Research Article

Omega-3 Ameliorates Effects of Letrozole-Induced Polycystic Ovary Syndrome in Adult Female Rats

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Abstract

Background and aim: Polycystic ovary syndrome (PSOS) is a common disease of multifactorial etiology causing dangerous complications for women. The pathophysiology and intrinsic mechanisms underlying PCOS are complex and not fully determined. This study is designed to explore the mechanisms involved in PCOS in rats and to investigate the possible role of omega-3 in prevention of PCOS. Methods: 24 adult female albino rats were randomly classified into (1) Control groups (C): either non-treated (5 rats) or treated with the vehicle (3 rats), (2) Letrozole group (L): (8 rats), rats received letrozole orally (1mg/kg for 21 days) to induce PCOS and (3) Letrozole and omega-3 group (LO): (8 rats), in which PCOS was induced by letrozole and rats simultaneously received omega-3 (240 mg/kg orally) for 3 weeks. Histopathological examination of ovaries and biochemical analysis for serum was done. Results: In L group, ovaries showed features of polycystic ovary syndrome. The body weight, lipid peroxides, serum levels of insulin, testosterone, TNF-glucose, TGs, TC, and LDL were significantly increased associated with a significant decrease in serum HDL and FSH. In LO group the body weight, lipid peroxides and serum levels of insulin, testosterone, TNF-α, glucose, TGs, TC and LDL were significantly decreased associated with a significant increase in serum HDL. Microscopic examination of the ovaries revealed that LO group showed improvement of polycystic features. Conclusion: Administration of omega-3 led to improvement of PCOS as regard to the histopathological and biochemical changes most probably via its anti-oxidant and anti-inflammatory effects.

Key Words: Polycystic ovary, Omega-3, Oxidative stress, Anti-oxidant.

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects almost 15% of women of reproductive age. As this disease is a syndrome, it affects different aspects of women health; it is much more prevalent in obese patients and affects different body systems leading to reproductive and metabolic complications\(^1\). It is also the most common cause of chronic anovulation and hyperandrogenism in young women\(^2\). It is also the most common cause of infertility in women of reproductive age\(^3\).

PCOS is typically first identified during the early reproductive years, its clinical manifestations usually include: oligo or anovulation, hyperandrogenism (either clinical or biochemical), presence of polycystic ovaries. In adult women, clinical evidence of hyperandrogenism includes hirsutism, alopecia, and acne; these should be considered as indicating a condition of excess androgen production\(^4\). Women with PCOS have an increased proportion of primordial follicles and a corresponding increase in activated growing (primary) follicles. Small follicles do not develop into ovulatory follicles because growth of these follicles is arrested before they mature. In PCOS, there is an abnormal follicular development and apparent failure to select a dominant follicle results in anovulation\(^1\). PCOS is associated with long term-health complications, including diabetes, obesity, heart disease and endometrial hyperplasia or cancer\(^5\).
Reactive oxygen species (ROS) are formed during normal metabolism of oxygen and are produced as by-products of aerobic metabolism. A certain amount of ROS production is necessary for gene expression and cell signaling. Scavenging antioxidant systems are indispensable for maintaining an adequate amount of ROS. The balance between the generation and elimination of ROS is a key factor required for almost every metabolic function in mammals. Maintenance of this balance is an important constitutive process and has a particular influence on cell proliferation, differentiation, apoptosis, and death. When ROS production overwhelms the scavenging ability of antioxidants, oxidative stress (OS) occurs. Regarding the female reproductive system, ROS and antioxidants have been recognized as key factors involved in ovarian physiological metabolism. Many studies have investigated the presence of antioxidants and their transcripts in the female reproductive tract. ROS in the follicular fluid are involved in follicular growth, oocyte maturation, and ovarian steroid biosynthesis. OS is considered one of the most important mechanisms by which PCOS could be developed. In addition, chronic inflammation may be also one of the possible causes of this syndrome.

The current study is an attempt to investigate the prophylactic effect of a known antioxidant, omega-3 on letrozole-induced PCOS in rats.

Materials and Methods

Animals:
Twenty four adult female albino rats from the local strain, of body weight ranging from 100-150 grams at the beginning of this study were used. The age of these rats was about 8 weeks at the start of the experimental work. They were housed in wire mesh cages at room temperature with natural light/dark cycles for one week acclimatization to lab conditions. Rats were fed a standard diet of commercial rat chow and tap water ad libitum. Experimental procedures and care of animals were carried out according to the guidelines of the Animal Care and Use Committee of Faculty of Medicine, Minia University.

Experimental protocol: The rats were randomly divided into three groups:
(1) Control groups (C):
a. Control group (5 rats): in which rats didn’t receive medications.
b. Control group treated with vehicle (3 rats): in which rats received the vehicle, carboxy methyl cellulose (CMC) vehicle for letrozole.
(2) Letrozole group (L) (8 rats): In which rats received letrozole (aromatase inhibitor) (Sigma-Aldrich, USA) orally at a dose level 1mg/kg for 21 days prepared by dissolving the medicine in 1% aqueous solution of CMC as 1mg letrozole dissolved in 2ml CMC. The induction of PCOS was evidenced by histopathological findings from a preliminary study.
(3) Letrozole and omega-3 group (LO) (8 rats): Induction of PCOS was done as previously, at the same time rats received omega-3 (Sigma Aldrich, USA) at a dose level of 240mg/kg orally.

At the end of the experiment, rats were sacrificed by decapitation after an overnight fasting and blood samples were collected, allowed to clot at room temperature, and then centrifuged at 3000 rpm for 15 min. The serum was collected, labeled and stored at -20°C till the time of biochemical assays. The abdomen was dissected, then the ovaries were removed and one ovary was weighed for determination of lipid peroxides concentration. The other ovary was sent for histopathological examination. Peritoneal omental fat was removed as the whole gastrocolicomentum and weighed.

Biochemical measurements:
1- Determination of serum glucose:
Serum glucose level was measured by enzymatic colorimetric method using commercially available kit (Biodiagnostic, Egypt) following the manufacturer protocol procedures.
2- Determination of serum insulin:
Serum insulin was measured by united biotech ink (UBI) MAGIWEL 	extsuperscript{TM} Insulin Enzyme-Linked Immunosorbet assay (ELISA) according to the manufacturer protocol (13).

3- Determination of total serum cholesterol:
Total serum cholesterol level was measured by enzymatic colorimetric method using commercially available kit (Biodiagnostic, Egypt) following the manufacturer protocol procedures (14).

4- Determination of serum triglycerides:
Serum triglycerides level was measured by enzymatic colorimetric method using commercially available kit (Biodiagnostic, Egypt) following the manufacturer protocol procedures (15).

5- Determination of serum Low-Density Lipoprotein cholesterol:
Serum Low-Density Lipoprotein cholesterol level was measured by enzymatic colorimetric method using commercially available kit (Biodiagnostic, Egypt) following the manufacturer protocol procedures (16).

6- Determination of serum high Density Lipoproteins cholesterol:
Serum high Density Lipoproteins cholesterol level was measured by enzymatic colorimetric method using commercially available kit (Biodiagnostic, Egypt) following the manufacturer protocol procedures (17).

7- Determination of serum TNF-α concentration:
Serum TNF-α concentration was measured by (Boster biological technology U.S.A) Enzyme-Linked Immunosorbert assay (ELISA) according to the manufacturer protocol (18).

8- Determination of lipid peroxides concentration in ovaries:
Lipid peroxides level was measured in ovarian tissues by enzymatic colorimetric method using commercially available kit (Biodiagnostic, Egypt) following the manufacturer protocol procedures (19).

9- Determination of serum Testosterone:
Serum Testosterone was measured by Modular Analytics E170 (Cobas) reagent depending on Competition principle in which a high affinity monoclonal antibody (sheep) specifically directed against testosterone (20).

10- Determination of serum FSH:
Serum FSH was measured by Modular Analytics E170 (Cobas) reagent depending on Sandwich principle (21).

Histopathological examination:
The ovaries of the sacrificed animals of different groups were fixed in 10% buffered formalin. Processing of ovarian tissues was done for hematoxylin and eosin (H&E) staining following the standard techniques. The slides were examined by a pathologist unaware by the sources of the ovaries using Olympus light microscope to assess the ovarian activity (22).

Statistical analysis of the data:
Data are presented as means ± SEM. The results were analyzed by one way analysis of variant (ANOVA) followed by student t-test with P ≤ 0.05 selected as the criterion for statistical significance using Software GraphPad Prism Version 5 (GraphPad Software Inc, La Jolla, CA, USA).

Results
1- Effect of PCOS with or without treatment on body weight:
At the beginning of the experiment, there was no significant difference in weights of all rats. In PCOS group (L), there was a significant increase in the body weight as compared to C group at the end of third week (figure 1)

In PCOS treated with omega-3, the body weights were significantly decreased at the end of the third week as compared to L group but, they were still significantly higher than the C group (figure 1).
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2- Effect of PCOS with or without treatment on gastro-colic fat:
In L group, there was a significant increase in the weight of gastro-colic fat relative to the C rats (figure 2).

Figure (1): Body weight in PCO treated and non-treated groups at the end of the third week. C: control, L: letrozol induced PCOS, LO: PCOS treated with omega-3. Data represent Mean ± S.E. of eight observations per group. ○: Significant difference from C group, P≤ 0.05. ●: Significant difference from L group, P≤ 0.05.

Figure 2 showed that administration of omega-3 led to significant reduction in the weight of gastro-colic fat in comparison with L rats, but it was still significantly higher in comparison with C rats.

Figure (2): Weight of gastro-colic fat in PCOS treated and non-treated groups. C: control, L: letrozol induced PCOS, LO: PCOS treated with omega-3 GCF: gastro-colic fat. Data represent Mean ± S.E. of eight observations per group. ○: Significant difference from C group, P≤ 0.05. ●: Significant difference from L group, P≤ 0.05.
3- Effect of PCOS with or without treatment on serum glucose, insulin and insulin resistance (IR):
In L group, there was a significant increase in IR and serum levels of glucose and insulin as compared to C rats (table1).

Table (1): Serum glucose, insulin and IR in different groups:

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<th>C</th>
<th>L</th>
<th>LO</th>
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<tr>
<td>Glucose (mg/dl)</td>
<td>63.4±2.0</td>
<td>123.3±3.5○</td>
<td>91.8±2.0○●</td>
</tr>
<tr>
<td>Insulin (µIU/ml)</td>
<td>33.4±0.5</td>
<td>45.5±0.7○</td>
<td>39.8±0.8○●</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>5.2±0.1</td>
<td>13.8±0.4○</td>
<td>8.9±0.2○●</td>
</tr>
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</table>

C: control, L: letrozol-induced PCOS LO: PCOS treated with omega-3, HOMA-IR: homeostasis model assessment of insulin resistance. Data represent Mean ± S.E. of eight observations per group. ○: Significant difference from C group, P≤ 0.05. ●: Significant difference from L group, P≤ 0.05. HOMA-IR = serum glucose (mg /dl) X serum insulin (µIU/ml) / 405.

4- Effect of PCOS with or without treatment on serum lipid profile:
In L group, there was a significant increase in the serum levels of total cholesterol (TC), triglycerides (TGs), and low density lipoprotein (LDL) associated with a significant decrease in serum high density lipoprotein (HDL) as compared to C group (table 2).

Table (2): Serum lipid profile in different groups:

<table>
<thead>
<tr>
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<th>C</th>
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<th>LO</th>
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<tbody>
<tr>
<td>TC (mg/dl)</td>
<td>135.3±3.4</td>
<td>252.6±5.0○</td>
<td>202.9±5.3○●</td>
</tr>
<tr>
<td>TGs (mg/dl)</td>
<td>96.2±1.0</td>
<td>227.5±3.9○</td>
<td>147.0±5.8○●</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>69.9±2.7</td>
<td>176.5±5.9○</td>
<td>134.6±5.1○●</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>47.2±1.1</td>
<td>29.9±1.7○</td>
<td>37.9±3.0○●</td>
</tr>
</tbody>
</table>

C: control, L: letrozol induced PCOS, LO: PCOS treated with omega-3 LDL: low density lipoprotein, HDL: high density lipoprotein, TC: total cholesterol and TG: triglycerides. Data represent Mean ± S.E. of eight observations per group. ○: Significant difference from C group, P≤ 0.05. ●: Significant difference from L group, P≤ 0.05.

5- Effect of PCOS with or without treatment on serum tumor necrosis factor alpha (TNF-α) and ovarian lipid peroxides:
In LO group, there was a significant reduction in IR and serum levels of glucose and insulin as compared to L group, but they were still significantly higher relative to the C group. (table1).

In L group, there was a significant increase in serum levels of glucose and insulin as compared to C rats (table1).

Administration of omega-3 significantly decreased the serum levels of TC, TGs and LDL as compared to L group, but their levels were still significantly higher than C rats. On the other hand, serum HDL was significantly increased as compared to L rats, but its level was significantly lower than the C rats (table 2)

Figure 3 showed that in L group, there was a significant increase in serum levels of TNF-α as compared to C rats.

Administration of omega-3 significantly decreased serum levels of TNF-α as compared to L rats, but still significantly higher in comparison with the C rats.
As regard to ovarian lipid peroxides, In L group, there was a significant increase in the level of ovarian lipid peroxides as compared to the C group. Administration of omega-3 led to significant decrease in the level of ovarian lipid peroxides as compared to L non-treated rats but its level was still significantly higher than the level of C rats as shown in figure (4).

6- Effect of PCOS with or without treatment on testosterone and follicular stimulating hormone (FSH): In L group, there was a significant increase in serum level of testosterone associated with a significant decrease in serum level of FSH as compared to C rats (figure 5 and 6).
Figure (5) showed that administration of omega-3 led to a significant reduction in serum levels of testosterone as compared to L rats, but still significantly higher in comparison with the C group. However, serum levels of FSH in LO group were insignificant as compared to L rats, but they were still significantly lower as compared to the control group (figure 6).

**Figure (5): Serum testosterone in PCOS treated and non-treated groups.** C: control, L: letrozole induced PCOS, LO: PCOS treated with omega-3 T: testosterone. Data represent Mean ± S.E. of eight observations per group. ○: Significant difference from C group, P≤ 0.05. ●: Significant difference from L group, P≤ 0.05.

**Figure (6): Serum FSH in PCOS treated and non-treated groups.** C: control, L: letrozole induced PCOS, LO: PCOS treated with omega-3. Data represent Mean ± S.E. of eight observations per group. ○: Significant difference from C group, P≤ 0.05

7- Histopathological Results:
Different types of follicles (i.e. cystic follicles, primordial follicles, growing follicles, oocytes and corpus luteum) were examined depending on their granulosa cell morphology, thickness of theca cells and corpus luteum presence. They are all counted and presented in Table (3). In normal ovaries of the control rats there were ovarian follicles at different developmental stages suggestive of normal ovarian physiology and several fresh corpora lutea. 

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Table (3): Percentage of ovarian follicles in different groups:

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<tr>
<td>Cystic follicles</td>
<td>11.1%</td>
<td>100%</td>
<td>38.5%</td>
</tr>
<tr>
<td>Corpus luteum</td>
<td>16.67%</td>
<td>-----</td>
<td>23.1%</td>
</tr>
<tr>
<td>Graffian follicles</td>
<td>38.89%</td>
<td>-----</td>
<td>19.2%</td>
</tr>
<tr>
<td>Primordial follicles</td>
<td>22.2%</td>
<td>-----</td>
<td>15.38%</td>
</tr>
<tr>
<td>Oocytes</td>
<td>11.1%</td>
<td>-----</td>
<td>3.8%</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

In PCOS non-treated rats (L group) there were only cystic follicles with no corpus luteum or other types of follicles. In LO group it was found that Cystic follicles decreased in number, the number of growing follicles of different stages of Graafian follicle maturation pathway increased. Primordial follicles, oocytes and corpus luteum counts were found to be increased in the LO group. Corpus luteal increase in number indicates marked attenuation of the cystic condition (table 3 and figure 7).

Figure (7): Slides examined from normal control group (A), polycystic ovary group (B) and group treated with omega-3 (C) (original magnification was 200x). Primordial follicles (PF), growing follicles of different stages of maturing Graafian follicles (GF), corpus luteum (CL), cystic follicles (CF) and oocytes (O).
Discussion
Polycystic ovary syndrome (PCOS) is a complex endocrine disorder characterized by hyperandrogenism, oligo-/anovulation, and polycystic ovarian morphology \[^{24}\]. It is also presented with features of the metabolic syndrome including obesity, insulin resistance (IR), and dyslipidemia; these latter symptoms are considered predisposing factors to the development of diabetes and cardiovascular disorders in PCOS individuals \[^{25}\].

In the present study, induction of PCOS was performed by using the aromatase inhibitor, letrozole. During PCOS induction, rats were treated by omega-3.

In letrozole induced PCOS group (L), in comparison with the control group (C), the body weight, gastro-colic fat weight, ovarian tissue lipid peroxides, insulin resistance (IR) and serum levels of glucose, insulin, tumor necrosis factor-alpha (TNF-\(\alpha\)), total cholesterol (TC), triglycerides (TGs), low density lipoprotein (LDL) and testosterone, were significantly increased associated with significant decrease in both serum high density lipoprotein (HDL) and FSH.

In the current study, decreased aromatase activity in the ovary may be the cause of PCOS development. Letrozole, the non-steroidal aromatase inhibitor, reduces conversion of androgens to estrogens in the ovary, resulting in increased testosterone and decreased estrogen production. In addition, the low estrogen level weakens the negative feedback on LH production in the pituitary, resulting in increased LH levels, which further stimulates ovarian theca cells to secrete testosterone \[^{26}\]. This is compatible with the increased serum testosterone in L group.

Excess testosterone was hypothesized to cause PCOS directly in L group. Previous study documented that excess androgen deteriorates maturation of developing follicles. It also can arrest follicular development indirectly via oxidative stress and pro-inflammatory cytokines which enhance apoptosis in granulosa cells resulting in bad oocyte quality and finally progressive atresia of ovarian follicles \[^{27}\]. This was confirmed by the histopathological picture of the ovaries in L group in the present study.

The higher body weight in L group might occurred secondary to the anabolic effect of testosterone and excess visceral fat accumulation. Several studies reported that testosterone enhanced the process of lipolysis via upregulation of \(\beta3\) adrenergic receptors and hormone sensitive lipase expression in visceral adipose tissue \[^{28}\]. But (2) reported that the ability of testosterone to reduce visceral adiposity was enhanced by aromatase enzyme, and inhibition of aromatase activity is associated with upregulation of the key genes involved in lipogenesis in visceral fat \[^{30}\] also mentioned that androgen excess in women with PCOS may be the cause of obesity specially the central abdominal obesity as it could impair adipocyte proliferation and differentiation via androgen receptors which may lead to adipocyte hypertrophy as a compensatory mechanism to increase adipose tissue mass.

This is compatible with the increased gastrocolic fat weight in L group in our study.

There are several possibilities that could explain the mechanism of IR and hyperglycemia in L group. One of them may be related to the ability of testosterone to change directly the muscle structure in female rats with PCOS. It may decrease the amount of highly oxidative insulin sensitive type I muscle fibers, increase the amount of glycolytic type II less insulin sensitive muscle fibers, and inhibit glycogen synthase enzyme \[^{31}\]. In addition, excess visceral fat accumulation is responsible for an increase in circulating adipocytokines, which have implications for IR, hyperglycemia and dyslipidemia \[^{32}\]. IR may be also related to TNF-\(\alpha\) induced serine phosphorylation of insulin receptor, leading to inhibition of signaling \[^{33}\].
Excess testosterone in L group could stimulate androgen receptors in endothelial cells and increase nuclear factor kappa B (NF-κB), nuclear translocation and pro-inflammatory gene expression. Moreover, excess cytokines may originate from androgen stimulated circulating mononuclear cells. This was evident in the present study by the increased serum TNF-α in L group and this is compatible with other study of the present study.

The IR is usually associated with hyperinsulinemia as a compensatory response as detected in the present study and by other study. Hyperinsulinemia itself also appears to be a contributor to IR via down-regulation of insulin receptors which acts as a kind of positive feedback, increasing the need for insulin.

The resulting hyperinsulinemia has a stimulatory effect on the ovaries that leads to enhanced androgen synthesizing enzyme in ovarian theca cells and decreases sex hormone binding globulin production by the liver. Moreover, Insulin may increase adrenal androgen secretion in PCOS via enhancing adrenal sensitivity to ACTH. Therefore, IR and the associated hyperinsulinemia resulted in hyperandrogenemia. Finally, IR and hyperandrogenemia continuously stimulates each other creating a vicious cycle.

In the present study, oxidative stress was augmented in L group and it was evidenced by significantly increased level of lipid peroxides in ovarian tissues. The mechanism of oxidative stress may be related to IR induced hyperglycemia which enhanced reactive oxygen species (ROS) production and lipid peroxidation. In addition, lipid peroxidation also may be resulted from excess proinflammatory cytokines production as evident by increased serum TNF-α in the present study. Excess ROS production may contribute to a local inflammatory state in ovarian theca cells that augment androgen production, IR and ovarian dysfunction.

Hyperandrogenemia and excess visceral fat may be the causes of lipid profile disturbances as found in the present study and by other investigators. The mechanism of decreased HDL in female rats with PCOS may be related to the ability of testosterone to upregulate scavenger receptor B1 (SR-B1) and hepatic lipase (HL) genes involved in HDL catabolism. SR-B1 mediates the selective uptake of HDL lipids by hepatocytes. HL catalyzes the hydrolysis of phospholipids on the surface of HDL, facilitating its clearance.

The serum level of LDL was significantly increased in L group. Certain study documented that androgen could interact with androgen receptors (AR) and decrease the catabolic removal of LDL by attenuating estrogen receptor (ER) mediated induction of low density lipoprotein receptor (LDLR) activity. One potential mechanism could be that AR directly blocks ER activation. Another possibility is the existence of a cofactor shared by AR and ER, whereby dominance of AR prohibits the activation of ER induced genes.

In the present study TC is significantly increased in L group as compared to C group this result is correlated with who confirmed that the increase in TC may be secondary to letrozole treatment which increases 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase) activity. Moreover, IR and hyperinsulinemia could increase denovo lipogenesis. Resistance to insulin action may result in hypertriglyceridemia as found in the present study and by others. IR could enhance TGs production from the liver and decreased its catabolism by lipoprotein lipase in peripheral tissues.

The frequency of pulsatile gonadotropin releasing hormone (GnRH) release may be the cause of reduced FSH level in the present study. In L group, the increased frequency of GnRH pulse favors the transcription of LH over FSH. The mechanism of GnRH secretion deregulation may be related to the weak peripheral aromatization of the androgen which affects blood level of sex hormone which may...
increase sensibility of GnRH receptors as well as pituitary sensibility to GnRH\(^{(44)}\).

Thus, oxidative stress, hyperandrogenemia, hyperinsulinemia, increased insulin resistance, increased ovarian lipid peroxides and reduced FSH in the L group is reflected on the microscopic picture of the ovaries in the present study.

In LO group treated with omega-3, the body weight, gastrocolic fat weight, ovarian tissue lipid peroxides, IR and serum levels of insulin, glucose, TNF-α, TC, TGs, LDL, and testosterone, were significantly decreased as compared to L group, associated with a significant increase in serum HDL. There was no significant change in serum FSH level as compared to the L group.

The decreased body weight and gastrocolic fat weight in LO group may occur secondary to the ability of omega-3 to decrease body fat deposition in adipose tissues by suppressing lipogenic enzymes and increasing \(β\)-oxidation so led to lipolysis and weight loss\(^{(45)}\).

The balance between omega-3 and omega-6 is of established importance, and members of these fatty acid families compete for the glycerophospholipids positioning on cell membrane and leads to subsequent metabolism to form eicosanoids, which are potent fatty acid–derived metabolites.

The mechanism by which omega-3 decreases testosterone level is indirect through the competition between it and omega-6. The increase in omega-3 leads to decrease concentrations of omega-6 mediated eicosanoid formation and steroidogenic acute regulatory (StAR) proteins thus, decreasing steroid-ogenesis and testosterone formation\(^{(46)}\).

The decrease in serum level of testosterone and visceral fat weight explains the increase in insulin sensitivity, reduction in hyperinsulinemia and reduction in hyperglycemia. IR also might be corrected due to the ability of omega-3 to increase adiponectin level\(^{(47)}\) which is produced by adipocytes and plays a role in reducing the plasma concentration of free fatty acid (FFA). Adiponectin also corrects hyperglycemia and this most likely mediated by decreasing hepatic gluconeogenic enzymes expression and increasing glucose uptake in myocytes via peroxisome proliferator-activated receptor alpha (PPAR-\(α\))\(^{(48)}\).

Omega-3 is associated with downregulation of NF-\(κB\), this can be explains the reduction in serum TNF-\(α\) level in LO group in comparison with the L group\(^{(49)}\). The reduction in serum TNF-\(α\) level led to the improvement of insulin signaling and sensitivity.

Omega-3 is a strong antioxidant as it has been shown to form micelles which scavenge free radicals, and to reduce hydroxyl radical and superoxide radical production. It also has been shown to downregulate the expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a major contributor to oxidative stress. Thus, it decreased ROS production and lipid peroxidation\(^{(50)}\). The reduction in proinflammatory cytokines, oxidative stress and testosterone level led to improvement of the quality and maturation of oocytes which explain the histopathological improvement in omega-3 treated rats.

Reduction of hyperandrogenemia and correction of IR by omega-3 led to the correction of dyslipidemia and decrease TGs level as discussed before. There was no significant change in serum FSH level in LO group in the present study.\(^{(51)}\)\(^{10}\)'s study showed similar results in contrast with\(^{(10)}\)'s results probably because they used omega-3 for longer duration.

**Conclusion**

The present study demonstrated that polycystic ovary syndrome is a common disease of multifactorial etiology. Administration of omega-3 led to improvement as regard to ovarian histopathological changes and metabolic syndrome manifestations of...
polycystic ovary syndrome. These results encourage us to recommend administration of omega-3 for longer duration in further studies as it may achieve better results and to suggest combinations of omega-3 with other medications to be tried in further studies to achieve the best combination for prevention and improvement of PCOS.

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