Nutrition with polyunsaturated fatty acid and lower carbohydrate diet has controlled poly cystic ovarian syndrome, on poly cystic ovarian (PCO) induces rats

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Abstract: PCOS 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. To confirm the role of nutrition with omega-3 polyunsaturated fatty acid on controlled of experimental PCO induced by estradiol-valerat (PPA) in rats, this study was done. Wistar female rat (n=50) were allocated into control (n=10) and test groups (n=40), test group subdivided into groups of 4, G1, received omega-3 (60 mg/rat/orally/daily), G2 induced PCO by single injection of estradiol valerate (4mg/rat/IM), G3 and G4 groups were induced PCO by single injection of estradiol-valerate (4mg/rat/IM), G3 & G4 groups was received omega-3 (60 mg/rat) and G4, received lower carbohydrate feeding, for 60 consequence day. In sixty day 5cc blood samples and ovarian tissues of Rats in whole groups were removed and prepared to biochemical pathological analysis. Hemorrhage, hyperemia and fibrosis were seen in pco groups, these side effects in groups that received omega-3 and lower carbohydrate feeding, significantly decreased (p<0.05) in comparison to experiment groups and ovarian weights in both experimental and control group were similar (p<0.05). Results revealed that administration of omega-3 plus lower carbohydrate food significantly controlled PCO syndrome.


Key words: Carbohydrate, Omega-3, Nutrition, Ovary, PCO.

1. Introduction

PCOS is associated with many disorders such as overweight or obesity, successful weight loss programs are one of most sufficient method in restoring normal ovulation/menstruation, but many women find it very difficult to achieve and sustain significant weight loss. Low-carbohydrate diets and sustained regular exercise (Leeman and Acharya, 2009) may help. Some experts recommend a low GI diet in which a significant part of total carbohydrates are obtained from fruit, vegetables and whole grain sources. (Sambuc et al., 2001) PCOS is a heterogeneous endocrine and metabolic disorder recognized as the primary cause of infertility in women of the reproductive age. The syndrome is associated with ovulatory dysfunction, abdominal obesity, hyperandrogenism, and profound insulin resistance (Kuscu & Var., 2009). Women are usually diagnosed when in their 20s or 30s, but polycystic ovary syndrome may also affect teenage girls. The symptoms often begin when a girl's periods start. Women with this disorder often have a mother or sister who has symptoms similar to those of polycystic ovary syndrome (Araf et al., 2011). PCOS is characterized by enlarged ovaries with multiple small cysts, an abnormally high number of follicles at various states of maturation, and a thick, scarred capsule surrounding each ovary. Follicles are sacs within the ovaries that contain eggs. Normally, one or more eggs are released during each menstrual cycle. This is called ovulation. In polycystic ovary syndrome, the eggs in these follicles do not mature and are not released from the ovaries. Instead, they can form very small cysts in the ovary (Duleba et al., 2004). Antioxidants just took a hit that could threaten their place upon the throne of nutritional impeccability, Antioxidants work because they neutralize destructive molecules called "reactive oxygen species." (ROS) Under stress (Kwintkiewicz et al., 2006). ROS are involved in modulation of signal transduction pathways, including regulation of tissue growth and apoptosis. Researchers discovered that ovulation relies on reactive oxygen species by treating ovarian follicles first with luteinizing hormone, which triggers ovulation, and then with hydrogen peroxide, a reactive oxygen species (Bourre, 2005). Omega-3 fatty acids (popularly referred to as ω−3 fatty acids or n-3 fatty acids) are fats commonly found in marine and plant oils (Kwintkiewicz et al., 2006). This study was designed to evaluate antioxidants effects of Omega-3 and lowed carbohydrate feeding on ovarian tissues in experimental induces PCO in rats.
2. Material and methods

2.1. Animals

Forty adult 8 weeks old Wistar albino female rats of 250±10 grams were obtained from Animal Facility of Pasture Institute of Iran. Rats were housed in temperature controlled rooms (25°C) with constant humidity (40-70%) and 12h/12h light/dark cycle prior to use in experimental protocols. All animals were treated in accordance with the Principles of Laboratory Animal Care [NIH]. The experimental protocol was approved by the Animal Ethical Committee in accordance with the guide for the care and use 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 rat (n=50) were allocated into control (n=10) and test groups (n=40), test group subdivided into groups of 4, G1, received omega-3 (60 mg/rat/orally/daily). G2 induced PCO by single injection of estradiol-valerate (4mg/rat/IM), G3 and G4 groups were induced PCO by single injection of estradiol-valerate (4mg/rat/IM), G3 & G4 groups was received omega-3 (60 mg/rat), and G4, received lower carbohydrate feeding ,for 60 consequence day. In sixty day 5cc blood samples and ovarian tissues of Rats in whole groups were removed and prepared to biochemical pathological analysis. Animals were kept in standard conditions.

2.2. Lower carbohydrate feed

To demonstrate the effect of carbohydrate on protein utilization, the test diet contained 40% of the appropriate protein material, 40% carbohydrate, 5% fat,5% cellulose and 10 % mineral and vitamins, per kg diet. Changes in true protein digestibility, biological value, and net protein utilization were calculated and recorded. To control groups feeding contained 30% of the appropriate protein material, 50% carbohydrate, 5% fat,5% cellulose and 10 % mineral and vitamins, per kg diet.

2.3. Induces PCO

Thirty days before the experimental procedure, twenty rats were each given a single intra muscular (i.m.) injection of 4 mg EV (Riedeldehaen, Germany) in 0.2 ml oil in order to induce PCO (PCO group).

2.4. Surgical Procedure

In the 60th day, (at the end of the treatment period), the blood samples in control & experimental groups were immediately obtained.

2.5. Histopathology of Ovary

The ovarian tissues were fixed in 10% buffer formalin and embedded in paraffin wax. Five micron thick sections were obtained and prepared than stained with hematoxylen and eosin (H&E). The specimens were examined using an Olympus 3H light microscope.

2.5. Statistical analysis

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

3. Results

results of ovarian tissue weights ,percentages of Hemorrhagic area per 100 microscopic cross sections ,artery hyperemia and percent of cysts all PCO induces groups were significantly increased (P<0.05), in compartment to control and Omega−3 groups, this side effect was controlled in groups of PCO witch receiving lowed carbohydrate fed (P<0.001), (Table-1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Omega−3, fatty acids</th>
<th>PCO</th>
<th>PCO plus, 60 mg/rat/orally/daily Omega−3/rat</th>
<th>PCO plus60 mg/rat/orally/daily Omega−3 and lower carbohydrate fed /rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>ovary (gr)</td>
<td>1.09 ±0.55</td>
<td>1.88 ±0.54</td>
<td>0.07 ±0.55*</td>
<td>0.09 ±0.55</td>
<td>0.08 ±0.55</td>
</tr>
<tr>
<td>Hemorrhagic (%)</td>
<td>0.01±0.55</td>
<td>0.01±0.01</td>
<td>4.11±0.55*</td>
<td>1.60 ±0.56*</td>
<td>1 ±0.56**</td>
</tr>
<tr>
<td>Hyperemia (%)</td>
<td>0.01±0.55</td>
<td>0.02 ±0.11</td>
<td>4.90 ±0.55*</td>
<td>2.1 ±0.55*</td>
<td>1.1 ±0.55**</td>
</tr>
<tr>
<td>Percent of cysts (%)</td>
<td>0.02±0.11</td>
<td>0.01 ±0.11</td>
<td>10.87 ±0.11*</td>
<td>5.01 ±0.11*</td>
<td>3.01 ±0.11*</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE.

*Significant different at P< 0.05 level, (compared with the control group).

Data are presented as mean ± SE.

**Significant different at P< 0.001 level, (compared with the control group).
3.2. Pathology results

The Results obtained from this study showed that in the $\omega - 3$, group ovarian follicles was similar to control group but blood vessels were increased in the corpus luteum (Figure-A,B). In the PCO group the arteric follicles were increased in comparison to control group and fibrosis was seen. The results obtained from PCO group that received $\omega - 3$, showed that ovarian contains of primary and multilayer follicles and also ovary contains of several blood vessels in comparison to control group. The histological changes in this group showed that the collagen fibers increased in the tunica adventitia and in the external theca of follicles, in group that received lower carbohydrate, recovery were shown and number of cysts were decreased (Figure-C,D).

3.3. Photomicrograph

Figure A) photomicrograph of ovary tissue in control group show normal structure of follicle, H&E staining, X160. Figure B) photomicrograph of ovary tissue in of $\omega - 3$ group show normal structure of follicle, T.C staining, X160. Figure C) photomicrograph of ovary tissue in of PCO group show cyst (arrow), inflammation cells(arrow), and hyperemia (star), H&E staining, X160. Figure D) photomicrograph of ovary tissue in of PCO group that treated with $\omega - 3$ and lower carbohydrate fed, show decreasing of cystic follicle and fibrosis (arrow) and present of connective (bold arrow) H&E staining, X160.

4. Discussion

One important point evolved from early studies regarding the relationship between fat and carbohydrates: “That carbohydrate utilization depends upon an animal’s previous nutritional state has long been recognized and is supported by an
overwhelming amount of evidence. Thus glucose (blood sugar) utilization is depressed in the fasted animal and in the animal fed a diet containing little or no carbohydrate. One’s habitual dietary intake is a controlling factor in regards to any change in future dietary pattern. This means, for example, that if one consumes a high-carbohydrate diet, conditioned to using carbohydrates. A switch, then, to a high-fat diet creates a metabolic disruption which continues until sufficient time has elapsed for the body to make its adjustments to the dietary switch. Other findings support this idea, generally, dietary carbohydrates increase liver lipogenesis (fat-making from carbohydrate) and the activities of enzymes related to lipogenesis, whereas dietary fats or starvation have the opposite effect. The liver’s capacity for lipogenesis (fat-making) was different from the capacity of adipose tissue. They concluded: Feeding a high-carbohydrate diet stimulated lipogenesis in adipose (fat tissue), which considerably exceeded that found in the liver. The assumption that adipose tissue is the main site of lipogenesis is supported by the observation of an unimpaired lipogenesis in animals without livers. And, the rate of lipogenesis from available carbohydrates seems to be regulated by the carbohydrate content of the diet. There are now health experts who consider that an inadequate supply of the Omega 3 fats in our diet is one of the most serious health issues confronting our generation. And studies are showing that a low level of the Omega 3 fatty acids in our diet is as serious a health problem as other high risk issues like a high intake of the trans fats, or a low intake of fruit and vegetables (Rizk et al., 2005). Under normal conditions, antioxidants act to oppose ROS production, scavenge existing free radicals, and promote theremap of ROS-induced damage to cell structures. Nonenzymatic antioxidants include vitamin C, vitamin E, selenium, zinc, beta carotene, carotene, taurine, hypotaurine, cysteamine, and glutathione. Enzymatic antioxidants include SOD, catalase, GSH-Px, glutaredoxin and glutathione reductase (Masha et al., 2009). The degree of antioxidant defense present is often expressed as total antioxidant capacity (TAC) (Boure., 2005). Other radical species overrides the scavenging capacity by antioxidants, either due to the excessive production of ROS or an inadequate availability of antioxidants. Thus, oral antioxidant supplementation may serve to prevent its contribution to the pathogenesis of obstetrical disease such as preeclampsia and recurrent pregnancy loss and gynecological disorders such as PCO, and endometriosis. Our results showed ω−3, could rebound hyperemia and decrease hemorrhage in experimental PCO groups, these finding clearly confirmed beneficial effects of ω−3, such as anti-oxidant in preventing cells in cell injury conditions and this results were agree with others research’s that done before (Rizk et al., 2005). The relationship between oxygen spaces (OS) and cyclical changes in the endometrium is well-established. OS-promoting alterations in ROS and SOD levels have been demonstrated just prior to menstruation, during the late-secretory phase (Masha et al., 2009). Our previous research confirmed ω−3 has beneficial effect on Pco by decreasing fibrosis (Ouladsahebmadarek et al., 2012). Estrogen and progesterone withdrawal in endometrial cells in vitro has been associated with a decrease in SOD activity, resulting in the unopposed activity of ROS (CAO et al., 2010). Elevated lipid peroxide and decreased SOD in the endometrium during the late secretory phase may modulate endometrial breakdown, leading to menstruation. Other researches showed using fiber in daily diet can controled Pco. Fiber helps in two ways with PCOS. The first way they help is by slowing down the digestion of sugars in the body, so there is no spike in insulin. The second way they help is by removing excess estrogens from the body, which may also help to reduce fibroids. Great sources of fiber are: broccoli, celery, whole grains, Ezekiel bread, apples, and dark leafy greens. This research our data showed lower value of carbohydrate (equal carbohydrate and protein) in daily diet can control increasing side effect of PCO as we demonstrated in our previous research.

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