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Hormonal and metabolic effects of polyunsaturated fatty acid (omega-3) on polycystic ovary syndrome induced rats under diet

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ABSTRACT

Objective(s): PCOS (polycystic ovary syndrome) produces symptoms in approximately 5% to 10% of women of reproductive age (12–45 years old). It is thought to be one of the leading causes of female subfertility. This study aimed to confirm the role of nutrition containing omega-3 (polyunsaturated fatty acid) on control of experimental PCO induced by estradiol-valerat in rats.

Materials and Methods: Wistar female rats (n=40) were allocated into control (n=10) and test groups (n= 30), test group was subdivided into 3 groups: G1, received omega-3 (240 mg/kg/orally/daily); G2 and G3 groups were induced PCO by single injection of estradiol-valerate (16 mg/kg/IM). Group 3 received omega-3 (240 mg/kg/orally/daily) and low carbohydrate feeding for 60 subsequent days; on sixtieth day 5 ml blood samples and ovarian tissues of all rats in the group were removed and prepared for biochemical and hormonal analysis.

Results: Catalase, GPX (Glutathione peroxidase), SOD (Superoxide dismutase) in groups that received omega-3 showed higher levels, but MDA (malondialdehyde) level was significantly decreased (P<0.05) in comparison with other experimental groups. Ovarian weights in both experimental and control groups were similar (P<0.05). Level of serum FSH (follicle stimulating hormone) was decreased, but level of testosterone was significantly increased (P<0.05) in PCO group in comparison with control and omega-3 groups.

Conclusion: Results revealed that administration of omega-3 plus lower carbohydrate food significantly controlled PCO syndrome and balanced FSH and testosterone.

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Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder affecting one in 15 women worldwide. The etiology of this hormonal disorder is unknown, but studies suggest a complex of lifestyle, environmental, and genetic factors. Some metabolic and clinical changes are reported in PCOS cases, including obesity, abnormal menstrual cycle, insulin resistance, cardiovascular diseases, hyperandrogenism symptoms (i.e. hirsutism), acne, and hormonal changes. In this disease, there is elevated level of estrogen but decreased level of progesterone (1-5). Many studies have demonstrated the impact of OS (oxidative stress) on subfertility in both female and male. OS generation in response to hyperglycemia increased in PCOS which may contribute to a pro inflammatory state, so insulin resistance and hyperandrogenism are common in women with PCOS (6, 7).

Since many environmental pollutants cause OS, life style is the most important factor in both sick and healthy individuals (6). OS is significantly associated with excess weight, so weight loss causes improvement of ovulation/menstruation. Low-carbohydrate diets and regular exercise are efficient in weight loss (8, 9). Omega-3 fatty acids (ω−3 fatty acids) are antioxidants found in plant and marine oils. Omega-3 is one of the polyunsaturated fatty acids, the anti-oxidative effect on experimental samples of which, many studies have demonstrated (10, 11). This experiment was designed to evaluate the effect of omega-3 on hormonal and biochemical factor changes in PCO induced rats under diet.

Materials and Methods

Animals and diet

Forty adult 8 week old Wistar albino female rats of 250±10 g were obtained from Animal Facility of Pasture Institute of Iran. Rats were housed in

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temperature controlled rooms (25°C) with constant humidity (40%–70%) and 12 hr/12 hr light/dark cycle prior to use in experimental protocols. The experimental protocol was approved by the Animal Ethical Committee in accordance with the Guide for the Care and Usage of Laboratory Animals prepared by Tabriz Medical University. All rats were fed a standard diet and water. The daily intake of animal water was monitored at least one week prior to start of treatments in order to determine the amount of water needed per experimental animal. Wistar female rats (n=40) were allocated into control (n=10) and test groups (n=30), test groups subdivided into groups of 3, G1 received Omega-3 (240 mg/kg/orally/daily), G2 and G3 were induced PCO by single injection of estradiol-valerate (16 mg/kg/IM), G3 also received Omega-3 (240 mg/kg) and lower carbohydrate feeding for 60 consecutive days. To demonstrate the effect of carbohydrates on protein utilization, the test diet contained 40% appropriate proteins, 40% carbohydrates, 5% fat, 5% cellulose, and 10 % minerals and vitamins per kg diet. Changes in true protein digestibility, biological value, and net protein utilization were calculated and recorded. For control group, feeding contained 30% appropriate proteins, 50% carbohydrates, 5% fat, 5% cellulose, and 10 % minerals and vitamins per kg diet. On sixtieth day 5 ml blood samples and ovarian tissues of all rats in the groups were removed and prepared for biochemical pathological analysis. Animals were kept in standard conditions; on the 60th day (at the end of the treatment period), blood samples from control and experimental groups were obtained.

Inducing PCO

Thirty days before the experimental procedure, twenty rats were each given a single intra muscular (IM) injection of 16 mg/kg estradiol-valerate (Riedeldehaen, Germany) in 0.2 ml oil in order to induce PCO (PCO group).

Glutathione peroxidase (GPX) activity measurement in serum

GPx activity was quantified by following the decrease in absorbance at 365 nm induced by 0.25 mM H_2O_2 in the presence of reduced glutathione (10 mM), NADPH, (4 mM), and 1 U enzymatic activity of GR (12).

Super oxide dismutase (SOD) activity measurement in serum

The activity of superoxide dismutase (SOD) was measured by following the method of Beyer and Fridovich (13).

Catalase (CAT) activity measurement in serum

Serum catalase activity was determined according to the method of Beers and Sizer (14) and measured the decrease in absorbance at 240 nm due to the decomposition of H_20 in a UV recording spectrophotometer. The reaction mixture (3 ml) contained 0.1 ml of serum in phosphate buffer (50 mM, pH 7.0) and 2.9 ml of 30 mM H_2O_2 in phosphate buffer pH 7.0. Molar extinction coefficient for H_2O_2 cm-1 was used for calculation. The specific activity of catalase was expressed as moles of H_2 reduced per min per mg protein at 240 nm of 40.0 M-1, cm-1 was used for calculation. The specific activity of catalase was expressed as moles of H_2O_2 reduced per min per mg protein.

Malondialdehyde(MDA)concentration measurement in serum

Free radical damage was determined by specifically measuring malondialdehyde (MDA). MDA was formed as an end-product of lipid peroxidation, which was treated with thiobarbituric acid to generate a colored product measured at 532 nm (MDA detection kit from Nanjing Jiancheng Bioengineering Institute, China).

Determination of blood glucose level

Blood samples were collected from the tail vein of male rats in all groups. Basal glucose levels were determined by using an automated blood glucose analyzer (Glucometer Elite XL).

Measurement of FSH hormones

Serum concentrations of FSH were measured with a two-site chemiluminescence (sandwich) immunoassay using two antibodies specific for the intact FSH molecule.

Statistical analysis

Statistical analysis and test for comparison of data in the control group with the experimental groups was done using the ANOVA. The results were expressed as mean ± SEM (standard error of means). *P*-values less than 0.05 were considered significant and are written in parentheses.

Results

Malondialdehyde (MDA) activity in serum

Administration of (240 mg/kg/orally/daily) omega-3 for sixty consecutive days significantly decreased malondialdehyde (MDA) concentration in experimental group as compared with the PCOS group ($P< 0.05$), (Figure 1).

Super oxide dismutase (SOD) activity in Serum

Administration of (240 mg/kg/orally/daily) omega-3 for sixty consecutive days significantly increased super oxide dismutase (SOD) activity in experimental group as compared with the PCOS group ($P< 0.05$), (Figure 2).

Glutathione peroxidase (GPX) activity in serum

Administration of (240 mg/kg/orally/daily) omega-3 for sixty consecutive days significantly increased glutathione peroxidase (GPX) activity in experimental group as compared with the PCOS group (P< 0.05), (Figure 3).

Catalase (CAT) activity in serum

Administration of (240 mg/kg/orally/daily) omega-3 for sixty consecutive days significantly increased catalase (CAT) activity in experimental group as compared with the PCOS group (P< 0.05), (Figure 2).

Testosterone level in serum

Administration of (240 mg/kg/orally/daily) omega-3 for sixty consecutive days significantly decreased testosterone concentration in experimental group as compared with the PCOS group (P< 0.05), (Figure 3).

FSH level in serum

Administration of (240 mg/kg/orally/daily) omega-3 for sixty consecutive days significantly increased FSH concentration in experimental group as compared with the PCOS group (P< 0.05), (Figure 3).

Blood glucose level

Administration of (240 mg/kg/orally/daily) omega-3 for sixty consecutive days significantly decreased blood glucose concentration to 1.75 ± 0.01 g/l in experimental group as compared with the PCOS group (P< 0.05), (Figure 4).

Table 1. Effect of Omega-3, fatty acids on lipid peroxidation in polycystic ovarian (PCO) rats with lower carbohydrate fed (P<0.05)

Groups	Control	PCO+ Omega3+Low carbohydrate diet	PCO	Omega-3
MDA (nmol/ml)	4.5±1.2	4.2±1.4	6.2±1.2*	3.0±0.8*

Table 2. Effect of Omega-3, fatty acids on antioxidant activities in polycystic ovarian (PCO)rats with lower carbohydrate fed (P<0.05)

Groups	Control	PCO+ Omega3+Low carbohydrate diet	PCO	Omega3
SOD (u/ml)	2.58±0.045	2.14±0.05	1.89±0.032*	2.94±0.05
Cat (u/ml)	71.4±16.7	68.3±15.9	56.24±15. 4*	92.3±18.2*
GPX (u/ml)	0.52±0.004	0.48±0.004	0.41±0.002*	0.84±0.005*

Table 3. Effect of Omega-3,fatty acids on hormonal levels in polycystic ovarian(PCO)rats with lower carbohydrate fed.(P<0.05)

Groups	Control	PCO+ Omega3+Low carbohydrate diet	PCO	Omega-3
Testosterone (ng/ml)	0.45±0.01	1.12±0.05	2.88±0.08*	0.40±0.01*
FSH (ng/ml)	1.15±0.01	0.85±0.01	0.55±0.01*	1.11±0.01

Table 4. Effect of Omega-3,fatty acids on blood glucose in polycystic ovarian(PCO)rats with lower carbohydrate fed (P<0.05)

Groups	Control	PCO+ Omega3+Low carbohydrate diet	PCO	Omega-3
Blood glucose (nmol/ml)	1.11±0.01	1.75±0.01	2.75±0.01*	1.05±0.01

Discussion

The impact of dietary composition has been recently evaluated on many diseases such as PCOS; studies showed PCOS outcomes can be affected by diet. An appropriate diet may restore ovulatory function and fertility in obese women with PCOS (15–16). Since dietary carbohydrates increase liver lipogenesis (fat-making) and the activities of enzymes related to lipogenesis, a high-carbohydrate diet induces lipogenesis in adipose tissue (fat tissue) and liver. Hence, in obese women with PCOS, a low-carbohydrate diet could be efficient; besides, recently relation between low carbohydrate diet and modulation of PCOS has been evaluated psychologically, and has been shown to be significant (17-19).

This study was designed in order to evaluate therapeutic effects of omega-3 along with low-carbohydrate diet on PCO-induced rats. We subdivided rats into a control group and three experimental groups; group 1 just received omega-3 for 60 days; groups 2 and 3 were induced PCOS by estradiol-valerate , group 3 received omega-3 along with low-carbohydrate diet for 60 days as well. After 60 days, biochemical results in group 3 in comparison to the other groups demonstrated positive effects of omega-3 and low-carbohydrate on improvement and normalization of antioxidant enzymes and reduction of oxidative species as well as testosterone. Although ROS have physiological roles in female reproduction, elevated level of ROS by active metabolism and steroidogenesis may cause DNA damage (20–21). Antioxidant enzymes restrict oxidative species to appropriate amounts for physiological processes of the body; antioxidant enzymatic defenses include SOD, GPx, and CAT. Non-enzymatic antioxidants can be represented by ascorbic acid (vitamin C), a-tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids, and other antioxidants (22). Superoxide dismutase (SOD), GPx,

and catalase have main roles to protect reproductive health, for instance SOD is closely associated with oocyte maturation (23). Oxidative stress and depletion of the antioxidants cause apoptosis (a type of physiological or active cell death) in many systems. Previous works showed that antioxidants prevented apoptosis effectively (24).

Polyunsaturated fatty acids omega-3(PUFA), especially α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), are bioactive lipids that positively impact signaling pathways involved in the improvement of cardiovascular diseases. Omega-3 may increase high density lipoprotein cholesterol (HDL), but it may decrease the triglyceride levels (25) .In women with PCOS, it can modify the adiponectin (a soluble matrix protein) level, insulin resistance, and lipid profiles, so it has a reducing effect on liver fat content (26). Studies showed modulating effect of PUFAS on hormonal, lipid profiles, and metabolic function in women with PCOS. Omega-3 also has a modulating effect on major risk factors of metabolic syndrome, especially adiposity, dyslipidaemia, insulin resistance, diabetes, hypertension, oxidative stress, and inflammation (27–28).

This syndrome (PCO) is also caused by hormonal imbalances that have a role in ovulation. PCOS usually causes a decrease in the level of follicle stimulating hormone (FSH) but increases the level of luteinizing hormone (LH). FSH is the hormone that is responsible for stimulating the growth of follicles with maturing eggs in ovaries. After lack of FSH for a long time, the follicles will not mature and release their eggs which results in infertility. Thereafter, the immature follicles in ovaries develop into small cysts and PCO syndrome will happen. Additionally high levels of LH cause the body to produce too much estrogen, androgens (male hormones) , testosterone, and DHEAS (dihydroepiandrosterone sulfate); this imbalance can cause pathological appearances in endometrial tissue and much thickening of the uterus, which can lead to heavy and/or irregular periods (29). Our results indicate that after treatment with omega-3 the mean of FSH was increased, but testosterone was significantly decreased (P<0.05).

Conclusion

he PCO-induced rats receiving omega-3 plus lower carbohydrate diet showed increased antioxidant levels and FSH, but decreased levels of testosterone and MDA, whereas PCO-induced rats not fed omega-3 showed decreased FSH and antioxidant levels but increased levels of testosterone and MDA. Therefore, results of this and the other studies demonstrate omega-3 plus lower carbohydrate feeding could be an efficient therapeutic choice so as to modulate PCOS side effects.

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