Effects of administration of omega-3 fatty acids with or without vitamin E supplementation on adiponectin gene expression in PBMCs and serum adiponectin and adipocyte fatty acid-binding protein levels in male patients with CAD

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Abstract

Objective: Adiponectin is a unique anti-atherogenic adipocytokine. Regulation of adiponectin secretion is dysfunctional in cardiovascular diseases. The current trial study assessed the effects of omega-3 fatty acids with or without vitamin E on adiponectin gene expression in peripheral blood mononuclear cells and serum adiponectin and adipocyte fatty acid-binding protein (A-FABP; also called ap2 and FABP4) levels in patients with coronary artery disease (CAD).

Methods: This randomized, double-blind, placebo-controlled trial included 67 male patients with CAD. First of the four group of participants received 4 g/day omega-3 fatty acids plus 400 IU/day vitamin E (OE), second group 4 g/day omega-3 fatty acids plus vitamin E placebo (OP), or both omega-3 fatty acid and vitamin E placebos (PP) for 8 weeks. Adiponectin gene expression and serum adiponectin and FABP4 levels were evaluated.

Results: The combination of omega-3 fatty acids and vitamin E in patients with CAD affected their serum adiponectin and FABP4 levels and the adiponectin/FABP4 ratio significantly. In the OP group, serum adiponectin levels did not change significantly. Consumption of omega-3 fatty acids with and without vitamin E had no significant effect on adiponectin gene expression.

Conclusion: Omega-3 fatty acids with or without vitamin E improve adiponectin levels in patients, without any significant changes in adiponectin gene expression. This nutritional intervention may prevent complications in patients with CAD because of increased adiponectin levels.
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Key words: coronary artery disease, omega-3 fatty acids, vitamin E, adiponectin, adipocyte fatty acid-binding protein

Introduction

Cardiovascular diseases (CVDs) are the leading cause of mortality and the fifth leading cause of disability and morbidity in the world. According to the World Health Organization (WHO), an estimated 17.3 million people died from CVDs in 2008, representing 30% of all global deaths (1). During the 20th century, the percentage of all deaths due to CVDs has increased from 10% to 30% and it is estimated that almost 23.6 million people will die from CVDs by 2030 if the current rates remain unchanged (2). In Iran, nearly 15 million people have been suffering from CVDs and nearly 317 of the daily 750 deaths occur on account of CVD (3). In fact, CVDs are one of the most life-threatening diseases and are the underlying cause in approximately 70% of all deaths in people over 75 years and 25% of deaths in people over 30 years (4). CVDs account for nearly half of the deaths due to non-communicable diseases in Iran (4, 5). Studies have revealed that dietary factors play a major role in the prevention and management of CVDs (2, 6-10). Consumption of omega-3 fatty acids from plant and marine resources appears to be useful because they improve endothelial function and reduce inflammatory responses, thrombosis risk, arrhythmias, and atherosclerotic plaque growth (8, 11-13).

Adiponectin is an adipocytokine secreted by adipose tissues (adipocytes); it has 244 amino acids and was first discovered in 1995. Adiponectin is a unique anti-atherogenic and anti-inflammatory adipocytokine. Studies have shown that secretion of

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adiponectin and expression of its receptor are reduced in patients with CVDs (11, 14-16). The gene responsible for adiponectin is located on chromosome 3q27. Adiponectin circulates in blood plasma in the form of low-molecular-weight (LMW) trimers, medium-molecular-weight (MMW) hexamers, and high-molecular-weight (HMW) multimers (14, 16-20). Epidemiological studies have shown that decreased adiponectin levels are associated with increased risk of CVDs (19), hypertension, and blood lipid abnormalities (21). *In vitro* studies have shown that adiponectin prevents the progression of atherosclerosis by inhibiting the production of pro-inflammatory agents through the inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and phospho-Akt and plays a central role in the regulation of macrophages switching from the M1 to M2 phenotype with an increase of anti-inflammatory agents (17). Laboratory studies have demonstrated that lower adiponectin levels can result in an increase in the inflammatory processes in CVDs (17, 22). Vitamin E is a fat-soluble vitamin with antioxidant properties. Of the 8 natural vitamers of vitamin E, α-tocopherol and γ-tocopherol are the most common forms in the plasma (21). In addition to its antioxidant properties, vitamin E plays a critical role in the regulation of gene expression involved in cell cycle, inflammation, cell adhesion, cell signaling, lipid uptake, and gene expression of adiponectin (21).

Adipocyte fatty acid-binding protein (A-FABP), also called ap2 and FABP4, belongs to the large family of intracellular lipid-binding proteins with an average molecular weight. It is produced by adipose tissues and then released into the blood circulation (22, 23). Studies have indicated that this compound is possibly associated with insulin resistance, hyperglycemia, lipid abnormalities, and increased pro-inflammatory factors (23). In fact, A-FABP is able to bond with hydrophobic compounds such as saturated fatty acids (SFAs), which results in the accumulation of cholesterol esters, inducing abnormalities of lipid metabolism and inflammation (23). Adiponectin and A-FABP are involved in the pathogenesis of atherosclerosis in different manners; adiponectin, as a compound with anti-inflammatory properties, and A-FABP, as a pro-inflammatory factor, can contribute to the incidence and progression of CVDs (22). The A-FABP/adiponectin ratio has been shown to be a more useful marker in CVDs than either of these parameters alone (22). Therefore, the current trial study was conducted to investigate the effects of administration of omega-3 fatty acids with or without vitamin E supplementation on adiponectin gene expression in peripheral blood mononuclear cells (PBMCs) and serum adiponectin and A-FABP levels in patients with coronary artery disease (CAD).

Methods

Study design and participants

This study was a randomized, double-blind, placebo-controlled trial that included 67 male patients suffering from CVDs with more than 50% stenosis proven by at least 1 coronary angiogram in the last 3 months. Non-smoking patients with a body mass index (BMI) of ≤30 and no infections, allergies, thyroid deficiencies, diabetes, or kidney and liver diseases were selected. The participants were recruited between July 2012 and June 2013 from the Tehran University of Medical Sciences Educating Hospital. The participants first signed an approval and were then familiarized with the study scope and methodology. Using a randomized permuted block method, the patients were assigned to receive 8 weeks of 4 g/day of omega-3 fatty acids and vitamin E resembling placebo (OP), 4 g/day of omega-3 fatty acids and vitamin E (OE), or omega-3 fatty acid and vitamin E placebo softgels (PP) with their lunch and dinner. Blood samples were collected at baseline and at the end of week 8. Nutritionist IV and Version 18.0; SPSS Inc., Chicago, USA, software were used for the analysis of dietary data and statistical analyses, respectively.

The study was approved by the Ethical Committee of Tehran University of Medical Sciences (reference no.: 86686). The study was registered in the Iranian Registry of Clinical Trials (IRCT) (IRCT2013080514273N1).

Information questionnaires

A general information questionnaire including questions such as age and disease period was provided to the participants. Twenty-four-hour food recalls were collected at the beginning and end of the study on 2 nonconsecutive days. Physical activities were assessed using the International Physical Activity Questionnaire (IPAQ). The physical activity questionnaire evaluates physical activity levels in terms of metabolic equivalent of task (MET). In this study, physical activity was categorized in to 3 different levels according to METs: low: <600 MET-min/week; moderate: 600-3000 MET-min/week; and severe: >3000 MET-min/week).

Anthropometric measurements

Height, weight, and waist and hip circumferences were measured before and after the study. All anthropometric measurements were performed according to the methods approved by the WHO. Weight was measured to the nearest 0.01 kg using a digital scale (Seca, Clara 803, Germany) with the patient minimally clothed and barefoot. Height was measured to the nearest 0.1 cm using a stadiometer (Seca, Germany) with the patient barefoot. Waist and hip circumferences were measured using a non-stretch tape (Seca, 201, Germany) and the mean of 2 measurements was recorded. BMI was calculated by dividing the patient's weight in kilogram by the squared height in meter. The waist-to-hip ratio (WHR) was calculated by dividing the waist circumference by the hip circumference.

Omega-3 fatty acid supplementation and placebos

Omega-3 fatty acid softgels containing 180 mg of eicosapentaenoic acid (EPA) and 120 mg of docosahexaenoic acid (DHA) and placebo softgels containing paraffin were provided by Minoo Pharmaceutical, Cosmetic and Hygienic Co., Iran. Placebos were phenotypically similar to the supplements.

Blood sampling

Venous blood (10 mL) was collected from the patients' antecubital vein under sterile conditions after 12-14 h of fasting before and after the study. The blood sample (8 mL) was trans-

Table 1. Baseline characteristics of the patients*

	OP (n=22)	OE (n=20)	PP (n=20)	*P*#
Triglyceride, mg/dL	159.95±12.31	176.50±17.99	189.22±19.54	0.45
Total cholesterol, mg/dL	166.16±7.21	185.15±9.12	161.88±7.38	0.10
LDL cholesterol, mg/dL	101.82±4.61	109.10±4.59	102.35±5.17	0.49
HDL cholesterol, mg/dL	32.85±1.57	31.05±1.57	32.42±1.61	0.78
Systolic blood pressure, mm Hg	124.23±3.85	124.45±3.70	128.80±2.72	0.58
Diastolic blood pressure, mm Hg	80.59±2.63	81.60±2.51	78.85±2.19	0.73

HDL - high-density lipoprotein; LDL - low-density lipoprotein; OE - omega-3 fatty acid & vitamin E; OP - omega-3 fatty acid & placebo; PP - placebo & placebo.*Mean±SE; #ANOVA

ferred to a sterile heparinized tube. Serum was separated from the whole blood sample and stored at -80°C until use. Adiponectin levels were assessed using an enzyme-linked immunosorbent assay (ELISA) kit (Mediagnost, Germany). Serum A-FABP levels were determined using a commercially available ELISA kit (Bioassay Technology Laboratory, China).

Isolation of PBMCs for assessment of adiponectin gene expression

First, 8 mL of phosphate-buffered saline (PBS) were added to 8 mL of heparinized blood in a 45-mL sterile tube and vortexed. Then, the mixture was slowly poured in a tube containing 8 mL of Ficoll®, followed by centrifugation at 2500 rpm for 2 min. The buffy coat layer was transferred to another sterile tube. Then, the same volume of PBMCs was taken and PBS was added to wash. It was centrifuged at 1600 rpm for 15 min. To dissolve the cell sediment, 150 mL of PBS was added to the cells and pipetted several times and then transferred to an RNase-free microtube.

Isolation of RNA and cDNA synthesis

RNA was extracted using the RNeasy® Plus Mini kit (Qiagen, Germany) according to the manufacturer's instructions. cDNA was extracted from RNA by the QuantiTect® reverse transcriptase kit (Qiagen, Germany) and stored at -20°C until use. To assess the intensity of gene expression, the StepOne® real-time PCR system (Applied Biosystems, USA) was used. PCR was performed in a final volume of 20 µL, including 0.5 µL of each primer, 7 µL of SYBR Green® Power PCR Master Mix (2×) (Applied Biosystems, USA), and sufficient amount of sterile distilled water. To calculate the gene expression, the following formula for $2^{-\Delta\Delta ct}$ was used: $2^{-[(\text{CT of the target gene after trial-CT of } \beta\text{-actin after trial})]-[(\text{CT of the target gene before trial-CT of } \beta\text{-actin before trial})]}$.

Primer design method

Target genes and their mRNA transcript sequences were obtained from the Ensembl website. Primers were modified according to the optimal PCR conditions. Primers for the housekeeping gene β-actin were selected and modified on the basis of previous studies. The primer sequences used in the current study were as follows: adiponectin: AGGCCGTGATGGCAGGAGATG (forward) and CTGAATGCTGAGCGGTATACATAAG (reverse) and β-actin: CCTGGCACCCAGCACAATGAAG (forward) and CTAAGTCATAGTCCGCCTAGAAG (reverse).

Statistical analysis

SPSS, version 18.0 (SPSS Inc., Chicago, USA) software was used for statistical analyses. Data were presented as mean±standard error (SE). Kolmogorov-Smirnov test was used to check the normality of data distribution. A paired t-test was used to compare the means of the variables before and after the study in each group. One-way analysis of variance (ANOVA) was used to compare the mean of the variables between the groups. The chi-square test was used to compare ordinal and categorical data between the groups. The reported p values were 2-sided and p values of <0.05 were considered significant.

Results

Sixty-seven male patients with CAD were equally divided into the OP, OE, and PP groups. During the study, 3 patients were hospitalized for heart surgery and 2 patients withdrew on account of personal reasons. Therefore, 62 patients completed the study, including 22, 20, and 20 patients in the OP, OE, and PP groups, respectively. The mean±SD age was 54.68±1.27 years, 56.30±1.62 years, and 58.50±1.33 years in the OP, OE, and PP groups, respectively. No significant differences were seen between the groups in terms of age (p=0.16). The duration of the disease in the OP, OE, and PP groups was 3.28±0.93 years, 2.66±0.88 years, and 5.29±1.39 years, respectively, with no statistically significant difference between the groups (p=0.22). The baseline characteristics of the patients, such as serum lipid profiles and blood pressure, are shown in Table 1.

Anthropometric variables

The anthropometric variables, including height, weight, BMI, waist and hip circumferences, and WHR, are shown in Table 2. No significant differences were seen in the anthropometric findings between the experimental groups. The differences were not significant between the values at the beginning and end of the study for any of the anthropometric variables.

Nutrient intake

The means±SE of intake are described in Table 3. No significant differences were observed in energy, macronutrient, and other nutrient intake between the groups at the beginning and end of the study. Comparison of the mean differences in energy and macronutrients between the beginning and end of the study showed no significant changes between the 3 groups.

Table 2. Anthropometric parameters among the groups at baseline and week 8*

		OP (n=22)	OE (n=20)	PP (n=20)	*P*#
Height, cm	Before	168.27±1.01	169.98±1.21	167.57±1.56	0.40
Weight, kg	Before	79.09±2.08	77.47±2.32	77.28±2.61	0.82
	After	79.24±2.09	77.66±2.30	77.25±2.56	0.80
	Difference	0.145±0.306	0.195±0.324	-0.35±0.248	0.85
	P value§	0.64	0.55	0.88	
BMI, kg/m^2	Before	27.97±0.76	26.80±0.72	27.47±0.81	0.55
	After	28.01±0.74	26.85±0.86	27.46±0.79	0.53
	Difference	0.039±0.113	0.053±0.109	-0.014±0.088	0.89
	P value§	0.73	0.63	0.86	
WC, cm	Before	97.46±1.96	95.05±1.61	97.35±1.84	0.55
	After	97.95±1.95	94.92±1.45	97.80±1.86	0.41
	Difference	0.318±0.412	-0.125±0.566	0.45±0.380	0.65
	P value§	0.45	0.82	0.25	
HC, cm	Before	101.73±1.38	99.65±1.18	99.95±1.22	0.45
	After	101.91±1.284	100.12±1.25	100.30±1.22	0.53
	Difference	0.181±0.408	0.475±0.566	0.350±0.357	0.89
	P value§	0.66	0.41	0.34	
WHR	Before	0.95±0.01	0.95±0.01	0.97±0.01	0.43
	After	0.96±0.01	0.95±0.01	0.97±0.01	0.21
	Difference	0.001±0.004	-0.005±0.005	0.001±0.003	0.44
	P value§	0.78	0.32	0.64	

BMI - body mass index; HC - hip circumference; OE - omega-3 fatty acid & vitamin E; OP - omega-3 fatty acid & placebo; PP - placebo & placebo; WHR - waist-to-hip ratio; WC - waist circumference; *Mean±SE; #ANOVA; §paired t-test

Fatty acid intake

The dietary intake of omega-3 fatty acids, omega-6 fatty acids, and SFAs is described in Table 4. The distribution of omega-3 and omega-6 fatty acids shifted to normal after logarithmic transformation. No statistical differences or significant changes were seen in the intake of omega-3 fatty acids, omega-6 fatty acids, and SFAs between the experimental groups.

Serum adiponectin and FABP4 levels

The mean±SE serum adiponectin and FABP4 levels at baseline and week 8 are shown in Table 5. At the end of the study, serum adiponectin levels were significantly different between the OP and PP groups (p=0.02) and between the OE and PP groups (p<0.001). However, serum adiponectin levels were not significantly different between the OP and OE groups (p=0.21). At the beginning and end of the study, no significant differences in serum FABP4 levels were seen between the 3 groups. However, FABP4 levels in the OP group were significantly decreased at the end of the study compared with the baseline (p=0.04) and OE group (p=0.02). The difference was not significant between the OP and OE groups.

Adiponectin gene expression

Adiponectin gene expression changes are shown in Table 6. After 2 months of study, no significant changes were seen in adiponectin gene expression in any of the 3 groups. Comparison of the mean difference in adiponectin gene expression between the beginning and end of the study showed no significant changes in the 3 groups.

Physical activity

No significant differences were reported in physical activity of the participants in all groups before and after the study (Table 7). Data showed that the participants' physical activity did not change significantly.

Discussion

The current study showed that combined omega-3 fatty acid and vitamin E supplementation for 2 months in CAD men affected serum adiponectin and A-FABP levels, and the adiponectin/A-FABP ratio significantly. However, consumption of omega-3 fatty acids did not significantly increase adiponectin levels. Omega-3 fatty acids with or without vitamin E supplementation had no effects on adiponectin gene expression. Adiponectin is mainly secreted by adipocytes and certain blood, colon, and small intestine cells (24-26). Usually, this protein is present in healthy human plasma at levels of 3-30 µg/mL (16-18). Adiponectin levels in women are 40% higher than those in men. This protein is present in various forms in plasma and comprises nearly 0.01% of total plasma proteins (18,

Table 3. Dietary intake among the groups at baseline and week 8*

		OP (n=22)	OE (n=20)	PP (n=20)	*P*#
Energy, Kcal	Before	1506.24±125.16	1900.33±161.37	1633.12±142.94	0.14
	After	1647.18±150.41	1890.29±135.31	1522.21±76.53	0.13
	Difference	14.19±15.81	-8.87±18.60	-11.04±14.52	0.54
	P value§	0.37	0.45	0.96	
Carbohydrate, g	Before	230.86±18.48	316.36±36.03	264.70±26.17	0.09
	After	260.37±25.69	284.73±20.40	222.50±16.02	0.13
	Difference	29.51±27.90	-31.63±35.41	-42.18±28.25	0.20
	P value§	0.30	0.15	0.38	
Protein, g	Before	56.23±5.67	64.84±4.94	67.13±7.93	0.42
	After	54.62±4.56	58.46±4.29	53.01±5.07	0.70
	Difference	-1.60±7.62	-6.37±5.29	-14.12±8.63	0.48
	P value§	0.83	0.11	0.24	
Fat, g	Before	42.84±6.75	45.96±2.73	37.36±4.33	0.49
	After	46.92±5.57	55.37±5.80	38.08±3.91	0.07
	Difference	4.07±4.82	9.40±6.16	0.714±6.14	0.56
	P value§	0.40	0.90	0.14	
Zinc, mg	Before	5.91±0.697	6.12±0.442	5.45±0.568	0.71
	After	5.38±0.560	5.99±0.691	38.08±3.91	0.47
	Difference	-0.536±0.971	-0.134±0.708	-0.491±0.689	0.93
	P value§	0.58	0.85	0.48	
Vitamin E, mg	Before	3.41±0.809	2.80±0.335	2.64±0.668	0.67
	After	3.59±0.914	3.65±1.03	3.00±0.582	0.84
	Difference	0.18±1.38	0.855±1.09	0.354±0.771	0.90
	P value§	0.89	0.44	0.65	
Selenium, mg	Before	0.062±0.008	0.067±0.006	0.068±0.008	0.67
	After	0.055±0.008	0.063±0.010	0.062±0.007	0.84
	Difference	-0.007±0.012	-0.004±0.012	-0.005±0.011	0.98
	P value§	0.54	0.73	0.63	
Folate, µg	Before	193.69±21.86	252.86±29.78	179.48±24.57	0.11
	After	203.20±22.53	240.37±41.10	159.34±19.11	0.15
	Difference	9.51±33.19	-12.48±52.19	-20.13±30.13	0.85
	P value§	0.77	0.81	0.51	
Vitamin C, mg	Before	84.23±1.26	96.92±2.35	93.10±1.57	0.87
	After	111.53±17.78	114.56±21.15	81.25±14.35	0.37
	Difference	27.290±22.46	17.63±21.06	-11.57±12.17	0.34
	P value§	0.23	0.41	0.35	

OE - omega-3 fatty acid & vitamin E; OP - omega-3 fatty acid & placebo; PP - placebo & placebo; *Mean±SE; #ANOVA; §paired t-test

25). Previous studies have shown that adiponectin is a unique anti-inflammatory and anti-atherogenic adipocytokine. Regulation and secretion of this protein are decreased in patients with CAD (14-16). Plasma adiponectin levels are negatively associated with BMI, age, and C-reactive protein (CRP) levels. It has been shown that adiponectin increases in people who have lost weight (27). However, adiponectin is linked to smoking and gender and the levels are lower in males possibly on account of androgenic effects (14). The level of this hormone increases with the consumption of Mediterranean foods and increased physical activity. Indeed, adiponectin is a protective factor in CVDs and therefore assessment of serum adiponectin levels is important. In fact, low level of adiponectin is one of the strongest predictors of CAD (27). The suggested mechanisms by which adiponectin protectively

Table 4. Fatty acid intake among the groups at baseline and week 8*

		OP (n=22)	OE (n=20)	PP (n=20)	*P*#
Omega-3 fatty acids, g	Before	0.16±0.08	0.12±0.004	0.11±0.06	0.66
	After	0.19±0.01	0.17±0.02	0.14±0.05	0.32
	Difference	0.03±0.04	0.05±0.01	0.03±0.03	0.78
	P value§	0.25	0.41	0.27	
Omega-6 fatty acids, g	Before	12.65±1.12	12.02±1.87	11.06±1.63	0.14
	After	14.55±1.41	15.10±1.98	13.08±1.69	0.11
	Difference	1.9±1.58	3.08±1.7	2.02±1.73	0.86
	P value§	0.28	0.22	0.28	
Saturated fatty acids, g	Before	10.50±2.43	11.08±0.831	9.37±1.17	0.77
	After	9.88±0.888	9.20±0.857	9.22±1.09	0.84
	Difference	-0.702±2.42	-1.88±1.41	-0.149±1.13	0.82
	P value§	0.77	0.19	0.93	

OE - omega-3 fatty acid & vitamin E; OP - omega-3 fatty acid & placebo; PP - placebo & placebo; *Mean±SE; #ANOVA; §paired t-test

Table 5. Serum adiponectin levels, serum FABP4 levels, and the adiponectin/FABP4 ratio among the groups at baseline and week 8*

		OP (n=22)	OE (n=20)	PP (n=20)	*P*#
Serum adiponectin, ng/mL	Before	6.18±0.396	6.08±0.963	4.66±0.362	0.17
	After	6.50±0.492	8.20±1.09	3.91±0.307	<0.001
	Difference	0.328±0.556	2.12±0.372	-0.845±0.472	<0.001
	P value§	0.56	<0.001	0.09	
Serum FABP4, ng/mL	Before	3.20±0.584	2.81±0.443	2.40±0.406	0.51
	After	2.35±0.329	1.80±0.169	2.18±0.274	0.34
	Difference	-0.856±0.410	-0.101±0.400	-0.214±0.163	0.25
	P value§	0.04	0.02	0.69	
Adiponectin/FABP4 ratio	Before	2.86±0.323	2.88±0.569	3.06±0.380	0.93
	After	2.90±0.394	4.80±0.568	3.28±0.339	0.009
	Difference	0.042±0.294	1.92±0.263	0.225±0.280	<0.001
	P value§	0.88	<0.001	0.43	

OE - omega-3 fatty acid & vitamin E; OP - omega-3 fatty acid & placebo; PP - placebo & placebo; *Mean±SE; #ANOVA; §paired t-test

affects the process of atherosclerosis include suppression of TNF-α-induced endothelial adhesion molecule expression, macrophage-to-foam cell transformation, and TNF-α expression in macrophages (27).

In the current study, combined omega-3 fatty acid and vitamin E supplementation significantly increased adiponectin levels. However, consumption of omega-3 fatty acids did not significantly increase adiponectin levels. Based on the insignificant changes in adiponectin levels in the OP group, it appears that the effect of omega-3 fatty acids on serum adiponectin levels cannot be ignored. Because other factors, including dose, duration of study, and diet, can also induce some effects, diet was assessed in the study. However, no significant differences in diet were observed between the baseline and end of the study. Stress and inflammation are other factors that may be involved; however, they were not assessed in the current study. Therefore, changes in stress, doses, or duration of the study may cause omega-3 fatty acids to induce no effects on serum adiponectin levels. A significant difference between the OP and OE groups was seen at the end of the study compared with the placebo group. However, the variation difference of this index was statistically significant at the end of the study compared with the baseline. According to the findings of the current study, it appears that the combination of omega-3 fatty acids and vitamin E may result in a marked increase in adiponectin levels.

In 2005, an *in vitro* study of ob/ob mice by Suganami et al. (28) showed that EPA may increase adiponectin secretion by reducing the secretion of TNFα. No significant increases were seen in the mRNA expression of adiponectin in adipose tissues. This indicated that EPA could halt NF-κB activity, decrease the mRNA expression of TNF-α, and decrease the activation of macrophages, resulting in an increase in adiponectin secretion rather than in its mRNA expression. In another study in animal models by Mazaki-Tovi et al. (29) in 2014, fish oil supplementation significantly increased circu-

Table 6. ΔCT of adiponectin gene and changes in the gene expression of adiponectin among the groups at baseline and week 8*

		OP (n=22)	OE (n=20)	PP (n=20)	*P*#
ΔCT of adiponectin gene	Before	21.02±1.51	18.49±1.03	21.57±0.708	0.28
	After	19.41±0.924	18.13±1.55	20.29±1.23	0.62
	Difference	-1.61±1.19	-0.36±2.26	-1.27±1.39	0.91
	P value§	0.21	0.88	0.37	
Changes in the gene expression of adiponectin (ΔΔCT)		10.26±3.91	8.81±6.51	71.91±56.38	0.61

OE - omega-3 fatty acid & vitamin E; OP - omega-3 fatty acid & placebo; PP - placebo & placebo; *Mean±SE; #ANOVA; §paired t-test

Table 7. Comparison of physical activity levels of the participants at baseline and week 8*

Physical activity		Low	Moderate	Severe	*P*§
Before intervention	OP	12 (54.5)	8 (36.4)	2 (9.1)	0.27
	OE	16 (51.6)	13 (41.9)	2 (6.5)	
	PP	9 (45)	9 (45)	2 (10)	
After 2 months of intervention	OP	10 (45.5)	9 (40.9)	3 (13.6)	0.41
	OE	12 (60)	7 (35)	1 (5)	
	PP	13 (65)	6 (30)	1 (5)	

OE - omega-3 fatty acid & vitamin E; OP - omega-3 fatty acid & placebo; PP - placebo & placebo; *Qualitative variables are shown by number (percentage); §chi-square test; ∞Because of the low number in the severe group, the moderate and severe groups were merged and the incorporated *P* value was reported.

lating adiponectin levels in healthy non-obese dogs. In the present study, omega-3 fatty acids and a combination of omega-3 fatty acids and vitamin E failed to increase the expression of genes in PBMCs in male patients with CAD. The adiponectin-induced suppression of monocyte adhesion has been reported to be inhibited by a selective AMP-activated protein kinase (AMPK) inhibitor compound C in animal models and cell cultures (30). The reduced expression and/or function of the adhesion molecules, integrins, may underlay the mechanism contributing to reduced monocyte adhesion following AMPK activation (31). These findings have suggested that the adiponectin expression of monocytes in PBMCs may play an important role in atherogenesis (32). However, studies in animal models and cell cultures have shown that overexpression of adiponectin in macrophages topically produces adiponectin in macrophage cells. This overexpression can also reduce foam cell production in arteries and prevent the cells from adhering to fibronectin-coated surfaces through the activation of 5-AMPK (32, 33). These results provide additional evidence that reduced circulating adiponectin levels and decreased adiponectin expression in PBMCs may increasingly be involved in atherogenesis (32). Neschen et al. (34) showed that consumption of fish oil for 2 weeks may increase the expression of adiponectin mRNA in epididymal adipose tissue and increase the secretion of this hormone in the blood circulation of rats. These data suggest that fish oil is a naturally occurring potent regulator of adiponectin secretion *in vivo*, acting through peroxisome proliferator-activated receptor gamma (PPARγ). However, Itoh et al. (15) did not report such an effect in their studies. These contradictory results could be due to the differences in the amount of drug administered or differences between the potential anti-inflammatory effects (34). Another study by Deemer et al. (33) showed no effects of omega-3 fatty acid supplementation on total serum adiponectin levels in healthy women. The current study showed no effects of omega-3 fatty acids alone, used for 2 months, on serum adiponectin levels, whereas the combination of omega-3 fatty acids and vitamin E increased adiponectin levels significantly at the end of the study. In general, α- and γ-tocopherols increase the expression of adiponectin and this effect is independent of the vitamin's antioxidant properties. Landrier et al. (35) have shown that tocopherol controls adiponectin expression through the regulation of gene expression (21).

Induction of PPARγ by vitamin E can increase the available PPARγ for binding to PPRE in the promoter region of adiponectin (21). Furthermore, PPARγ is required for adiponectin gene transcription and vitamin E plays a role in the PPARγ ligand-dependent transcriptional activity, which is associated with PPARγ target genes such as adiponectin (21). Iwaki et al. (36) have shown that the expression of the adiponectin gene is induced and maintained by endogenous or exogenous PPARγ ligands. They identified a functional PPRE in the adiponectin promoter, which plays a significant role in the transcriptional activation of the adiponectin gene in adipocytes by PPARγ. Apparently, combined omega-3 fatty acids and vitamin E may have a stronger synergistic effect on adiponectin gene expression. Furthermore, differences in this gene expression affection in various studies may be linked to differences in the administrated doses or duration of intervention. Adiponectin is suggested to exist in different forms in the blood circulation, including as LMW, MMW, and HMW forms; of these, the predominant form is LMW. However, between these 3 forms, HMW adiponectin plays an important biological role in relation to metabolic risk factors and CVDs (14, 16-18). In the present study, total adiponectin levels were assessed as well (37). Dodecamer adiponectin can bind multiple biotin-conjugated secondary antibodies, leading to over calculation. This over calculation could be the cause of low levels of LMW adiponectin in patients with high total adiponectin levels (>7 μg/mL). Measurement of total adiponectin levels is more

stable when evaluating absolute adiponectin levels, irrespective of the multimer size (37).

In this study, omega-3 fatty acids alone or combined with vitamin E for 2 months were shown to significantly reduce serum FABP4 levels in male patients with CAD. Combined intake of omega-3 fatty acids and vitamin E further reduced serum FABP4 levels. However, this difference was not statistically significant. FABP, as a new adipocytokine secreted by the adipose tissue, plays an important role in the development of metabolic syndrome and CVDs (17). Emerging evidence suggests that A-FABP plays an important role in metabolism and development of atherosclerosis and is positively associated with the severity of coronary artery stenosis in patients with CVDs. Furthermore, A-FABP may cause increased insulin resistance, hyperglycemia, and lipid abnormalities (17, 22). Recent studies have shown that A-FABP plays an essential regulatory role in energy metabolism and inflammation. A-FABP may affect atherosclerosis by dysregulation of metabolism in the adipose tissue as a result of macrophage activation. A-FABP expression in macrophages has been reported to be induced by oxidized low-density lipoprotein (LDL). Moreover, A-FABP dysregulates toll-like receptor (TLR) activators (38). In addition, it has been reported that an inhibitor of A-FABP markedly reduces atherosclerotic lesions (38). Adiponectin and A-FABP are involved in the pathogenesis of atherosclerosis. Adiponectin includes anti-inflammatory properties. In contrast, A-FABP is a pro-inflammatory factor in the development of lipid abnormalities and insulin resistance and contributes to the formation of foam cells (22). Studies have shown that the adiponectin/A-FABP ratio may be more useful for indicating CAD than A-FABP or adiponectin alone. There is a stronger correlation between the Gensini score and adiponectin/A-FABP ratio than adiponectin or A-FABP alone in both genders. Previous findings indicate that the adiponectin/A-FABP ratio shows metabolic and inflammatory conditions more comprehensively and it is less affected by age, sex, BMI, and confounding factors (22).

In the current study, serum A-FABP levels decreased by 2 months of supplementation with omega-3 fatty acids alone and combination of omega-3 fatty acids and vitamin E. It could consequently decrease the progression of cardiovascular complications. In this study, consumption of omega-3 fatty acids and vitamin E increased the adiponectin/A-FABP ratio. At the end of week 8 of the study, the adiponectin/A-FABP ratio was significantly different between the 3 groups. Furthermore, the difference from the baseline to the end of the study was statistically significant. However, various studies have shown that the genetic involvement in regulating adipocytokine could be associated with atherosclerosis (38). The A-FABP allele T87C has been shown to be associated with a decreased risk of CVDs (38). However, communication and direct interaction of A-FABP polymorphism with atherosclerosis has not been fully explained. The effects of A-FABP on lipid and glucose metabolisms may be an important mechanism for the progression of atherosclerosis (38). In 2011, Doi et al. (38) showed that increased plasma A-FABP levels in non-elderly men had a significant association with the presence of CAD. However, this association was not detected in patients aged ≥65 years old. This shows the power of predictive risk factor for CVDs in relation with age. Moreover, Doi et al. (38) reported a decrease in the effects of A-FABP on the progression of atherosclerosis in older people. Therefore, the effects of increased A-FABP levels can be considered as a risk factor for CVDs in patients under the age of 65 years. Furthermore, most studies on CVDs are conducted in men because the male gender is a risk factor for CAD (39). Previous studies have shown that serum A-FABP levels in female patients with CAD are significantly higher than those in male patients with CAD. Usually, A-FABP levels in men are lower than those in women. These inconsistent differences in serum A-FABP levels in women could make it difficult to analyze the survey findings in both genders (39). Therefore, only men were included in the current study. It should be noted that in the present study, nutrient intake were evaluated to show constant levels during the study. In this study, food intake, using 24-h food recalls, and physical activity were assessed regularly. At the end of week 8, no significant changes were seen compared with the baseline and patients did not change their diet and physical activity. In summary, the major highlights of the current study include a homogenous population study, assessment of total serum adiponectin levels, and study of adiponectin-encoding genes using real-time PCR.

Study limitations

The relatively short duration of the study and low doses of omega-3 fatty acid supplementation are some of the limitations. These factors may induce a significant influence on adiponectin gene expression. Therefore, additional large-scale studies with longer duration and higher doses are needed to better investigate the subject.

Conclusion

Administration of omega-3 fatty acids with or without vitamin E supplementation improves adiponectin levels in patients with CAD; however, it results in insignificant changes in adiponectin gene expression in PBMCs. This nutritional intervention may prevent complications in such patients occurring on account of decreased adiponectin levels.

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