Research Article

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The effects of vitamin D, fish oil and exercise on cardiovascular parameters in ovariectomized rats

[Overiyektomize sıçanlarda vitamin D, balık yağı ve egzersizizin kardiyovasküler parametreler üzerine etkileri]

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Abstract

Objectives: This study compared effects of vitamin D, fish oil and exercise on cardiovascular parameters in ovariectomized rats.

Materials and methods: This is an experimental study conducted with 45 female Wistar Albino rats; consisted of one sham (n=8) and four intervention (n=37) groups. Rats (n=37) were oophorectomized and, randomly divided into four groups at the end of the first week following wound healing. Interventions were applied to the groups of oophorectomize+Dvit, oophorectomize+fish oil and oophorectomize+exercise for 12 weeks. In analyzing the data, ANOVA and Tamhane’s T2 tests were used (p<0.05).

Results: The levels of total-cholesterol (66.7 ± 7.6 mg/dL), HDL-cholesterol (33.8 ± 2.1 mg/dL), TOS (7.3 ± 1.2 μmol H2O2 Eq/L), TAS (4.0 ± 0.5 mmol Trolox Eq/L), OSI (0.2 ± 0.1) and total-cholesterol/HDL-cholesterol (2.0 ± 0.3) were obtained significant in exercising oophorectomized rats according to the oophorectomized rats (p<0.05). The estrogen levels of rats using vitamin D and fish oil and exercising were found to be higher than those in menopause.

Conclusions: It was concluded that exercising oophorectomized rats had a lower risk of cardiovascular disease. In this context, exercise/physical activity should be recommended and must be supported for practicing in order to protect the cardiovascular health of postmenopausal women.

Keywords: exercise; fish oil; menopause; vitamin D; women health.

Amaç: Bu çalışma, ooforektomize sıçanlarda vitamin D, balık yağı ve egzersizizin kardiyovasküler parametreler üzerine etkilerini karşılaştırdı.

Gereç ve Yöntem: Bu çalışma 215-257 gr ağırlığındaki 45 dişi Wistar Albino sıçanla yapılan deneyesel bir çalışmadır ve bir sham (n=8) ve dört müdahale (n=37) grubundan oluşmaktadır. Müdahale grubundaki sıçanlar (n=37) ooforektomize edildi ve yara iyileşmesini takiben ilk hafta sonunda rastgele dört gruba ayrıldı. Daha sonra; ooforektomize+Dvit, ooforektomize+balık yağı ve ooforektomize+egzersiz gruplarına 12 hafta boyunca müdahaleler uygulandi. Verilerin analizinde ANOVA ve Tamhane T2 testleri kullanıldı (p<0.05).

Bulgular: Egzersiz yapan ooforektomize sıçanların total kolesterol (66.7 ± 7.6 mg/dL), HDL-kolesterol (33.8 ± 2.1 mg/dL), TOS (7.3 ± 1.2 μmol H2O2 Eq/L), TAS (4.0 ± 0.5 mmol Trolox Eq/L), OSI (0.2 ± 0.1) ve total-cholesterol/HDL-cholesterol (2.0 ± 0.3) düzeyleri ooforektomize edilen sıçan gruplarına göre istatistiksel olarak anlamlı bir farka sahipti (p<0.05).

Amaçlar: Bu çalışma, ooforektomize sıçanlarda vitamin D, balık yağı ve egzersizinin kardiyovasküler parametreler üzerine etkilerini karşılaştırdı.

Materyal ve Yöntem: Bu çalışma, 45 dişi Wistar Albino sıçanla yapılmış bir deneyesel çalışmadır ve bir sham (n=8) ve dört müdahale (n=37) grubundan oluşmaktadır. Müdahale grubundaki sıçanlar (n=37) ooforektomize edildi ve yara iyileşmesi takiben ilk hafta sonunda rastgele dört gruba ayrıldı. Daha sonra; ooforektomize+Dvit, ooforektomize+balık yağı ve ooforektomize+egzersiz gruplarına 12 hafta boyunca müdahaleler uygulandı. Verilerin analizinde ANOVA ve Tamhane T2 testleri kullanıldı (p<0.05).

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Introduction

Cardiovascular health is important during menopause and can be a cause of death and disability. The World Health Organization (WHO) states that cardiovascular diseases (CVD) is the first cause of death worldwide [1], and most people die from CVD more than other causes [2]. According to WHO data, it is estimated that 47.9 million people die from CVD, with 31.0% of deaths worldwide [1], fatal or non-fatal myocardial infarction or CVD female mortality of 4.17% [3].

The risk of CVD in women is very low because estrogen protects them. This trend is reversed in menopause, and the CVD risk in women increases by 50% compared to men [4]. Therefore, estrogen has an important protective role on women’s health. Estrogen deficiency is believed to be a key factor in increasing the risk of postmenopausal CVD [5]. The incidence of CVD in women increases 50 times at the age of 50–59 compared to the age of 30–34 and exceeds men after the age of 65. While the preventive measures taken provided a decrease in the incidence of CVD in men, a decrease was not achieved in women after menopause due to estrogen deficiency [2, 4]. Estrogen has antioxidant properties and reduces the risk of CVD through various mechanisms. Estrogen protects from CVD risk by enhancing endothelial function and endothelium-dependent vasodilation and preventing platelet aggregation and adhesion [6]. Estrogen affects lipid metabolism and increases high-density lipoprotein cholesterol (HDL-C), which have a protective role in the cardiovascular system, lowers the risk of CVD by lowering low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC). With the decrease in estrogen level in the postmenopausal period, HDL-C decreases gradually, while TC, triglycerides and LDL-C increase [2, 4].

Omega 3 fatty acids are abundant in deep-sea fish. Docosahexaenoic acid (DHA), which is an omega 3 fatty acid forms an important part of the structure of tissues such as the gray matter of the brain, retina and nerves. In addition, the presence of DHA in breast milk sheds light on the structural importance of this nutrient element [7, 8]. Long chain omega 3 fatty acids have antioxidant and anti-inflammatory properties [9]. Omega 3 fatty acids are effective in preventing and treating heart diseases as in many diseases. It is stated that omega 3 fatty acids are effective in the treatment of heart diseases due to their anti-inflammatory, analgesic, anti-rombotic, vasodilator, and antiimotogenic effects [7, 8, 10].

Vitamin D is a fat-soluble secosteroid prohormone and is taken from plants (D2), animal tissues (D3) through diet, made endogenously from sunlight. In vitamin D deficiency, there is an increased risk of hypertension and CVD [7, 8, 11, 12]. Vitamin D increases the total antioxidant capacity in ovariectomized rats and therefore can prevent cardiac structural and apoptotic changes by increasing antioxidant activity. In addition, vitamin D has therapeutic effect in preventing postmenopausal cardiovascular disease [13]. It is stated that vitamin D and exercise improve the lipid profile in ovariectomized rats [14]. In addition, vitamin D and exercise develop lipid profiles of menopausal women [15]. Exercise has a protective effect against CVD and is one of the most important and effective tools for reducing the risk of CVD [16, 17]. Exercise has the effect of increasing total antioxidant capacity, protecting body from the harmful effects of free radicals [18].

It is thought that vitamin D, fish oil (omega 3) and exercise therapy will prevent cardiovascular diseases in the postmenopausal period. There are studies in the literature on rats and ovariectomized rats [19, 20]. However, no studies were found examining the cardiovascular effects of vitamin D, fish oil, and exercise in ovariectomized rats together. Therefore, the aim of this study is to examine the effects of vitamin D, fish oil and exercise on cardiovascular parameters in ovariectomized rats.

Materials and methods

This research is an experimental study. In the study, 50 adult female Wistar Albino rats, weighing between 215 and 257 g, raised in Aydın Adnan Menderes University Experimental Animal Production Laboratory were used. Rats were kept at room temperature, on a 12 h day/night cycle, and were fed with normal tap water and standard laboratory feed. When the rats were 3–4 months old, they were divided into five groups with 10 in each group: “Group that is closed by making an abdominal incision (SHAM)”, “Oophorectomized group (OVX)”, “Oophorectomized group given vitamin D (OVX+vitD)”, “Oophorectomized group given fish oil (OVX+F0)”, “Oophorectomized group were applied exercises-OVX+E”. Oophorectomy was performed under general anesthesia on 40 rats comprising the other four groups except the SHAM group. During the study period, a total of five the rats died (2 in SHAM group, 2 in OVX group and 1 in OVX+vitD group.) and the study continued with 45 rats.
Oophorectomized rats were randomly divided into four groups at the end of the first week following wound healing. After that; interventions were made to OVX+FO, OVX+vitD and OVX+E groups for 12 weeks [19].

The intervention procedures applied to the rats included in the study are as follows:

1. SHAM group (n=8): the group that is closed without removing the ovaries by making an abdominal incision.
2. OVX group (n=8): the group that underwent oophorectomy but did not undergo any other procedures for 12 weeks.
3. OVX+vitD group (n=9): the group that was given 1000 IU/kg vitamin D (Devâ® Devit-3 oral solution) orally once a week for 12 weeks following oophorectomy [21].
4. OVX+FO group (n=10): the group given 0.8 mL/kg fish oil (Solgar® 700 gel capsule) once a week by gavage method for 12 weeks following oophorectomy [20].
5. OVX+E group (n=10): the group in which treadmill exercises were applied for 45 min 3 days a week for 12 weeks following oophorectomy [22].

The rats in all groups were followed up by weekly weight measurements during the 12 weeks of study. According to the weight measurements, vitamin D and the fish oil dose of the rats was calculated every week and the rats were given fish oil and vitamin D according to the calculation made every week. Blood specimens were taken from all groups for biochemical evaluations at the end of 12 weeks.

Serum samples were obtained by centrifugation at 1,500 g for 10 min (Hettich 320R, DE), 1 h after blood samples were taken. The serum samples were stored at –80 °C until they were studied. Biochemical parameters examined in serum samples of rats to determine the effects of vitamin D, fish oil and exercise on oxidative systems and protective antioxidant systems, which are risk factors in rats, are as follows; malondialdehyde (MDA) and total oxidant status (TOS) as oxidant stress parameters, total antioxidant status (TAS) as a measure of antioxidant defense system, nitric oxide (NO) as a marker of nitrosative stress interleukin-1 (IL-1), interleukin-6 (IL-6) as inflammation markers, as well as omega 3 and 25 hydroxy vitamin D levels, TC, triglyceride and HDL-C were studied in the Clinical Biochemistry Laboratory, TAS (Rel Assay, TR), TOS (Rel Assay, TR), IL-1 (Elabscience, USA), IL-6 (Elabscience, USA), LDL-C, HDL-C and TC/HDL-C parameters were significantly different between OVX group in favor of SHAM group (p<0.05).

Triglyceride, TC, LDL-C and TC/HDL-C parameters were significantly different between OVX+vitD group and OVX group in favor of OVX+vitD group (p<0.05).

Triglyceride, TC, LDL-C, TAS and TC/HDL-C parameters were significantly different between OVX+FO group and OVX group in favor of OVX+FO group (p<0.05).

The biochemical values of groups (mean ± SD) and the differences between groups are shown in Table 2 (p<0.05).

NO determination: nitrite+nitrate, the metabolites of nitric oxide, were determined as total nitrite by the method of Navarro Gonzalez et al. [24]. According to this method, cadmium (Fluka) granules were used. Glycine-NaOH buffer was prepared using glycine (Merck) and sodium hydroxide (Prolabo). CuSO4 solution in glycine NaOH buffer was prepared using glycine, NaOH and copper sulfate (Riedel).

Sulfanilamide solution was prepared with hydrochloric acid 37% (Merck) and sulfonilamide (Sigma). NED solution was prepared using N-(1-naphthyl) ethyl-enediaime dihydrochloride (Aldrich). Standards were prepared using sodium nitrite (Sigma). Samples and standards were read on an ELISA microplate reader at 540 nm (Thermo Scientific Multiskan Spectrum 1500, USA). Imprecision of all the methods used were less than 10%.

Data were analyzed with SPSS (Version 22.0, SPSS Inc., Chicago, IL, USA; license, University of Aydın Adnan Menderes, Turkey). In the statistical analyzes of the biochemical data obtained, one way analysis of variance/ANOVA (significance test), and Tamhane’s T2 test (post-hoc test) were used to determine whether there were differences between the groups. A p value <0.05 was considered statistically significant.

**Results**

The average body weight of the rats at the beginning and end of the 12-week study period is shown in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pre-test (g)</th>
<th>Post-test (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>215 ± 8</td>
<td>252 ± 8</td>
</tr>
<tr>
<td>OVX</td>
<td>224 ± 19</td>
<td>300 ± 10</td>
</tr>
<tr>
<td>OVX + vitD</td>
<td>239 ± 17</td>
<td>325 ± 19</td>
</tr>
<tr>
<td>OVX + FO</td>
<td>239 ± 17</td>
<td>321 ± 17</td>
</tr>
<tr>
<td>OVX + E</td>
<td>222 ± 10</td>
<td>302 ± 13</td>
</tr>
</tbody>
</table>

OVX, Oophorectomized group; OVX + vitD, Oophorectomized group given vitamin D; OVX + FO, Oophorectomized group given fish oil; OVX + E, Oophorectomized group were applied exercises.
Table 2: Biochemical results of groups (mean ± SD).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SHAM (n=8)</th>
<th>OVX (n=8)</th>
<th>OVX + vitD (n=9)</th>
<th>OVX + FO (n=10)</th>
<th>OVX + E (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EST (ng/L)</td>
<td>38.7 ± 4.9</td>
<td>28.7 ± 10.1</td>
<td>33.2 ± 9.2</td>
<td>30.4 ± 7.6</td>
<td>29.5 ± 8.4</td>
</tr>
<tr>
<td>VitD(25OH3) (ng/mL)</td>
<td>0.2 ± 0.3</td>
<td>0.2 ± 0.10</td>
<td>0.6 ± 0.3</td>
<td>0.5 ± 0.4</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>NO (mol/L)</td>
<td>148.3 ± 33.0</td>
<td>89.8 ± 21.9</td>
<td>111.8 ± 23.7</td>
<td>128.0 ± 27.8</td>
<td>126.6 ± 25.6</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>63.3 ± 22.6</td>
<td>104.6 ± 36.3</td>
<td>38.4 ± 5.4</td>
<td>44.1 ± 7.6</td>
<td>49.7 ± 15.7</td>
</tr>
<tr>
<td>LC (mg/dL)</td>
<td>67.3 ± 3.4</td>
<td>81.3 ± 8.8</td>
<td>65.1 ± 3.9</td>
<td>64.6 ± 12.1</td>
<td>66.7 ± 7.6</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>16.3 ± 9.5</td>
<td>34.9 ± 8.2</td>
<td>30.4 ± 1.9</td>
<td>28.5 ± 9.1</td>
<td>27.3 ± 5.4</td>
</tr>
<tr>
<td>IL-1 (pg/mL)</td>
<td>344.6 ± 268.5</td>
<td>220.1 ± 165.2</td>
<td>195.5 ± 142.4</td>
<td>90.8 ± 50.2</td>
<td>383.9 ± 533.8</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>172.9 ± 59.8</td>
<td>228.8 ± 68.6</td>
<td>171.4 ± 37.8</td>
<td>169.5 ± 33.9</td>
<td>243.3 ± 69.7</td>
</tr>
<tr>
<td>TOS (μmol H/sub 2/O/sub 2 Eq/L)</td>
<td>10.7 ± 1.3</td>
<td>9.9 ± 1.4</td>
<td>8.6 ± 3.6</td>
<td>7.7 ± 1.5</td>
<td>7.3 ± 1.2</td>
</tr>
<tr>
<td>TAS (mmol Trolox Eq/L)</td>
<td>3.0 ± 0.3</td>
<td>2.9 ± 0.5</td>
<td>3.8 ± 0.9</td>
<td>3.8 ± 0.2</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td>OSI</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.3</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>MDA (μmol/mL)</td>
<td>10.4 ± 3.2</td>
<td>8.5 ± 1.7</td>
<td>10.8 ± 5.0</td>
<td>9.0 ± 1.5</td>
<td>9.9 ± 2.5</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>2.2 ± 0.2</td>
<td>3.1 ± 0.4</td>
<td>2.2 ± 0.2</td>
<td>2.2 ± 0.5</td>
<td>2.0 ± 0.3</td>
</tr>
</tbody>
</table>

EST, estrogen; NO, nitric oxide; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; IL-1: interleukin-1, IL-6: interleukin-6, TOS: total oxidant Status; TAS, total antioxidant status; OSI, oxidative stress index; MDA, malondialdehyde. *Significant difference with OVX group (p<0.05). †Significant difference with SHAM group (p<0.05). ‡Significant difference with OVX+E group (p<0.05).

HDL-C parameter was significantly different between OVX+E group, OVX+vitD group OVX+FO group in favor of OVX+E group (p<0.05).

Discussion

Our data suggest that vitamin D, fish oil and exercise can significantly prevent CVD risk. Vitamin D, fish oil and exercise produced positive changes in the cardiovascular parameters of ovariectomized rats, but no changes occurred in the cardiovascular parameters of SHAM group and OVX group rats. These results are very important in terms of revealing the beneficial effects of vitamin D, fish oil and exercise against cardiovascular risk.

In our study, there was an increase in the estrogen levels of the experimental groups, although it was not statistically significant. Vitamin D can alter the synthesis of steroid hormones such as estrogen [25]. Vitamin D provides its biological functions via the vitamin D receptor (VDR). VDR is found in most organs such as brain, heart, intestine, skin, prostate, breast, placenta, uterus, placenta, testis, pituitary, as well as in ovaries [26]. There are studies showing an increase in estrogen levels after vitamin D supplementation [27, 28]. These results suggest that vitamin D supplementation may increase estrogen levels. Other randomized controlled studies can be planned to confirm this hypothesis. In the study of Tartibian et al. [29], estrogen level was 14.5 ± 6.6 pg mL⁻¹ (change from baseline=2.2 ± 3.2) in postmenopausal women given omega-3 fish oil, 15.2 ± 6.4 pg mL⁻¹ (change from baseline=7.3 ± 1.1) in women who exercised and reported that fish oil and exercise increase the estrogen levels. According to our study, which is supported by the results of these researches, in addition to vitamin D, both fish oil and exercise increase the estrogen levels and contribute to the protection of cardiovascular health.

While triglycerides, TC and LDL-C increase in menopause, HDL-C decreases gradually [30] As the estrogen umbrella was closed in OVX rats, the elevated triglyceride and TC levels decreased significantly in rats treated with vitamin D, FO and SHAM and this was found to be consistent with the literature [11, 19]. Similar to the results of our study, Cappellari et al. [31] stated that fish oil reversed changes in lipid composition and reduced vascular oxidative stress in OVX rats and there are also similar studies [32, 33].

LDL-C is also known as the “bad cholesterol”, it is recognized as harmful for the whole body and especially cardiovascular health. LDL-C levels of ovariectomized rats were higher than SHAM group and other groups. Although not statistically significant, the LDL-C levels of rats given vitamin D, omega3 and exercised decreased compared to those in menopause [14, 15, 20, 34]. Similarly, Tang et al. [35] reported that exercise significantly reduced LDL-C levels and ameliorating cardiac damage caused by oxidative stress in OVX rats.

HDL-C is known as the “good cholesterol”, it is recognized as beneficial for the whole body and especially...
cardiovascular health. In our study, there were significant differences between the HDL-C levels of the ovariectomized rats and all the groups, and the ovariectomized rats had the lowest levels, suggesting that both vitamin D, omega 3, exercise and SHAM will be beneficial for heart disease risk. At the same time, a significant difference was observed between the exercise group and the vitD, omega 3, SHAM groups, which shows that exercise is more effective than vitamin D and omega 3 in preventing the risk of heart disease. The effect of exercise on HDL-C level is a positive result supported by the results of other studies [14, 15, 34, 36].

In the study of Babaei et al. [14] in rats; TC, triglyceride, LDL-C, HDL-C values in OVX+E Group, OVX+VitD Group and OVX Group are consistent with the results of our study and it is supported by the study of Abadi [15]. Mohammed et al. [13] also reported that vitamin D has a therapeutic effect against postmenopausal cardiovascular disease.

Oxidative stress (OS) is the imbalance between oxidant and antioxidant molecules. It was observed that TOS value was high and TAS value was low in OVX group rats. This shows us that menopause has an oxidant effect. The TOS values of the exercised rats were significantly lower than the other ovariectomy groups. This shows that exercise is more effective in reducing the risk of CVD than vitamin D and omega 3. TAS values of rats that were exercised and given omega 3 were significantly higher than OVX group. This shows that exercise and omega 3 are more effective in reducing the risk of CVD than vitamin D. These results are supported by the literature [11, 12, 36]. Parallel to TOS and TAS, the OSI values of the rats that exercised were lower and significantly different from the OVX group rats. These data is a desired positive result [7, 8, 20, 37]. In addition, Tang et al. [35], also found out that exercise improved OS status in OVX rats. Cappellari et al. [31], Zanetti et al. [32] and Marinho et al. [33] also reported positive effects of FO on OS in OVX rats.

TC/HDL-C used as cardiovascular risk factor were significantly lower in vitD, FO and E groups rats than OVX rats. This reinforced the effectiveness of vitD, FO and E, and moreover, the lower level of TC/HDL-C in the exercise group revealed once again that exercise can be used as a priority in preventing cardiovascular risk factor in postmenopausal women [32, 34]. There are also other positive features of exercise/physical activity, such as (1) exercise/physical activity is non-invasive, (2) exercise/physical activity requires no extra costs, (3) exercise/physical activity allows the postmenopausal woman to take care of herself by exercising, (4) exercise/physical activity could be practiced on their own, does not require dependency on someone else.

There was not significant difference in the levels of NO [38], IL-1 beta and IL-6 [39] and MDA [40] which can be counted as an indicator of endothelial dysfunction, in vitD, FO and E groups. Suppression of the activities of proinflammatory cytokines with the effect of fish oil, vitamin D and exercise in the experimental groups and estrogen in the SHAM group is an expected situation. Therefore, IL-1 and IL-6 levels of VitD, FO, E and SHAM groups are thought to be similar.

Conclusions

Postmenopausal period can cause cardiovascular effects in rats. In conclusion, estrogen levels of rats in the vitamin D, fish oil and exercise groups were higher, although not significantly, compared to the postmenopausal group. This result is an indication of a lower risk of CVD exposure. Because fish oil, vitamin D and exercise have protective effects against oxidative stress.

Therefore, fish oil, vitamin D and exercise are beneficial and we recommend for the protection of cardiovascular health and prevention of CVD in postmenopausal women. Exercise/physical activity is the best protective method against the effects of menopause on the cardiovascular system. To do exercise/physical activity at least three days in a week will reduce the risk of CVD. Therefore, exercise/physical activity should be recommended and must be supported for practicing in postmenopausal women.

Conflict of interests: None.

Çıkar çatışması: Yok.

References


