The impact of vitamin D3 administration and of high fat diet on oxidative stress and inflammation in experimentally induced polycystic ovary syndrome

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Abstract

Background. Polycystic ovary syndrome (PCOS) is commonly associated with obesity and may be exacerbated by the lack of vitamin D3.

Aim. The study aimed to investigate the effects of vitamin D3 administration in female rats with PCOS and prolonged high fat diet (HFD).

Methods. Forty-four female Wistar rats, 180-200 g, 10 weeks old, were randomly allocated into 2 groups (n=22) that received a single dose intramuscular injection of: sesame oil (group I), or estradiol valerate (5 mg) in sesame oil (group II). After 4 weeks, intraovarian cysts developed in group II, as evidenced by ultrasonography. In the next step, half of rats from each group received standard diet (SD) and the other half high fat diet, through oral gavage, for 17 weeks, the following groups being obtained: Control (SD), HFD, PCOS (PCOS+SD) and PCOS+HFD. Next, all the rats received, for 5 weeks, 500 UI/kg/day vitamin D3, through oral gavage. Lipid peroxidation was assessed through malondialdehyde level in the ovary and periovarian tissue and the inflammation was quantified in ovary by NFkB, pNFkB, NRF2 and SOD1 expressions. Ovaries from all groups were collected for histopathological analysis. Blood samples were taken to evaluate the basal insulin, triglycerides and total cholesterol levels throughout the experiment.

Results. Both groups with PCOS recorded significant increases of malondialdehyde in ovaries ($p<0.001$) and in periovarian tissue, especially in PCOS+HFD ($p<0.05$), even after vitamin D3 administration. PCOS+HFD group treated with vitamin D3 showed a high degree of inflammation in ovarian histopathology but with decreased pNFkB expression ($p<0.01$) while PCOS group recorded an increased SOD1 expression ($p<0.05$). Additionally, vitamin D3 treatment attenuated the insulin level ($p<0.001$) in PCOS and in HFD groups and the total cholesterol level in PCOS+HFD group, but triglycerides recordings were without statistical significance ($p>0.05$). HFD induced inflammation in ovaries, evidenced histologically and through increases of COX2 expressions ($p<0.05$) without significant influences on oxidative stress and on cholesterol levels.

Conclusions. Polycystic ovary syndrome is associated with oxidative stress and inflammation in the ovary tissue and in blood with increased levels of insulin, total cholesterol and triglycerides that might be partially mitigated by vitamin D3 oral administration.

Keywords: polycystic ovary syndrome, high fat diet, obesity, vitamin D3

Introduction

Polycystic ovary syndrome (PCOS) represents an endocrine disorder manifested by hyperandrogenism, ovulatory dysfunction and polycystic ovarian morphology, which involves oxidative stress and inflammation [1] that act over a genetic inherited predisposition [2]. It is frequently associated with insulin resistance and dyslipidemia, conditions that may lead to diabetes mellitus and obesity development [3]. PCOS is a heterogeneous syndrome with uncertain etiology (genetic, hormonal disruption or environmental) [4] which continues to arouse the researchers' interest, despite the numerous different studies investigating it.

Dyslipidemia accompanies PCOS and includes high levels of total cholesterol, triglycerides and LDL (low-density lipoproteins) [5], and decreased HDL (high-density lipoproteins) levels [6] that may lead to atherosclerosis or heart impairments as complications of disease.

Women with PCOS are predisposed to type 2 diabetes mellitus due to alteration of insulin actions on glucose metabolism. The resulted hyperinsulinism and hyperglycemia promote the oxidative stress even in women with normal body weight [7-9]. Abnormal secretions of hormones, including hyperandrogenism, high levels of luteinizing hormone (LH), normal or low levels of follicle stimulating hormone (FSH), and increased estrone levels due to aromatization of androgens in adipose tissue [10], were found in PCOS. Some changes in ghrelin and leptin levels and alteration of food intake were also noticed [11].

Oxidative stress, important pathological mechanism of PCOS, occurs in different bodily compartments where it initiates or aggravates the cellular modifications. Different parameters were investigated for the evaluation of oxidative stress in serum and high levels of nitric oxide (NO), xanthine oxidase (XO) or malondialdehyde (MDA) were recorded [1]. Large amounts of pro-oxidants compounds like homocysteine (Hcy), asymmetric dimethylarginine (ADMA), or of other markers [12], but also the reduced efficacy of antioxidant enzyme SOD (superoxide dismutase) in serum, are common features in PCOS [13].

Inflammation is a frequent outcome of PCOS, related or not to obesity, and the data from the literature mentioned the increase of serum C-reactive protein (CRP) levels, IL-6 [14] secretion, and altered expression of NFkB [15].

Vitamin D3 possesses anti-inflammatory, antioxidant and antiangiogenic properties. It was administered to patients with PCOS in several clinical studies [16] or it was used in different *in vitro* experiments [17] leading to diverse results, even contradictory.

The purpose of the present study was to evaluate the dynamics of serum metabolic parameters in female Wistar rats with experimentally induced PCOS, fed with HFD for 17 weeks and then treated with vitamin D3 for 5 weeks. At the end of the experiment, the ovaries and periovarian adipose tissue were collected for the investigation of the oxidative stress and inflammation.

Methods

Animals

For the experiment, 44 female Wistar rats, 10 weeks old, 180-200 g were purchased from Iuliu Hatieganu University of Medicine and Pharmacy Biobase and had one week for environmental accommodation. They were hosted in individual cages, in conditions of 65% humidity, temperature 21°C, 12 hours day/night cycle. The food and water were *ad libitum*. The experiments were performed with the approval of the Ethics Committee on Animal Welfare of the Iuliu Hatieganu U.M.Ph (213/02.04.2020) and in accordance with Directive 86/609/ EEC.

Substances administrated

The sesame oil, estradiol valerate and vitamin D3 were purchased from Sigma-Aldrich Co. LLC, Germany.

High Fat Diet

The administered high fat diet was produced by Cantacuzino National Medico-Military Institute for Research and Development, Bucharest, Romania. It contained 59% lipids, 25.9% carbohydrates, 14.9% proteins, and provided 5.4 Kcal/g.

Experimental design

The female rats were randomly allocated into 2 groups: I (n=22) - control group and II (n=22) - PCOS (polycystic ovary syndrome) group. Before the treatment administration, blood samples were collected from all rats.

The animals received a single dose intramuscular administered of 0.4 mL sesame oil -group I or 5 mg of estradiol valerate (EV) in 0.4 mL of sesame oil - group II. After 4 weeks, blood samples were taken, echography was performed in all rats, and two rats from each group were used for ovaries collection (under deep anesthesia with 5 mg/100 gbw ketamine 10% and 100 mg/100 gbw of xylazine hydroxychloride 2%) to confirm, through histopathological analysis, the development of ovary cysts in group II.

In the next step, the obesity was induced in half of rats from both groups (I and II) through administration, for 17 weeks, of high fat diet (HFD). The remaining rats of both groups received standard diet (SD). In this way, 4 groups (n=10) emerged: Control (standard diet), HFD (high fat diet) - from group I; PCOS (polycystic ovary syndrome with standard diet), PCOS+HFD (polycystic ovary syndrome with high fat diet) - from group II. At the end of these 17 weeks, blood samples were collected.

For the next 5 weeks, vitamin D3 treatment was daily administrated to all rats' groups, 500 UI/kg, through oral gavage. At the end of the experiment, blood samples were taken.

The blood was taken under mild anesthesia (2.5 mg/100 gbw ketamine 10% and 50 mg/100 gbw of xylazine hydroxychloride 2%) from the retroorbital plexus for metabolic markers (total cholesterol, triglycerides and basal insulin) evaluation.

The periovarian adipose tissue and ovarian samples were collected under deep anesthesia to evaluate the oxidative stress. From the ovarian tissue, the measurement of inflammatory markers was also performed (Figure 1).

Metabolic markers investigation

The metabolic markers were investigated through colorimetric assay kits obtained from Elabscience China. Insulin levels were assessed by ELISA assay according to the manufacturer's protocol and the results were expressed as pg/mg protein.

Ultrasound examination

For the ovaries morphologic evaluation, at four weeks after the EV administration, abdominal echography was performed using Ultrasonix Sono Touch Series ultrasound diagnostic system with a linear transducer, 1.5 cm aperture, wide frequency selection range (8-40 MHz), 40 MHz operating frequency, depth of focus <1 cm, spatial resolution <0.1 mm. Transversal sections were performed to measure the ovaries in all the animals of groups I and II. The maximum dimensions of identified ovarian cysts were also measured.

Western blot analysis

The ovary inflammation level and antioxidant status were investigated through Western blot analysis. Protein levels of nuclear factor kappa B (NFkB) and phosphorylated form (p-RELA/NFkB p65-pNFkB), nuclear factor erythroid 2 (NRF2), cyclooxygenase 2 (COX2) and superoxide dismutase 1 (SOD1) were evaluated by Western Blot, using primary antibodies against NFkB acquired from Cell Signaling Technology (Danvers, USA), and the rest from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The corresponding secondary antibody marked with horseradish peroxidase anti-mouse was from Promega Corporation, (Madison, USA) and anti-rabbit from Cell Signaling Technology. β actin was used as a protein loading control, anti-β actin antibodies were purchased from Santa Cruz Biotechnology. Briefly, the tissue lysates, (20 µg protein/lane) were separated by electrophoresis on SDS PAGE gels using Bio-Rad Mini protean system and transferred to polyvinylidene difluoride membranes, using the Trans Blot Turbo™ Transfer System (Bio-Rad, Hercules, USA). After blocking, membranes were incubated overnight at 4°C with primary antibodies, then washed and incubated with the corresponding secondary antibodies for two hours at RT. Proteins were detected using Clarity Western Blot substrate (Bio-Rad) and a Chemi Doc Imaging system equipped with Image Lab analysis software (Bio-Rad).

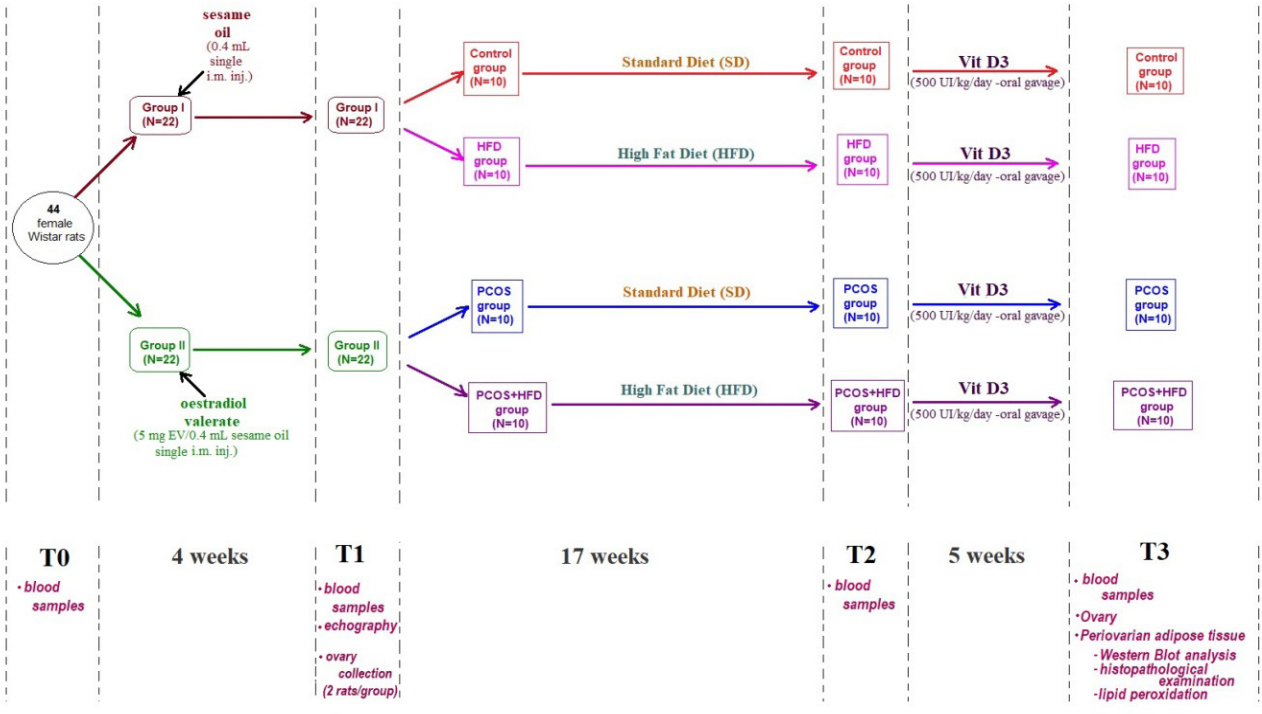


Figure 1. Experimental design.

Oxidative stress investigation

Lipid peroxidation in the ovary and in periovarian adipose tissue samples was investigated through malondialdehyde (MDA) by using the Conti's method [18]. Bradford method was used to quantify the proteins contained in tissue homogenates [19].

Histopathological analysis

At the end of the experiments, the ovaries from two animals of each experimental group (Control, HFD, PCOS, PCOS+HFD) were collected for histopathology analysis in routine haematoxylin eosin (HE) stain. Ovaries were fixed in 4% buffered formaldehyde for 24 hours at 4°C and then were embedded in paraffin and serial sections of 5 mm were used for H&E staining. Histological images of the ovaries were captured using an Optika B383LDI fluorescence microscope with a 2 MP CCD camera.

Statistical analysis

Oxidative stress and inflammatory parameters were analyzed statistically with GraphPad Prism version 9.0.0 for Windows, GraphPad Software Dotmatics (Boston, MA, USA), one-way ANOVA followed by the Tukey post-test. The metabolic markers variations throughout the experiment were statistically evaluated with two-way ANOVA multiple comparisons test. The threshold significance was set at $p<0.05$ (*$p<0.05$; **$p<0.01$; ***$p<0.001$).

Results

The female Wistar rats that received HFD increased their body weight to 420 ± 38 g.

Metabolic markers

The blood was taken from the female rats before the beginning of the experiment (T0 moment), after 4 weeks from the intramuscular injection of sesame oil or EV (T1 moment), after 17 weeks of standard diet or high fat diet, according to the group (T2 moment) and at the end of the experiment, after 5 weeks of vitamin D3 daily administration (T3 moment).

Basal insulin levels presented significant variations throughout the experiment.

At moment T1, insulin was significantly decreased ($p<0.001$) in group II (with EV administration), compared to group I.

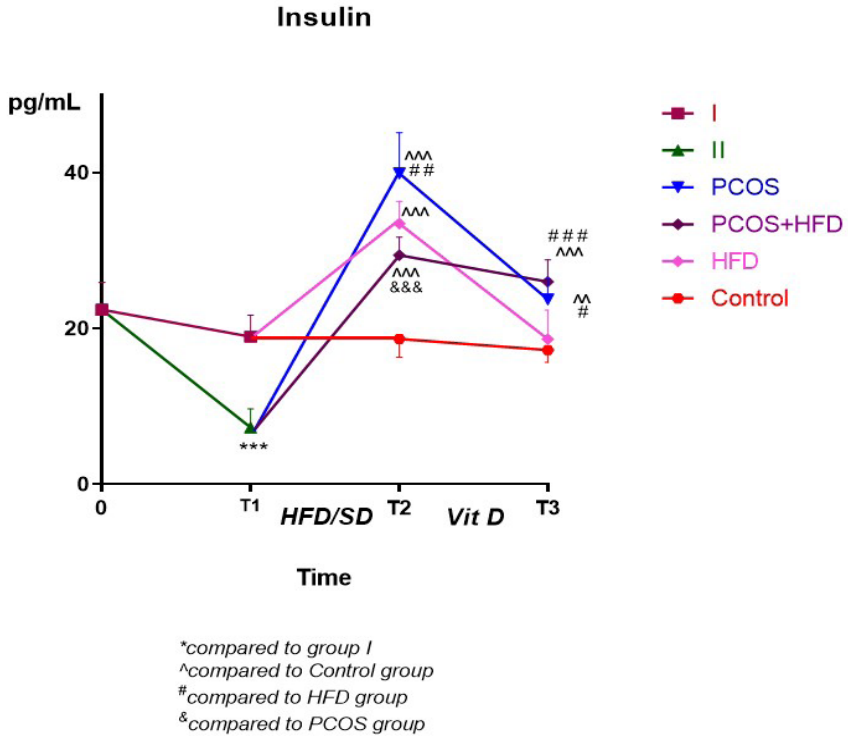


Figure 2. Basal insulin levels throughout the experiment: T1: at 4 weeks after EV/sesame oil administration; T2: after 17 weeks of HFD/SD; T3: after 5 weeks of vitamin D3 administration.

The results were statistically analyzed by two-way ANOVA multiple comparisons tests, using GraphPad Prism version 9.0.0 software (GraphPad, San Diego, CA, USA).

The parameters were expressed as means ± SD. (***$p<0.001$ compared to group I, ^^^$p<0.001$ compared to Control group, ^^$p<0.01$ compared to Control group, ###$p<0.001$ compared to HFD group, ##$p<0.01$ compared to HFD group, #$p<0.05$ compared to HFD group, &&&$p<0.001$ compared to PCOS group).

After 17 weeks of HFD or SD, at T2 moment, insulin levels were significantly increased ($p<0.001$) in HFD, PCOS and PCOS+HFD groups, compared to Control group. In comparison with HFD group, basal insulin was significantly increased in PCOS group ($p<0.01$). Significant alterations were recorded also between the groups with PCOS ($p<0.001$), HFD lowering the basal insulin levels.

At the end of the experiment, after the 5 weeks with oral administration of vitamin D3, blood samples taken at T3 moment showed significant increases of insulin levels in PCOS+HFD ($p<0.001$) and PCOS ($p<0.01$) groups, compared to Control group. In comparison with HFD group, insulin levels were significantly amplified in PCOS ($p<0.05$) and PCOS+HFD ($p<0.001$) groups (Figure 2).

The statistical comparisons realized per each group between T2 and T3 moments showed significant decreases of insulin secretion ($p<0.001$) in HFD and in PCOS groups (not mentioned in figure 2).

The levels of ***triglycerides*** were modified significantly during the experiment.

At moment T1, triglycerides level was significantly increased ($p<0.05$) in group II (with EV administration), compared to group I.

At T2 moment (after 17 weeks of standard diet or HFD), triglycerides levels were significantly enhanced ($p<0.001$) in female rats with PCOS but also in rats with only HFD, compared to Control group. Significant decreases of triglycerides were recorded in PCOS group, in comparison with HFD group ($p<0.001$). Between the groups of rats with PCOS, significant increases were seen in PCOS+HFD group, compared to PCOS group ($p<0.01$).

At T3 moment (after 5 weeks with oral administration of vitamin D3) triglycerides were significantly increased in HFD, PCOS and PCOS+HFD groups, compared to Control group ($p<0.001$).

Between the T2 and T3 moments, non-significant variations of triglycerides levels were recorded per each group (Figure 3).

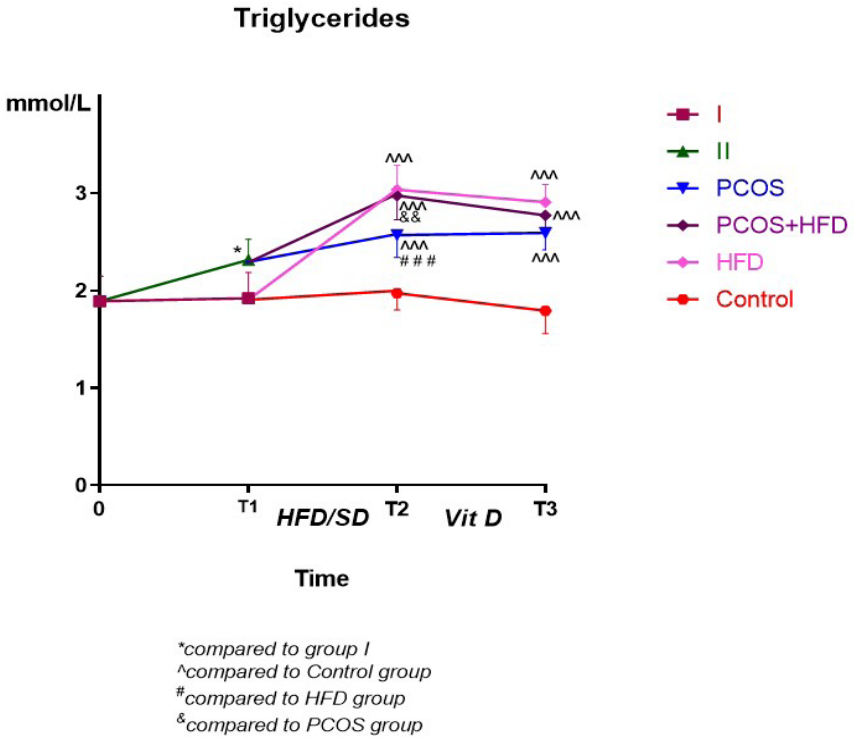


Figure 3. Triglycerides levels throughout the experiment: T1: at 4 weeks after EV/sesame oil administration; T2: after 17 weeks of HFD/SD; T3: after 5 weeks of vitamin D3 administration.
The results were statistically analyzed by two-way ANOVA multiple comparisons tests, using GraphPad Prism version 9.0.0 software (GraphPad, San Diego, CA, USA).
The parameters were expressed as means ± SD. (*$p<0.05$ compared to group I, ^^^$p<0.001$ compared to Control group, ###$p<0.001$ compared to HFD group, &&$p<0.01$ compared to PCOS group).

Total cholesterol evaluation showed the following significant modifications throughout the experiment.

At T1 moment, total cholesterol diminished significantly in rats with PCOS (group II) ($p<0.01$) compared to the rats treated with sesame oil (group I).

At T2 moment of the experiment (after 17 weeks of HFD or SD), significant increases of total cholesterol levels were recorded only in PCOS+HFD group when compared to Control group ($p<0.01$) or in comparison with PCOS group ($p<0.001$).

No significant modifications of cholesterol levels were recorded in blood samples taken at T3 moment (after vitamin D3 treatment) (Figure 4).

The statistical evaluation per each group between T2 and T3 moments presented significant decreases of cholesterol levels ($p<0.05$) in PCOS+HFD group (not mentioned in Figure 4).

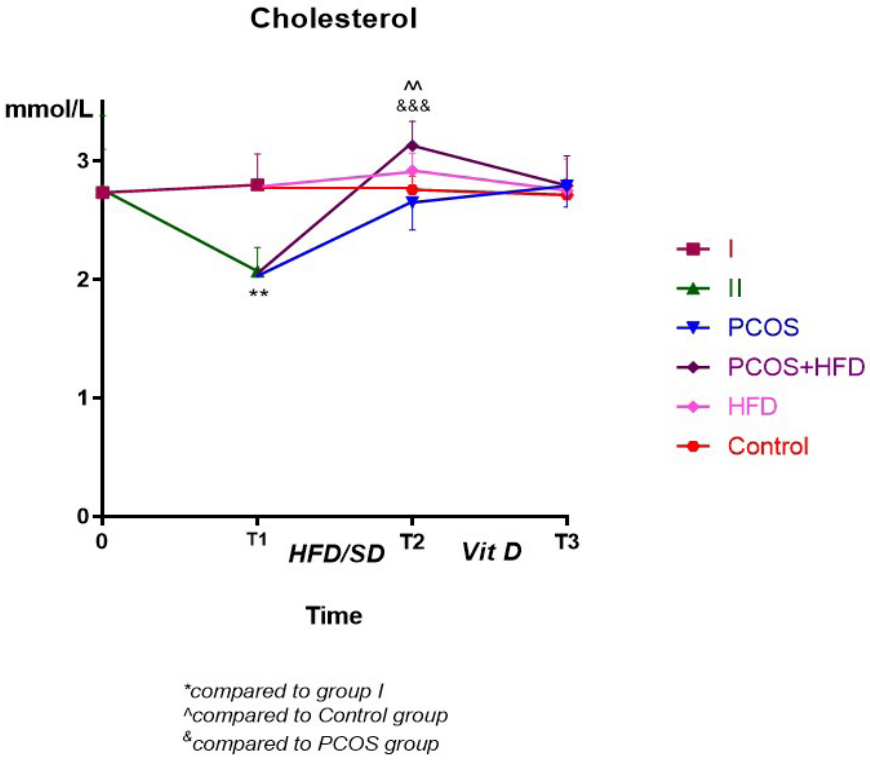


Figure 4. Cholesterol levels throughout the experiment: T1: at 4 weeks after EV/sesame oil administration; T2: after 17 weeks of HFD/SD; T3: after 5 weeks of vitamin D3 administration.
The results were statistically analyzed by two-way ANOVA multiple comparisons tests, using GraphPad Prism version 9.0.0 software (GraphPad, San Diego, CA, USA).
The parameters were expressed as means ± SD. (**$p<0.01$ compared to group I, ^^$p<0.01$ compared to Control group, $^{\&\&\&}p<0.001$ compared to PCOS group).

Ultrasound examination

At four weeks after the EV administration, in animals of group I, normal ultrasound morphologies of ovaries were recorded, with mean dimensions of 2.38±0.56/1.15±0.25 mm and no cystic modifications (Figure 5).

The rats in EV-induced PCOS group (II) had ovaries with mean diameters of 3.85±1.13/1.58±0.55 mm and cystic modifications varying between 0.19-1.87 mm (Figure 6a, 6b).

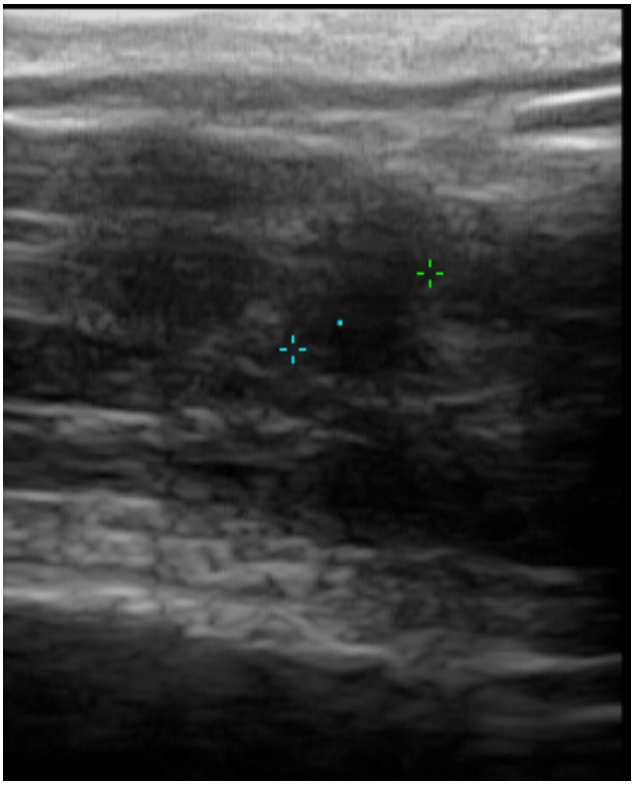
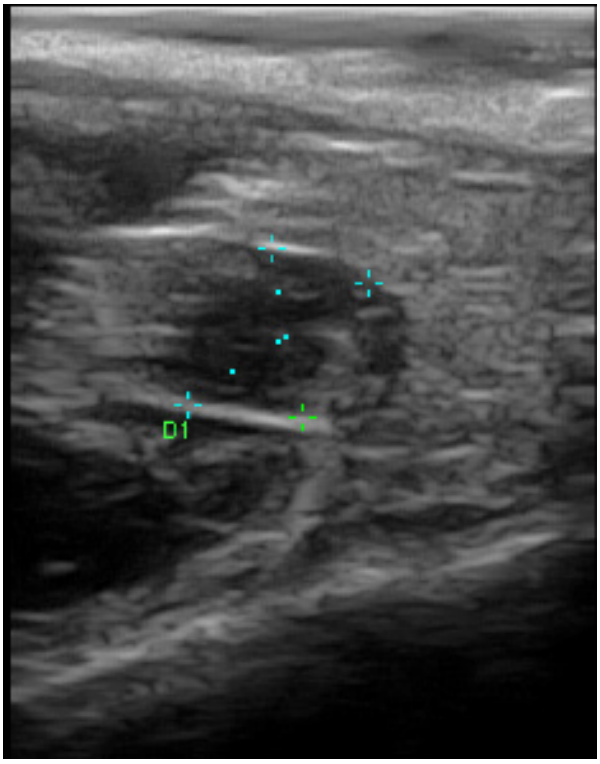
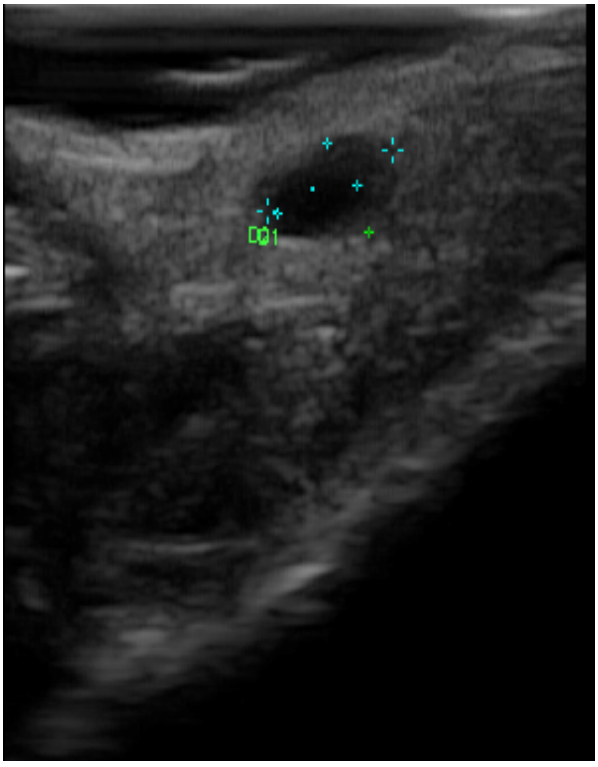


Figure 5. Ultrasound examination of ovaries in animals of group I: normal morphological aspect, no cysts identified.



(a)



(b)

Figure 6. Ultrasound examination of ovaries in animals of group II: increased dimensions of ovaries and cysts were identified.

Western Blot analysis

In the ovarian tissue, after 5 weeks of vitamin D3 administration, the western blot analysis showed, compared to the Control group, significant decreases of pNFkB expression in PCOS+HFD group ($p<0.01$), significant increases of COX2 protein in HFD group ($p<0.05$) and of SOD1 expression in PCOS group ($p<0.05$) (Figure 7).

Lipid peroxidation analysis in ovary and periovarian adipose tissue

In ovarian tissue, after 5 weeks of vitamin D3 administration, malondialdehyde (MDA) level was significantly increased in rats with PCOS, with or without HFD, compared to Control group ($p<0.001$). In comparison with HFD group, MDA was significantly enhanced in PCOS ($p<0.001$) and in PCOS+HFD ($p<0.01$) groups.

In periovarian adipose tissue, at the end of the experiment, the lipid peroxidation was significantly elevated only in PCOS+HFD group ($p<0.05$), compared to Control group (Figure 8).

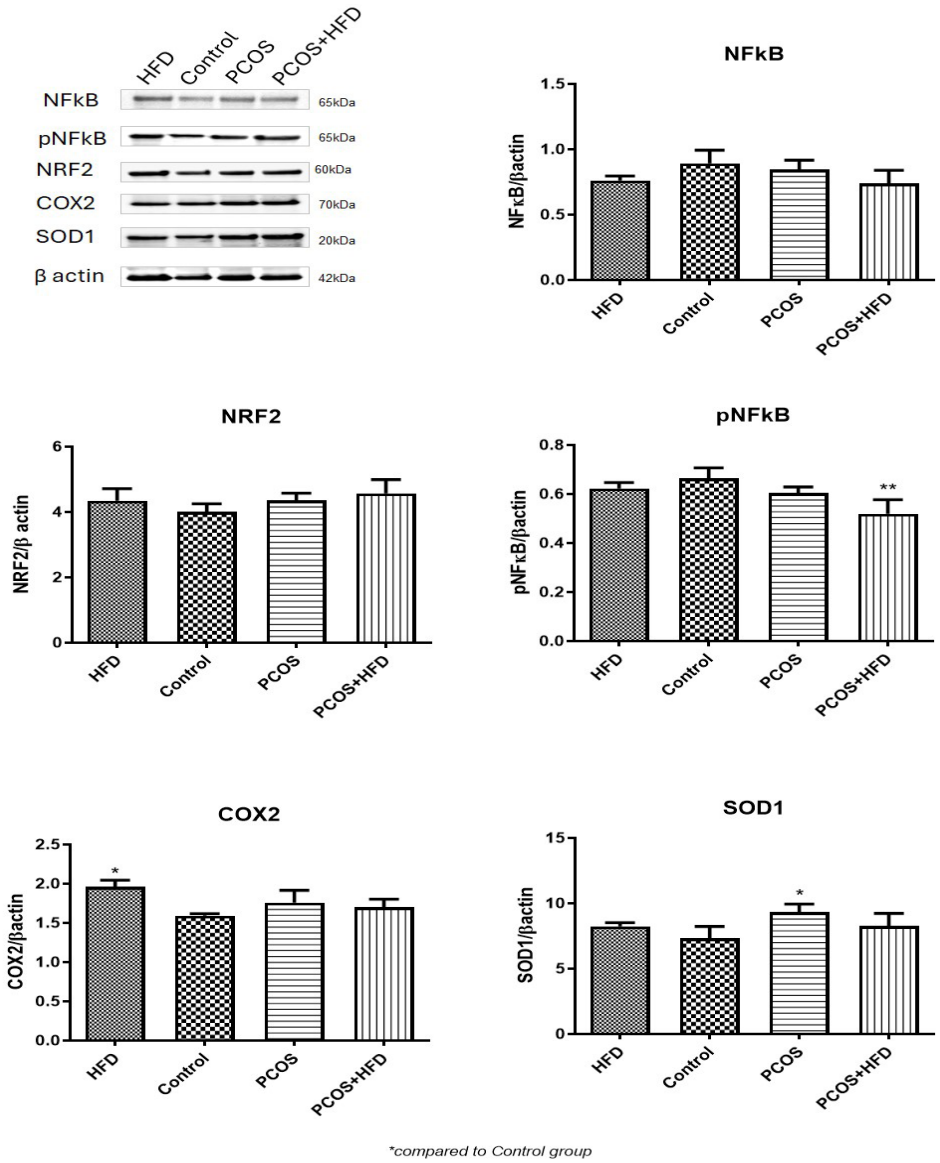


Figure 7. Western Blot analysis from ovarian tissue. The results were analyzed statistically with GraphPad Prism version 9.0.0 for Windows, GraphPad Software Dotmatics (Boston, MA, USA), one-way ANOVA followed by the Tukey post-test. The parameters were expressed as means ± SD. (**$p<0.01$ compared to Control group, *$p<0.05$ compared to Control group).

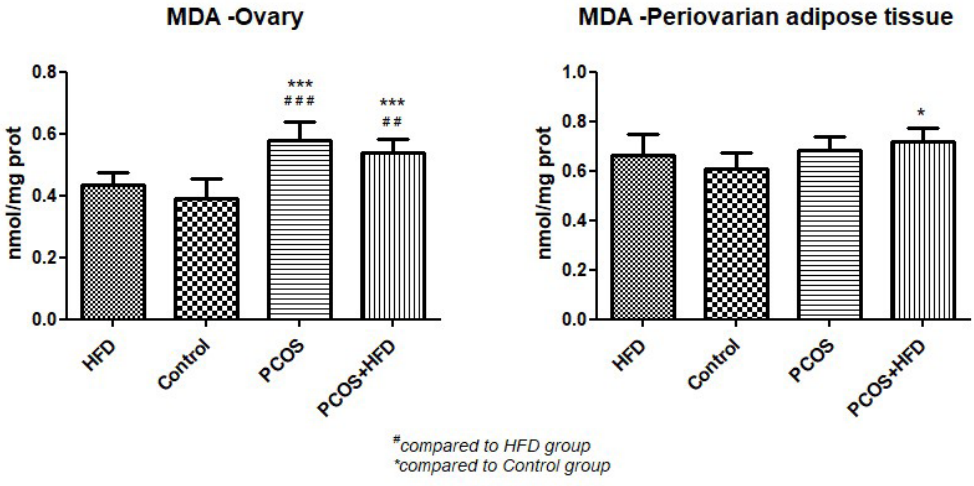


Figure 8. Lipid peroxidation in ovarian and in periovarian adipose tissue. The results were analyzed statistically with GraphPad Prism version 9.0.0 for Windows, GraphPad Software Dotmatics (Boston, MA, USA), one-way ANOVA followed by the Tukey post-test. The parameters were expressed as means ± SD. (***p<0.001 compared to Control group, *p<0.05 compared to Control group, $^{\#\#\#}p$<0.001 compared to HFD group, $^{\#\#}p$<0.01 compared to HFD group).

Histopathological analysis

In the Control group, figure 9a left image, the low-magnification image showed an overview of ovarian tissue. The structure included follicles at various stages of development, some possible atretic (degenerating). There were stromal tissues visible as well. In figure 9a right image, the higher magnification of ovarian tissue showed dense stromal cells with some slight infiltration of immune cells without pathological features.

In PCOS+HFD group, similarly to Control group, the low magnification (Figure 9b left image) showed ovarian tissue, likely focusing on the formation and development of cystic formations. The arrangement of follicles and stromal tissue suggested that ovaries became polycystic, and the inflammation features were also visible as mononuclear cell infiltration in ovarian stromal tissue. Thus, the higher magnification image (Figure 9b right image) presented a detailed view of the ovarian cortex with follicles surrounded by a dense cellular infiltrate, which could suggest inflammation.

Animals with high fat diet (HFD group) developed less organized follicles and there may be signs of degeneration (Figure 9c, left image). The high-magnification image (Figure 9c right image) revealed significant cellular infiltration within the stromal tissue, possibly indicating a more intense inflammatory response but the polycystic appearance of the ovary was not notable, the appearance being comparable to the control. However, ovary degeneration was found with degenerating pigment depositions (red-green pigment like lipofuscin) in perifollicular areas and stromal tissue.

In PCOS group, the ovarian tissue presented a normal aspect at low magnification (Figure 9d left image) while the higher magnification image (Figure 9d right image) showed a dense cellular infiltrate, likely composed of inflammatory cells within the stroma, which could indicate chronic inflammation or an immune response.

The ovarian sections in the set showed a combination of normal follicular structures and areas with significant inflammatory cell infiltration, particularly in the higher-magnification images. The most prominent changes were noted in the PCOS+HFD (cystic formations, inflammation) followed by PCOS and HFD groups, which were associated with cystic formations and low inflammation (PCOS) or active inflammatory processes without notable cystic formations (HFD).

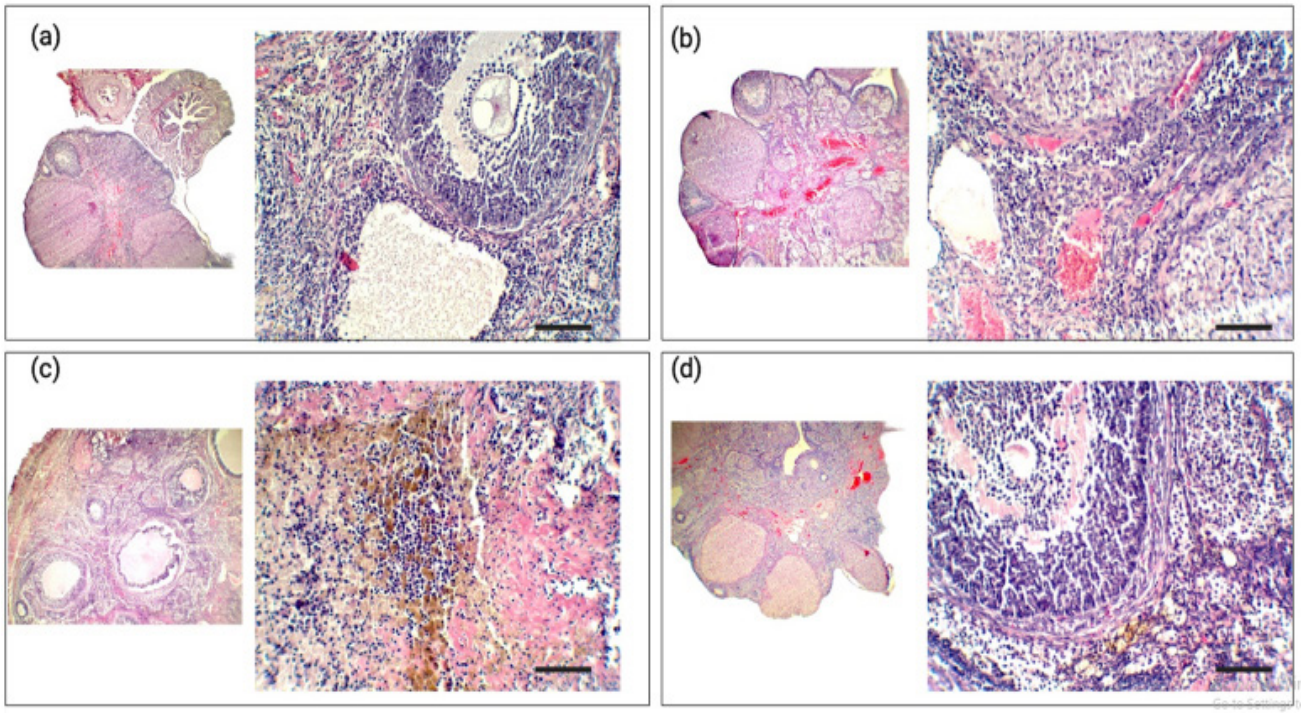


Figure 9. Histological examination of ovaries stained with hematoxylin and eosin (H&E): Control group (a), PCOS+HFD group (b), HFD group (c), PCOS group (d).
(Scale bar = 20 μm; Col. H&E).

Discussion

PCOS is a heterogeneous endocrine disorder defined by hyperandrogenism, ovulatory dysfunction and polycystic ovarian morphology, associated with metabolic and endocrine disorders. In our study, the results showed high levels of lipid peroxides in ovarian and periovarian tissues in PCOS group, especially after HFD administration, even in animals treated with vitamin D3. In parallel, HFD administration in rats with PCOS increased the inflammation in ovaries, but the activation of NFkB was decreased, probably the consequence of vitamin D3 treatment. In animals with PCOS, the treatment with vitamin D3 increased SOD1 expression and attenuated insulin and total cholesterol levels in blood. The same effect on cholesterol levels had vitamin D3 in PCOS+HFD group or in HFD group. Additionally, rats fed with HFD associated inflammation and high expression of COX2 in the ovarian tissue.

The first part of the experiment accomplished the development of PCOS in group II after the administration of estradiol valerate (EV), and the blood investigation showed significant decreased values of insulin, compared to group I (sesame oil injection). This finding is similar to those noticed by Millán and Castañeda in a study performed in 20 women with EV administration for 3 months, treatment that decreased insulin levels with 54.5% [20]. The cholesterol levels in group II were decreased, condition that was also observed by Zhang et al. in a study realized in postmenopausal females with estrogen supplementation [21]. In our study, EV administration for PCOS induction associated only high triglycerides levels with low values of cholesterol or insulin, situation in agreement with values found by Marbaniang et al. in women with PCOS [22]. The same trend of changes in metabolic parameters was also mentioned by Bittner in a clinical study in healthy postmenopausal women [23]. In our study, one dose of EV administration induced changes of triglyceride levels probably due to early assessment of metabolic changes.

It is known that in animals with PCOS, lipid diet can alter additionally the metabolic markers. Thus, the animals with PCOS+HFD or HFD had high levels of triglycerides and cholesterol suggesting that HFD potentiated the metabolic disorders noticed in PCOS. Hypersecretion of insulin observed in PCOS+HFD or PCOS groups can explain the resistance to insulin noted in this disease and is confirmed by the results obtained by Blázquez and Quidaja in Wistar male rats that received HFD [24] or Barnard et al [25]. Moreover, the triglycerides and cholesterol increased in PCOS+HFD compared to PCOS with SD, results that are concordant with literature data [3] and are explained by the high lipids intake for 17 weeks.

Vitamin D3 administration, for 5 weeks, reduced insulin levels in PCOS but the values were maintained

increased compared to those of control or HFD groups, suggesting the beneficial role of vitamin D3 supplementation in the mechanisms involved in the insulin receptor expression, as Mohan et al. found [16]. Vitamin D3 maintained the cholesterol levels close to those of the control group, but did not have the same effects on triglycerides. However, the relationship between vitamin D and metabolic parameters are contradictory and sometimes difficult to be explained. In a cross-sectional analysis on 15600 patients, Gholamzad et al. exhibited that between vitamin D and total cholesterol or between vitamin D and triglycerides no significant relationships were noticed [26] while other researchers assigned protective roles to vitamin D on metabolic parameters [16,17].

In the PCOS group, SOD1 expression in ovarian tissue increased adaptive as result of free radicals' production and of generation of redox imbalance. Talat et al. confirmed in a meta-analysis performed on patients with PCOS an increase of SOD expression [27] due to inducible effect of vitamin D3 on SOD1 synthesis. Moreover, Hajiluian et al. found similar findings in male Wistar rats fed with HFD and treated with vitamin D [28]. Although in histological examinations, PCOS group associated inflammation, in western blot analysis, vitamin D exerted a protective role on NRF2 and NFkB activations, suggesting its antioxidant effect. Lipid peroxidation was also noticed in ovaries in PCOS group, results that are in concordance with literature data that showed the increase of serum MDA in PCOS [1].

In the PCOS+HFD group, vitamin D3 slightly improved insulin level and cholesterol concentration in serum, with non-significant decreases between T2 and T3 moments, probably due to the low dose of vitamin D3 or short treatment time. Several researchers recommend for treatment, higher doses or a combination of vitamin D with omega-3-fatty acids, as Jamilian et al. mentioned in their study [29]. Cholesterol was significantly reduced ($p<0.05$) by vitamin D3 in PCOS+HFD group, effect observed also by Gao et al. in women with PCOS [30]. A reduced pNFkB expression after vitamin D3 administration in rats with PCOS+HFD was common finding found in this treatment [31,32]. The same degree of inflammation with mononuclear cells infiltrates and a high number of cysts were also noticed by Rakic et al. in ovaries of Wistar rats with PCOS and HFD in parallel with increased lipid peroxidation in serum [33].

In the HFD group, vitamin D3 decreased significantly ($p<0.001$) the insulin levels, effect reported by Belenchia et al. in obese adolescents [34] without influencing the cholesterol and triglycerides levels in serum, in agreement with other results from literature [26,35]. Generally, COX2 expression increased in ovaries of animals fed with HFD suggesting its implication in obesity-induced inflammation [36] but in our study the inflammation seen in ovaries was probably not related to COX2 activation [37]. An argument in this regard is the lack of oxidative stress changes associated with HFD in presence of vitamin D3, signifying the beneficial role of vitamin D3 in HFD [38,39].

Vitamin D3 administration in patients with PCOS may improve the lipid metabolism, may have beneficial effects on insulin resistance, but also on the oxidative stress and inflammation in ovaries and in periovarian tissue, even if the women are on a hyperlipid diet.

This nutrition type could increase the risk of atherosclerosis, diabetes mellitus, heart diseases and of other physiological mechanisms impairments, pathological conditions that may be mitigated by oral administration of vitamin D3.

Conclusions

Vitamin D3 administration decreased significantly the insulin levels in rats with HFD and in those with PCOS. Efficacy of this treatment was also seen on the cholesterol level but only in the animals with both conditions (PCOS+HFD), while on the triglyceride's levels, in all experimental groups, slight non-significant decreases were recorded.

PCOS and HFD are associated with oxidative stress and inflammation in ovaries, pathological processes that were partially mitigated by vitamin D3 administration, especially on NFkB activation.

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