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The effects of coenzyme Q10 supplementation on gene expression related to insulin, lipid and inflammation in patients with polycystic ovary syndrome

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ABSTRACT

Objective: This research was conducted to assess the effects of coenzyme Q10 (CoQ10) intake on gene expression related to insulin, lipid and inflammation in subjects with polycystic ovary syndrome (PCOS).

Methods: This randomized double-blind, placebo-controlled trial was conducted on 40 subjects diagnosed with PCOS. Subjects were randomly allocated into two groups to intake either 100 mg CoQ10 ($n=20$) or placebo ($n=20$) per day for 12 weeks. Gene expression related to insulin, lipid and inflammation were quantified in blood samples of PCOS women with RT-PCR method.

Results: Results of RT-PCR shown that compared with the placebo, CoQ10 intake downregulated gene expression of oxidized low-density lipoprotein receptor 1 (LDLR) ($p<0.001$) and upregulated gene expression of peroxisome proliferator-activated receptor gamma (PPAR- γ) ($p=0.01$) in peripheral blood mononuclear cells of subjects with PCOS. In addition, compared to the placebo group, CoQ10 supplementation downregulated gene expression of interleukin-1 (IL-1) ($p=0.03$), interleukin-8 (IL-8) ($p=0.001$) and tumor necrosis factor alpha (TNF- α) ($p<0.001$) in peripheral blood mononuclear cells of subjects with PCOS.

Conclusions: Overall, CoQ10 intake for 12 weeks in PCOS women significantly improved gene expression of LDLR, PPAR- γ , IL-1, IL-8 and TNF- α .

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Q10; gene expression; insulin; lipid; inflammation; polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is a prevalent heterogeneous complication related to disorders of reproductive, metabolic and endocrine function [1]. The prevalence of PCOS among reproductive-aged women was reported 6–25%, depending on defined criteria [2]. Previous evidences show that PCOS is associated with hyperandrogenism and metabolic variations including insulin resistance and lipid disorders [3]. In addition, a relation has been observed between pro-inflammatory genotypes and PCOS, linked to polymorphism of genes coding for tumor necrosis factor alpha (TNF- α) and interleukin 6 (IL-6) [4].

Coenzyme Q10 (CoQ10) is a nutrient that plays an important function in the production of cellular energy as well as acts in scavenging free radicals and inhibiting lipid and protein oxidation as an antioxidant [5]. Previously, beneficial effects of CoQ10 on gene expression related to insulin, lipid and inflammation among subjects without PCOS have evaluated. Tarry-Adkins et al. [6] found that postweaning dietary intake of recuperated animals with CoQ10 prevented the programmed reduction in insulin receptorsubstrate-1 and p110- β and the programmed increased in IL-6. In addition, in another study was seen that CoQ10 administration at a dosage of 20 mg/kg increased insulin sensitivity and had antidiabetic properties via increasing activity of phosphatidylinositol kinase (PI3K) in rats fed a high-fat, high-fructose diet [7]. Mediterranean diet with CoQ10 also modified gene

expression of pro-inflammatory markers among elderly men and women for four weeks [8].

This evidence might support the importance of CoQ10 administration in women with PCOS. According to the best of our knowledge, data on the effects of CoQ10 intake on gene expression related to insulin, lipid and inflammation in subjects with PCOS are limited. The objective of our study was to determine the effects of CoQ10 on gene expression related to insulin, lipid and inflammation in subjects with PCOS.

Subjects and methods

Trial design and participants

This randomized double-blind placebo-controlled clinical trial, registered in the Iranian website for registration of clinical trials (<http://www.irct.ir>: IRCT201605225623N80), was done among 40 subjects with PCOS diagnosed according to the Rotterdam criteria [9] aged 18–40 years old who referred to the Kosar Clinic in Arak and the Persian Gulf Martyrs Hospital in Bushehr, Iran, between May 2016 and September 2016. We excluded pregnant, elevated levels of prolactin, endocrine diseases and no hormonal treatments in the previous 6 months in the study. This research was approved by the ethics committee of Arak University of Medical Sciences (AUMS) and informed consent form was taken

from all subjects. All subjects were matched according to age, phenotypes of PCOS and BMI at the study baseline. Subjects were then randomly divided into two groups to receive either CoQ10 ($n=20$) or placebo ($n=20$) for 12 weeks. CoQ10 and its placebos (cellulose) were provided by Nature Made Pharmaceutical Company (New York, NY, USA) and Barij Essence Pharmaceutical Company (Kashan, Iran), respectively.

Treatment adherence

To evaluate the compliance, we counted the remaining supplements. To increase compliance, all women received short messages every day to remind them about taking the capsules.

Assessment of anthropometric measures

Weight and height of participants were determined in an overnight fasting status using a standard scale (Seca, Hamburg, Germany) at the beginning of the study and after 12-weeks' intervention. BMI was calculated as weight in kg divided by height in meters squared.

Assessment of outcomes

In our study, gene expression of peroxisome proliferator-activated receptor gamma (PPAR- γ) and glucose transporter 1 (GLUT-1) were considered as the primary outcome and gene expression of lipoprotein(a) [Lp(a)], oxidized low-density lipoprotein receptor 1 (LDLR), interleukin-1 (IL-1), interleukin-8 (IL-8), tumor necrosis factor alpha (TNF- α) and transforming growth factor beta (TGF- β) were considered as the secondary outcomes.

Isolation of lymphocyte cells

At baseline and endpoint of the intervention, 10 ml samples of venous blood were taken after overnight fasting at Arak reference laboratory. Then, lymphocyte cells were extracted from blood samples of subjects with PCOS by the use of a 50% percoll (Sigma-Aldrich, Dorset, UK). Samples were taken for cell

count and viability testing by trypan blue, RNA and DNA extraction [10].

RNA extraction and real-time PCR

To RNA extraction, we used the RNX-plus kit (Cinnacolon, Tehran, Iran). RNA suspension was frozen in -20°C until cDNA making. Following extraction of the total RNAs from each sample, RNA quantification were performed by UV spectrophotometer. Each samples OD 260/280 ratio between 1.7 and 2.1 was intended that shows no contamination with both protein and DNA [10]. The isolated RNA was reverse transcribed to cDNA library using moloney murine leukemia virus (MMLV) reverse transcriptase (RT). Gene expression of PPAR- γ , GLUT-1, Lp(a) and LDLR were evaluated by quantitative RT-PCR, using the LightCycler technology (Roche Diagnostics, Rotkreuz, Switzerland) with SYBR green detection and Amplicon Kit (Table 1). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers were used as housekeeping gene. To design primers, Primer Express Software (Applied Biosystems, Foster City, CA, USA) and Beacon designer software (Takaposizt, Tehran, Iran) were used. Relative transcription levels were calculated by the method of Pfaffi or $2^{-\Delta\Delta\text{CT}}$.

Randomization

Randomization assignment was done using computer-generated random numbers as blindness by a trained staff at the gynecology clinic.

Statistical methods

To establish normal data distribution, we used the Kolmogorov-Smirnov test. To establish differences in anthropometric measures as well as in macro- and micro-nutrient dietary intakes between the two groups, we applied independent samples t -test. To determine the effects of CoQ10 supplementation on gene expression involved in lipid, insulin and inflammation signaling pathway, we used independent samples t -test. The $p < 0.05$ were considered statistically significant. All data entry and

Table 1. Specific primers used for real-time quantitative PCR.

Gene	Primer	Product size (bp)	Annealing temperature ($^{\circ}\text{C}$)
GAPDH	F: AAGCTCATTCTGGTATGACAACG	126	61.3
	R: TCTTCTCTTGCTCTTGCTGG		
PPAR- γ	F: ATGACAGACCTCAGACAGATTG	210	54
	R: AATGTTGGCAGTGGCTCAG		
GLUT-1	F: TATCTGAGCATCGTGCCAT	238	62.1
	R: AAGACGTAGGGACCACACAG		
Lp(a)	F: GACACAGCACGTTTCATCCA	200	55
	R: ACACCCCTACAATGCTTC		
LDLR	F: ACTTACGGACAGACAGACAG	223	57
	R: GGCCACACATCCCATGATTC		
IL-1	F: GCTTCTCTGGTCCTTGG	174	56
	R: AGGGCAGGGTAGAGAAGAG		
IL-8	F: GCAGAGGGTTGTGGAGAAGT	150	56
	R: ACCCTACAACAGACCCACAC		
TNF- α	F: GTCAACCTCCTCTGCCAT	188	52
	R: CCAAAGTAGACCTGCCAGA		
TGF- β	F: TTGAGACTTTCCGTTGCCG	227	56
	R: CGAGGCTGGGAAAAGTCT		

GAPDH: glyceraldehyde-3-Phosphate dehydrogenase; GLUT-1: glucose transporter 1; IL-1: interleukin-1; IL-8: interleukin-8; Lp(a): lipoprotein(a); LDLR1: oxidized low-density lipoprotein receptor 1; PPAR- γ : peroxisome proliferator-activated receptor gamma; TNF- α : tumor necrosis factor alpha; TGF- β : transforming growth factor beta.

statistical analyzes were conducted using the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, IL, USA).

Results

At baseline, we were invited 55 women; however, 15 women were excluded from the study because of not meeting inclusion criteria. In the current study, 40 women [placebo ($n=20$) and CoQ10 ($n=20$)] completed the trial (Figure 1).

Mean age, height, and weight and BMI at baseline and end-of-trial were not statistically different between the two groups (Table 2).

Results of RT-PCR indicated that compared with the placebo, CoQ10 intake downregulated gene expression of LDLR ($p < 0.001$), but did not affect gene expression of LP(a) in peripheral blood mononuclear cells of subjects with PCOS (Figure 2).

We found that CoQ10 intake, compared with the placebo, upregulated gene expression of PPAR- γ ($p = 0.01$), but unchanged gene expression of GLUT-1 in peripheral blood mononuclear cells of subjects with PCOS (Figure 3).

Compared with the placebo, CoQ10 intake downregulated gene expression of IL-1 ($p = 0.03$), IL-8 ($p = 0.001$) and TNF- α ($p < 0.001$), but did not influence gene expression of TGF- β in peripheral blood mononuclear cells of subjects with PCOS (Figure 4).

Discussion

However, in another study, we have previously shown beneficial effects of CoQ10 on insulin metabolism and some lipid profiles in patients with PCOS [11], to our knowledge, this trial is the first evaluating effects of CoQ10 on gene expression related to insulin, lipid and inflammation among women with PCOS. We demonstrated that CoQ10 intake for 12 weeks among subjects

with PCOS had beneficial effects on few gene expression related to insulin, lipid and inflammation.

Subjects with PCOS are susceptible to some metabolic disorders and inflammation [12,13]. We found that taking CoQ10 for 12 weeks in subjects with PCOS downregulated LDLR expression, but did not affect Lp(a) expression. Tsai et al. [14] seen that CoQ10 rescued dephosphorylation of AMP-activated protein kinase (AMPK) caused by Ox-LDL. In addition, CoQ10 attenuated the Ox-LDL-induced generation of reactive oxygen species (ROS) [15]. Prior genetic and epidemiologic studies have shown that Ox-LDL and Lp(a) are risk factors of atherosclerotic diseases such as CHD [16–18]. CoQ10 intake mat decrease Ox-LDL-induced endothelial oxidative injuries by the modulation of LOX-1-mediated ROS generation via the AMPK/PKC/NADPH oxidase signaling pathway [14].

This research demonstrated that CoQ10 supplementation for 12 weeks in subjects with PCOS upregulated PPAR- γ expression, but unchanged GLUT-1 expression. However, data on the effects of CoQ10 intake on gene expression of PPAR- γ and GLUT-1 are scarce; few studies have evaluated the effects of CoQ10 on gene expression related to insulin. Lee et al. [19] found that CoQ10 increased gene expression of PPAR- α at both the mRNA and protein levels in 3T3-L1preadipocytes. In addition, supplementation with CoQ10 induced gene expression of PPAR- α in SAMP1 mice [20]. In another study, CoQ10 supplementation increased p110 β protein expression both liver and skeletal muscle [6]. This is consistent with prior studies indicating that administration of a much higher dose of CoQ10 (20 mg/kg) affects insulin sensitivity and had antidiabetic properties via increasing the activity of phosphatidylinositol kinase (PI-3Ks) in rats fed with a high-fat, high-fructose diet [7]. PPAR- γ is primarily present in adipocytes, which plays an important function in glucose and insulin metabolism [21]. CoQ10 intake may induce PPAR- γ expression thorough the calcium-mediated AMPK signal pathway and suppressing differentiation-induced adipogenesis [19].

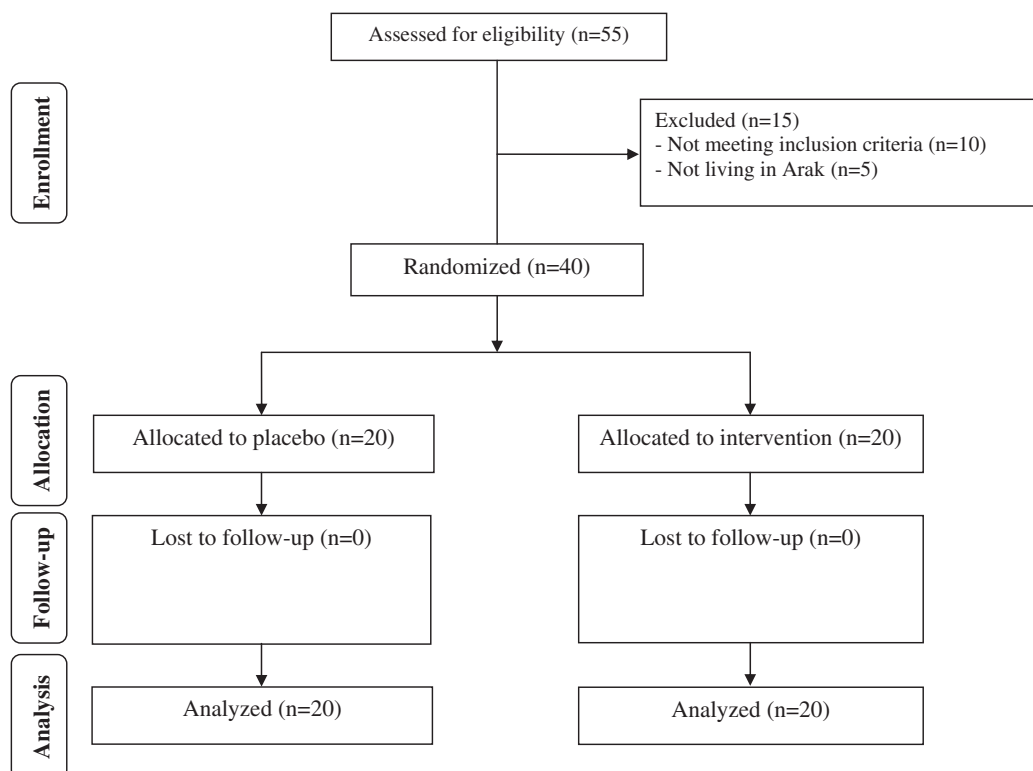


Figure 1. Summary of patient flow diagram.

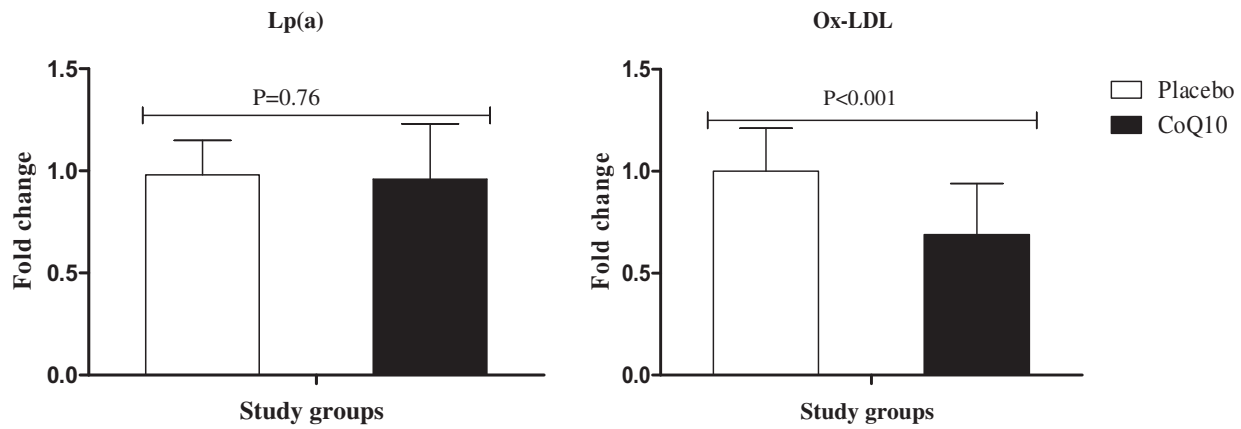


Figure 2. Effect of 12-week supplementation with CoQ10 or placebo on expression ratio of Ox-LDL and Lp(a) gene in blood mononuclear cells of PCOS women.

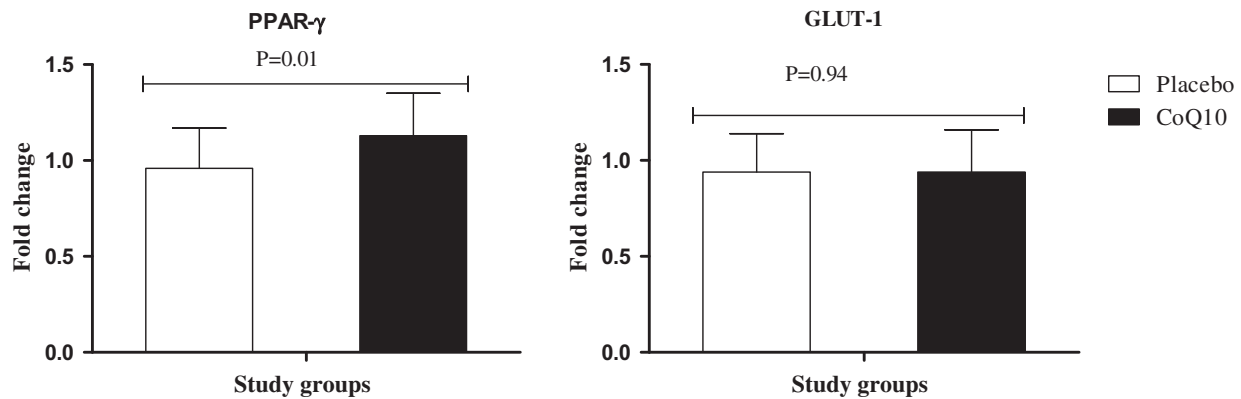


Figure 3. Effect of 12-week supplementation with CoQ10 or placebo on expression ratio of PPAR- γ and GLUT-1 gene in blood mononuclear cells of PCOS women.

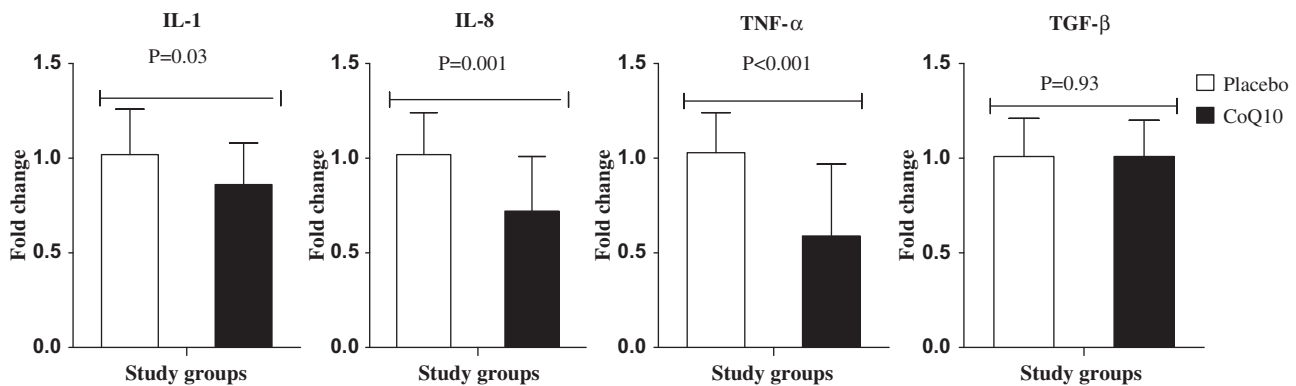


Figure 4. Effect of 12-week supplementation with CoQ10 or placebo on expression ratio of IL-1, IL-8, TNF- α and TGF- β gene in blood mononuclear cells of PCOS women.

Table 2. General characteristics of study participants.

	Placebo group (n = 20)	CoQ10 group (n = 20)	<i>p</i> ^a
Age (y)	24.7 \pm 5.3	24.9 \pm 3.7	0.91
Height (cm)	162.5 \pm 6.6	160.8 \pm 7.8	0.47
Weight at study baseline (kg)	76.5 \pm 17.1	71.6 \pm 11.1	0.29
Weight at end-of-trial (kg)	76.1 \pm 17.7	71.9 \pm 10.8	0.36
Weight change (kg)	-0.4 \pm 1.0	0.3 \pm 5.7	0.63
BMI at study baseline (kg/m ²)	28.9 \pm 6.6	27.7 \pm 3.6	0.44
BMI at end-of-trial (kg/m ²)	28.8 \pm 6.7	27.8 \pm 3.6	0.54
BMI change (kg/m ²)	-0.1 \pm 0.3	0.1 \pm 2.1	0.61

Data are means \pm SDs.

^aObtained from independent *t*-test.

We found that taking CoQ10 for 12 weeks in subjects with PCOS downregulated gene expression of IL-1, IL-8 and TNF- α compared with the placebo, but did not affect gene expression of TGF- β . Yoneda et al. [22] demonstrated that CoQ10 reduced gene expression of interleukin-1 β , TNF- α and nuclear factor- κ B (NF- κ B) in rats after 8 days. In another study, CoQ10 supplementation significantly reduced the IL-6 protein and TGF- β 1 and Lep mRNA levels [6]. In addition, our study was in agreement with findings in cardiac tissue [23], and CoQ10 is known to have anti-inflammatory properties in mouse liver [24] and human plasma [25]. Also, gene expression of TNF- α were significantly decreased following CoQ10 intake in an experimental model of multiple sclerosis for three weeks [26]. Increased inflammatory cytokines would result in the development of insulin resistance via the inhibition of insulin signaling through activation of the inhibitory- κ B kinase- β and c-Jun N-terminal kinase pathways [27]. Moreover, inflammation in subjects with PCOS render them at an increased risk for the development of atherosclerosis and infertility [28]. Due to its antioxidant and radical scavenging activity, CoQ10 can reduce ROS production and free radicals, which in turn could affect gene expression of TNF- α via the NF- κ B pathway [29].

The current study had few limitations. We did not evaluate CoQ10 levels at baseline and at the end-of-trial. Furthermore, in the current study, we considered PPAR- γ and GLUT-1 as gene expressions involved in the insulin signaling pathway. However, GLUT-1 is not directly influenced by insulin, it is not surprising whether we did not observe any variation of GLUT-1 gene expression following the supplementation of CoQ10. We agree that other insulin-dependent transporters such as GLUT-4 or other post-receptor steps including insulin receptor substrate 1 or-2 and PKB are more important than GLUT-1. Unfortunately, we did not assess gene expression of these pathways. Therefore, measurement of these pathways is suggested in future studies.

Overall, CoQ10 supplementation for 12 weeks in PCOS women significantly improved gene expression of LDLR, PPAR- γ , IL-1, IL-8 and TNF- α , but did not affect gene expression of Lp(a), GLUT-1 and TGF- β .

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Disclosure statement

No potential conflict of interest was reported by the authors.

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