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Beneficial effects of omega-3 and vitamin E coadministration on gene expression of SIRT1 and PGC1α and serum antioxidant enzymes in patients with coronary artery disease

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Abstract *Background and aim:* SIRT1 and PGC1α are two important genes, which play critical roles in regulating oxidative stress and inflammation processes. The study aimed assess the effects of coadministration of omega-3 and vitamin E supplements on SIRT1 and PGC1α gene expression and serum levels of antioxidant enzymes in coronary artery disease (CAD) patients.
Methods and results: Participants of this randomized controlled trial included 60 CAD male patients who were categorized into three groups: Group 1 received omega-3 (4 g/day) and vitamin E placebo (OP), group 2 omega-3 (4 g/day) and vitamin E (400 IU/day; OE), and group 3 omega-3 and vitamin E placebos (PP) for 2 months. Gene expression of SIRT1 and PGC1α in peripheral blood mononuclear cells ($PBMC_S$) was assessed by reverse transcription polymerase chain reaction (RT-PCR). Furthermore, serum antioxidant enzyme and high-sensitivity C-reactive protein (hsCRP) levels were assessed at the beginning and end of the intervention. Gene expression of SIRT1 and PGC1α increased significantly in the OE group ($P = 0.039$ and $P = 0.050$, respectively). Catalase and hsCRP levels increased significantly in the OE and OP groups. However, glutathione peroxidase (GPX) and superoxide dismutase (SOD) levels did not statistically change in all groups. The total antioxidant capacity (TAC) increased significantly in the OE group ($P = 0.009$) but not in OP and PP groups.
Conclusion: Supplementation of omega-3 fatty acids in combination with vitamin E may have beneficial effects on CAD patients by increasing gene expression of SIRT1 and PGC1α and improving oxidative stress and inflammation in these patients.

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Introduction

Sirtuins are a class of nicotinamine adenine dinucleotide (NAD)-dependent histone deacetylase proteins, which can transfer acetyl from acetyllysine residue of histones to adenosine diphosphate (ADP)-ribose of NAD [1,2]. Sirtuins deacetylate various substrates such as NF-kB, forkhead box O (FOXO), PGC-1α, and peroxisome-activated proliferator receptors (PPARs) [3]; therefore, they can influence a wide variety of cell pathways such as apoptosis, inflammation, and aging process and also extension of life span during calorie restriction conditions [4–6]. Sir2a or SIRT1 belongs to the sirtuin family and can regulate oxidative stress by affecting p53 [7,8]. In fact, deacetylation of this tumor suppressor by SIRT1 may have beneficial effects on cellular senescence by inhibiting the expression of growth suppressive genes involved in cellular senescence, thus reducing oxidative stress [8–10]. PGC1α is a key regulator of mitochondrial respiration and plays an important role in metabolism and energy homeostasis. Furthermore, it can increase the gene expression of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase. Therefore, interventions increasing the expression of PGC1α protect the body from oxidative stress [11]. Studies have shown that inflammation and oxidative stress play essential roles in the pathogenesis of cardiovascular diseases [12]. Therefore, strategies that can regulate oxidative stress and inflammation can improve pathologic conditions and overall health of cardiovascular patients.

Cell culture studies have shown that omega-3 fatty acids can elevate SIRT1 gene expression by increasing expression, phosphorylation, and activation of AMP-activated protein kinase (AMPK) in macrophages. Furthermore, they can reduce the gene expression of pro-inflammatory cytokines [13]. In fact, SIRT1 deacetylates NF-kB after activation of AMPK and results in inhibition of NF-kB signaling and expression of inflammatory genes [14]. Vitamin E, as an antioxidant agent, can protect cells from oxidative stress and increase the gene expression of antioxidant enzymes [15]. Moreover, vitamin E can activate AMPK and therefore increase the expression of sirtuins [16]. Due to the anti-inflammatory and antioxidant effects of omega-3 fatty acids and vitamin E and the potential role of sirtuins and PGC1α in protecting cells from oxidative stress and inflammation, this study was designed to assess the effects of coadministration of omega-3 and vitamin E supplement on SIRT1 and PGC1α gene expression as well as serum levels of antioxidant enzymes in coronary artery disease (CAD) patients.

Materials and methods

The participants of this randomized double-blind placebo-controlled clinical trial included 60 male CAD patients with at least 50% stenosis in one coronary artery proven by angiography in the past 3 months. These volunteers were selected from the Heart Medical Center, Tehran, Iran, between June 2012 and July 2013. An informed consent was obtained from them prior to the commencement of the study. The study was approved by the Tehran University of Medical Sciences Ethical Committee (ID: 23605) and registered inwww.clinicaltrial.org (registration number: NCT02011906). The participants were divided into three random groups by permuted block randomization; group 1 received omega-3 and vitamin E (OE), group 2 omega-3 and vitamin E placebo (OP), and group 3 omega-3 and vitamin E placebos (PP). The OE group received 4 g/day of omega-3 fatty acids and 400 IU of vitamin E. The OP group received 4 g/day of omega-3 fatty acids and vitamin E placebo. The PP group received both omega-3 fatty acids and vitamin E placebo softgels with lunch and dinner for 2 months. Each 1 g of omega-3 softgels contained 180 mg of eicosapentaenoic acid (EPA) and 120 mg of docosahexaenoic acid (DHA). Omega-3, vitamin E, and placebos were produced by Minoo Pharmaceutical, Cosmetic and Hygienic Company, Tehran, Iran. Height, hip, and waist circumference were measured before and after the intervention to the nearest centimeter, and weight was measured to the nearest kilogram. Body mass index (BMI) was calculated as the weight divided by the square of height, and waist to hip ratio (WHR) was calculated as the waist circumference divided by the hip circumference. All patients did not consume omega-3 and vitamin E supplements or fish oil in the past 3 months before starting the study, and we preferred not to change their dietary patterns during intervention.

In the beginning of the study and after 2 months of intervention, blood samples of 15 ml were collected after 12–14 h of overnight fasting. For peripheral blood mononuclear cell (PBMC) isolation using Ficoll separation technique, 10 mL of the blood samples was used and the remaining for serum separation. Blood serum was separated by centrifugation and stored at −80 °C until use. RNA was extracted using RNeasy Plus Mini Kit and then cDNA was synthesized using Qiagen Reverse Transcriptase Kit (Qiagen, Germany). Real-time polymerase chain reaction (PCR) was carried out based on the protocols described in previous studies [17]. β-actin was used in real-time PCR as the housekeeping gene. Primer sequences used in real-time PCR are described in Table 1. Serum levels of total antioxidant capacity (TAC) was assessed using 2,2′-azinobis3-ethylbenzthiazoline-6-sulfonic acid (ABTS) [18]. Catalase activity was assessed according to Hugo Aebi's method [19]. Serum levels of GPX and SOD were assessed by the methods prescribed by Paglia et al. and Sun et al., respectively [20,21]. Statistical analysis was carried out using SPSS Software v.18. Data were shown as mean ± SE

Table 1 Primers used in the current study.

Primer	Sequence
SIRT-1 Forward	GCCGGAAACAATACCTCCAC
SIRT-1 Reverse	ACACCCCAGCTCCAGTTAG
PGC-1A Forward	CTTGGCAGAGTATGACGATG
PGC-1A Reverse	TAGTGCAAGTAGAAACACTGC
β-actin Forward	CCTGGCACCCAGCACAATGAAG
β-actin Reverse	CTAAGTCATAGTCCGCCTAGAAG

(standard error). The Kolmogorov–Smirnoff test was used for determining normality of the parameters. One-way analysis of variance (ANOVA) test was used to compare the mean of the variables between the groups and paired *t*-test for comparison between groups before and after the supplementation. A *P*-value of ≤0.05 was considered statistically significant.

Results

Sixty-five male CAD patients initially participated in the current study; five of them discontinued the supplement consumption due to personal reasons and hence were excluded from the study. Therefore, at the end of intervention, the patients (total: 60) were placed in three groups as follows: OE: 21, OP: 20, and PP: 19. No statistically significant differences were observed between the mean values of patients' age and their disease duration within the groups at the beginning of the study ($P = 0.079$ and $P = 0.299$, respectively). Table 2 shows the baseline and post-intervention anthropometric parameters of the patients. No significant differences were observed between the anthropometric parameters within the various groups at the beginning of the intervention. Neither omega-3 nor omega-3 and vitamin E supplementation had significant effects on anthropometric parameters. Table 3 describes dietary intakes of the study groups at the beginning and end of the intervention based on their recall analyses. As shown, no significant differences in energy and macronutrient intakes were observed between the study groups at the baseline and end of the intervention. Furthermore, no statistical differences were observed between dietary intakes of vitamin E and fatty acids in the study groups during the 2-month intervention. Patients in all groups did not change their dietary patterns during the intervention.

Serum antioxidant enzymes and high-sensitivity C-reactive protein

As shown in Table 4, omega-3 alone and a combination of omega-3 and vitamin E supplementations significantly increased serum levels of catalase and decreased high-sensitivity C-reactive protein (hsCRP). Omega-3 and vitamin E supplementation increased serum level of GPX, but it was not statistically significant ($P = 0.086$). Furthermore, TAC increased significantly in the OE group ($P = 0.009$) but not in the OP and PP groups.

Gene expression findings

Results of this study showed that the gene expressions of SIRT1 and PGC1α based on $2^{-\Delta\Delta ct}$ calculation were statistically different between the study groups ($P = 0.039$ and $P = 0.050$, respectively). Post hoc analysis (Tukey's test) revealed a significant difference between the gene expressions of SIRT1 and PGC1α in the OE and PP groups ($P = 0.037$ and $P = 0.043$, respectively) but not in the OP and PP groups. Apparently, omega-3 in combination with vitamin E supplementation can increase the expression of SIRT1 and PGC1α genes in CAD patients (Table 5).

Discussion

The current study was the first to investigate the effects of nutrients on gene expression of SIRT1 and PGC1α in humans. We used PBMCs of CAD patients for studying the effects of omega-3 and vitamin E on gene expressions

Table 2 Anthropometric parameters of the study groups before and after the intervention.

Treatment group		OP ($n = 20$)	OE ($n = 21$)	PP ($n = 19$)	*P*-value*
Height (cm)	Baseline	169.04 ± 1.36	170.32 ± 1.19	170.92 ± 1.58	0.623
Weight (kg)	Baseline	79.95 ± 2.68	78.54 ± 2.17	78.35 ± 1.87	0.864
	Post-intervention	80.13 ± 2.70	78.85 ± 2.14	79.23 ± 1.82	0.916
	difference	0.18 ± 0.33	0.30 ± 0.30	0.88 ± 0.40	0.322
	P-value#	0.591	0.335	0.139	
BMI (kg/m^2)	Baseline	27.95 ± 0.83	27.08 ± 0.70	26.85 ± 0.61	0.530
	Post-intervention	28.00 ± 0.81	27.17 ± 0.66	27.14 ± 0.58	0.616
	difference	0.05 ± 0.12	0.09 ± 0.10	0.29 ± 0.13	0.318
	P-value#	0.687	0.370	0.170	
Waist circumference (cm)	Baseline	98.72 ± 2.11	95.76 ± 1.58	96.18 ± 1.88	0.479
	Post-intervention	98.30 ± 2.01	95.64 ± 1.45	96.42 ± 1.88	0.556
	difference	−0.42 ± 0.45	−0.12 ± 0.53	0.24 ± 0.39	0.618
	P-value#	0.359	0.827	0.548	
Hip circumference (cm)	Baseline	101.12 ± 1.59	100.33 ± 1.15	99.63 ± 0.80	0.701
	Post-intervention	100.75 ± 1.42	100.78 ± 1.23	99.37 ± 0.83	0.644
	difference	−0.37 ± 0.52	0.45 ± 0.54	−0.26 ± 0.45	0.455
	P-value#	0.481	0.411	0.567	
WHR	Baseline	0.97 ± 0.01	0.95 ± 0.01	0.96 ± 0.01	0.463
	Post-intervention	0.97 ± 0.01	0.95 ± 0.01	0.97 ± 0.01	0.214
	difference	−0.001 ± 0.005	0.005 ± 0.005	0.005 ± 0.004	0.304
	P-value#	0.850	0.325	0.191	

OP, omega-3 fatty acid and placebo; OE, omega-3 fatty acid and vitamin E; PP, omega-3 and vitamin E placebos; BMI, body mass index; WHR; waist–hip ratio; *mean ± SE; *ANOVA; #paired *T*-test.

Table 3 Dietary intakes of the study groups before and after the intervention.

Treatment group		OP ($n = 20$)	OE ($n = 21$)	PP ($n = 19$)	*P*-value*
Energy (Kcal)	Baseline	1450.74 ± 114.37	1469.10 ± 93.33	1528.49 ± 111.25	0.867
	Post-intervention	1684.55 ± 131.39	1649.48 ± 122.47	1508.20 ± 130.59	0.596
	difference	233.81 ± 169.99	180.38 ± 162.53	32.36 ± 173.82	0.688
	P-value#	0.185	0.281	0.854	
Carbohydrate (g)	Baseline	231.47 ± 22.93	228.44 ± 16.93	264.01 ± 18.52	0.800
	Post-intervention	271.56 ± 25.45	259.51 ± 21.96	238.00 ± 21.37	0588
	difference	40.09 ± 26.02	31.06 ± 25.68	−8.01 ± 30.04	0.426
	P-value#	0.140	0.241	0.793	
Protein (g)	Baseline	62.25 ± 7.75	69.64 ± 8.76	62.27 ± 7.71	0.770
	Post-intervention	60.44 ± 5.74	66.16 ± 6.98	59.30 ± 6.49	0.722
	difference	−1.81 ± 10.58	−3.48 ± 11.49	3.42 ± 10.64	0.992
	P-value#	0.866	0.765	0.751	
Fat (g)	Baseline	33.05 ± 2.79	33.45 ± 2.96	35.08 ± 3.82	0.895
	Post-intervention	42.88 ± 3.75	41.80 ± 4.10	37.01 ± 3.86	0.538
	difference	9.82 ± 4.97	8.34 ± 5.28	1.93 ± 5.53	0.538
	P-value#	0.063	0.131		
Vitamin E (mg)	Baseline	2.70 ± 0.55	2.72 ± 0.74	2.29 ± 0.65	0.427
	Post-intervention	4.19 ± 1.04	4.04 ± 0.97	2.77 ± 0.45	0.311
	difference	1.48 ± 1.29	1.33 ± 1.35	0.48 ± 0.78	0.971
	P-value#	0.265	0.338	0.456	
Omega-3 fatty acids (g)	Baseline	0.12 ± 0.03	0.13 ± 0.04	0.11 ± 0.04	0.963
	Post-intervention	0.21 ± 0.10	0.11 ± 0.05	0.10 ± 0.03	0.464
	difference	0.09 ± 0.11	−0.01 ± 0.06	−0.01 ± 0.05	0.570
	P-value#	0.428	0.821	0.781	
Omega 6 fatty acids (g)	Baseline	11.47 ± 0.93	10.80 ± 1.17	10.89 ± 1.76	0.926
	Post-intervention	13.76 ± 1.95	13.58 ± 2.15	12.87 ± 2.19	0.148
	difference	2.29 ± 2.21	2.78 ± 2.59	1.98 ± 2.91	0.534
	P-value#	0.273	0.380	0.506	
Saturated fatty acids (g)	Baseline	8.17 ± 6.28	9.73 ± 1.05	10.33 ± 1.19	0.333
	Post-intervention	9.16 ± 0.80	8.98 ± 0.77	10.11 ± 1.13	0.649
	difference	0.99 ± 1.07	−0.75 ± 1.28	−0.22 ± 1.65	0.643
	P-value#	0.367	0.566	0.897	

OP, omega-3 fatty acid and placebo; OE, omega-3 fatty acid and vitamin E; PP, omega-3 and vitamin E placebos; *mean ± SE; *ANOVA; #paired *T*-test.

Table 4 Serum antioxidant enzymes, TAC, and hsCRP values of the study groups before and after the intervention.

Treatment group		OP ($n = 20$)	OE ($n = 21$)	PP ($n = 19$)	*P*-value*
Catalase (mg/dl)	Baseline	71.35 ± 2.22	64.43 ± 2.16	65.00 ± 2.67	0.073
	Post-intervention	76.00 ± 2.13	71.19 ± 2.62	66.58 ± 2.83	0.042
	difference	4.65 ± 1.74	6.76 ± 1.94	2.44 ± 2.16	0.303
	P-value#	0.015	0.002	0.273	
SOD (mg/dl)	Baseline	175.05 ± 25.97	139.47 ± 7.46	194.85 ± 26.63	0.183
	Post-intervention	167.48 ± 18.31	134.68 ± 6.40	183.34 ± 25.35	0.164
	difference	−7.55 ± 13.11	−4.16 ± 6.04	−8.94 ± 7.55	0.936
	P-value#	0.571	0.499	0.252	
GPX (mg/dl)	Baseline	1.74 ± 0.65	1.70 ± 0.045	1.85 ± 0.056	0.163
	Post-intervention	1.81 ± 0.068	1.81 ± 0.045	1.79 ± 0.057	0.959
	difference	0.068 ± 0.072	0.12 ± 0.065	−0.052 ± 0.078	0.243
	P-value#	0.353	0.086	0.514	
TAC (mg/dl)	Baseline	112.26 ± 23.04	113.91 ± 21.15	79.40 ± 3.13	0.342
	Post-intervention	116.30 ± 22.81	119.32 ± 21.07	81.88 ± 3.50	0.316
	difference	4.04 ± 2.61	7.58 ± 2.62	2.45 ± 3.15	0.418
	P-value#	0.138	0.009	0.446	
hsCRP (mg/dl)	Baseline	2.76 ± 0.48	3.12 ± 0.55	3.34 ± 0.45	0.720
	Post-intervention	1.80 ± 0.18	1.60 ± 0.23	3.57 ± 0.64	0.001
	difference	−0.96 ± 0.46	−1.21 ± 0.48	0.23 ± 0.64	0.132
	P-value#	0.050	0.008	0.716	

OP, omega-3 fatty acid and placebo; OE, omega-3 fatty acid and vitamin E; PP, omega-3 and vitamin E placebos; hsCRP, high-sensitivity C-reactive protein; *mean ± SE; *ANOVA; #paired *T*-test.

Table 5 Gene expression of SIRT1 and PGC1α in the study groups[#].

	OP ($n = 22$)	OE ($n = 20$)	PP ($n = 20$)	P-value*
Gene expression of SIRT1	1.44 ± 0.31	2.77 ± 0.79	0.95 ± 0.16	0.039
Gene expression of PGC1α	5.28 ± 1.58	10.81 ± 3.71	2.24 ± 0.98	0.050

OP, omega-3 fatty acid and placebo; OE, omega-3 fatty acid and vitamin E; PP, omega-3 and vitamin E placebos; [#]mean ± SE; *ANOVA; the values were reported based on $2^{-\Delta\Delta ct}$ calculation.

because these cells can travel through blood and enter various tissues such as the adipose tissue [22]. Furthermore, these cells can reflect the metabolic and immune responses of adipocytes or hepatocytes to dietary interventions at the level of gene transcription [23,24] and also have critical roles in the development of atherosclerosis [25]. Results of this study showed that vitamin E and omega-3 can increase the expression of SIRT1 and PGC1α genes in CAD patients. Previous cell culture studies have shown that omega-3 fatty acids can increase SIRT1 gene expression by increasing phosphorylation and activation of AMPK, thereby resulting in the suppression of pro-inflammatory genes [13,14]. Increase in SIRT1 gene can also modulate endothelial nitric oxide synthase (eNOS) and p53 activity and promote vascular function by affecting smooth muscle cells of blood vessels [26]. Furthermore, omega-3 can increase the expression of genes involved in mitochondrial biogenesis such as PGC1a and also increase oxidation of fatty acids via induction of PPARα [27–29]. Fatty acids are the primary energy source of heart in adulthood [30,31]. Expression of PGC1a in cardiac cells can affect a wide variety of enzymes involved in many biological pathways such as Krebs cycle, fatty acid oxidation, and lactate and ketone body metabolisms [32]. Therefore, PGC1α may affect cardiac cells by increasing their efficiency of oxygen consumption and adenosine triphosphate (ATP) production [33]. Elevation of reactive oxygen species (ROS) levels is a common feature in cardiovascular disease and results in endothelial dysfunction [34,35]. Overexpression of PGC1α in endothelial cells also decreases ROS levels by enhancing antioxidant enzymes such as manganese (Mn)SOD, catalase, and thioredoxin [36]. Vitamin E is a powerful antioxidant agent, and its deficiency may aggravate oxidative stress as observed in cardiovascular diseases. Therefore, it is plausible that vitamin E supplementation can affect the level of antioxidant enzymes in CAD patients. In the current study, omega-3 alone and combined omega-3 and vitamin E supplementations resulted in a significant increase in serum catalase levels. However, serum SOD and GPX levels did not increase. Although omega-3 supplementation did not change the mean level of TAC, coadministration of omega-3 and vitamin E resulted in a statistically significant increase in TAC in the OE group. In a research study, supplementation with 400 IU/day of vitamin E for 12 weeks did not alter serum SOD levels in chronic obstructive pulmonary disease (COPD) patients [37]. Kolahi et al. reported no significant changes in SOD, GPX, and TAC levels in rheumatoid arthritis (RA) patients receiving omega-3 supplements with or without vitamin E [38]. Sarbolouki et al., in their study on diabetic patients, showed that vitamin E alone and a combination of EPA and vitamin E increased significantly serum TAC; however, only EPA showed significant effects on increasing levels of SOD and GPX, and catalase levels increased only in the group receiving EPA and vitamin E [39].

Previous studies have shown that AMPK can impose inhibitory effects on NF-kB signaling through the induction of deacetylase activity of SIRT1 and subsequent suppression of pro-inflammatory gene expression [14]. Xue et al. have shown that SIRT1 is required for stimulating anti-inflammatory effects of omega-3 fatty acids for antagonizing NF-kB signaling in macrophages [13]. Several studies have shown that omega-3 fatty acids are anti-inflammatory agents and can reduce inflammation by inhibiting the production of cytokines such as interleukin (IL)-1, 1L-2, and tumor necrosis factor (TNF)-α [40–42]. Moreover, they can reduce serum CRP levels [43,44]. Vitamin E can decrease the release of interleukins such as IL-6 and reduce serum CRP levels by lowering pro-inflammatory cytokines such as IL-1β [45–47]. Recently, Saboori et al. have revealed that supplementation with vitamin E in the form of either α- or γ-tocopherol can reduce serum CRP levels significantly [48]. In the present study, serum hsCRP levels decreased significantly in the OP and OE groups, but the OE group experienced more decline in serum CRP levels compared to OP. Similarly, Ramezani et al. have reported that omega-3 alone and combined omega-3 and vitamin E supplementation significantly decreased hsCRP levels in CAD patients [49]. Hence, it is evident that omega-3 fatty acids in combination with vitamin E significantly reduce inflammatory processes by decreasing CRP. To the best of the authors' knowledge, this is the first human study investigating the effects of nutrients on gene expression of SIRT1 and PGC1α in CAD patients. However, further studies are warranted to reveal the exact mechanisms by which omega-3 and vitamin E influence these key genes in humans. In conclusion, results of the current study showed that omega-3 fatty acids and vitamin E supplementation increased gene expression of SIRT1 and PGC1α in PMBCs along with beneficial effects on some antioxidant enzymes such as catalase and may relieve inflammation by decreasing hsCRP levels.

Conflicts of interest

The authors declare no conflict of interest.

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