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Evaluating Effect of Omega-3 and Zinc Supplements on Inflammation, Lipid Profile and Antioxidant Capacity in Type 2 Diabetic Patients

Mehdi Khakian, Majid Manafi*, Setareh Alibakhshi, Peyvand Mohammadi

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Abstract

Background: The aim of this study was to evaluate and compare the effect of dietary supplements of zinc and omega 3 separately and together on the lipid profile, inflammatory condition (hs-Crp) and total antioxidant capacity (TAC) in type 2 diabetic patients. **Methods:** In this, double-blind, randomized controlled trial, 100 type 2 diabetic patients were randomly divided into four groups: omega-3 (n =25 patients, 1,000mg of omega-3), zinc group (n =25, 30 mg zinc gluconate), zinc plus omega-3 group (n =25) and placebo group (n =25). They were followed for a two-month period. **Results:** After 2 months, a significant increase in total antioxidant capacity was observed in all the intervention groups (P <0.001). On the other hand, it can be stated that the highest amount of total antioxidant capacity increase was observed in omega-3 plus zinc supplementation group (0/313 units). Intra-group comparisons showed significant reduction of hs-CRP levels before and after intervention in intervention groups. Also, LDL blood levels in the groups receiving zinc and zinc plus omega-3, were significantly decreased after the study (P = 0.000, P = 0.0001, respectively). **Conclusion:** Our study results suggest that zinc supplementation plus omega-3 can have a beneficial effect on increasing the antioxidant capacity of diabetic patients. Zinc supplementation also enhances the effect of omega-3 in reducing triglycerides (TG) and Low-density lipoprotein (LDL) in the blood. Also taking omega-3 alone or with zinc supplements can help reduce inflammation in the body.

Key words: Omega-3, Zinc, Diabetes, Supplement, Inflammation, Antioxidant Capacity

Introduction

Diabetes mellitus (DM) is a common metabolic disorder that can be due to a defect in insulin secretion or insulin function in target cells (Hales, & Barker, 1992). With insulin resistance, pancreatic beta cells begin to produce more insulin in the body to counteract the rise in blood sugar levels, which, in the long term, results in the destruction of these cells and can lead to conditions such as high blood sugar levels and Diabetes Mellitus Type 2 (T2DM) (Alberti, & Zimmet, 1998). According to the World Health Organization (WHO), in 2014, more than 422 million people worldwide have diabetes, and the incidence of diabetes has increased from 4.7% in 1980 to 8.5% in 2014 (Roglic, 2016). The zinc element plays a role in over 300 types of enzymes, hormone, protein, and cell membrane activity (Maret & Sandstead, 2006). The blood zinc level in diabetic patients decreases significantly, which leads to increased complications of diabetes due to the presence of free radicals and intracellular radicals and reduction in zinc-dependent antioxidant enzymes (Al-Maroof & Al-Sharbatti, 2006, RAHIMI and et al, 2008). Chronic oxidative stress due to long-term increase in blood sugar level, especially after eating food and producing Reactive Oxygen Species (ROS), contribute to progressive loss of beta cell function and ultimately T2DM (Selvaraj and et al, 2008). Omega-3 fatty acids are known as essential fatty acids in the body and include α-linoleic acid (ALA), Eicosa Pentaenoic Acid (EPA) and Docosa Hexaenoic Acid (DHA) (Carpentier and et al, 2006). Omega-3 fatty acids are at risk of oxidation due to dual bounding, but human and animal studies have shown contradictory results about the effect of supplementation with omega-3 on the oxidant/antioxidant status (Song, & Miyazawa, 2001; Frenoux and et al, 2001; Wander & Du, 2000; Higdon and et al, 2000). It has also been shown that supplementation with omega-3 fatty acids increases the activity of superoxide dismutase (SOD) and reduces the formation of malondialdehyde (MDA) in the brain (Wu and et al, 2016). Studies on diabetes have shown that there is a significant relationship between elevated levels of acute phase inflammatory markers, especially C-reactive protein, with elevated blood sugar levels and T2DM (Festa and et al, 2000; Nakanishi and et al, 2005). EPA inhibits the production of fourth generation leukotriene and the second generation prostaglandins that are both inflammatory. On the other hand, DHA also causes specific responses to inflammatory cytokines through gene expression, which indicates the beneficial effects of omega-3 fatty acids on inflammation (Mahan and et al,

Mehdi Khakian, Majid Manafi*, Setareh Alibakhshi
MSc in Nutrition Sciences, Urmia University of Medical Sciences, Urmia, Iran.

Peyvand Mohammadi
Endocrinology sub-specialist, Urmia University of Medical Sciences, Urmia, Iran.

***Email**: majidmanafi@yahoo.com

2007). Lipid disorders are important features of diabetes pathology. Major changes in lipid levels include increase in serum LDL level, increase in TG, and decrease in High-density lipoprotein (HDL) (Taskinen, 2005; Goldberg, 1981). Zinc can play a vital role in blood lipids level in the body, as in a study by Afkhami (2002) in Iran on diabetic patients, zinc supplementation significantly reduced blood cholesterol (Afkhami-Ardekani and et al, 2008). However, in the Hernandez study in Mexico (2006), there was no significant effect of zinc supplements on LDL levels in diabetic patients (Partida-Hernandez and et al, 2006). On the other hand, omega-3 fatty acids reduce the plasma triglyceride concentration and prevent thrombosis, but there is no agreement on their effect on plasma cholesterol, although some studies have shown that LDL concentration increases with Omega- 3 supplement (Carpentier and et al, 2006; Toorang and et al, 2015). The desaturase enzyme plays a role in the efficient conversion of short chain fatty acids to omega-3 fatty acids, while, the zinc element acts as a cofactor in the activity of desaturase enzyme, thus, due to zinc deficiency, desaturase enzyme activity declines and causes EPA and DHA deficiency in the blood (Maes, M and et al, 1999). Given the importance of blood lipid levels, oxidative stress and inflammation in diabetic patients, and given the controversy of the results of studies, we assume that omega-3 fatty acids supplementation plus zinc can have beneficial effects in improving the health of individuals. Therefore, this study was the first study to be conducted using zinc and omega-3 fatty acids in a complementary form with the aim of determining the effects of these two nutrients on inflammatory status, lipid profiles and antioxidant capacity in type 2 diabetic patients.

Methods

This study was a prospective, randomized, double-blind, controlled clinical trial designed to determine the effect of dietary supplements of omega-3 fatty acids and zinc together and separately on the inflammatory status, lipid profiles and antioxidant capacity in type 2 diabetic patients for two months.

At the beginning of the study, diabetic patients referred to endocrine clinic of Imam Khomeini hospital in Urmia who had inclusion criteria were selected. After considering the inclusion and exclusion criteria for the study, qualified people were provided with explanations on the goals and methods of doing the study. Then, in the case of the patients' willingness to participate in this study, they filled written informed consent. Inclusion criteria of the study included diabetic patients who have had their disease for at least two years, taking metformin glucose or glibenclamide or similar medicines by the patients, age range of 18-65 years, and taking blood lipid lowering medicines including statins, blood pressure lowering medicines including calcium channel blockers, and ACE-I by the patients. Exclusion criteria included insulin injections, not taking 80% of supplements in each period, omega-3 allergy, pregnancy and lactation, taking omega-3 supplement and zinc in the last three months, having a weight over 150 kg, or Body Mass Index (BMI) above 40, because BMI outside this range can affect blood sugar levels and insulin resistance (Hossain and et al, 2009). The study was adopted at the Human Research Ethics Committee of Urmia University of Medical Sciences (IR.UMSU.REC.1395.371) and registered in Iran's Clinical Trials Registry (IRCT20170214032571N2).

Intervention and Randomization

In this study, 100 patients with type 2 diabetes were randomly divided into four intervention and control groups: omega-3 group, Zn group, Zn and omega-3 group and control group. Randomization was assigned through a random assignment list. The capsules used in the control group were similar to the capsule of the intervention groups in terms of shape and color. Considering the different results of studies with different doses of omega-3 and the usefulness of doses up to 1000 mg in these individuals (Crochemore and et al, 2012) and, given that a study using omega-3 at a dose of 1000 mg has not been done, the dose used in this study was 1000 mg (including 180 EPA mg, 120 DHA mg and 700 mg other omega-3 fatty acids). Also, due to the usefulness of 30 mg dose of zinc in previous studies (Parham and et al, 2008) zinc dosage used in this study, contained 30 mg of zinc gluconate (Dineh Pharmaceuticals Company of Iran). The omega-3 and Zn supplements were taken with meals at noon and evening, respectively. Supplements were delivered at the beginning of each month in the form of 30 packs. People were contacted to help them better remember the use of supplements. In order to ensure the use of supplements by the participants, in addition to the weekly phone calls, all individuals were asked to contact the Imam Khomeini Hospital on the 30th and 60th day after the intervention and bring with them the blank sheets of supplements. In each of the follow-up studies, participants were monitored for the positive or negative effects of supplements.

Study measures

BMI was calculated by dividing the weight (kg) by the square of height (m^2). Also, height with an accuracy of 0.1 cm and weight with an accuracy of 100g were measured using standard Bioelectrical Impedance Analysis *(*BIA*)* (In body 770, made in Korea) in all patients under standard conditions. To collect the demographic data of the participants in the study, a general information questionnaire was used.

Collection of Dietary Information

All subjects were asked not to change their diet and lifestyle during the intervention. In order to evaluate the dietary changes, 24-hour dietary recall was used at the beginning and the end of the study in 3 days of the week (two days off and one day off). Dietary information received was evaluated through recall using N4 software version 3.5.2.

Blood Collection:

Blood samples (5 cc) were taken once at the beginning of the study and once at the end of the study, while the patients were fast for 12-hour, and after centrifugation at a rate of 3000 r/min for 15 minutes, the sera were isolated and used for testing biochemical. The sera were stored at -80°C to analyze hs-CRP and TAC. It should be noted that all biochemical tests were carried out at the beginning and at the end of the study by the same subjects under the supervision of the laboratory scientist.
Measurement of lipid profiles was done by enzymatic method and using Pars test kit.

*TAC***:**

In order to determine the Total Antioxidant Capacity (TAC), a colorimetric method was used with a Zell Bio (Germany)-ELISA reader kit. At the end and during of the experiment, the absorbance at 492 nm was measured 2 times by the ELISA reader (biotech epoch, American). Finally, the serum samples TAC concentration (Mm) of each individual was determined according to the absorbance chart (ΔOD = OD2-OD1) of standard solution to its concentration by Microsoft Excel 2010 software. The determined TAC concentration range by the manufacturer was 0.2-125 Mm.

Hs-CRP:

Serum hs-CRP concentration was measured by nephelometric method. All samples were diluted 40 times with a special nephlorometric diluent solution. The amount of 20 µl of each serum sample was poured into a cuvette with a magnetic stirrer and was placed in the nephelometer reservoir, and then 400 µl of the special hs-CRP buffer and 40 µl of hs-CRP reagent were added to the cuvette and simultaneously were placed in pressure device. In this reaction, existed hs-CRP in serum with existed antibodies in micro particles, has created antigen and an antibody polystyrene complex, and created complexes were placed against the light of the device. The laser light subtraction that is measured by the device is proportional to the hs-CRP concentration in the samples.

Sample Size and Statistical Analysis:

A total of 100 type-2 diabetic patients were randomly divided into four intervention and control group. In this study, a single-sample Kolmogorov-Smirnov test was used to check the normal distribution of data. In order to identify confounding variables, the literature review method was used and the four groups with Chi-square and Kruskal-Wallis single-valued tests were compared. The variables that were less than 0.25 were selected as confounding variables. Finally, the confounding variables used in multivariate statistical models were as follows:

Age, level of education, job, change in dietary energy, dietary fat change, dietary SFA changes, dietary PUFA changes, dietary MUFA changes, dietary fiber changes, and insoluble fiber changes. In addition, the ANOVA analysis model was used for multivariate modeling along with regulating the effect of variables. In all cases, the variable "the change over time" is considered. In order to investigate the main effects and mutual effects in the ANOVA analysis model (the main effect of omega-3 supplement, the main effect of Zn supplement and the mutual effect of omega-3 and Zn supplements), two separate models were used as follows:
Model 1: In this raw model, except for the base values of dependent variables, the effect of any of the confounding variables is not regulated.

Model 2: In this model, in addition to the base values of dependent variables, the effect of other confounding variables is also regulated.
The mean, standard deviations, median, inter-quartile domain and frequency distribution tables (abundance and percentage) have been used in order to describe the quantitative data for frequency data.
It should be noted that all stages of statistical analysis were performed using SPSS software version 22, under the significance level of 0.05.

Results

Of the 100 patients who participated in the study, 83 patients completed the intervention. In Table 1, some demographic variables are compared in four experimental groups. Kruskal-Wallis test showed that there was no significant difference between the four groups in terms of age, duration of disease and BMI ($P < 0.05$); however, the difference between the groups was considerable in terms of age

(P=0.089). In addition, the results of Chi-square test showed that there was no significant association between sex, education level, type of occupation of patients and place of residence and type of experimental group. However, the difference between groups was considerable in terms of level of education and the type of job (P=0.126, P=0.147).

Table 1- Comparison of the demographic and background variables of the patients with diabetes type 2 in the groups receiving zinc and omega 3 supplementation

Variable	Category	Experimental group				P-value *
		Omega-3	Zinc	Omega-3 and zinc	Control	
Age (year)	-	58.0 **(13.0)	(8.0) 57.0	(7.0) 53.0	(8.0) 54.5	0.089
Years of affliction with the disease	-	(5.0) 5.0	(2.0) 4.0	(3.0) 4.0	(2.0) 4.5	0.553
BMI (kg/m2)	-	(34.34) 4.56	(31.57) 3.88	(30.25) 4.34	(31.89) 4.27	0.319
Gender	Female	**(52.2%) 12	(57.1%) 12	(57.1%) 12	(61.1%) 11	0.953
	Male	(47.8%) 11	(42.9%) 9	(42.9%) 9	(38.9%) 7	
Educational level	Illiterate	(8.7%) 2	(23.8%) 5	(33.3%) 7	(50.0%) 9	0.126
	Elementary or junior school	(39.1%) 9	(38.1%) 8	(23.8%) 5	(16.7%) 3	
	High school and above	(52.2%) 12	(38.1%) 8	(42.9%) 9	(33.3%) 6	
Job	Housekeeper	(52.2%) 12	(57.1%) 12	(57.1%) 12	(50.0%) 9	0.147
	Retired	(39.1%) 9	(9.5%) 2	(28.6%) 6	(16.7%) 3	
	Other jobs	(8.7%) 2	(33.3%) 7	(14.3%) 3	(33.3%) 6	
Place of living	City	(95.7%) 22	(100.0%) 21	(95.2%) 20	(94.4%) 17	0.778
	Village	(4.3%) 1	(0.0%) 0	(4.8%) 1	(5.6%) 1	

*Kruskal–Wallis test was used to compare quantitative variables in the groups and chi-square test was used to compare qualitative variables.

**Median (interquartile range) was used to describe quantitative variables and frequency (percentage) was used to describe qualitative variables.

Table 2 shows the dietary intake of patients with type 2 diabetes in the four groups under study. The ANOVA analysis showed that there was a statistically significant difference between the four groups in terms of receiving SFA, PUFA, MUFA, dietary fiber and insoluble fiber (P <0.05).

Table 2- Comparison of the received amount of nutrition by the patients with diabetes type 2 in the groups receiving zinc and omega 3 supplementation

Variable	Experimental group												P-value*
	Omega3 (n=23)			Zinc (n=21)			Omega3 and zinc (n=21)			Control (n=18)			
	before	after	change	before	after	change	Before	after	change	Before	after	change	
	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean±SD	Mean± SD	Mean± SD	Mean± SD	
Energy Kcal	3.21±229.1912	53.78±178.1765	9.43±235.-146	6.85±427.1990	9.66±286.1890	6.19±365.100-	6.52±178.1882	2.95±201.1841	5.57±239.-40	6.27±223.1951	4.11±151.1766	1.16±237.-185	156.0
Protein g/d	9.36±13.68	2.33±11.69	3.97±10.0	5.11±11.68	6.55±12.67	87.56±1.0	1.08±11.69	9.36±10.71	1.28±11.2	7.60±10.66	4.14±9.70	7.53±8.3	638.0

CHO g/d	7.37±40.236	4.42±43.231	6.94±43.-4	9.86±33.228	8.58±40.238	7.72±34.9	7.490±47. 240	5.43±37.244	6.93±40.3	5.59±29.237	3.93±17.227	5.66±31.9	351.0
Fat g/d	7.77±14.74	2.89±12.67	8.88±15.-6	9.54±16.72	8.46±9.66	1.07±18.6	4.67±15.72	1.83±10.63	2.84±14.-8	8.30±12.74	2.36±13.71	6.93±15.-2	248.0
SFA g/d	6.72±3.17	8.51±3.17	2.20±5.-0	1.85±3.16	8.61±3.17	4.76±4.0	3.38±3.18	6.04±3.17	9.34±5.-1	1.67±3.14	6.09±2.14	0.57±4.-0	010.0
PUFA g/d	8.10±6.21	8.16±10.29	7.05±10.8	5.74±15.23	2.35±13.22	3.38±5.1	4.92±8.23	3.95±4.20	3.97±6.-2	3.24±13.29	1.48±9.25	8.76±8.-3	001.<0
MUFA g/d	6.62±9/25	8.72±8.25	2.1±7.0	21/82±5/6	6.31±5.21	1.51±3.-0	71±6.21	2.77±5.19	9.93±4.-1	6.16±6.21	7.30±4.18	4.86±7.-2	019.0
Glucose g/d	63/48±21/1	61/77±15/2	-1/71±21/3	60/95±18/2	63/84±17/5	2/89±21/3	52/19±17/7	59/49±16/7	7/30±9/9	52/61±14/8	55/40±17/2	2/79±7/3	0/676
Cholesterol Mg/d	6.44±136.237	3.54±131.258	2.09±69.21	2.99±82.211	7.34±57.217	8.34±76.5	8.83±96.237	6.82±78.227	4.0±87.-1	2.78±111.233	4.05±109.252	3.26±89.18	405.0
Dietary fiber g/d	14/55±3/3	14/36±3/4	-0/19±2/7	17/10±5/2	17/69±5/0	0/59±6/4	15/11±3/9	15/73±3/1	0/62±4/1	14/18±3/9	13/52±2/7	-0/65±2/7	0/035
Insolubl e fiber g/d	6.46±1.3	1.15±1.3	7.31±1.-0	7.39±3.6	2.88±1.2	2.50±4.-3	3.40±1.2	6.13±0.2	4.27±1.0	9.46±0.2	9.44±0.2	3.03±1.-0	008.0
Zinc Mg/d	9.33±2.10	9.40±2.10	2.06±1.0	7.67±3.10	5.00±4.11	3.33±3.0	4.54±4.11	5.41±4.11	4.13±6.-0	9.23±2.10	0.39±3.10	3.16±3.0	955.0

*The ANCOVA analysis model with modification of the effects of the basic values was used for comparing the four groups.

Table 3 compares the physical activity level of patients with type 2 diabetes in different groups receiving omega-3 and Zn supplements. The ANOVA analysis model (by regulating the effect of base values) showed no significant difference between the four groups in terms of the average change in physical activity (P=0.546).

Table 3- omparison of the rate of physical activity in the patients with diabetes type 2 in the groups receiving omega3 and zinc supplementation (MET.h/day)

Omega-3 (n=23)			Zinc (n=21)			Omega-3 and Zinc (n=21)			Control (n=18)			P-value**
Before	after	Change	before	After	Change	before	after	change	before	after	change	

Mean± SD	Mean± SD	Mean± SD	P-value*	Mean± SD	Mean± SD	Mean± SD	P-value*	Mean± SD	Mean± SD	Mean± SD	P-value*	Mean± SD	Mean± SD	Mean± SD	P-value*	
41.1±16.6	43.0±17.7	1.9±6.3	0.581	39.8±18.5	42.2±20	2.4±5.5	0.607	38.3±2.14	39.2±19.5	0.9±5.3	0.866	40.0±15.7	43.6±17.8	2.1±6.7	0.394	0.546

* shows the comparison the before and after values in each group.

** shows the comparison between the four groups in terms of the average value change.

Table 4 compares the lipid profiles of type 2 diabetic patients in different groups receiving omega-3 and zinc supplements. The covariance analysis model (by modulating the effect of all confounding variables) showed that the effect of omega-3 supplementation (alone or with zinc supplementation) on the mean change in TG was insignificant, but noticeable (P=0.075); similarly, The effect of using zinc supplementation (alone or with omega-3 supplementation) on the mean change in TG was insignificant, but noticeable (P=0.068); similarly, the effect of using zinc supplementation (alone or with the omega-3 supplementation) on the mean change in serum cholesterol was insignificant and, of course, noticeable (P=0.061). Blood cholesterol levels in the intervention group with omega-3 showed insignificant but noticeable decrease after intervention (P = 0.081). On the other hand, in the intervention group with zinc, the cholesterol decreased significantly after the study (P = 0.038).

Based on the Wilcoxon test, LDL levels significantly decreased in the zinc group and zinc plus omega-3 before and after the study (P = 0.000 and P = 0.001, respectively), as well, the covariance analysis model (after modulating the effect of confounding variables) showed that the effect of taking zinc supplement alone or with omega-3 on LDL changes was significant (P = 0.001), so that, the reduction in LDL level was 15.262 units, however, in groups not consuming zinc, the reduction was 0.626 units. Also, in the omega-3 consumer groups, the reduction in LDL level was 8.926 units. No significant finding was observed regarding the main effect of omega-3 supplementation, the main effect of zinc supplementation, as well as the interaction of zinc supplementation and omega-3 supplementation on the mean change in HDL level (P >0.05).

Table 4- Comparison of lipid profiles of type 2 diabetic patients in omega-3 and zinc supplement recipients groups.

Model 2 (by modulating the effect of all confounding variables)			Model 1 (without modulating the effect of all confounding variables)			**Control (18=n)**				omega-3 and zinc **(21=n)**				Zinc **(21=n)**				omega-3 **(23=n)**				**Variable**
						change		**after**	**before**	**change**		**after**	**before**	**change**		**after**	**before**	**change**		**after**	**before**	
P value ++3	P value +2	P value **1	P value ++3	P value +2	P value **1	P value *	SD±mean	SD±mean	SD±mean	P value *	SD±mean	SD±mean	SD±mean	P value *	SD±mean	SD±mean	SD±mean	P value *	SD±mean	SD±mean	SD±mean	
0.971	0.068	0.075	0.875	0.116	0.067	0.885	0.88±25.6	160.00±85.4	159.11±85.4		-46.80±51.4	162.80±76.9	209.61±95.4	0.090	-21.28±54.8	161.19±98	189.47±96.3	0.235	15.26±59.9	136.13±65.7	151.39±62.8	TG
0.756	0.061	0.642	0.149	0.103	0.924	0.707	-1.38±15.4	181.50±43.1	182.88±45.1	0.114	-11.76±32.6	168.09±41.1	179.85±50.9	0.038	-27.47±56.5	165.66±36.5	193.14±55.9	0.081	-14.4±36.8	173.13±38.6	187.17±44.2	Cholesterol
0.825	0.001	0.654	0.347	0.269	0.756	0.737	0.72±8.9	102.72±32	102±32.3	0.001	12.14±14.2	79.04±25.2	91.19±32	0.000	12.71±13.1	90.38±32.2	103.9±29.8	0.601	3.47±31.4	97.65±26.8	101.13±32.2	LDL
0.613	0.741	0.428	0.827	0.716	0.241	0.026	-2.77±4.8	43.88±5.5	46.66±6.5	0.123	-2.23±6.3	4.66±7.8	42.90±8.1	0.599	-0.95 ±8.1	43.09±9.4	44.4±11.6	0.008	-4.08±6.7	42.82±6.9	42.91±7.4	HDL

* The significance of before and after comparison of dependent variables within each experimental group using the Wilcoxon test.
** The significance of the main effect of omega-3 supplement on the mean change in responses (compares the groups receiving omega-3 supplements with other groups).
+ The significance of the main effect of zinc supplement on the mean change in responses (compares the groups receiving zinc supplements with other groups).
++ The significance of interaction between the omega-3 supplements and zinc supplements on the mean change in responses.

Table 5 shows the total antioxidant capacity of type 2 diabetic patients in different groups receiving omega-3 and zinc supplements. In all intervention groups with zinc and omega-3, total antioxidant capacity was significantly increased after study ($p < 0.001$). The covariance analysis model (by modulating the effect of all confounding variables) showed that the interaction of omega-3supplement and zinc supplement on the mean total antioxidant capacity change was significant ($P < 0.001$); that is, it could be stated that the highest total antioxidant capacity was observed in the omega-3 supplementation and zinc supplementation group (0. 313 unit), while the lowest total antioxidant capacity was observed in the control group (0.026 unit).

Table 5- Comparison of total antioxidant capacity of type 2 diabetic patients in omega-3 and zinc supplement recipients groups.

Model 2 (by modulating the effect of all confounding variables)			Model 1 (without modulating the effect of all confounding variables)			**Control (18=n)**				omega-3 and zinc (**21=n)**				Zinc **(21=n)**				omega-3 **(23=n)**			
						Change		after	before	change		after	before	Change		after	before	change		after	before
P value ++3	P value +2	P value **1	P value ++3	P value +2	P value **1	P value *	SD±mean	SD±mean	SD±mean	P value *	SD±mean	SD±mean	SD±mean	P value *	SD±mean	SD±mean	SD±mean	P value *	SD±mean	SD±mean	SD±mean
<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.903	-0.00±0.04	0.27±0.04	0.27±0.02	<0.001	0.30±0.06	0.56±0.06	0.26±0.03	<0.001	0.27±0.1	0.52±0.1	0.24±0.02	<0.001	0.25±0.09	0.52±0.08	0.26±0.04

* The significance of before and after comparison of dependent variables within each experimental group using the Wilcoxon test.
** The significance of the main effect of omega-3 supplement on the mean change in responses (compares the groups receiving omega-3 supplements with other groups).
+ The significance of the main effect of zinc supplement on the mean change in responses (compares the groups receiving zinc supplements with other groups).
++ The significance of interaction between the omega-3 supplements and zinc supplements on the mean change in responses.

In Table 6, the hs-CRP level of type 2 diabetic patients in the different groups receiving omega-3 and zinc supplements was compared. The hs-CRP levels in intervention groups with omega-3, zinc and omega-3 groups plus zinc significantly decreased after the study ($P < 0.004$, $P < 0.001$ and $P < 0.003$, respectively), However, after modulating the effect of all confounding variables, no significant finding was observed regarding the main effect of omega-3supplementation, the main effect of zinc supplementation, as well as the interaction of zinc supplementation and omega-3 supplementation on the mean change in hs-CRP level ($P < 0.05$).

Table 6- Comparison of hs-CRP of type 2 diabetic patients in omega-3 and zinc supplement recipients groups

Model 2 (by modulating the effect of all confounding variables)	Model 1 (without modulating the effect of all confounding variables)	Control (18=n)			omega-3 and zinc (21=n)			Zinc (21=n)			omega-3 (23=n)		
		change	after	before	change	after	before	change	after	before	change	after	before

P value ++3	P value +2	P value **1	P value ++3	P value +2	P value **1	P value *	SD±mean	SD±mean	SD±mean	P value *	SD±mean	SD±mean	SD±mean	P value *	SD±mean	SD±mean	SD±mean	P value *	SD±mean	SD±mean	SD±mean
0.202	0.484	0.352	0.994	0.678	0.006	0.105	-0.9±2.3	6.31±2.3	7.26±0.3	0.003	-2.72±3.6	5.97±3.3	8.7±5.0	<0.001	-1.59±0.9	7.42±0.9	9.02±1.1	0.004	-2.60±3.9	5.47±4.4	8.07±6.5

* The significance of before and after comparison of dependent variables within each experimental group using the Wilcoxon test.

** The significance of the main effect of omega-3 supplement on the mean change in responses (compares the groups receiving omega-3 supplements with other groups).

+ The significance of the main effect of zinc supplement on the mean change in responses (compares the groups receiving zinc supplements with other groups).

++ The significance of interaction between the omega-3 supplements and zinc supplements on the mean change in responses.

Discussion and Conclusion

This study was a prospective, randomized, double-blind, controlled clinical trial designed to determine the effect of dietary supplements of omega-3 fatty acids and zinc together and separately on the lipid profiles (LDL, HDL, CHOL, TG) and inflammatory status (hs-Crp) and total antioxidant capacity in type 2 diabetic patients for two months. According to our data, this study was the first study to evaluate and compare the effect of omega-3 fatty acids and zinc supplements together and separately on lipid profile, inflammatory status (hs-Crp) and total antioxidant capacity (TAC) in type 2 diabetic patients. Increasing blood sugar can result in sugar oxidation and glycation of proteins, these proteins in the brain increase the production of free oxygen radicals and oxidative stress (Fakher and et al, 2007). Omega-3 fatty acids may be at risk for oxidation due to dual bounding and can lead to lipid peroxidation, but according to Jazaery study, omega-3 fatty acids can increase the antioxidant activity, this happens when these supplements are consumed for a long time (Sarbolouki and et al, 2010). Similarly, in the present study, omega-3 fatty acids could significantly increase antioxidant capacity in diabetic patients, while the Toorang study did not show a significant effect of omega-3 fatty acids on the antioxidant status of diabetic patients (Toorang and et al, 2016). In the study conducted by Yessouf, received omega-3 fatty acids increased antioxidant activity in the body as well (Yessoufou and et al, 2006). LI in another study showed that receiving omega-3 by increasing the superoxide activity of motuzas could have an effect on improving the antioxidant activity (Li and et al, 2006). Omega-3 fatty acids can enhance antioxidant defense by increasing levels of catalase in peroxisomes and cytoplasm. On the other hand, omega-3 supplementation results in replacing these fatty acids with other long-chain fatty acids and can strengthen the antioxidant defense (Toorang and et al, 2016; Masters, 1996) . Zinc supplementation at 30 mg dose significantly increased antioxidant activity in diabetic patients before and after study ($P < 0.001$). Zinc can be a potent protective agent against oxidative stress due to its role in cellular activity (Powell, 2000). Zinc has a structural role in the activity of the superoxide dismutase enzyme, it can also act to protect sulfhydryl groups against oxidation and to participate in inhibiting the production of free radicals. On the other hand, the zinc complex of Metallothionein in pancreatic cells can play a protective role against free radicals produced by immune system cells (Ohly and et al, 2000) Dervis et al. Showed that zinc can play a vital role in modulating and protecting oxidative stress in diabetic patients (Özcelik and et al, 2012). Other studies have shown that taking 30 mg of zinc supplement can reduce the effects and risks of oxidation by increasing antioxidant potency (Faure and et al, 1995; Anderson and et al, 2001). This study showed that taking omega-3 plus zinc has boost effect on antioxidant activity, in a way that the highest increase in TAC was observed in the omega-3 plus zinc group after the study.

Lipid disorders are one of the most important variables of cardiovascular disease even more important than blood pressure and blood sugar in diabetic patients (Shepherd, 2007). In this study, as in the Foster study (Foster and et al, 2013), a significant decrease in serum HDL concentration was observed in patients receiving zinc supplement after two months, but in Afkhami study (Afkhami-Ardekani and et al, 2008), the level of HDL concentration increased significantly at the end of the study. Studies have shown that HDL particles, independent of the role that have in cholesterol homeostasis, can have an effect in insulin sensitivity (Barter, 2011). Studies have shown that triglyceride levels are reduced after omega-3 consumption (Harris, 1989; Harris, 1996), while HDL changes were variable after omega-3 consumption (Harris, 1989; Harris, 1996). These results can be due to the difference in consumed dosages, consumed fat, and the type and severity of lipoprotein disorder. In the present study, serum HDL levels significantly decreased in the omega-3 group before and after the study, which is consistent with the results of the Farah Bakhsh study (Farsi and et al, 2014). While HDL values were increased in the study conducted by Toorang (Toorang and et al, 2015); this could be due to differences in the consumed dose of omega-3 in studies. However, the covariance analysis model (by modulating the effect of all confounding variables) showed no significant finding regarding the main effect of omega-3 supplement, the main effect of zinc supplement, as well as the interaction of zinc

supplement and omega-3 supplement on the mean change of HDL level in the present study (P>0.05). Overall, the effects of omega-3 fatty acids on HDL depend on the dose and duration of consuming supplement (Mensink & Katan, 1992). Supplementation with higher doses or longer period may have more beneficial effects. Serum LDL levels decreased significantly before and after the study (P = 0.000) in the pre-and post-test zinc group as it is seen in Afkhami's study (Afkhami-Ardekani and et al, 2008). As well, in Parham's study, receiving zinc supplement at 30 mg reduced the LDL levels in diabetic patients after three months (Parham and et al, 2008). However, blood levels of LDL increased after Zn supplementation in the Hernandez study (Partida-Hernandez and et al, 2006). The covariance analysis model showed that the effect of Zn supplement alone or with omega-3 on LDL changes was significant (P = 0.001). In general, weight loss can be considered as an important factor in lowering the blood levels of LDL and HDL, which was also observed in this study. According to a study conducted in 2007, triglyceride levels in blood were found to be an independent risk factor for cardiovascular disease, even after moderating the effects of HDL (Sarwar and et al, 2007). The consumption of omega-3 fatty acids leads to the removal of free fatty acids by the liver and their oxidation, and thus the synthesis of triglycerides reduces due to the absence of free fatty acids (Shidfar and et al, 2007). On the other hand, omega-3 fatty acids increase triglyceride degradation and reduce liver triglyceride absorption by activating the lipoprotein lipase enzyme (Kelley and et al, 2007). In the present study, an insignificant decrease in triglyceride levels was observed in the groups consuming omega-3 and zinc supplement separately, but a decrease in triglyceride levels in the group consuming omega-3 plus zinc was significant (P <0.001), in other words, the simultaneous intake of omega-3 and zinc enhances their effect in reducing triglyceride levels. In this study like most other studies, blood cholesterol levels decreased in the intervention groups (Afkhami-Ardekani and et al, 2008, Partida-Hernandez and et al, 2006). There is a direct correlation between increased blood sugar levels and inflammatory factors, especially the C-reactive protein in the body (Festa and et al, 2000, Nakanishi and et al, 2005). CRP is a highly susceptible marker for systemic inflammation, and its high levels in the blood can cause endothelial dysfunction in the blood vessels (Shepherd, 2007; Foster and et al, 2013). There was an inverse relationship between the hs-CRP blood levels and the omega-3 fatty acid plasma levels (Micallef and et al, 2009). Omega-3 fatty acids have anti-inflammatory properties, and the addition of omega-3 fatty acids in the form of fish oil to the diet can reduce symptoms in inflammatory patients (Tull and et al, 2009; Tayyebi-Khosroshahi and et al, 2012). In the present study as in most studies (Micallef and et al, 2009; Tayyebi-Khosroshahi and et al, 2012), the hs-CRP blood levels significantly decreased in the omega-3 intervention group after study (P = 0.004). The peroxisome proliferator activated receptors (PPARs) act as mediators for lipoprotein metabolism, inflammatory processes and glucose homeostasis (Blaschke and et al, 2006). By attaching to these receptors and activating them, the zinc element can play a significant role in setting up inflammatory and protective procedures (Reiterer and et al, 2004). Studies have shown that zinc supplements can be effective in reducing inflammation (Shen and et al, 2007). In the present study, the blood hs CRP levels significantly decreased in the intervention group with zinc (P <0.001), which was similar to that of Usman et al (Khan and et al, 2013). The results of this study indicate that omega-3 and zinc can have a boost effect in reducing inflammation, so that the reduction of hs-crp in the simultaneous intervention group was more than the separate intervention groups.

Conclusions

Considering the lack of essential omega-3 fatty acids and zinc in the diet and the usefulness of these elements in the body and according to the present study, it is recommended to use omega-3 and zinc supplements, especially in diabetic patients. As well, this study showed that taking omega-3 and zinc together can enhance their effects in reducing lipid levels and increasing the antioxidant capacity of the body.

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