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Original research

Omega-3 fatty acids and vitamin E supplementation can affect gene expressions of SIRT1, FOXO1 and UCP-2 in coronary artery disease patients

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ABSTRACT

Aims: This randomized controlled trial (RCT) study was carried out to assess effects of omega-3 supplementation alone and in combination with vitamin E on gene expressions of SIRT1, SIRT3, SIRT6, FOXO1, FOXO3 and UCP-2 in patients with coronary artery disease (CAD).

Methods: Participants of this RCT included 60 male CAD patients who classified into three study groups using block randomization, including 1) OE receiving omega-3 fatty acids (EPA: 720 mg, DHA: 480 mg) and vitamin E (400 IU); 2) OP receiving omega3 fatty acids (EPA: 720 mg, DHA: 480 mg) and vitamin E placebo; and 3) PP receiving omega-3 and vitamin E placebos for two months.

Results: In compared to PP group, gene expression of SIRT1 increased significantly in OE group ($P = 0.037$) while this group experienced significant decrease in gene expression of UCP-2 ($P = 0.035$). The expression of FOXO1 increased significantly in both OE and OP groups in compared to PP group ($P = 0.021$ and $P = 0.035$, respectively). However, gene expressions of SIRT3, SIRT6 and FOXO3 were not different between study groups.

Conclusion: The current study has shown that omega-3 fatty acids and vitamin E supplementation can increase gene expressions of SIRT1, FOXO1 while it can decrease gene expression of UCP-2 in CAD patients.

1. Introduction

Sirtuins or SIRTs are NAD-dependent histone deacetylase proteins involved in various pathways in cells, including cell functions and metabolisms, and consist of seven members, Sirt1–7 (Lavu et al., 2008a; Masri and Sassone-Corsi, 2014). These proteins can affect many pathways including oxidative stress, inflammation, energy metabolism in calorie restriction conditions, apoptosis and glucose and lipid metabolisms through deacetylating several substrates such as forkhead box O (FOXO), NF-kB, glucokinase and PGC-1a (Lavu et al., 2008b; Kitada et al., 2013). Recently, sirtuins have been described as novel target molecules for controlling chronic diseases (Dali-Youcef et al., 2007). Notably, sirtuins can up-regulate several members of forkhead box O (FOXO) transcription factors including FOXO3 and FOXO1 as they activate expression of some target genes such as antioxidant enzymes (Brunet et al., 2004). Furthermore, FOXO proteins are involved in regulation of insulin signaling pathway and glucose metabolism (Barthel et al., 2005). Gene expression of FOXO1 in islet cells of diabetic patients is more than that of non-diabetic patients (Del Guerra et al., 2005). Un-coupling proteins are a five-member family of mitochondrial transport proteins (UCP1-5) founded in the inner membrane of mitochondria which have suggested roles in regulating thermogenesis and protecting body from free radicals (Arsenijevic et al., 2000). Expression of UCP-2 occurs in many tissues such as immune system, spleen and especially pancreatic β-cells. This protein has critical roles in glucose and lipid metabolisms (Diano and Horvath, 2012). Studies have shown that UCP-2 negatively regulates insulin secretion and overexpression of UCP-2 in β-cells can result in inhibition of glucose-stimulated insulin secretion (GSIS) (Chan et al., 2001; Saleh et al., 2006).

Studies have shown that supplementation with omega-3 fatty acids can improve glucose and insulin homeostasis in overweight or obese subjects (Browning et al., 2007; Dangardt et al., 2012). Vitamin E, as a potent antioxidant and anti-inflammatory agent, includes beneficial effects on cardiovascular diseases by neutralizing free-radicals and

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decreasing anti-inflammatory factors such as hs-CRP, IL-1β and IL-6 (Singh et al., 2005; Saboori et al., 2015). In a study by Xue et al., omega-3 fatty acids were shown to antagonize macrophage inflammation via elevating gene expression of SIRT1 and activating AMPK/SIRT1 pathway (Xue et al., 2012). The aim of this study were to assess the effects of omega-3 fatty acid supplementation alone and in combination with vitamin E on gene expressions of UCP-2, FOXOs and SIRTs in patients with coronary artery disease.

2. Materials and methods

2.1. Study design and participants

This randomized controlled trial (RCT) study included 65 45–65 year-old male CAD patients with at least 50% stenosis in one coronary artery. The stenosis had been proven using angiography within the last three months of study commencement. All patients were selected from Tehran Heart Center, Tehran, Iran, after they agreed to participate in the study by signing an informed consent. The study was approved by the Ethical Committee of Tehran University of Medical Science, Tehran, Iran (No. 23605) and registered in www.clinicaltrial.org (No. NCT 02011906). The inclusion criteria were 1) BMI < 30 kg/m^2; 2) No consumption of supplements including omega-3, vitamin E and fish oil within the three months before the study commencement; 3) No history of diabetes, liver, kidney and thyroid diseases; and 4) Maintaining dietary patterns during intervention.

2.2. Intervention

The participants were assigned into three study groups using permuted block randomization. These three groups included 1) OE receiving omega-3 fatty acids (EPA, 720 mg; DHA, 480 mg) and vitamin E (400 IU); 2) OP receiving omega3 fatty acids (EPA, 720 mg; DHA, 480 mg) and vitamin E placebo; and 3) PP receiving omega-3 and vitamin E placebos for two months. All supplements and placebo softgels were purchased from Minoo Pharmaceutical, Cosmetic and Hygienic Company, Tehran, Iran.

2.3. Measurements

Anthropometric parameters were measured in the beginning and after two months of supplementation. Fifteen milliliters of the participant venous blood were collected in the baseline and post-intervention and 10 ml of the blood samples were used for Peripheral Blood Mononuclear Cell (PBMC) isolation using Ficoll®. Cell RNA was extracted using RNeasy Plus Mini Kit® (Qiagen, Germany). Qiagen Reverse Transcriptase Kit® (Qiagen, Germany) was used for cDNA synthesis. Real-time polymerase chain reaction (real-time PCR) was carried out using SYBR Green® Power PCR Master Mix in the StepOne system (Applied Biosystems, USA). Primer sequences used for real-time PCR were designed by Primer Express 3 and Beta-actin encoding gene was used as housekeeping gene (Table 1).

The PCR was performed using 48-well reaction plates containing 20 μl reaction mixture which was composed of 0.5 μl of each forward and reverse primers, 10 μl Power SYBR Green PCR Master Mix, 7 μl DEPC water and 2 μl cDNA of each gene. After a two-step amplification confirmed by melting curve, the relative changes of gene expressions were calculated based on $2^{-\Delta\Delta ct}$ formula in which ΔCT calculated as CT of target gene subtracted from the CT value of reference gene and ΔΔCT is ΔCT test sample – ΔCT calibrator sample.

2.4. Statistical analysis

All statistical analyses were carried out using SPSS Software v.18 and data were shown as mean ± standard error. Kolmogorov-Smirnoff test was used for determining normality of the parameters distribution.

Table 1
Primer sequences used in this study.

Primer	Sequence
Forward β-actin	CCTGGCACCCAGCACAATGAAG
Reverse β-actin	CTAAGTCATAGTCCGCCTGAAG
Forward SIRT1	GCCGGAAACAATACCTCCAC
Reverse SIRT1	ACACCCCAGCTCCAGTTAG
Forward SIRT3	GCATTCCAGACTTCAGATCG
Reverse SIRT3	AAGCAGCCGGAGAAAGTAG
Forward SIRT6	CAAGTGTAAGACGCAGTACGT
Reverse SIRT6	ATGTACCCAGCGTGATGGAC
Forward FOXO1	CAGATCTACGAGTGGATGGTC
Reverse FOXO1	AACTGTGATCCAGGGCTGTC
Forward FOXO3	CTACGAGTGGATGGTGCGTTG
Reverse FOXO3	TCTGGACCCGCATGAATCGAC
Forward UCP-2	ACAAGACCATTGCCCGAGAG
Reverse UCP-2	CATGAGGTTGGCTTTCAGGAG

One-way ANOVA test and paired *t*-test were respectively used for comparing mean of the variables between and within the groups at baseline and after the intervention. In all analyses, *P*-values less than 0.05 were considered as statistically significant.

3. Results

Of 65 patients initially participated in this study, three patients withdrew due to hospitalization for heart surgery and two due to failure of consuming at least 90% of the total supplements. Therefore, 60 participants were grouped in OE (n = 21), OP (n = 20) and PP (n = 19) groups. Baseline anthropometric parameters of the study groups are shown in Table 2. Anthropometric parameters were not significantly different between the study groups at the beginning of the supplementation. No significant differences were seen between the study groups in terms of age, disease duration and dietary intakes of energy, macronutrients, omega-3 fatty acids and vitamin E at the beginning and end of the intervention (Saboori et al., 2016a).

The gene expression results are demonstrated in Table 3. Based on $2^{-\Delta\Delta CT}$ formula, the gene expression rates of SIRT1, FOXO1 and UCP-2 were significantly different between the study groups. Tukey test showed that in compared to PP group, gene expression of SIRT1 increased significantly in OE group (P = 0.037) while this group experienced significant decrease in gene expression of UCP-2 (P = 0.035). The expression of FOXO1 increased significantly in both OE and OP groups in compared to PP group (P = 0.021 and P = 0.035, respectively). However, gene expressions of SIRT3, SIRT6 and FOXO3 were not different between study groups.

4. Discussion

To the best of the authors' knowledge, this is the first RCT study to assess effects of omega-3 supplementation alone and in combination with vitamin E on gene expressions of SIRTs, UCP-2 and FOXO transcription factors. Result of our previous study have shown that supplementation with omega-3 fatty acids in combination with vitamin E results in significant decrease in serum insulin and HOMA-IR, compared to that in CAD patients who received omega-3 fatty acids (OP) alone (Saboori et al., 2016b). Studies have shown that omega-3 fatty acids can improve insulin sensitivity via stimulating PPAR-α and PPAR-γ and hence mutation of PPAR-γ gene can result in insulin resistance in humans (Stumvoll and Haring, 2002; Deckelbaum et al., 2006). Vitamin E can act as selective PPAR modulator and enhance the interaction between PPAR-α and PGC-1α co-activator peptides (Fang et al., 2010). Results of the present study have shown that supplementation with omega-3 fatty acids in combination with vitamin E can increase gene expression of SIRT1 in treatment patients, compared to that in placebo patients. Furthermore, gene expression of FOXO1 increased

Table 2
Anthropometric parameters of the study groups at baseline.

Treatment group	OE (n = 21)	OP (n = 20)	PP (n = 19)	P-value*
Height (cm)	170.32 ± 1.19	169.04 ± 1.36	170.92 ± 1.58	0.623
Weight (kg)	78.54 ± 2.17	79.95 ± 2.68	78.35 ± 1.87	0.864
BMI (kg/cm^2)	27.08 ± 0.70	27.95 ± 0.83	26.85 ± 0.61	0.530
Waist circumference (cm)	95.76 ± 1.58	98.72 ± 2.11	96.18 ± 1.88	0.479
Hip circumference (cm)	100.33 ± 1.15	101.12 ± 1.59	99.63 ± 0.80	0.701
WHR	0.95 ± 0.01	0.97 ± 0.01	0.96 ± 0.01	0.463

OP, omega-3 fatty acid and placebo; OE, omega-3 fatty acid and vitamin E; PP, placebo and placebo; BMI, body mass index; WHR; waist to hip ratio; *ANOVA analysis (with permission of Iran J Public Health, Vol. 45, No.11, Nov 2016, pp.1465–1472).

Table 3
Gene expression of SIRTs, FOXOs and UCP-2 in the study groups.

Treatment group	OP (n = 20)	OE (n = 21)	PP (n = 19)	P-value*
SIRT1	1.44 ± 0.31	2.77 ± 0.79	0.95 ± 0.16	**0.039**
SIRT3	1.97 ± 0.49	2.80 ± 0.59	1.22 ± 0.26	0.068
SIRT6	2.66 ± 0.67	2.11 ± 0.57	1.00 ± 0.27	0.213
FOXO1	1.21 ± 0.27	2.82 ± 0.68	1.07 ± 0.27	**0.013**
FOXO3	2.36 ± 0.96	3.52 ± 1.25	1.06 ± 0.32	0.251
UCP-2	1.79 ± 0.46	1.18 ± 0.31	3.54 ± 1.03	**0.038**

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; Values reported based on $2^{-\Delta\Delta CT}$ calculation.

significantly in both OP and OE groups, compared to that in placebo group. Recently, studies have shown that omega-3 fatty acids can activate SIRT1 by up-regulating gene expression via AMP-activated protein kinases in macrophages (Xue et al., 2012). The SIRT1 can deacetylate and up-regulate gene expressions of FOXO1 and FOXO3. Furthermore, studies have demonstrated that FOXOs play an important role in protecting body from oxidative stress. The FOXO3 can increase mn-SOD in mitochondria which scavenges superoxide free radicals and increases production of antioxidant enzyme catalase (Lu et al., 2013). Similar to FOXO3, FOXO1 can increase expression of genes involved in protecting cells from oxidative stress and inflammation. An example includes protection of β-cells in pancreas from oxidative stress; hence, FOXO1 can improve insulin resistance (Kitamura et al., 2005). Although SIRTs can up-regulate several members of forkhead box O (FOXO) transcription factors including FOXO3 and FOXO1, this up-regulation could be influenced by several confounding factors. Some studies showed that subcellular localization of FOXO3 could be affected by factors including the presence of growth factor, menadione and oxidative status (Brunet et al., 2004). Transcriptional activity of FOXOs via SIRT1-mediated deacetylation also could be affected by some factors such as amount of resveratrol (Frescas et al., 2005). SIRT3 also can regulate FOXO3 activity through reversible acetylation and overexpression of this deacetylase could increase gene expressions of FOXO3-dependent antioxidant enzymes (Jacobs et al., 2008). It is possible that these different mentioned factors in study groups could be explained different gene expression responses after omega 3 and vitamin E co-supplementation in this study.

The possible relationship between UCP-2 and insulin secretion was firstly described in 2001 in an experimental study showing that UCP-2 deficient mice had increased levels of glucose-mediated insulin secretion (Zhang et al., 2001). This protein negatively regulates insulin secretion. Conversely, an increase in UCP-2 gene expression in β-cell line can result in a decrease in glucose stimulated insulin secretion (Chan et al., 2001). Results of the current study have shown that omega-3 fatty acid plus vitamin E supplementation can significantly decrease UCP-2 gene expression in treatment group, compared to that in placebo group. Interestingly, omega-3 fatty acids are PPAR inducers as experimental studies on rats have demonstrated that fish oil feeding can up-regulate mRNA of UCP-2 gene by five-folds (Tsuboyama-Kasaoka et al., 1999). However, studies on PPAR-knockout mice have suggested that gene expression of UCP-2 can be regulated in a PPAR-independent manner (YOUNG et al., 2001). A culture study has revealed that failure in insulin secretion seen in SIRT1 knockdown cell lines is resulted from elevated UCP-2 gene expression in these cell lines. The SIRT1 can bind to the UCP-2 gene promoter and positively affect insulin secretion by repressing UCP-2 gene expression (Bordone et al., 2005).

5. Conclusions

In conclusion, the current study had shown that omega-3 fatty acid and vitamin E supplementation can increase gene expressions of SIRT1 and FOXO1 while they can decrease gene expression of UCP-2 in CAD patients. Further studies are necessary to reveal the exact mechanisms by which, these supplements affect human metabolism through these highlighted genes.

Conflicts of interest

The authors declare that there is no conflict of interest with respect to this manuscript.

Author contributions

SS and MD: designing the study; EY, SG, EN and SS: sampling and data collection; MD: Resource and Supervision; SS and EF: Data analysis &/or interpretation; EY and SS: drafting the manuscript; EF and MD: revising the article for important intellectual content. All authors have approved the final manuscript.

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