diabetic by the Association's physician. The inclusion criteria were not taking omega-3 or other nutritional supplements; not taking insulin; absence of renal, hepatic, and cardiovascular diseases or cancer. Data on anthropometric measurements and 24-hour recalls was taken by a nutritionist before and after treatment. A questionnaire was designed to obtain demographic information and medical and therapeutic regimen history, and fulfilled by face-to-face interview at baseline and at the end of the study. Fasting blood samples (after 12-14 hours of overnight fasting) were taken before and after the intervention between 8 and 10 a.m. before taking any hypoglycemic drugs.

Based on the results of previous studies on omega-3 supplementation on diabetic patients, a dosage of 2714 mg per day was prescribed for two mo (14). It was provided in the form of three capsules, one with every meal. Capsules were made in PBL company, US and totally provided 2714mg (EPA=1548 mg, DHA=828 mg and 338 mg of other omega=3 fatty acids) per day. Placebo was provided especially for our study by Zakaria Company, Iran. It contained 2100 mg sunflower oil (12% SFA, 65% linoleic acid, 23% MUFA), which is the main oil used in the study population (15).

Twenty-four-hour dietary recall data were analyzed by Food Processor ver.2 software. The subjects were asked periodically if they were following study protocols and if there were any changes in food habits, physical activity or therapeutic regimen. Changes would result in exclusion from the study, but no changes were reported.

Biochemical analysis

Red blood cells were separated by centrifugation at 3000 rpm for 10 min and washed with 9 g/l NaCl solution for three times. The hemolysates were stored at -70 °C for testing for enzymes. Catalase activity was measured by Abei method (16). Glutathione peroxidase was determined by paglia method (17) and glutathione reductase by the method of Goldberg (18). The superoxide dismutase (SOD) activity in the red blood cells was measured with a RANDOX SOD kit using the manufacturer's instructions (RANDOX Laboratories, Antrim, UK). Total antioxidant activity was tested based on TAC method (19). Glycated hemoglobin was determined by D-10 kits.

Statistical analysis

Normality was assessed by Kolmogrov-Smirnov test and configuring histogram chart. All values are expressed as mean±S.D. Differences between the two groups were tested by independent sample T-test before and after intervention. Differences within a group before and after intervention were analyzed by paired sample *t*-test. A *P*-value<0.05 was considered to be statistically significant. All data were analyzed using the SPSS 11.5 software (Chicago, IL, USA).

Ethical consideration

The study protocol was approved by Ethics Committee on Human Experimentation of Tehran

University of Medical Science. Written informed consent was fulfilled by the all participants.

Results

Nine out of 90 subjects did not follow the study but none of them due to complications related to the supplement**.** Therefore, 81 individual with a mean age of 54.83 ± 10.05 years completed the study. There were 41 subjects in the intervention group, including 21 females and 20 males. The control group consisted of 40 subjects, 22 females and 18 males. The two study groups were similar as regards duration of diabetes, age and the enzymes at baseline (Table 1, 2). Energy and macro- and micronutrients intakes were similar between the two groups at baseline and did not change (Table 3).

Glycated hemoglobin showed a significant reduction in the intervention group (7.9±0.2 vs. 7.25±0.17, *P*<0.00), and a significant increase in controls (7.64±0.2 vs.7.84±0.2, *P*<0.02) (20). SOD, GPX, GR, catalase and TAC did not change significantly in either group (Table 2).

Table 1: Characteristics of participants at baseline

Groups	Age (yr)	Duration of diabetes (years)	Metformin (mg/day in users) (% of group using it)	glibenclamide (mg/day in users) (% of group using it)
Treatment group (n=41)	56.38±9.24	8.72±3.4	2.06± 0.2(55)	2.51± 0.4(51)
Control group (n=40)	52.7±10.65	8.02±2.9	2.44± 0.3(51)	2.71± 0.5(49)
P value	NS	NS	NS	NS

Data are expressed as mean±SD
Statistical test was independent t-test (*P*<0.50)

Table 2: Antioxidant enzymes activity of participants before and after treatment

Variable	Treatment group (n=41)			Control group (n=40)		
	Before	After	*P* value	Before	After	*P* value
TAC (mg/dl)	3.62±0.1	3.79±0.1	0.107	3.66±0.1	3.78±0.1	0.295
SOD (u/gHb)	417.16±11.9	449.22±14.1	0.66	447.58±20.6	460.23±20.0	0.608
Catalase(k/gHb)	150.72±9.6	151.86±12.6	0.941	180.42±14.3	177.41±13.8	0.858
GPx(u/gHb)	20.85±1.68	19.23±6.18	0.413	19.7±2.2	18.16±1.7	0.505
GR(u/gHb)	28.51±2.05	27.87±1.5	0.749	48.79±15.8	43.68±14.5	0.068
HbA_1c (%)	7.90±0.2	7.25±0.17	0.00	7.64±0.2	7.84±0.2	0.02

Data are expressed as mean±SD.
Independent *t*-test did not show any significant difference between two groups at baseline (*P*<0.05)
There were no changes in antioxidant enzymes status after treatment within groups using paired t-test (*P*<0.05)

Discussion

Daily supplementation of 2714 mg mega-3 for two months resulted in a significant reduction in HbA1c level in type2 diabetic patients with no effects on antioxidant enzymes activity. Dietary components, weight, physical activity and therapeutic regimen did not change during the study, which means that any absorbed effects were solely due to omega-3 supplements. However, we did not assess omega-3 status before and after supplementation.

Table 3: Weight and nutritional intakes of participants before at after treatment

Variable	Treatment group (n=41)		Control group (n=40)	
	Before	After	Before	After
Weigh (kg)	79.62±3.6	79.12±3.7	73.48±2.8	73.00±2.8
Energy (kcal)	1612.88±270.2	1718.65±407.33	1703.77±352.91	1793.92±423.12
Carbohydrate (g)	322.61±46.9	326.88±68.6	338.50±68.01	341.09±54.2
Protein (g)	93.5±16.3	87±12.6	89.07±11.8	86.9±13
Fat (g)	26.1±21.44	27.56±20.7	23.6±17.06	22.88±25.8
Saturated fatty acids(g)	9.6±7.09	9.8±6.9	9.01±5.3	9.9±4.6
polyunsaturated fatty acids(g)	7.23 ±5.9	7.19±5.9	7.50±5.4	7.06±4.7
Vitamin A (RE)	722.46±12.07	554.13±121.3	867.66±423.7	292.57±35.4
Vitamin C (mg)	91.32±15.6	101.35±20.7	81.52±15.2	83.10±14.8
Vitamin E (mg)	4.95±0.6	5.6±0.8	4.17±1.9	4.18±0.4
Selenium (mcg)	117.6±8.0	112.66±10.2	138.77±15.2	127.11±11.3
Zinc (mg)	8.1±0.45	7.76±0.6	8.98±0.5	9.21±0.7

Data are expressed as mean±SD.
Independent *t*-test did not show any significant difference between two groups at baseline ($P<0.05$).
There were no changes after treatment within groups using paired t-test ($P<0.05$)

Certainly, such assessment would have helped to a deeper understanding of the effects of omega-3 supplements on antioxidant enzymes activity. However, omega-3 fatty acids intakes are known to be low in the studied population and are mainly from vegetable oils. Fish and long chain omega-3 fatty acids intakes, too, are lower than recommendations (21).

Hyperglycemia brings about glucose auto-oxidation and protein glycation. Advanced glycation end products interact with their receptors on macrophages, resulting in increased production of oxygen-free radicals which leads to oxidative stress and its related diseases (22). This oxidative stress may further lead to increased activity of GPx and GR but the activity of SOD will decrease. However, changes in activity level of erythrocyte antioxidant enzymes are controversial and to some extent depend on the duration of diabetes and the glycemic control by increasing fluidity of membrane and GLUT4 (23-25). Diabetes is related with oxidative stress followed by harmful effects on antioxidant defenses. Therefore, the oxidant/ antioxidant balance will deteriorate in diabetic patients (23). Glycemic control is associated with antioxidant enzymes activity in diabetic patients and could be used as a marker of glycemic control (3, 24). However, Pearson correlation test did not show any relation between glycemic controls measured by HbA_1c with non-antioxidant enzyme status in none of the studied groups before and after intervention. However, it should be mentioned that our sample size was not large enough to assess this relation.

Omega-3 supplement in some studies leads to increased antioxidant activity, but when supplementation continued for longer periods (26). Moreover, it may improve inflammatory markers in diabetic patients (27). Some researchers believe that it will decrease NO production resulting in suppressing inflammation (28). On the other hand, some studies have shown higher oxidative stress after EPA supplementation. However, in those studies, doses were higher (2g EPA) and subjects were athletes (29).

On the whole, it is believed that omega-3 supplementation in diabetic patients is advisable, which also decreases triglycerides levels and probably would not deteriorate glycemic control and oxidative stress (9).We achieved the same results, as triglyceride level decreased without any significant changes in total cholesterol, LDL and HDL (30). However, the LDL cholesterol should be monitored carefully (9).

Conclusion

Omega-3 supplementation in type2 diabetic patients has no effect on antioxidant enzymes activity in diabetic patients, although it results in a significant reduction in HbA1c level.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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