Effects of ω-3 Fatty Acids and Estrogen Replacement Therapy on Ovariectomy Induced Osteoporosis in Female Albino Rats

NAHED S. MOHAMED, M.D.*; MOHAMED A. SALEH, M.D.*; AYMAN S. SOLIMAN, M.D.** and AHMED M. SIDDIQ, M.Sc.**
The Department of Physiology, Faculty of Medicine, Cairo* and Beni-Suef** Universities, Egypt

Abstract

Background: Fatty acids especially omega 3 unsaturated fatty acids has known anti-inflammatory effects. There are different mechanisms by which dietary fatty acids may affect bone, but their protective effect against postmenopausal osteoporosis remains a controversial issue.

Material and Methods: This study was applied on 50 female albino rats that were divided into 5 equal groups: Sham operated group, ovariectomy group, ovariectomy + flaxseed oil group (0.4g/kg/day), ovariectomy + fish oil administered group (0.4g/kg/day) and ovariectomy + estrogen replacement therapy group (30 µg/kg 5 days a week). The study proceeded for 12 weeks then femurs Bone Mineral Density (BMD) were measured with Dual-energy X-ray Absorptiometer (DXA) scan and serum osteoprotogerin (OPG), bone specific alkaline phosphatase (B-ALP) and tumour necrosis factor alpha (TNF-α) were measured.

Results: There was a significant (p<0.01) increase in the BMD of the treated groups compared with the ovariectomy group. There were significant (p<0.001) differences in the means of serum bone markers measures of the treated groups versus the ovariectomy group in the form of significant increase in OPG and a significant (p<0.001) decrease in B-ALP and TNF-α of the treated groups compared with the ovariectomy group. This study showed a significant negative correlation between BMD and B-ALP (r = -0.375, p<0.001).

Conclusion: According to this study the chronic administration of omega 3 fatty acids (flaxseed oil-fish oil) has a preventive effect against osteoporosis and suppression of bone turn over process appears to have a role in this preventive effect.

Key Words: Osteoporosis – Omega 3 fatty acids – Bone mineral density – Osteoprotogerin – Tumour necrosis factor alpha – Alkaline phosphatase.

Introduction

OSTEOPOROSIS is a disease characterized by porous bones and a reduced bone mass. The associated structural changes predispose the bone to fracture. Osteoporosis usually refers to the most common forms, senile and post-menopausal osteoporosis [1]. Primary osteoporosis, the most common type of osteoporosis, is more common in women than men [2]. Primary osteoporosis is diagnosed when no disease causing low bone mineral density other than osteoporosis and no secondary osteoporosis are observed [3]. Secondary osteoporosis results from medical conditions or treatments that interfere with the attainment of peak bone mass and/or may predispose to accelerated bone loss [4]. Recently it has been demonstrated that, in contrast to primary osteoporosis which is associated with age, gender, and family history, secondary osteoporosis shows a prevalence in men similar to that in women [4].

Post-menopausal osteoporosis due to estrogen deficiency is a major health problem, because of the severe morbidity and mortality associated with osteoporotic fractures [5]. Estrogen deficiency is associated with large increases in bone resorption caused by increased osteoclast numbers (due to enhanced osteoclast formation and reduced osteoclast apoptosis) and by increased osteoclast activity [6]. Estrogen and/or hormone replacement therapies are able to prevent osteoporotic bone loss, however, accompanied by adverse side-effects, such as uterine, ovarian and breast cancer and increased risk of cardiovascular diseases. Therefore, diet therapies that minimize bone loss would be an ideal alternative [5].

ω-6 and ω-3 are two classes of essential Polyunsaturated Fatty Acids (PUFA). The distinction between ω-6 and ω-3 Fatty Acids (FA) is based on the location of the first double bond, counting from the methyl end of the FA molecule. ω-6 and ω-3 FA are essential because humans, like all
mammals, cannot make them and must obtain them in their diet [7]. Higher ω-3 FA and lower ω-6/ω-3 FA level in bone marrow phospholipids could maintain higher Bone Mineral Density (BMD) in estrogen deficient condition and lower incidence of osteoclastogenesis in mice [8]. Their supplementation was accompanied by reduced production of pro-inflammatory cytokines and increased production of anti-inflammatory mediators [8]. Flax (Linum usitatissimum L.) is a multi-purpose crop. Its seeds containing about 36 to 40% of oil, have long been used in human and animal diets and in industry as a source of oil. Flax oil is the richest plant source of linoleic (18:2 ω-6, 18-24%) and linolenic (C 18:3 ω-3, 36-50%) PUFA. Flax oil is also a source of oleic acid (18:1 ω-9, 16-24%) [8]. The fatty acid composition of freshwater fish oil shows differences compared with marine fish, marine fish oil has higher level of EPA (4.1-24.2%) and DHA (6.5-34.3%) [9].

Given the potential health benefits of PUFA, this study was designed to investigate the possible beneficial effects of estrogen and different types of ω-3 PUFA separately on ovariectomy-induced osteoporosis in female albino rats, through assessment of bone mineral density, serum alkaline phosphatase as indicators of osteoblastic activity and bone resorption.

Material and Methods

Experimental animals:

Fifty adult female albino rats (4-6 months), weighing 150-200g were used in the current study. They were obtained from Animal House Unit, Kasr Al-Ainy Faculty of Medicine, Cairo University (Egypt) and were housed at room temperature in cages with ordinary light/dark cycle, and left to acclimatize to environment for two weeks prior to inclusion in the experiment. The experiment was done during the year 2015.

Ovariectomy:

The animals were anesthetized with sodium thiopental (EIPICO, Egypt) 40mg/kg intraperitoneal. The area of surgery was cleaned with ethanol. A small transverse peritoneal incision of 0.4-0.6cm was made with surgical scalpel blade no.11 on the middle part of the abdomen slightly towards right, just near to the second right nipple of the rat. The ovary and associated fat were located and exteriorized by gentle retraction. A braided silk suture 3/0 was performed around the area of the distal uterine horns, that was sectioned thereafter, and the ovaries were removed. The uterine horn was returned to the peritoneal cavity after the removal of ovaries. The wound was closed using sterile sutures and povidone iodine was applied on the area to disinfect the skin after suturing. Regarding the sham operated group, the same incision was made and closed in 2 layers by the same suture types and the same disinfection measures but without ovariectomy procedures [10].

Experimental design:

The fifty rats were randomly divided into 5 groups, each is 10 rats, as following:

Group I (Sham operated control): The animals were accessed to standard diet of commercial rodent chow and tap water ad libitum for 12 weeks.

Group II (OVX): The animals were bilaterally ovariectomized and accessed to standard diet of commercial rodent chow and tap water ad libitum for 12 weeks.

Group III (OVX + FX): The animals were bilaterally ovariectomized received 0.4g/kg/day flax-seed oil (Sigma-Aldrich, Dorset, UK) orally by gavage tube as a source of ω-3 PUFA for 12 weeks [11].

Group IV (OXV + FS): The animals were bilaterally ovariectomized received 0.4g/kg/day fish oil (Sigma-Aldrich, Dorset, UK) orally by gavage tube as a source of ω-3 PUFA for 12 weeks [11].

Group V (OVX + E): Received subcutaneous injections of 30 µg/kg/day 17β-estradiol valerate (Sigma-Aldrich, Dorset, UK) for 12 weeks [12].

At the end of the experiment, rats were sacrificed, blood samples were collected and femurs were dissected, harvested and frozen.

Biochemical assays:

Serum Bone Alkaline Phosphatase (B-ALP) levels were measured using enzyme immunoassay (Alkaphase-B kit, Metra Biosystems). The enzyme activity of the captured BAP is detected with a p-Nitrophenyl phosphate substrate [13].

Tumour Necrosis Factor Alpha (TNF-α) and Osteoprotegerin (OPG) were determined using specific rat ELISA kits (R & D Systems) following the manufacturer instructions.

Femur Bone Mineral Density (BMD) measurement:

Rats were sacrificed at 12 weeks and both femurs from each rat were harvested. BMD was measured in the distal ends of the femurs with a Lunar Prodigy dual-energy X-ray absorptiometer (DXA) using the small-animal programme in Prof.
Nahed S. Mohamed, et al. 317

Dr. Omar Hussien’s Osteo-Test centre in Cairo-Egypt.

Statistical analysis:
The results were analyzed using the IBM SPSS computer software Version 22. Quantitative data were expressed as mean ± Standard Deviation (SD). Differences between groups means were analyzed by one way ANOVA test, and comparison between groups were analyzed by Tukey HSD test as a post hoc test.

Values of $p$$>$0.05 were considered statistically non-significantly different.

Values of $p$$<$0.05 were considered significantly different.

Values of $p$$<$0.001 were considered highly significantly different.

Pearson correlation coefficient ($r$) was used.

Results

Effects of ovariectomy, flaxseed oil, fish oil and estrogen replacement therapy on BMD:

Table (1) and Fig. (1) show the BMD changes based on DXA scan on the lower end of the femur in the studied groups. A significant ($p$$<$0.05) decrease was detected in the mean BMD of Ovariectomy (OVX) group ($0.145±0.034$ gm/cm$^2$) versus control group ($0.2206±0.031$) gm/cm$^2$. Regarding treatment groups, a significant increase in the BMD means was detected in ovariectomized rats fed flaxseed oil ($0.23±0.074$ gm/cm$^2$) and fish oil ($0.242±0.058$ gm/cm$^2$) and estrogen injected ovariectomized rats ($0.2436±0.052$ gm/cm$^2$) versus OVX group ($0.145±0.034$ gm/cm$^2$) ($p$$<$0.01). Regarding the same treatment groups a non-significant ($p$$>$.05) difference was detected in their means versus control group. Also, a non-significant difference was detected between the means of OVX + FX, OVX + FS and OVX + E.

Effects of ovariectomy, flaxseed oil, fish oil and estrogen replacement therapy on serum OPG:

Table (2) compares the serum OPG mean levels in the studied groups. A significant ($p$$<$0.001) decrease was detected in the mean serum OPG level of OVX group ($91.09±18.48$ pg/ml) versus control group ($248.14±36.60$ pg/ml). As regards treatment groups a significant increase in the serum OPG means was detected in ovariectomized rats fed flaxseed oil ($158.86±24.10$ pg/ml) and fish oil ($157.5±24.32$ pg/ml) and estrogen injected ovariectomized rats ($174±21.64$ pg/ml) versus OVX group ($91.09±18.48$ pg/ml) ($p$$<$.01). A significant ($p$$<$0.01) decrease was detected in the means of the same treatment groups versus control group ($248.14±36.60$ pg/ml). However, a non-significant ($p$$>$.05) difference was detected between the means of OVX + FX, OVX + FS and OVX + E.

Effects of ovariectomy, flaxseed oil, fish oil and estrogen replacement therapy on serum TNF-α:

Table (3) shows a comparison between the serum TNF-α level changes in the studied groups. A significant ($p$$<$0.001) increase was detected in the mean serum TNF-α level of OVX group ($121.69±15.72$ pg/ml) versus control group ($31.19±2.51$ pg/ml). Regarding treatment groups a significant ($p$$<$0.001) decrease in the serum TNF-α means was detected in ovariectomized rats fed flaxseed oil ($92.43±10.61$ pg/ml) and fish oil fed ($92.55±14.82$ pg/ml) and estrogen injected group ($85.26±11.41$ pg/ml) versus OVX group ($121.69±15.72$ pg/ml). Regarding the same treatment groups a significant ($p$$<$0.001) increase was detected in their means versus control group. No significant ($p$$>$.05) difference was detected between the means of OVX + FX, OVX + FS and OVX + E.

Correlation between the studied parameters:

Pearson’s correlation coefficient ($r$) between BMD, OPG, TNF-α and B-ALP was represented in Fig. (6). It shows a significant positive correlation between TNF-α and B-ALP ($r=0.734$, $p$$<$0.001) Fig. (6A). A significant negative correlation was detected between BMD and ALP ($r=−0.375$, $p$$<$0.001) Fig. (6B), between OPG and TNF-α ($r=0.819$, $p$$<$0.001) Fig. (6C) and between OPG and B-ALP.
Effects of ω-3 Fatty Acids & Estrogen Replacement Therapy

(r=0.788, p<.001) Fig. (6D). However, a non-significant positive correlation between BMD and OPG was detected (r=0.277, p>.05) Fig. (6E), and a non-significant negative correlation between BMD and TNF-α was detected (r=0.236, p>.05) Fig. (6F).

Table (1): Effect of 12 weeks administration of flaxseed oil, fish oil and estrogen on BMD in ovariectomized female rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
<th>p-value in comparison with control</th>
<th>p-value in comparison with OVX</th>
<th>p-value in comparison with OVX + E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.2206±0.031</td>
<td>&lt;.05*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVX</td>
<td>0.145±0.034</td>
<td>.05</td>
<td>&lt;.01*</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>OVX + FX</td>
<td>0.23±0.074</td>
<td>&lt;.05</td>
<td>&lt;.01*</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>OVX + FS</td>
<td>0.242±0.058</td>
<td>&lt;.05</td>
<td>&lt;.01*</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>OVX + E</td>
<td>0.243±0.052</td>
<td>&lt;.05</td>
<td>&lt;.01*</td>
<td>&gt;.05</td>
</tr>
</tbody>
</table>

*p-value is significant (p<.05).

Table (2): Effect of 12 weeks administration of flaxseed oil, fish oil and estrogen on serum OPG in ovariectomized female rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
<th>p-value in comparison with control</th>
<th>p-value in comparison with OVX</th>
<th>p-value in comparison with OVX + E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>248.14±36.60</td>
<td>&lt;.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVX</td>
<td>91.09±18.48</td>
<td>&lt;.001*</td>
<td>&lt;.001*</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>OVX + FX</td>
<td>158.86±24.10</td>
<td>&lt;.001*</td>
<td>&lt;.001*</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>OVX + FS</td>
<td>157.5±24.32</td>
<td>&lt;.001*</td>
<td>&lt;.001*</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>OVX + E</td>
<td>174±21.64</td>
<td>&lt;.001*</td>
<td>&lt;.001*</td>
<td>&gt;.05</td>
</tr>
</tbody>
</table>

*p-value is significant (p<.05).

Table (3): Effect of 12 weeks administration of flaxseed oil, fish oil and estrogen on serum TNF-α in ovariectomized female rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
<th>p-value in comparison with control</th>
<th>p-value in comparison with OVX</th>
<th>p-value in comparison with OVX + E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>31.19±2.51</td>
<td>&lt;.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVX</td>
<td>121.69±15.72</td>
<td>&lt;.001*</td>
<td>&lt;.001*</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>OVX + FX</td>
<td>92.43±10.61</td>
<td>&lt;.001*</td>
<td>&lt;.001*</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>OVX + FS</td>
<td>92.55±14.82</td>
<td>&lt;.001*</td>
<td>&lt;.001*</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>OVX + E</td>
<td>85.26±11.41</td>
<td>&lt;.001*</td>
<td>&lt;.001*</td>
<td>&gt;.05</td>
</tr>
</tbody>
</table>

*p-value is significant (p<.05).

Table (4): Effect of 12 weeks administration of flaxseed oil, fish oil and estrogen on serum B-ALP in ovariectomized female rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
<th>p-value in comparison with control</th>
<th>p-value in comparison with OVX</th>
<th>p-value in comparison with OVX + E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>68.85±10.82</td>
<td>&lt;.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVX</td>
<td>148.72±24.30</td>
<td>&lt;.001*</td>
<td>&lt;.001*</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>OVX + FX</td>
<td>101.8±15.97</td>
<td>&lt;.001*</td>
<td>&lt;.001*</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>OVX + FS</td>
<td>107.29±11.48</td>
<td>&lt;.001*</td>
<td>&lt;.001*</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>OVX + E</td>
<td>105.16±8.93</td>
<td>&lt;.001*</td>
<td>&lt;.001*</td>
<td>&gt;.05</td>
</tr>
</tbody>
</table>

*p-value is significant (p<.05).

Fig. (1): Comparison of BMD among studied groups control, OVX (ovariectomy), OVX-FX (ovariectomy + flaxseed oil), OVX-FS (ovariectomy + fish oil) and OVX-E (ovariectomy + estrogen).

Fig. (2): Comparison of OPG serum levels among studied groups control, OVX (ovariectomy), OVX-FX (ovariectomy + flaxseed oil), OVX-FS (ovariectomy + fish oil) and OVX-E (ovariectomy + estrogen).
Fig. (3): Comparison of TNF-\(\alpha\) serum levels among studied groups control, OVX (ovariectomy), OVX-FX (ovariectomy + flaxseed oil), OVX-FS (ovariectomy + fish oil) and OVX-E (ovariectomy + estrogen).

Fig. (4): Comparison of ALP-b serum levels among studied groups control, OVX (ovariectomy), OVX-FX (ovariectomy + flaxseed oil), OVX-FS (ovariectomy + fish oil) and OVX-E (ovariectomy + estrogen).

Fig. (5): DXA scan on lower end of the femur of the control (A) OVX (B), OVX + FX (C), OVX + FS (D) and OVX-E group (E).
Discussion

The present study was designed to explore the potential protective effects of ω-3 PUFA in flaxseed oil and fish oil on ovariectomy induced osteoporosis in female albino rats, and to compare these potential effects with the effect of estrogen replacement therapy.

The current results showed a significant decrease in BMD of the distal ends of the femurs in 12-week OVX rats. In agreement with the present study, [14] showed a significant decrease in BMD of rats proximal femoral metaphysis of the 12-week ovariectomized group compared to the 12-week Sham group. They reported that the BMD of L5 vertebral body was significantly lower in the OVX group than that in the Sham group. Administration of ω-3 PUFAs in the form of flaxseed oil and fish oil appears to have a protective effect against osteoporosis as there is a significant increase in BMD of OVX rats. These results were in accordance with the findings of [15] who showed that ω-3 and ω-6 FA mixture combined with calcium, ω-3 FA fortified dairy products, and a high α-Linolenic Acid (ALA) diet resulted in statistically
significant positive effects on BMD and decreasing fracture risk. Another study showed that the ratio of total \( \omega-6 \) to \( \omega-3 \)FA was inversely associated with BMD at the hip in both men and women regardless of hormone therapy status and at the lumbar spine in women not using hormone therapy [16]. The results of [17] showed positive correlation of a specific \( \omega-3 \)FA, namely DHA, with both BMD at 22y of age and changes in BMD of the spine between 16 and 22y in healthy men. Another cross-sectional study provided evidence of a marginally significant positive association between dietary \( \omega-3 \)FA (EPA, DHA, and Steardonic Acid (SDA)) and BMD of the femoral neck among older adults in the United States population. The results of that study also showed that supplement use of DHA, EPA, SDA, or their combination was overtly significantly associated with better spine BMD [18].

In addition, there was a significant increase in BMD of estrogen replacement therapy group. Estrogen is the major hormonal regulator of bone metabolism in women and men. Estrogen inhibits the activation of bone remodeling, most likely via the osteocytes, and also inhibits bone resorption, not only by direct actions on osteoclasts, but also by modulation of osteoblasts/osteocyte and T-cell regulation of osteoclasts formation and activity [19].

Regarding the inhibiting effect of \( \omega-3 \) PUFAs on bone resorption, increasing OPG transcription and secretion is one of the mechanisms by which \( \omega-3 \) PUFAs decrease bone resorption and prevent osteoporosis [20]. The down regulating effect of \( \omega-3 \) PUFAs on TNF-\( \alpha \) provides another mechanism for the preventive effect of \( \omega-3 \) PUFAs against osteoporosis. Results of the present study showed a significant decrease in serum OPG level in the OVX group with a significant increase in serum OPG level in the OVX + FX, OVX + FS and OVX + E groups [21,22]. These results are supported by evidences from [23] study that showed a decrease in OPG level in OVX rats. These results are also supported by [24] who found that estrogen acts on osteoblastic cells to increase the secretion of OPG. Estrogens stimulate OPG secretion from osteoblasts and inhibit RANKL production. This effect is attributed to the anti-resorbing property of estrogen. Thus, the production of estrogen is proportional to OPG secretion and so estrogen withdrawal enhances RANKL-RANK signal by down-regulating OPG expression [25]. This explains the decreased serum OPG level in OVX group in this study, and the significant increase in OPG level in OVX + E group.

A study done by [20] reported enhanced OPG mRNA expression in fish oil fed mice compared to corn oil fed mice. It was reported by [26] that in addition to the known role of Cbfa1 in promoting the differentiation of mesenchymal stem cells to form mature osteoblasts, expression of Cbfa1 appears capable of specifically increasing the production of OPG, which in turn could interfere with the interaction of RANK ligand with its receptor, RANK, on osteoclast precursors, thereby leading to an inhibition of osteoclast differentiation. Also [27] reported regulatory effects of PUFAs on Cbfa1 expression in fetal murine calvarial osteoblasts as EPA stimulated Cbfa1 expression after 14 days of treatment.

Regarding TNF-\( \alpha \) levels, the present study showed a significant increase in serum TNF-\( \alpha \) level in OVX rats and a significant decrease in the OVX + FX, OVX + FS and OVX + E groups. These results of increased TNF-\( \alpha \) in OVX rats are supported by the study of [28] who revealed increased numbers of TNF producing T cells in OVX mice by about 5-fold. Moreover, [29] reported that estrogen down-regulate TNF-\( \alpha \) production. The mechanism of the regulatory effect of estrogen on TNF-\( \alpha \) is attributed to its inhibitory effect on the expression of TNF-\( \alpha \) gene. An estradiol inhibitory element was mapped to the TNF-\( \alpha \) gene promoter. ER\( \beta \) was more potent than ER\( \alpha \) at repressing the TNF-\( \alpha \) promoter [30].

In addition, [21] showed that marine-derived \( \omega-3 \) PUFAs supplementation had a significant lowering effect on fasting blood levels of C-Reactive Protein (CRP), IL-6 and TNF-\( \alpha \). In another previous study, the synthesis of IL-1\( \beta \), IL-1\( \alpha \), and TNF in human was suppressed by dietary supplementation with fish oil as a source of long-chain \( \omega-3 \) FA [22]. The dietary \( \omega-3 \) PUFA may play an important role in suppressing the production of bone resorption and pro-inflammatory cytokines as IL-6 and TNF-\( \alpha \). The anti-inflammatory effects of \( \omega-3 \) PUFA are due to the inhibition of transcription nuclear factor-\( \kappa \)B intracellular signaling activation, and act as an activator ligand for another transcription factor Peroxisome Proliferator-Activated Receptor-\( \gamma \) (PPAR-\( \gamma \)) [31]. Furthermore, the anti-inflammatory effects of \( \omega-3 \) PUFA may partly be explained by their effects on lymphocytes, as \( \omega-3 \) PUFA suppress T-cell proliferation and pro-inflammatory cytokine secretion [32].

Serum B-ALP activity, a marker of bone mineralization, showed a significant increase in OVX group. This finding is supported by the results of [33,34] who reported increased serum B-ALP in OVX rats. This increase can be explained by the
lack of inhibiting activity of estrogen on osteoclasts, which caused the increase in acid phosphatase activity and in consequence increase in bone resorption. Simultaneously with intensification of resorption processes bone formation process was also increased by enhancement of osteoblast activity as a compensatory mechanism, and it led to growth of ALP activity \([34]\).

Few data are available regarding the effect of \(\omega-3\) PUFA on serum B-ALP activity. It was found by \([35]\) that fish oil did not affect the increased levels of ALP activity in OVX rats. In addition, it was showed by \([36]\) that \(\omega-3\) PUFA administration increased plasma ALP in salt-loaded male rats.

As B-ALP is a marker of bone mineralization \([37]\), the decreased B-ALP in the \(\omega-3\) PUFA fed groups and estrogen replacement therapy group can be explained by the decrease in bone turnover and hence the osteoblastic activity and the need to remineralization.

In conclusion, the present study proved that \(\omega-3\) PUFAs have a protective effect against OVX-induced osteoporosis in female albino rats. The serum parameters measured showed significant improvement in the form of statistically significant increase in OPG level and decrease in TNF-\(\alpha\) and B-ALP levels. However, further studies are required to investigate the mechanisms through which \(\omega-3\) PUFAs affect bone turnover, and more clinical studies are needed to assess the efficacy of \(\omega-3\) PUFAs preventive effect on osteoporosis in human beings.

References

21. LI K., HUANG T., ZHENG J., et al.: Effect of marine-derived n-3 polyunsaturated fatty acids on C-reactive


الملخص العربي

في العقد الذي يعقب انقطاع الطمث تتناقص كثافة العظام لدى النساء، بصورة ملحوظة بسبب نقص هرمون الإستروجين، مما يؤدي إلى تزايد تعرض كسور العظام في هذه الفترة من العمر. وقد انتشر استخدام هرمون الاستروجين كعلاج توعيسي للوقاية من هشاشة العظام بعد سن الستة، ولكن استخدام هرمون الاستروجين كعلاج توعيسي لا يخلو من المخاطر والأثار الجانبية كازداعة إجمالية الإصابة بسرطان الثدي وسرطان الرحم. ومن هنا نشأت الحاجة إلى علاج وقائي قائم على التغذية للحماية من هشاشة العظام.

هدفت هذه الدراسة إلى بحث التأثيرات الوقائية والعلاجية المحتملة من إضافة الأحماض الدهنية الغير مشبعة من النوع أوميجا 3 إلى الطعام، ومقارنتها بتأثير علاج الإستروجين التوعيسي.

وقد أجريت هذه الدراسة على 50 من إناث الفئران البضاء واستمرت 12 أسبوعا. وقد تضمن البحث 5 مجموعات بحث قسمت بالتسلسل:

1- المجموعة الضابطة: هو تأثير لها جراحة صورية.
2- مجموعة أخرى لها جراحة إستئصال المبيضين.
3- مجموعة أخرى لها جراحة إستئصال المبيضين ثم تم إعطائها زيت بذرة الكتان عن طريق التجربة بـ 4 جرام يوميا.
4- مجموعة أخرى لها جراحة إستئصال المبيضين ثم تم إعطائها زيت السمك عن طريق التجربة بمعدل 4 جرام يوميا.
5- مجموعة أخري لها جراحة إستئصال المبيضين ثم تم إعطائها هرمون الاستروجين عن طريق الحقن بمعدل 20 ميكروجرام لكل كيلو جرام لفترة 6 أيام من كل أسبوع حتى إنتهاء التجربة.

بعد مرور 12 أسبوع على بدء التجربة تم قياس مؤشرات بناء وقدم النظام في المصل وهي أوميغابيرتنتيرجين (OPG) وعامل نخر العظام (ARF) وانزيم الفوسفاتاز القلى ALP، كما تم قياس كثافة العظام بالطرق السليمة لقياس كثافة العظام (DXA).

وقد أظهر الفحص بجهاز قياس كثافة العظام أن العلاج بالأحماض الدهنية الغير مشبعة أوميجا 3 سوياً في صورة زيت بذرة الكتان أو زيت السمك يحافظ على كثافة العظام ويمنع الإصابة من خلال زيادة إجمالية العظام على المجموعة التي أجريت لها عملية إستئصال المبيضين دون تقلق علاج.

كما لم تظهر نتائج قياس كثافة العظام فراغ ذات دلالة إحصائية بين المجموعات التي تلت علاج الإستروجين التوعيسي وتلك التي تمت تغذيتها على الأحماض الدهنية غير المشبعة أوميجا 3.

كما أظهرت النتائج إرتفاعاً دون دلالة إحصائية في مستوى الأوميغابيرتنتيرجين في المصل لدى المجموعات التي تغذت على الأحماض الدهنية غير المشبعة أوميجا 3 مقارنة بالمجموعة التي أجريت لها إستئصال المبيضين دون تقلق علاج.

كما أظهرت النتائج إرتفاعاً في مستويات معامل نخر الورم أوميغابيرتنتيرجين في الفئران التي تلت علاج الأحماض الدهنية غير المشبعة أوميجا 3 مقارنة بالفئران التي أجريت لها إستئصال المبيضين دون تقلق علاج.

ولم تظهر النتائج فرقاً ذات دلالة إحصائية في مستويات بناء والدهم العظام بالصلب بين الفئران التي تلت علاج الإستروجين التوعيسي وتلك التي تمت تغذيتها على الأحماض الدهنية غير المشبعة أوميجا 3.

وقد خلصت هذه الدراسة إلى أن الأحماض الدهنية الغير مشبعة لها آثار وقائية من مرض هشاشة العظام الناتج عن نقص الإستروجين.

ومع ذلك فإن هناك حاجة إلى المزيد من الدراسات السريرية على البشر لتقديم فعالية الأحماض الدهنية الغير مشبعة أوميجا 3 في الوقاية من هشاشة العظام.