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Original Article

The effect of Omega-3 fatty acids on serum paraoxonase activity, vitamins A, E, and C in type 2 diabetic patients

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Abstract

BACKGROUND: Diabetes mellitus is a heterogeneous metabolic disorder characterized by hyperglycemia. Studies showed paraoxonase activity, and vitamin C and A levels are decreased in diabetes. The effect of omega-3 fatty acids on serum paraoxonase activity and vitamins A, E, C in patients with type 2 diabetes is not fully understood. This study aimed to determine the effect of omega-3 fatty acids on paraoxonase activity, vitamins C, A and E levels in type 2 diabetic patients.

METHODS: In a double-blind, placebo controlled trial, 80 type 2 diabetic patients were randomly enrolled into the study. Study subjects received daily 2714 mg of omega-3 fatty acids or placebo for 8 weeks. Ten milliliter fasting blood was collected before and after treatments. Serum paraoxonase activity and vitamin C levels were measured by spectrophotometry. Vitamin A and vitamin E were measured using high performance liquid chromatography. Nutrient intake was estimated using 24-hours dietary recall questionnaire (for 2 days) before and after treatments. Dietary data were analyzed using FPII. To compare the means of variables between the two groups, independent t-test was employed. Differences between variables before and after interventions were calculated using paired t-test.

RESULTS: Serum levels of paraoxonase activity were significantly increased after omega-3 intake (126.47 IU/ml *vs.* 180.13 IU/ml). However, omega-3 intake caused no significant change in serum vitamin A, C, and E.

CONCLUSIONS: Supplementation of omega-3 fatty acids was found to increase paraoxonase activity in diabetic patients.

KEYWORDS: Paraoxonase, Diabetes mellitus, Vitamin C, Vitamin A, Vitamin E.

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Diabetes is a complex metabolic disease characterized by high blood glucose level.[1] Its macrovascular complications include atherosclerosis, and its microvascular complications include damage to the kidney, nerves and eyes.[2] Oxidative stress is increased in diabetes,[3] therefore, it is considered as a cause of development of atherosclerosis.[4] In diabetic patients, atherosclerosis is one of the most important causes of mortality.[5] Human paraoxonase (PON-1) which is an antioxidant (via scavenging of free radicals),[6] is synthesized in the liver and secreted into blood, where it is associated with high density lipoprotein (HDL) levels,[7] and serum paraoxonase levels are inversely related to the risk of cardiovascular disease.[8,9] Some studies found that PON-1 activity is decreased in cardiovascular and diabetes mellitus.[10,11]

Vitamin C is an important antioxidant in human,[12,13] however, several studies showed vitamin C level in diabetic patients is decreased.[14,15] Vitamin A is a hydrophobic antioxidant [16,17] which is decreased in diabetes causes retinopathy.[18] Also, vitamin E is a hydrophobic antioxidant which is found in membranes as well as lipoproteins and can afford primary as well as secondary stage protection

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against ROS activity.[12,19] Many studies showed the association between intake of omega-3 fatty acids and decreased risk of coronary heart disease[20,21] and its mortality.[21] Studies with evaluation of the metabolic effect of omega-3 fatty acids on serum vitamins A, E, C in patients with type 2 diabetes are rare in literature. In addition, the metabolic effect of omega-3 fatty acids in patients with type 2 diabetes is not fully clear. There is also no evidence about the effect of omega-3 fatty acids in patients with type 2 diabetes in Iranian population. The aim of this study was to determine the effect of omega-3 fatty acids on paraoxonase activity, as well as the levels of vitamin A, C and E in Iranian diabetic type 2 patients.

Methods

Study design

The present study was a randomized double blind placebo-controlled study financially supported by Tehran University of Medical Science (research project number: 2296). Study subjects included 80 type 2 diabetic patients (aged 45-80 years) with at least 2 years of diagnosed diabetes since onset of the condition. The subjects had a written consent to enroll into the study from Iranian Diabetes Association, Tehran, Iran using written announcement. They were divided randomly into two groups (experiment and control, n = 41 and n = 40, respectively) using computer's random numbers. Subjects had their routine medication regimens. Patients did not take omega-3 fatty acid supplements and/or medications interacting with lipid profile, did not have hepatic or renal disease, and had no history of cancer or myocardial infarction. Each patient received 3 capsules per day for 8 weeks. The dose of the supplements was chosen from other studies [22]. The supplement was obtained from PBL Company, US. Omega-3 fatty acid dose was 2714 mg (EPA= 1548 mg, DHA= 828 mg and 338 mg of other omega-3 fatty acids) via 3 gel capsules. Placebo consisted of 3 capsules containing 2100 mg sunflower oil (12% SFA, 65% linoleic acid, and 23% MUFA). Placebo capsules looked identical and were prepared for this study by Zakaria Inc., Tehran, Iran.

Data collection

Anthropometric data, medical and drug history were obtained via face-to-face interviews. Ten milliliter blood was collected from each patient after 12-14 hours overnight fasting, before taking oral hypoglycemia drugs, between 8-10 a.m., at the beginning and at the end of 8 weeks. Fasting blood sugar was measured. Paraoxonase (PON-1) activity and vitamin C were measured by spectrophotometry; and vitamin E and A were measured using HPLC. Daily nutrient intakes were estimated using 24-hour dietary recall questionnaires at the beginning and at the end of the study for 2 days. Dietary data were analyzed using Food Processor version 2 software. The patients were asked not to change their usual diets, activity and medications throughout the study.

Statistical analysis

Differences between the two groups of supplemented and control groups were calculated using independent t-test. Differences between variables before and after interventions were calculated using paired t-test. Statistical analyses were performed using SPSS 11 (compatible with Microsoft Windows). The results are expressed as mean ± SD (standard deviation). For statistical evaluations, P value of less than 0.05 was considered as a significant change.

Results

The clinical and demographic characteristics of subjects are shown in Table 1. Participants in case or control groups had equal age, sex, BMI, glucose and duration of diabetes at the beginning of the study ($P > 0.05$). As shown in Table 2, energy and nutrient intake before and after intervention were also equal. Table 3 shows that there was no significant difference in serum paraoxonase activity between the two groups before and after intervention, but after 8 weeks of omega-3 supplementation, serum paraoxonase activity was increased compared to its activity before treatment ($P=0.002$). Table 4

Table 1. Demographic and clinical characteristic of the study groups

Variables	Experiment n=41	Control n=40	P-value
Age (years)	56.47 ± 9.24	52.7 ± 10.65	0.86
BMI (kg/m^2)	27.78 ± 3.41	28.09 ± 5.03	0.28
Duration of diabetes (years)	8.76 ± 2.76	8.02 ± 3.07	0.89
Blood glucose levels (mg/dl)	170 ± 43.98	176 ± 32.09	0.76
Blood vitamin A levels (µg/dl)	1.56 ± 0.05	1.51 ± 0.05	0.98
Blood vitamin E levels (µg/dl)	15.98 ± 1.23	14.23 ± 0.81	0.39
Blood vitamin C levels (mg/dl)	0.48 ± 0.01	0.47 ± 0.01	0.24

Data are expressed as means ± SD
Independent t-test was used.

presents the serum concentration of vitamins (A, E and C) in subjects before and after supplementation. At the beginning of the study, 2 groups were similar by the means of vitamins level. Following 2 months of supplementation, serum levels of vitamins A, E, C were not significantly changed in the case group.

Table 2. Comparison of dietary intake of macro- and micro-nutrients between two groups of study

Dietary Intake	Experiment n = 41	Control n = 40	P-value
Total calorie:			
Before intervention	1342.48 ± 170.84	1368.77 ± 343.71	0.98
After intervention	1319.45 ± 576.38	1371.62 ± 313.92	0.76
Total fat (g/day):			
Before intervention	45.09 ± 12.35	46.07 ± 13.85	0.85
After intervention	43.64 ± 12.96	43.91 ± 13.08	0.94
Polyunsaturated fatty acids (g/day):			
Before intervention	7.15 ± 4.91	7.61 ± 4.49	0.43
After intervention	7.13 ± 5.74	7.46 ± 4.79	0.34
Cholesterol (g/day):			
Before intervention	160.13 ± 1.57	156.97 ± 6.67	0.67
After intervention	154.69 ± 1.37	154.04 ± 6.87	0.59
Fiber (g/day):			
Before intervention	18.63 ± 2.15	22.82 ± 2.79	0.82
After intervention	21.58 ± 1.28	21.58 ± 2.32	0.98
Vitamin A (RE):			
Before intervention	722.64 ± 120.15	822.82 ± 422.53	0.78
After intervention	554.14 ± 122.16	659.82 ± 385.81	0.59
Vitamin E (mg):			
Before intervention	4.94 ± 0.55	3.52 ±32.13	0.33
After intervention	5.64 ± 0.86	4.18 ± 43.81	0.34
Vitamin C (mg):			
Before intervention	94.54 ± 15.07	83.66 ± 31.09	0.78
After intervention	101.35 ± 20.86	85.49 ± 14.93	0.94

Data are expressed as means ± SD.
Independent t-test was used.

Table 3. Paraoxonase (arylesterase) (IU/mL) activity before and after 2 months omega-3 supplementation in type 2 diabetic patients

Paraoxonase (IU/ml)	Before intervention	After intervention	P-value (In each group)
Experiment	126.47 ± 9.07	180.13 ± 12.2	0.002
Control	154.78 ± 9.51	162.2 ± 11.63	0.054
P-value (Between groups)	0.164	0.125	

Data are expressed as mean ± SD.
Paired t-test was used.

Table 4 presents the serum concentration of vitamins (A, E and C) in subjects before and after supplementation. At the beginning of the study, two groups were similar by the means of vitamin levels. Following 2 months of supplementation, serum levels of vitamins A, E, C were not significantly changed in the case group.

Discussion

It is believed that oxidative stress plays an important role in the pathogenesis of type II diabetes.[23] Many studies on diabetes mellitus have showed that free radicals can cause chronic complications such as cardiovascular disease.[24] Paraoxonase protects low density lipoprotein (LDL) and high density lipoprotein (HDL) against oxidative modification.[25] It is believed that the oxidation of LDL is the first stage of development of atherosclerosis.[26] Mackness and Karabina found that paraoxonase activity decreases in cardiovascular disease as well as in diabetes mellitus.[10, 11] Omega-3 fatty acids are a class of polyunsaturated fatty acids that found in fish and fish oils.[27] Omega-3 fatty acids from seafood and plant sources can reduce coronary heart disease.[28] Our previous studies showed that omega-3 PUFA can decrease the inflammation in a cell culture model.[29] The possible mechanism of anti-inflammatory effect of omega-3 PUFA is the decreased levels of arachidonic acid inflammatory pathway in the cells.[30] However, it is believed that these fatty acids has no antioxidant activity and increase the utilization of the antioxidants.[30] In our study, it is found that paraoxonase activity increased following omega-3 supplements. The changes in paraoxonase activity were observed after 8 weeks of supplementation of omega-3 by Calabresi.[31] In other studies, it has been found that plasma concentration of HDL-bound antioxidant paraoxonase enzyme increased by 10% after omega-3 supplementation [31] which is in the same track of our study.

Table 4. Levels of vitamin A, E (μmol/L) and vitamin C (mg/dl) before and after 2 months omega-3 supplementation in type 2 diabetic patients

Vitamin	Before intervention	After intervention	P-value
Vitamin A (μmol/L):			
Experiment group	1.59 ± 0.47	1.63 ± 0.04	0.21
Control group	1.54 ± 0.05	1.58 ± 0.06	0.58
Vitamin E.(μmol/ L):			
Experiment group	15.83 ± 0.71	17.26 ± 0.78	0.39
Control group	14.19 ± 1.10	15.43 ± 0.83	0.71
Vitamin C (mg/dL):			
Experiment group	0.48 ± 0.01	0.51 ± 0.01	0.27
Control group	0.47 ± 0.01	0.46 ± 0.02	0.72

Data are expressed as mean ± SD.
Paired t-test was used.

Increased oxidative stress in diabetes could contribute to depletion of antioxidants.[32] Several studies showed decreased basal vitamin C levels (a potent water phase antioxidant) in diabetic patients.[33, 34] There are conflicting reports regarding plasma vitamin concentrations in diabetes mellitus. Merzouk found no alteration in the levels of serum vitamin C, whereas the levels of vitamin A and E were lower in type I and type II diabetes mellitus patients with or without complications than in control subjects.[35] Studies also reported that plasma vitamin C and E in type II diabetic patients were not significantly different from those in control groups, while vitamin A levels were significantly lower.[36, 37] On the other hand, Sundaram reported low levels of vitamin E and C in diabetic patients.[38] Basu showed that the concentration of serum vitamin A and its binding protein, RBP, were lower in patients with diabetes than in normal control subjects.[39] The present study demonstrated no significant change in vitamin A, C and E after consumption of omega-3 fatty acids. Zhou demonstrated that α-linolenic acid deficiency decreased DHA and elevated Osbond acid (OA) levels in tissue membranes, and dietary vitamin A deficiency had an important effect on membrane DHA and OA in rat tissues.[40, 41] However, the effect of omega-3 on vitamin A, C and E in diabetic patients has not been studied recently.

Conclusion

The present study demonstrated for the first time, that there was no significant change in vitamin A, C and E after consumption of omega-3 fatty acids. We concluded that omega-3 fatty acid supplementation (3 g/per day) in the form of capsules can increase paraoxonase activity in diabetic patients. The metabolic effect of omega-3 fatty acid in diabetic patients is less studied.

Conflict of Interests

Authors have no conflict of interests.

Authors' Contributions

All the authors have carried out the study, participated in the design of the study and acquisition of data performed the statistical analysis and wrote the manuscript. All authors read and approved the final manuscript.

References

1. Canepa ET, Llambias EB, Grinstein M. Studies on regulatory mechanisms of heme biosynthesis in hepatocytes from normal and experimental-diabetic rats. Role of insulin. Biochem Cell Biol 1990; 68(6): 914-21.
2. Reusch JE. Diabetes, microvascular complications, and cardiovascular complications: what is it about glucose? J Clin Invest 2003; 112(7): 986-8.
3. Evans M, Anderson RA, Smith JC, Khan N, Graham JM, Thomas AW, et al. Effects of insulin lispro and chronic vitamin C therapy on postprandial lipaemia, oxidative stress and endothelial function in patients with type 2 diabetes mellitus. Eur J Clin Invest 2003; 33(3): 231-8.
4. Neyestani TR, Shariatzadeh N, Gharavi A, Kalayi A, Khalaji N. Physiological dose of lycopene suppressed oxidative stress and enhanced serum levels of immunoglobulin M in patients with Type 2 diabetes mellitus: a possible role in the prevention of long-term complications. J Endocrinol Invest 2007; 30(10): 833-8.
5. Yamagishi K, Iso H, Date C, Fukui M, Wakai K, Kikuchi S, et al. Fish, omega-3 polyunsaturated fatty acids, and mortality from cardiovascular diseases in a nationwide community-based cohort of Japanese men and women the JACC (Japan Collaborative Cohort Study for Evaluation of Cancer Risk) Study. J Am Coll Cardiol 2008; 52(12): 988-96.
6. Valiyaveettil M, Alamneh Y, Biggemann L, Soojhawon I, Farag HA, Agrawal P, et al. In vitro efficacy of paraoxonase 1 from multiple sources against various organophosphates. Toxicol In Vitro 2011; 25(4): 905-13.

7. Hassett C, Richter RJ, Humbert R, Chapline C, Crabb JW, Omiecinski CJ, et al. Characterization of cDNA clones encoding rabbit and human serum paraoxonase: the mature protein retains its signal sequence. Biochemistry 1991; 30(42): 10141-9.
8. Costa LG, Cole TB, Jarvik GP, Furlong CE. Functional genomic of the paraoxonase (PON1) polymorphisms: effects on pesticide sensitivity, cardiovascular disease, and drug metabolism. Annu Rev Med 2003; 54: 371-92.
9. Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. Arterioscler Thromb Vasc Biol 2001; 21(4): 473-80.
10. Karabina SA, Lehner AN, Frank E, Parthasarathy S, Santanam N. Oxidative inactivation of paraoxonase--implications in diabetes mellitus and atherosclerosis. Biochim Biophys Acta 2005; 1725(2): 213-21.
11. Mackness MI, Durrington PN, Mackness B. The role of paraoxonase 1 activity in cardiovascular disease: potential for therapeutic intervention. Am J Cardiovasc Drugs 2004; 4(4): 211-7.
12. Lai MH. Antioxidant effects and insulin resistance improvement of chromium combined with vitamin C and e supplementation for type 2 diabetes mellitus. J Clin Biochem Nutr 2008; 43(3): 191-8.
13. Riccioni G, Bucciarelli T, Mancini B, Di IC, Capra V, D'Orazio N. The role of the antioxidant vitamin supplementation in the prevention of cardiovascular diseases. Expert Opin Investig Drugs 2007; 16(1): 25-32.
14. Dorchy H. Lower plasma vitamin C levels in young type I diabetic patients with microalbuminuria. J Diabetes Complications 1999; 13(2): 119.
15. Takahashi N, Morimoto S, Okigaki M, Seo M, Someya K, Morita T, et al. Decreased plasma level of vitamin C in chronic kidney disease: comparison between diabetic and non-diabetic patients. Nephrol Dial Transplant 2011; 26(4): 1252-7.
16. Dobrucki R, Radomska A. Retinol palmitate as a model substance to test antioxidant properties in vitro on the example of captopril. Pharmazie 2002; 57(9): 635-7.
17. Tesoriere L, D'Arpa D, Re R, Livrea MA. Antioxidant reactions of all-trans retinol in phospholipid bilayers: effect of oxygen partial pressure, radical fluxes, and retinol concentration. Arch Biochem Biophys 1997; 343(1): 13-8.
18. Johnson AT, Kretzer FL. Interstitial retinol binding protein in the developing human retina: a proposed explanation for vitamin E suppression of retinopathy of prematurity. Prog Clin Biol Res 1985; 190: 251-77.
19. Shirpoor A, Ilkhanizadeh B, Saadatian R, Darvari BS, Behtaj F, Karimipour M, et al. Effect of vitamin E on diabetes-induced changes in small intestine and plasma antioxidant capacity in rat. J Physiol Biochem 2006; 62(3): 171-7.
20. Bucher HC, Hengstler P, Schindler C, Meier G. N-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. Am J Med 2002; 112(4): 298-304.
21. Hu FB, Cho E, Rexrode KM, Albert CM, Manson JE. Fish and long-chain omega-3 fatty acid intake and risk of coronary heart disease and total mortality in diabetic women. Circulation 2003; 107(14): 1852-7.
22. Tanaka N, Sano K, Horiuchi A, Tanaka E, Kiyosawa K, Aoyama T. Highly purified eicosapentaenoic acid treatment improves nonalcoholic steatohepatitis. J Clin Gastroenterol 2008; 42(4): 413-8.
23. Carmeli E, Coleman R, Berner YN. Activities of antioxidant scavenger enzymes (superoxide dismutase and glutathione peroxidase) in erythrocytes in adult women with and without type II diabetes. Exp Diabesity Res 2004; 5(2): 171-5.
24. Song SH, Hardisty CA. Early-onset Type 2 diabetes mellitus: an increasing phenomenon of elevated cardiovascular risk. Expert Rev Cardiovasc Ther 2008; 6(3): 315-22.
25. Shih DM, Xia YR, Wang XP, Miller E, Castellani LW, Subbanagounder G, et al. Combined serum paraoxonase knockout/apolipoprotein E knockout mice exhibit increased lipoprotein oxidation and atherosclerosis. J Biol Chem 2000; 275(23): 17527-35.
26. Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, Edwards PA, et al. Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics. Circulation 1995; 91(9): 2488-96.
27. Calder PC, Yaqoob P. Omega-3 (n-3) fatty acids, cardiovascular disease and stability of atherosclerotic plaques. Cell Mol Biol (Noisy -le -grand) 2010; 56(1): 28-37.
28. Mozaffarian D, Ascherio A, Hu FB, Stampfer MJ, Willett WC, Siscovick DS, et al. Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men. Circulation 2005; 111(2): 157-64.
29. Saedisomeolia A, Wood LG, Garg ML, Gibson PG, Wark PA. Anti-inflammatory effects of long-chain n-3 PUFA in rhinovirus-infected cultured airway epithelial cells. Br J Nutr 2009; 101(4): 533-40.
30. Saedisomeolia A, Wood LG, Garg ML, Gibson PG, Wark PA. Supplementation of long chain N-3 polyunsaturated fatty acidsincrease the utilization oflycopene in cultured airway epithelial cells. Journal of Food Lipids 2008; 15(4): 421-32.
31. Calabresi L, Villa B, Canavesi M, Sirtori CR, James RW, Bernini F, et al. An omega-3 polyunsaturated fatty acid concentrate increases plasma high-density lipoprotein 2 cholesterol and paraoxonase levels in patients with familial combined hyperlipidemia. Metabolism 2004; 53(2): 153-8.

32. Maritim AC, Sanders RA, Watkins JB, III. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol 2003; 17(1): 24-38.
33. Chen MS, Hutchinson ML, Pecoraro RE, Lee WY, Labbe RF. Hyperglycemia-induced intracellular depletion of ascorbic acid in human mononuclear leukocytes. Diabetes 1983; 32(11): 1078-81.
34. Dyer RG, Stewart MW, Mitcheson J, George K, Alberti MM, Laker MF. 7-ketocholesterol, a specific indicator of lipoprotein oxidation, and malondialdehyde in non-insulin dependent diabetes and peripheral vascular disease. Clin Chim Acta 1997; 260(1): 1-13.
35. Merzouk S, Hichami A, Madani S, Merzouk H, Berrouiguet AY, Prost J, et al. Antioxidant status and levels of different vitamins determined by high performance liquid chromatography in diabetic subjects with multiple complications. Gen Physiol Biophys 2003; 22(1): 15-27.
36. Salonen JT, Nyyssonen K, Tuomainen TP, Maenpaa PH, Korpela H, Kaplan GA, et al. Increased risk of non-insulin dependent diabetes mellitus at low plasma vitamin E concentrations: a four year follow up study in men. BMJ 1995; 311(7013): 1124-7.
37. Yamada H, Yamada K, Waki M, Umegaki K. Lymphocyte and plasma vitamin C levels in type 2 diabetic patients with and without diabetes complications. Diabetes Care 2004; 27(10): 2491-2.
38. Sundaram RK, Bhaskar A, Vijayalingam S, Viswanathan M, Mohan R, Shanmugasundaram KR. Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. Clin Sci (Lond) 1996; 90(4): 255-60.
39. Basu TK, Tze WJ, Leichter J. Serum vitamin A and retinol-binding protein in patients with insulin-dependent diabetes mellitus. Am J Clin Nutr 1989; 50(2): 329-31.
40. Zhou D, Ghebremeskel K, Crawford MA, Reifen R. Vitamin A deficiency enhances docosahexaenoic and osbond acids in liver of rats fed an alpha linolenic acid-adequate diet. Lipids 2006; 41(3): 213-9.
41. Zhou D, Zaiger G, Ghebremeskel K, Crawford MA, Reifen R. Vitamin A deficiency reduces liver and colon docosahexaenoic acid levels in rats fed high linoleic and low alpha-linolenic acid diet. Prostaglandins Leukot Essent Fatty Acids 2004; 71(6): 383-9.