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Supplementation with omega-3 plus vitamin E and zinc plus vitamin C on metabolic syndrome components in postmenopausal women with type 2 diabetes

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Abstract

Background: The aim of this study was to find the influence of supplementation with omega-3 plus vitamin E and vitamin C plus zinc on metabolic syndrome components.

Methods: In a double-blind controlled clinical trial, 75 diabetic postmenopausal women were randomly assigned to one of the three therapeutic groups of daily supplementation of 1.8g omega-3 plus 400mg vitamin E (group A), 5mg zinc plus 300mg vitamin C (group B), or placebo (group C) for 12 weeks and the results were evaluated.

Results: One-way repeated measures ANOVA showed that systolic and diastolic blood pressure and waist circumference showed significant difference before and after the intervention (P= 0.0001, 0.001, and 0.045, respectively). Results of Univariate ANOVA showed that the level of plasma fasting blood sugar (FBS) and HDL-cholesterol significantly increased (P=0.01 and P=0.03, respectively) in patients who had been diagnosed as diabetic ≤7 years in group B. The plasma TG concentration significantly decreased (P=0.007) in patients who had been diagnosed as diabetic ≤7 years in group A. Systolic and diastolic BP significantly decreased (P=0.005 and P=0.04, respectively) in patients who had been diagnosed as diabetic ≤7 years in group A. The most effectiveness of nutraceutical supplementation was on patients that had been diagnosed as diabetic ≤7 years. Plasma TG and systolic BP decreased with omega-3 plus vitamin E; however, plasma FBS and HDL-cholesterol increased with zinc plus vitamin C supplementation.

Conclusion: Several factors, such as duration of diabetes, age, gender, pathophysiology status, genetic, and other anthropometric characteristics may influence the effectiveness of supplementation.

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Introduction

Numerous extensive clinical trials and meta-analyses revealed that the occurrence of metabolic syndrome, regardless of description, was greatly predictive of new-onset type 2 diabetes (T2DM) and cardiovascular disease in many different societies (1, 2). On the other hand, since micronutrient deficiencies are prevalent in metabolic syndrome, interventions to diminish this risk factor of metabolic syndrome would be beneficial (3). Many studies have revealed that people with the T2DM and metabolic syndrome have lower vitamins E and C levels (4). Supplementation with antioxidants may be valuable in these patients. It has been recently proposed that diabetic persons may have a damaged cellular antioxidant reaction against the oxidative stress generated by hyperglycemia (5).

On the other hand, replacement of n-3 poly unsaturated fatty acids (n-3PUFA) or fish oil with the safflower oil in dietary patterns improves insulin sensitivity and insulin function in skeletal muscles and liver (6). Dietary supplementation with n-3PUFA supplements could play an important role in the prevention and management of many illnesses such as coronary artery disease, hyperlipidemia, T2DM, metabolic syndrome, and high blood pressure. Nevertheless, several effects of n-3PUFAs supplementation on biological procedures in both humans and experimental animals have remained uncertain and require further investigations (7, 8). Moreover, clinical trials have revealed that supplementation with n-3PUFA and alpha tocopherol and other antioxidants ameliorate insulin sensitivity in patients with insulin resistance or T2DM. These supplements have an advantageous effect on atherogenesis and deteriorate T2DM complications (9, 10). Moreover, zinc supplementation in patients with T2DM improves cardiometabolic biomarkers (11, 12). Medical nutrition therapy is the first strategy in the management of diabetes and dietary interventions that modify low density lipoprotein (LDL) oxidative resistance may impact the development of vascular disease (13).

The consequences of a large-scale study authenticated that the relative risks of traditional cardiovascular risk factors for the incidence of myocardial infarction (MI) in all age groups of postmenopausal females were greater compared to males (14). Few studies approved predictor variables for better prediction of MI occurrence. A classification and regression tree (CART) model is capable of symbolizing interpretable clinical data. Hyperglycemia, hyperlipidemia, hypertension, and hyperuricemia are serious predictors for the incidence of MI in diabetic patients based on CART model (15). In previous studies, we revealed that the efficiency of nutraceutical supplements on cardiovascular biomarkers varies based on the kind of supplements or supplement pharmacogenomics, pathophysiologic status, and duration of diabetes (16). Therefore, in this investigation, we evaluated the influence of supplementation with omega-3 plus vitamin E and vitamin C plus zinc on metabolic syndrome components in postmenopausal women with T2DM.

Methods

Study Subjects

Seventy-five postmenopausal women with T2DM aged 50-65 years participated in a randomized, double-blind placebo-controlled clinical trial in the Iranian Diabetes Association. Similar to our previous study, patients were randomly assigned to three therapeutic groups. Each group comprises twenty-five diabetic patients to take 1) group A: 1.8 g of omega-3 (two capsules of 0.9 g per day) plus 400 mg

vitamin E (one capsule per day), 2) group B: 5mg zinc plus 300mg vitamin C (one capsule per day), 3) group C: 500mg canola oil as placebo (one capsule per day) for 12 weeks. In order to maintain equal intensity of treatment, all three supplements were placed in similar white plastic containers (16).

Eligibility and Study Design

The baseline protocol has been previously described in details (16). We evaluated the influence of supplementation with omega-3 plus vitamin E and vitamin C plus zinc on metabolic syndrome components such as fasting blood sugar (FBS), high density lipoprotein (HDL-) cholesterol, serum triglyceride (TG), systolic and diastolic blood pressure (BP), and waist circumference (WC) in diabetic patients compared with the control group. After randomization and before enrollment, each participant completed a written informed consent. Sequential sampling was carried out in the first visit.

The concealed administration of supplements began at the first visit and at 4-week intervals. The criteria for eligibility and exclusion criteria were previously described (16).

This study was registered in the Iranian Registry of Clinical Trials (IRCT138804312214N1). The review panels and ethics committees of each involved research center approved the protocol (Reference Number 5830/47/25). The funding administrations did not participate in the protocol of the project, analysis, or commentary on the data, or the writing of the manuscript.

Anthropometry Assessment

Waist circumference was measured to the nearest 0.1 cm using a non-stretchable tape at the narrowest point between the iliac crest and the lowest rib while the participant was lightly clothed and exhaled (NHANES protocol).

Dietary Intake Assessment

The patients completed three repeated 24-hour dietary recalls to confirm the total intake in the mid of intervention. In order to increase of recalls accuracy, trained research staff performed dietary intake assessment with the use of Food Album. Energy and nutrient intakes were determined by using the modified Nutrition IV database which modified for Iranian food substances in order to analyze 24-hour dietary recall forms (17).

Blood Pressure Measurements

Blood pressure was determined at the baseline and the end of the investigation by using a standard Mercurial Sphygmomanometer (ALP K2, Tanaka Sangyo, Japan) on the left or non-dominant arm after being in sitting position for 15 min. Measurements were repeated twice with 5 min rest between each reading, and an average reading was obtained for more BP accuracy.

Biochemical Measurement

After a 12-hour fasting, blood samples of patients were drawn into EDTA tubes at the beginning and the end of the investigation and instantly stored on ice until centrifugation at 3000rpm for 10 minutes. Plasma samples were stored at –80°C until an assay for cardiometabolic biomarkers could be accomplished. Levels of plasma glucose were measured in fresh samples by glucose oxidase through enzymatically colorimetric method (Pars Azmoon kit) with a sensitivity of 5mg/dl. Plasma HDL-cholesterol concentrations were

determined by enzymatically photometric method (Pars Azmoon kit) after sedimentation of apolipoprotein by the phosphotungstic acid solution. The sensitivity of plasma HDL-cholesterol measurement was 1mg/dl. Laboratory personnel were blind to the treatment groups.

Statistical Analysis

The sample size was calculated using the data of a study indicating that a minimum of 18 patients per group would be required to identify significant differences between groups (24). Statistical analysis was performed using SPSS software (SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, SPSS Inc.). The Kolmogorov-Smirnov test was applied in order to determine the normal distribution of biomarkers. Significance was assumed at $P < 0.05$. We applied ANOVA to determine significant mean differences between the beginning and the end of the investigation among three groups. Comparison of mean difference of each biomarker between the beginning and the end of the investigation in each group was analyzed by paired t-test. One-way repeated measures analysis of variance compared mean differences to clarify individual differences, diminish intragroup variances and increase power analysis. To achieve the desired results from the analysis, when there is no defined cutoff points for the selected independent variable, we divided groups into two parts (dichotomize) according to the selected independent variable such as duration of diabetes in participants. Surprisingly, mean and median of independent variable of duration of diabetes in participants (7years) were closely corresponded; then, this variable was dichotomized. Analysis of Covariance with the variable of duration of diabetes as a covariate was used. Then, general linear models (Univariate ANOVA) were used to examine the variation between metabolic syndrome components in terms of a linear combination of a predictor such as duration of diabetes in the beginning and the end of the investigation.

Supplement Characteristics

Eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA) capsules (PluShinzO-3 Cardio Omega-3) and placebos were donated by the Belgian Minami Nutrition Company. Vitamin C and Zinc capsules (EuRho OTC Pharma GmbH) and vitamin E capsules (Nature Made) were donated by Hakiman Teb Pharmaceutical Company and Iranian Poura Teb Medical and Pharmaceutical Company, respectively.

Results

Patients Characteristics

Sixty-nine patients (%92) completed the study in a 12-week intervention. Three participants withdrew from group A due to corticosteroid infusion (1 patient), having angioplasty (1 patient), and lack of motivation (1 patient). Two participants withdrew from group B due to the lack of motivation. One participant withdrew from group C due to having a trip. The mean (±SD) age of patients was 53.97 ± 2.14 years. The participants' mean and median duration of disease was 7 years. The mean (±SE) energy and nutrient intakes of diabetic participants in the three groups are shown in Table 1.

Table 1. Mean ± SE* of Energy and Nutrients Intake of Participants in the Studied Groups

	Groups			*P* value §
	Omega-3 plus Vitamin E (n=22)	Zinc plus Vitamin C (n=23)	Control (n=24)	
Energy (Kcal)	1195±76	1197±81	1352±66	0.23
Protein (g)	48.7±3.3	46.4±2.7	53.6±2.6	0.19
Carbohydrate (g)	171.2±10.0	166.8±9.6	214.6±12.2 ¶	0.003
Total fat (g)	37.9±3.4	40.9±4.9	34.6±2.4	0.48
Saturated fatty acids (g)	10.2±0.9	9.4±0.9	9.7±0.6	0.78
Mono unsaturated fatty acids (g)	11.1±1.2	12.0±1.8	9.8±0.8	0.51
Oleic acid (g)	10.1±1.4	11.8±2.1	8.0±1.0	0.22
Poly unsaturated fatty acids (g)	11.7±1.4	13.9±2.4	10.3±1.1	0.32
Linoleic acid (g)	9.8±1.4	12.7±2.4	8.8±1.1	0.23
Linolenic acid (g)	0.16±0.03	0.12±0.02	0.16±0.02	0.36
Eicosapentaenoic acid (mg)	0.009±0.006	0.002±0.000	0.012±0.004	0.19
Docosahexaenoic acid (mg)	0.029±0.015	0.009±0.002	0.037±0.013	0.20
Cholesterol (mg)	135±15	114±15	118±11	0.53
Fiber (g)	11±0.7	10±0.8	14±1.3	0.06
Soluble fiber (g)	0.4±0.1	0.4±0.1	0.4±0.1	0.84
Insoluble fiber (g)	2.0±0.2	2.1±0.3	2.4±0.3	0.50
Zinc (mg)	5.4±0.4	5.3±0.4	6.0±0.4	0.36
Vitamin C (mg)	94±7 ¶	66±7	77±6	0.03
Vitamin E (mg)	2.2±0.3	2.1±0.3	2.1±0.3	0.92

* One-way ANOVA was used to find the differences between energy and selected nutrient intake in the studied groups.

§ *P* value related to difference among three studied groups

¶ : *P* value < 0.05 one group in comparison with the other groups

Biochemical Measurement

There were no significant differences in any of metabolic syndrome components among the groups at the beginning of the intervention. Moreover, there were no significant differences in components among the groups after the end of the intervention except for plasma TG (Table 2). Table 2 also shows the mean (±SE) of metabolic syndrome components in diabetic women in every group at the beginning and at the end of the intervention. Plasma TG and systolic BP in patients of group A had a significant decrease at the end of the study. Systolic and diastolic BP in patients of group B had a significant decrease, but plasma FBS had a significant augment in this group at the end of the study.

Table 2. Mean ± SE* of Metabolic Syndrome Components of Participants in Three Groups as well as in Every Group at Baseline and at the End of Study

Biomarkers	Omega-3 plus Vitamin E (n=22)	*P* value #	Zinc plus Vitamin C (n=23)	*P* value #	Control (n=24)	*P* value #	*P value* §
Fasting blood sugar (mg/dl)							
Before	196±12	0.441	157±10	0.018	181±11	0.339	0.06
After	185±13		185±11		169±8		0.50
HDL-C‡ (mg/dl)							
Before	49.0±2.5	0.302	44.6±2.7	0.240	51.3±2.6	0.778	0.19
After	51.0±2.6		48.2±2.2		50.7±2.4		0.67
Plasma triglyceride (mg/dl)							
Before	152.6±7.8	0.009	182.8±14.0	0.867	162.9±7.4	0.022	0.12
After	138.3±5.7		180.3±18.0 ¶		148.5±7.0		0.04
Systolic blood pressure (mmHg)							
Before	125±4 ¶	0.000	127±3 ¶	0.001	129±4	0.012 ¶	0.84
After	116±3		116±2		119±2		0.59
Diastolic blood pressure (mmHg)							
Before	75±1	0.396	76±2 ¶	0.026	77±1 ¶	0.012	0.66
After	73±1		72±1		73±1		0.60
Waist circumference (cm)							
Before	96.2±2.3	0.853	100.1±1.8	0.245	101.3±2.5 ¶	0.045	0.25
After	96.1±2.3		99.9±8.9		101.0±2.5		0.29

*: One-way ANOVA was used to analyze the differences between metabolic syndrome components of participants in three groups and paired t-test was used to analyze the difference between metabolic syndrome components in every group at baseline and the end of the study.

§: *P* value related to the difference among three studied groups at baseline and at the end of the study.

#: *P* value showing difference between components in every group at baseline and at the end of the study.

¶: P value < 0.05 of one group in comparison with the other groups.

‡: High density lipoprotein-cholesterol

Table 3 displays the mean (±SE) differences of metabolic syndrome components variations of patients in three groups during the intervention. Some metabolic syndrome component variations diminished after 12-week intervention; however, there was a significant increase of plasma FBS in group B compared to the other groups. HDL-cholesterol levels in therapeutic groups increased at the end of intervention without any significant difference.

Table 3. Mean ± SE* of Metabolic Syndrome Components Variations of Participants in Three Groups at Baseline and at the End of Study

	Variations			P value§
	Omega-3 plus Vitamin E (n=22)	Zinc plus Vitamin C (n=23)	Control (n=24)	
Fasting blood sugar (mg/dl)	-11±14	28±11¶	-12±12	0.04
HDL-C‡ (mg/dl)	2.0±1.8	3.6±3.0	-0.6±2.0	0.44
Plasma triglyceride (mg/dl)	-14.4±5.0	-2.5±14.9	-14.5±5.9	0.61
Systolic blood pressure (mmHg)	-10±2	-12±3	-10±4	0.88
Diastolic blood pressure (mmHg)	-2±2	-5±2	-4±1	0.40
Waist Circumference (cm)	-0.02±0.12	-0.15±0.13	-0.27±0.13	0.38

*: One-way ANOVA was used to analyze the differences between metabolic syndrome components variation of participants in the three groups during the intervention

§: P.value related to difference between three studied groups at baseline and the end of the study

¶: P.value < 0.05 of one group in comparison with the other groups

‡: High density lipoprotein-cholesterol

The one-way repeated measures ANOVA showed that systolic, diastolic BP and WC were significantly different in two time stages (*P*= 0.0001, 0.001, and 0.045, respectively). However, interaction between these components and therapeutic groups was not significant. The levels of HDL-cholesterol and plasma TG in two time stages and interaction between these components and therapeutic groups were not significant. The level of plasma FBS was not significant in two time phases; however, the interaction between this component and the therapeutic group was significant (*P*= 0.04).

Results of Univariate ANOVA of metabolic syndrome components were shown in Table 4. The level of plasma FBS and HDL-cholesterol significantly increased (*P*=0.01 and *P*=0.03, respectively) in patients diagnosed with diabetes ≤7 years in group B. The level of plasma TG significantly decreased (*P*=0.007) in patients diagnosed with diabetes ≤7 years in group A. Systolic BP significantly decreased in all therapeutic groups; however, the highest decrease was shown (*P*=0.005) in patients diagnosed with diabetes ≤7 years in group A. Moreover, decrease of diastolic BP (*P*=0.04) was predominant in patients diagnosed with diabetes ≤7 years in group A.

Table 4. Univariate Analysis of Variances of Metabolic Syndrome Components of Participants in Three Groups at Baseline and at the End of Study

	Omega-3 plus Vitamin E (n=22) *		Zinc plus Vitamin C (n=23) *		Control (n=24) *		P value >7 years	P value ≤ 7 years
	>7 years (n=11)	≤ 7 years (n=11)	>7 years (n=13)	≤ 7 years (n=10)	>7 years (n=8)	≤ 7 years (n=16)		
Fasting blood sugar (mg/dl)								
Before	209.9±15.7	183.5±15.7	192.0±16.5	131.4±14.5¶	174.1±13.1	196.5±18.5	0.28	0.01
After	183.0±16.0	188.3±16.0	194.6±16.8	178.8±14.8	169.4±13.3	171.0±18.8	0.47	0.80
P value	0.21	0.82	0.83	0.01	0.77	0.21		
HDL-C‡ (mg/dl)								
Before	51.2±3.8	46.8±3.8	49.9±3.9	40.5±3.5	51.8±3.1	50.4±4.4	0.95	0.08
After	53.1±3.5	48.8±3.5	48.5±3.7	48.0±3.2	50.9±2.9	50.3±4.1	0.63	0.92
P value	0.55	0.37	0.80	0.03	0.77	0.97		
Plasma triglyceride (mg/dl)								
Before	145.8±15.0	159.5±15.0	183.3±15.7	182.4±13.8	166.1±12.4	156.8±17.6	0.25	0.41
After	138.7±17.2	137.8±17.2	170.7±18.0	187.6±15.8	148.9±14.3	147.6±20.2	0.38	0.13
P value	0.35	0.007	0.11	0.84	0.04	0.34		
Systolic blood pressure (mmHg)								
Before	126.5±6.1	125.3±6.1	126.9±6.4	127.9±5.6	129.0±5.1	130.1±7.2	0.95	0.86
After	119.1±4.0	112.8±4.0	116.4±4.1	114.9±3.6	116.1±3.3	125.4±4.6	0.82	0.13
P value	0.01	0.005	0.04	0.01	0.02	0.37		
Diastolic blood pressure (mmHg)								
Before	74.6±2.6	75.5±2.6	73.9±2.7	78.7±2.4	77.1±2.2	78.1±3.1	0.53	0.73
After	75.9±2.0	71.4±2.0	71.5±2.1	72.4±1.8	72.6±1.7	76.3±2.4	0.27	0.30
P value	0.64	0.04	0.28	0.06	0.15	0.44		
Waist circumference (cm)								
Before	92.4±3.1	100.0±3.1	101.4±3.3	99.2±2.9	98.1±2.6	107.8±3.7	0.07	0.25
After	92.2±3.1	100.1±3.1	101.2±3.3	99.0±2.9	97.9±2.6	107.4±3.7	0.08	0.28
P value	0.49	0.55	0.43	0.41	.13	0.22		

*: Mean ± SE of metabolic syndrome components in patients diagnosed as diabetic ≤7 and >7 years in three groups at baseline and the end of the study

¶: P.value < 0.05 for one group in comparison with the other groups

‡: High density lipoprotein-cholesterol

Results of Analysis of Covariance revealed that the interaction between the independent variable (the three exclusive therapeutic groups in relationship to supplementation) and the covariate variable of duration of diabetes was not significant. Therefore, the assumption of homogeneity of regression was met for all components. After controlling for the covariate, three therapeutic groups did not differ significantly in none of the metabolic syndrome components.

Discussion

The results of our study demonstrated that supplementation with omega-3 plus vitamin E has the most effect on reduction of plasma TG, systolic and diastolic BP in patients diagnosed with diabetes ≤7 years. These findings show that reduction of plasma TG with omega-3 plus vitamin E supplementation in comparison to TG increase in zinc plus vitamin C supplementation group is predominant. In a meta-analysis, supplementation with 3.5g omega-3 in patients with diabetes with the mean intervention of 8.9 weeks increased the plasma TG (18). In contrast, the other researchers showed that supplementation with 1.8g (19), 2.0g (20), 2.5g (21-23), and 4.0g EPA and DHA (24, 25) and 12.0g fish oil (26) decreased significantly plasma TG in patients with T2DM. Therefore, there is a negative association between omega-3 fatty acid intakes from fish oil and plasma TG level (27). Ble-Castillo and colleagues revealed that supplementation with vitamin E had not any effect on plasma TG level (28). In addition, supplementation with vitamin E plus vitamin C and zinc plus magnesium had not any influence on TG concentration in patients with diabetes (29). In our study, supplementation with water-soluble antioxidants such as zinc plus vitamin C had not any effect on TG plasma. This result was similar to the findings of another research (29). However, the effect of supplementation on metabolic syndrome components was related to the effect of omega-3 plus vitamin E simultaneously. Prevention of peroxidation of omega-3 was one of our minor purposes for supplementing vitamin E plus omega-3.

On the other hand, when we dichotomized samples based on duration of affecting by diabetes, the levels of FBS and HDL-cholesterol significantly increased in patients diagnosed with diabetes ≤7 years in the zinc plus vitamin C group. In some studies, supplementation with omega-3 significantly increased HDL-cholesterol level (19, 21), while, in other studies, there was not a significant variation in HDL-cholesterol level (23, 24). In addition, supplementation with vitamin E plus vitamin C and zinc plus magnesium increased HDL-cholesterol level by 24% in patients with diabetes after three- month intervention (29). In the present study, thinking of increasing or decreasing of a biomarker was related to paired-nutrient supplementation. Therefore, the effect of one nutrient may intensify or neutralize the effect of the other nutrients.

One limitation of the present study was that we could not evaluate the influence of supplementation on metabolic syndrome components based on Haptoglobin (Hp) genotypes in these participants. Decreased HDL-Cholesterol is a risk biomarker of metabolic syndrome. Since vitamin E significantly improves the quality of HDL in Hp 2-2 diabetic individuals and we did not detect the number of patients with the Hp 2-2 genotype, we could not assess the precise effect of vitamin E on decrease of oxidation of HDL. Nonetheless, another study revealed that vitamin E supplementation reduced oxidation of HDL and corrected HDL dysfunction in Hp 2-2 diabetic patients as compared with non-Hp 2-2 individuals (30). On the contrary, vitamin C supplementation increased the oxidative activity of Hp 2-2 bound to HDL and not only increased HDL oxidation, but also resulted in proinflammatory and proatherogenic status (30).

Moreover, systolic and diastolic BP reduction is more predominant in participants diagnosed with diabetes ≤7 years in omega-3 plus vitamin E supplementation than in the other subgroups of supplementation. Supplementation with 4g omega-3 did not show any significant variation in BP of patients with diabetes and hyperlipidemia (22). However, Axelrod and colleagues revealed that supplementation with 2.5g omega-3 during 6 weeks resulted in decreasing systolic BP (23). While, Ebbesson and colleagues demonstrated that dietary intake of omega-3 fatty acids inversely correlated with diastolic BP (27). In addition, another study showed that supplementation with vitamin E plus vitamin C and zinc plus magnesium, decrease systolic and diastolic BP in patients with diabetes (29).

In our study, one of the most important reasons was supplementation with omega-3 and vitamin E simultaneously due to decreasing oxidative stress from n-3PUFAs consumption. In other words, decrease or increase of a marker might be attributed to co-supplementation. The other reason in supplementation effectiveness was the duration of being affected by diabetes. Independent and effective variables such as body mass index and waist to hip ratio are valuable for dichotomizing studied samples (16). Therefore, in our study, duration of being affected by diabetes was a causative factor in supplementation efficiency on biochemical measurements. Pathophysiology status of patients in our study might have particular importance. Age, gender, obesity and predisposing disorders such as hypertension and dyslipidemia can diverse the results of studies.

Conclusions

The highest nutraceutical supplementation efficacy was observed in subjects diagnosed with diabetes ≤7 years. Plasma TG and systolic BP decreased with omega-3 plus vitamin E; however, plasma FBS and HDL- cholesterol increased with zinc plus vitamin C supplementation. Therefore, many factors such as duration of diabetes, age, gender, pathophysiology status, genetic, and the other anthropometric characteristics may influence the effectiveness of supplementation.

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Conflict of Interest

No potential conflict of interest relevant to this article is reported.

Authors' contributions

Mohammad Reza Mahmoodi developed the original idea and protocol and contributed in study design, data gathering and writing of the manuscript. Yadollah Mehrabi was the advisor and contributed in statistical analysis. The late Masoud Kimiagar was the supervisor and contributed in original idea and protocol and Asadollah Rajab was the clinical advisor and contributed in managing and supervision of diabetic patients in Iranian Diabetic society.

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