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Benefits of Omega-3 Fatty Acids Supplementation on Serum Paraoxonase 1 Activity and Lipids Ratios in Polycystic Ovary Syndrome

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a common endocrine disorder associated with increased risk of cardiovascular disease. The purpose of this study was to investigate the effects of omega-3 fatty acids on serum paraoxonase 1 activity and lipids ratios in polycystic ovary syndrome.

Methods: This double-blind randomized controlled clinical trial was conducted on 64 PCOS patients with 20-35 years old. Thirty two of the subjects had taken 4 g/day omega -3 fatty acids and 32 patients were given placebo for 8 weeks. Fasting blood samples, anthropometric measurements and dietary intakes were collected at the beginning and the end of the study. Serum total cholesterol, triglyceride, and HDL-C were measured using the enzymatic methods. LDL-C concentration was calculated by the Friedewald formula and arylesterase activity of serum PON1 was measured. Data were analyzed using SPSS software.

Results: Omega-3 fatty acids significantly decreased TC/HDL-C and LDL-C/HDL-C ratios ($P = 0.009$ for both) and significantly increased serum PON1 activity ($P = 0.048$) compared with placebo. Changes in TG/HDL-C ratio were not statistically significant in omega-3 fatty acids group at the end of the study in comparison to placebo group. Reduction in TC/HDL-C, LDL-C/HDL-C and TG/HDL-C ratios and increase in serum PON1 activity were also significant in omega-3 fatty acids group at the end of the study compared with baseline values ($P <0.001$, $P < 0.001$, $P = 0.004$, and $P = 0.001$, respectively).

Conclusion: Omega-3 fatty acids may decrease the risk for cardiovascular disease through the improvement in paraxonase-1 activity and reduction in some lipids ratio in PCOS women.

Keywords: Omega-3 fatty acids, Polycystic ovary syndrome, Paraoxonase 1, Lipids

Introduction

Polycystic ovary syndrome (PCOS) is one of the most frequent endocrine disorders in reproductive age women with a worldwide prevalence rate of 5%–10% [1]. PCOS is the main cause of female infertility due to anovulation [2]. PCOS and its associated symptoms have a negative effect on health-related quality of life and cause social and emotional stress in affected women [1].

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Hyperinsulinemia has an important role in pathogenesis of this disorder [3]. Several risk factors including obesity, insulin resistance, dyslipidemia and oxidative stress lead to early onset of cardiovascular disease and type 2 diabetes in these women. The risk of myocardial infarction has been reported to increase by 7.4-fold in PCOS patients than normal [4].

Oxidative stress arises from an imbalance between pro-oxidant molecules produced from cellular metabolism or exogenous sources and antioxidants [5]. Several enzymes, vitamins and other biomarkers constitute antioxidant defense of the body. Disturbance in this system may lead to molecular and cellular damage [6]. Oxidative stress may be involved in the pathogenesis of PCOS and female infertility [5, 6] and can affect a variety of physiological functions including folliculogenesis, androgen production and hyperinsulinemia in women with PCOS [5, 6].

Paraoxonase-1 (PON1) is an antioxidant enzyme exists in the circulation bound to high-density lipoproteins cholesterol (HDL-C) and prevents lipid peroxidation. These actions suggest a protective role of PON1 against atherosclerosis [7]. Serum PON1 activity was lower in women with PCOS compared with healthy women [4, 8, 9].

Increased dietary polyunsaturated fatty acids intake may be associated with improved metabolic and endocrine characteristics in women with PCOS [10]. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are long chain omega-3 polyunsaturated fatty acids found in fatty fish [11]. Growing body of evidence indicate that an increased intake of EPA and EDA could impact positively on the human health [12].

Omega-3 fatty acid supplementation had a beneficial effect on some cardiometabolic risk factors in women with PCOS [13]. The positive effects of omega-3 fatty acids on antioxidant enzymes are repoted [14, 15]. Fish oil supplementation caused remarkable increase in serum PON1 levels in familial combined hyperlipidemia [16]. However, little is known about the effects of omega-3 fatty acids on PON1 activity.

Considering that oxidative stress in combination with impaired lipid profile are contributed in metabolic complication of PCOS, and the effects of omega-3 fatty acids on serum PON1 activity in PCOS patients have not yet been investigated, the objectives of this study were to determine the effects of omega-3 fatty acids on serum paraoxonase 1 activity and lipids ratios in polycystic ovary syndrome.

Materials and Methods

Study population

Sixty-four PCOS patients were recruited in this double-blind randomized controlled clinical trial from outpatient Department of Obstetrics of Alzahra Hospital in Tabriz, Iran. The registration ID of this study in Iranian Registry of Clinical Trials was: IRCT201011083664N3.

The inclusion criteria were 20-35 yr old PCOS patients, with body mass index (BMI) ranging from 25 to 40 kg/m^2. The diagnosis of PCOS was established according to 2003 Rotterdam criteria, which require at least two of three features for diagnosis: chronic anovulation, clinical or biochemical signs of hyperandrogenism, and polycystic ovaries in ultrasonography [17]. The study exclusion criteria included smoking, pregnancy, using fish oil and other dietary supplements with the past 3 months and during the study, history of diseases including diabetes, liver, kidney and cardiovascular diseases, thyroid disorders, hyperprolactinemia, cushing's syndrome and use of any medications. Subjects were asked to maintain their usual dietary intakes and physical activity throughout the study.

Ethical Committee of Tabriz University of Medical Science approved the study protocol. Written informed consent obtained from each subjects prior to study.

Study design

The participants were randomly allocated in two groups using a block randomi-

zation procedure with matched subjects in each block based on BMI and age. A general questionnaire was completed for each subject. Body weight was measured using a scale (Seca, Germany), without shoes and wearing light clothing. Height was measured using a mounted tape without shoes. BMI was calculated as the weight in kg divided by the height in meters squared. Information about daily energy and macronutrient intakes were obtained by 24-hour recall method for 3 days, including 2 week day and 1 weekend. Amounts of all consumed foods were calculated in gram and data were interred in Nutritionsit 4 software. Then energy and macronutrient content of all foods were determined by software and three day averages of dietary intakes were calculated.

The blood sampling (5 ml) was conducted after 12 hours of fasting at 7 and 10 a.m. The serum was separated by centrifugation and stored at−70 °C until further analysis. Subjects in omega-3 fatty acids group (n=32) were taken 4 g daily of omega-3 fatty acids (4× 1000 mg capsules, each capsule contained 180 mg EPA and 120 mg DHA, Good Health Company, USA) for 8 weeks and placebo group (n=32) was given placebo capsules contained 500 mg paraffin oil for the same period. All anthropometric, dietary intakes and biochemical measurements were assessed again at the end of intervention period in both groups.

Biochemical measurements

Serum total cholesterol (TC), triglyceride (TG), and HDL-C were measured using the standard enzymatic methods by Pars Azmun kits (Karaj, Iran). Low-density lipoprotein cholesterol (LDL-C) concentration was determined by the Friedewald formula: LDL-C = TC − (HDL-C+TG/5) [19]. The TC/HDL-C, LDL-C/HDL-C and TG/HDL ratio were calculated for all subjects. Arylesterase activity of serum PON1 was measured by spectrophotometric method using phenyl acetate as the substrate and absorbance was monitored at 270 nm and 37 °C.

Three patients were excluded from the study because of personal reasons. Sixty one patients (n = 30 in omega-3 fatty acids group and n = 31 in placebo group) completed the study.

Statistical analysis

Data were analyzed using SPSS software (version 11.5; SPSS Inc., Chicago, IL) and the results are expressed as mean ± standard deviation. The normality of the distribution of variables was determined by the Kolmogorov-Smirnov test. Anthropometric measurements, dietary intakes and biochemical parameters of subjects in two groups at baseline were compared using independent sample *t*-test.

Analysis of covariance was used to identify any differences in biochemical parameters between two groups after intervention, adjusting for baseline measurements and covariates. The changes in anthropometric measurements, dietary intakes and biochemical parameters between the beginning and end of the study were compared by paired sample *t*-test. The percentage of changes in variables after intervention was determined by formula: [(after values − before values) / before values] × 100. *P* value of < 0.05 was considered statistically significant.

Results

Anthropometric characteristics and dietary intakes of participants at the beginning and end of the study are shown in Table 1. There were no significant differences between and within groups in weight and BMI at the beginning of the study and after 8 weeks of intervention. Cholesterol intake was significantly different between placebo and omega-3 fatty acids group at the beginning of the study ($P = 0.04$). No significant differences in energy and other dietary intakes were observed between two groups at baseline. Total energy and nutrient intakes also did not change significantly in any of the groups during the study.

Metabolic parameters of subjects at baseline and after 8 weeks intervention are shown in Table 2.

Table1: Characteristics and dietary intakes of women with PCOS at baseline and after 8 weeks intervention

Variable	Measurement period	Placebo (n=31)	Omega-3 fatty acids (n=30)
Age (yr)	Baseline	27.73 ± 4.53	27.33 ± 4.27
Weight (kg)	Baseline	74.93 ± 9.95	73.65 ± 8.87
	After intervention	75.08 ± 9.88	73.41 ± 8.88
BMI (kg/m2)	Baseline	28.77 ± 2.92	28.67 ± 3.21
	After intervention	28.83 ± 2.94	28.58 ± 3.30
Energy (kcal/day)	Baseline	1734.50 ± 408.08	1741.80 ± 419.13
	After intervention	1680.13 ± 367.75	1668.14 ± 415.30
Carbohydrate (g/day)	Baseline	214.63 ± 63.49	223.67 ± 65.90
	After intervention	216.10 ± 52.83	209.36 ± 51.50
Protein (g/day)	Baseline	62.81 ± 19.47	63.58 ± 19.19
	After intervention	62.10 ± 18.70	61.71 ± 21.93
Total Fat (g/day)	Baseline	66.97 ± 21.95	64.98 ± 21.18
	After intervention	65.75 ± 19.84	64.56 ± 20.06
SFA (g/day)	Baseline	17.74 ± 6.59	16.79 ± 6.20
	After intervention	17.27 ± 6.30	18.05 ± 6.82
MUFA (g/day)	Baseline	20.33 ± 6.20	18.25 ± 6.72
	After intervention	21.06 ± 7.11	19.73 ± 6.87
PUFA† (g/day)	Baseline	20.89 ± 8.65	19.97 ± 9.55
	After intervention	19.07 ± 7.62	17.76 ± 6.81
Cholesterol (mg/day)	Baseline	203.10 ± 39.36	183.36 ± 34.52*
	After intervention	187.61 ± 45.78	191.42 ± 38.06

BMI: body mass index, SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid
Data are presented as mean ± SD
† Excluding the PUFA from the supplement
*Significant difference between two groups at baseline ($P < 0.05$, independent sample *t*-test)

Table 2: Metabolic parameters of women with PCOS at baseline and after 8 weeks intervention

variable	Placebo (n=31)		Omega-3 fatty acids (n=30)	
	Baseline	after intervention	Baseline	after intervention
TC (mg/dl)	188.10 ± 29.21	186.63 ± 25.89	186.60 ± 32.45	170.33 ± 32.03 [a,b]
TG (mg/dl)	125.73 ± 28.49	120.23 ± 28.52	126.97 ± 29.50	119.13 ± 26.04 [a]
LDL-C (mg/dl)	117.41± 31.53	117.25 ± 27.44	118.06 ± 29.39	102.25 ± 29.59 [a,b]
HDL-C (mg/dl)	44.86 ± 6.11	45.33 ± 4.49	43.13 ± 6.55	45.86 ± 6.53 [a]
TC/HDL-C	4.29 ± 1.04	4.17 ± 0.84	4.40 ± 0.93	3.75 ± 0.70 [a,b]
LDL-C/HDL-C	2.71 ± 0.99	2.64 ± 0.79	2.80 ± 0.81	2.25 ± 0.64 [a,b]
TG/HDL-C	2.84 ± 0.73	2.67 ± 0.67	3.04 ± 0.97	2.66 ± 0.78
PON1(IU/ml)	146.58 ± 36.82	143.22 ± 37.07	138.66 ± 30.14	159.04 ± 34.17 [a,b]

TC: total cholesterol, TG: triglyceride, LDL-C: low density lipoprotein, HDL-C: high density lipoprotein, PON1: paraoxonase 1
Data are presented as mean ± SD
[a]Significant difference within groups after intervention (*P* < 0.05, paired sample *t*-test)
[b]Significant difference between groups after intervention (*P* < 0.05, analysis of covariance)

There was no significant difference between 2 groups in terms of serum lipids and PON1 activity at baseline.

Results of analysis of covariance showed statistically significant differences between two studied groups in serum levels of TC, LDL-C, PON1 activity (*P* = 0.002, *P* = 0.003 and *P* = 0.048, respectively), TC/HDL-C and LDL-C/HDL-C ratio (*P* = 0.009 for both) at the end of the study, adjusted for energy, PUFA and cholesterol intakes and baseline values. Changes in serum TG and HDL-C levels and TG/HDL-C ratio were not significant. Supplementation with omega-3 fatty acids resulted in 12.8% and 19.7% reduction in TC/HDL and LDL/HDL ratio and 16.3% increase in serum PON1 activity, in comparison to placebo group.

As shown in Table 2, significant decrease in serum levels of TC, LDL-C (*P* < 0.001 for both), TG (*P* = 0.024), TC/HDL-C and LDL-C/HDL-C ratio (*P* < 0.001 for both) and TG/HDL-C ratio (*P* = 0.004) was obtained in omega-fatty acids group at the end of the study compared with baseline values. Serum HDL-C levels and PON1 activity also increased significantly in omega-3 fatty acids group at the end of the intervention in comparison to the beginning of the study (*P* = 0.018 and *P* = 0.001, respectively).

Discussion

Based on results (Table 1) we did not find any significant changes in weight, BMI, energy and macronutrient intakes within omega-3 fatty acids and placebo groups during the study. Our results were in accordance with a previous study which reported that omega-3 supplementation by dose of 4 g/day for 8 weeks, had no significant effects on BMI of PCOS patients with nonalcoholic fatty liver disease [13]. Six week supplementation with 3.5 g/day fish oil in women with PCOS did not change weight and BMI sig-

nificantly [18]. No other published data exists about the effects of omega-3 fatty acids in PCOS patients. Woodman et al. and Tsitouras et al. reported similar findings in studies on type 2 diabetes and healthy older subjects, respectively [19, 20].

According to Table 1, overall energy intakes of subjects in both groups was not significantly different at the end of the study compared with baseline values, so no significant changes in weight and BMI of all subjects would be partially expected.

We observed that supplementation with omega-3 fatty acids lead to improvement in lipid profiles (Table 2). Cussons et al. and Vargas et al. reported significant reduction in serum TG levels in PCOS patients after supplementation with EPA and DHA, but serum TC, LDL-C and HDL-C concentrations remained unchanged [13, 18]. The beneficial effects of omega-3 fatty acids in reducing serum TG levels were demonstrated in other studies in obese subjects [21] and diabetic patients [19]. Supplementation with omega-3 fatty acids resulted in significant reduction in serum TG and increase in HDL-C levels in individuals with visceral obesity [22]. Fish oil supplementation reduced serum LDL-C levels in healthy subjects and diabetic patients, respectively [23, 24]. Our trial confirmed the results of mentioned studies.

Several mechanisms are suggested to explain the effects of omega-3 fatty acids on serum lipids. The omega-3 fatty acids are natural ligands for some metabolic nuclear receptors including PPARs (peroxisome proliferator- activated receptors). Activation of these receptors by EPA and DHA resulted in suppression of lipid synthesis and stimulation of fatty acid oxidation in liver and muscle [25]. Fish oil feeding reduces cholesterol absorption and LDL-C synthesis, improves LDL receptor activity in liver, and increases fractional rate of catabolism of LDL-C [20, 23, 26].

Increased incidence of atherosclerosis in patients with PCOS was reported in previous studies [8]. TC/HDL-C and LDL-C/HDL-C ratio have been considered as atherogenic indices and recently, the TG/HDL-C ratio has been proven to be a strong predictor of cardiovascular disease [27]. Based on results (Table 2) TC/HDL-C and LDL-C/HDL-C ratio were reduced in our patients after omega-3 fatty acids intervention. These findings indicate that omega-3 fatty acids might have protective role against atherosclerosis. The effects of omega- fatty acids on lipid profile are dose dependent [11]. So it seems that if dose or duration of supplementation in our study were more, significant differences in TG/HDL-C ratio also might be determined between 2 groups, at the end of the intervention.

PON1 has antioxidant properties and accounts as a new marker of lipid peroxidation [7]. PON1 prevents the generation of oxidized LDL and inactivates LDL-derived oxidized products when they are formed. PON1 is responsible for anti-atherosclerotic actions of HDL-C [28]. Serum PON1 levels were inversely associated to the risk of coronary heart disease [29]. Some human and animal investigations have shown that intake of omega-3 fatty acids could improve antioxidant status [14, 30, 31]. In our study, supplementation with omega-3 fatty acids increased PON1 activity (Table 2). There are limited studies on the effects of omega-3 fatty acids on serum levels or activity of PON1. Intake of 1.88 g EPA and 1.48 g DHA per day for 8 weeks in patients with familial combined hyperlipidemia increased serum PON1 levels by 10% compared with baseline values [16]. Fish oil intervention for 6 weeks in male Wistar rats elevated PON1 activity [32]. Long chain omega-3 PUFAs affect PON1 gene expression and synthesis of this enzyme in liver via activation of PPAR-γ. In addition changes in fatty acid composition of HDL-C by dietary omega-3 fatty acids may modulate fluidity of HDL-C and PON1 activity [33]. Our findings confirm the positive effects of omega-3 fatty acids on elevating PON1 activity as an important part of antioxidant system in PCOS patients.

This study had some limitation. We did not measure other antioxidant enzymes in our subjects, which could help us for more precise evaluation. In addition, we studied only overweight and obese PCOS patients so present results are not applicable to other PCOS women with different BMI range.

Conclusion

Oxidative stress and impaired serum lipids are contributed in early onset of cardiovascular disease and other long-term complications in PCOS patients. Our results suggest that omega-3 fatty acids could constitute a helpful approach to decrease cardiometabolic risks via both improvements in PON-1 activity as an antioxidant marker and reduction in some serum lipids ratios in these women.

Acknowledgments

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