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The impact of high-intensity interval training on insulin resistance, oxidative stress, and muscle function in a PCOS rat model

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ABSTRACT

Polycystic ovary syndrome (PCOS) is one of the most common endocrine and metabolic disorders . This study aimed to investigate the effects of high-intensity interval training (HIIT) on insulin resistance, oxidative stress, soleus muscle function, and myokine levels in a PCOS rat model. Female rats were assigned to four groups: Control, PCOS, PCOS+Exercise, and Exercise (n=15 each). PCOS was induced by subcutaneous administration of dehydroepiandrosterone (DHEA) for 3 weeks, and exercise groups underwent HIIT for 12 weeks. Insulin resistance (HOMA-IR), serum oxidative stress markers, hormone levels (FSH, LH), soleus myokine expression, and muscle function were analyzed. Results showed that the PCOS group exhibited increased blood pressure and insulin resistance compared to controls, with a significant reduction in FSH and LH levels in the PCOS+Exercise group. Exercise improved insulin sensitivity and reduced insulin resistance in the PCOS+Exercise group. Serum oxidative stress markers did not differ significantly between groups. Soleus muscle IL-6 levels were significantly reduced in the PCOS+Exercise group. Histological analysis revealed a larger cross-sectional area of the soleus muscle in the PCOS+Exercise group compared to the PCOS group, suggesting improved muscle morphology. Furthermore, exercise improved the functional capacity of soleus muscles, as evidenced by weightlifting performance. These findings indicate that HIIT has beneficial effects on insulin resistance, reproductive hormone levels in PCOS. Exercise also shows potential in slowing oocyte loss and improving follicle health, highlighting its role as a therapeutic intervention for reproductive health in PCOS. This study suggests that HIIT could be a beneficial approach for managing PCOS, and further research is needed to better understand its underlying mechanisms and potential long-term benefits.

1. Introduction

Polycystic ovary syndrome (PCOS) is a metabolic and endocrine condition that affects women throughout their lives [1]. In PCOS, not only many organs but also skeletal muscle is affected [2]. Insulin resistance and hyperinsulinemia are observed in 65–95 % of women with PCOS, regardless of whether they are overweight or have a normal weight [3]. Therefore, insulin resistance plays a crucial role in the pathophysiology of PCOS. Physical activity can increase skeletal muscle insulin sensitivity [4], and there is ongoing research on the relationship between skeletal muscle dysfunction associated with insulin resistance in PCOS and the potential benefits of high-intensity interval training (HIIT) in improving insulin sensitivity in PCOS patients [5].

About two-thirds of postprandial peripheral glucose uptake occurs in skeletal muscle. Various mechanisms underlie insulin resistance in skeletal muscle, including decreased glucose uptake in muscle tissue and target tissues [6]. Women with PCOS often experience postprandial dysglycemia, which reflects skeletal muscle insulin resistance [3]. Furthermore, fat accumulation in skeletal muscle [7] increased inflammatory cytokines [8], or disruptions in the insulin signaling pathway may [9] also contribute to the development of skeletal muscle insulin resistance in PCOS.

Exercise is a low-cost and accessible treatment option for people. Health professionals have reached a consensus on exercise that is

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beneficial [10]. HIIT, is characterized by short, repeated periods of intense submaximal and supramaximal exercise with rest or active recovery intervals [11]. HIIT protocols have been applied on different disorders [12]. Studies have shown that ten weeks of HIIT increased antioxidant parameters and improved insulin sensitivity in patients with type 2 diabetes [13]. In PCOS patients, HIIT has led to cardiovascular and hormonal improvements, including reduced insulin resistance, low-density lipoprotein (LDL) cholesterol levels, atherogenic index, and testosterone levels [14]. Colombo et al. displayed that menstrual regularity was improved by HIIT compared to moderate-intensity continuous training (MICT) in their meta-analysis [15]. Moreover, in a 12-week study by Aktaş et al., HIIT increased serum adiponectin levels more than MICT [16]. Anxiety, depression and stress scores were lower in the HIIT group compared to the MICT group in a 12-week mental health study by Patten et al. [17]. However, studies specifically investigating the effects of HIIT on skeletal muscle in PCOS patients are limited. Some researchers suggest that improving insulin sensitivity in slow-oxidative muscle fibers may have a more significant impact on metabolic health [18].

Recent research has focused on the role of skeletal muscles in the preventive and therapeutic effects of exercise on metabolic diseases. Skeletal muscle, as an active organ, releases cytokine-like molecules called myokines [19]. Myokines have diverse effects on muscle development, angiogenesis, and metabolism, and they can also influence other organs and systems [20]. For example, IL-6 is considered a myokine/cytokine and can indicate chronic inflammation, which generally has negative effects on glucose homeostasis [19]. Meteorin like peptid (METRNL) is a protein-structured myokine that plays a role in physiological and pathological processes, and its levels in serum and muscle have shown conflicting results in previous studies [21,22]. Serum Metrnl levels were elevated in patients with T2DM. Consequently, increased serum Metrnl concentrations may contribute to a heightened risk of T2DM by promoting insulin resistance, a finding consistent with the results reported by Wang et al. [21]. In contrast, the study by Dadmanesh et al. [22] found significantly lower serum Metrnl levels in patients with T2DM compared to the control group. Fibroblast Growth Factor 21 (FGF21) is another myokine that is secreted by the liver, pancreas, and adipose tissue. There are limited studies examining serum FGF21 levels in PCOS. While serum FGF21 levels were high in the study by Bednarska et al. [23], serum FGF21 levels were similar to controls in the study by Sahin et al. [24].

Angiopoietin-like 4 (ANGPTL4) is a myokine that affects insulin sensitivity, lipid metabolism, and adipogenesis pathways. The impact of ANGPTL4 overexpression on glucose metabolism remains controversial [25,26].While some studies have shown increased serum ANGPTL4 levels in both obese and non-obese PCOS patients [27], others have found no difference in serum ANGPTL4 levels between PCOS patients with a normal body mass index and healthy subjects [28]. Surprisingly, despite the previously mentioned studies exploring the roles of METRNL, FGF21, and ANGPTL4 in other contexts, there are no studies investigating their roles as myokines in PCOS.

In this study, we aimed to investigate the effects of 12 weeks of HIIT exercise on endocrine and metabolic changes and impaired skeletal muscle mechanisms in a PCOS rat model. Additionally, we planned to observe changes in muscle function and myokines in PCOS. We hypothesize that HIIT will positively impact PCOS metabolic parameters and soleus muscle parameters.

2. Methods

2.1. Animals

Sixty female Wistar albino rats (Rattus norvegicus) aged 21 days and weighing 40–50 g were used in this study. The rats were obtained from Ankara University, Medical Faculty Experimental Animal Breeding and Supply Laboratory. The study was approved by the Ankara University Experimental Animals Ethics Committee (approval number: 2020–12–106). Before commencing the experiments, they were brought to the Physiology Department of the Faculty of Medicine, where the exercise program would be applied, and they were given time to adapt. The rats were housed in a standard animal chamber with a 12-hour light - 12-hour dark cycle (lights on at 08:00AM at a controlled temperature of 23.5 ± 0,5 °C. They were provided with polycarbonate cages and had ad libitum access to food.

All treatments, grouping of animals, and behavioral tests were performed in a blinded manner. The rats were randomly divided into four groups: control group (control, $n = 15$), the exercise group (EX, $n = 15$), the PCOS group (PCOS, $n = 15$), and the PCOS + exercise group (PCOSEX, $n = 15$).

2.2. PCOS model

In the planned study, to induce PCOS, 21 days old female Wistar albino rats were administered a subcutaneous injection of 6 mg/100 g body weight of DHEA dissolved in 0.2 mL sesame oil for 3 weeks. The same amount of sesame oil was given subcutaneously to the control and exercise groups as a control [29]. To diagnose PCOS, a microscopic evaluation of the cell types found in the animals' vaginal smears was performed. Vaginal smears were performed daily to confirm the estrous stages of the rats, ensuring accurate phase identification before the experimental procedures. Starting from the 7th day of model preparation, vaginal swabs were taken for 2 weeks and evaluated every morning between 9.00 and 10.00 based on cell type and number. If an estrous phase lasts for 4–5 days, the rat is diagnosed with PCOS. In our study, we observed rats in a constant diestrous phase, as all animals were monitored for regular estrous cycles prior to the experiments.Female rats showing irregular cycles were excluded from the study to maintain consistency after applying DHEA.

2.3. Female rat incremental load exercise program

The animals in the exercise groups were included in the adaptation program for two weeks on the treadmill, following the 21-day DHEA application. Then, exercise groups were trained for 12 weeks according to the individual exercise protocol determined. The age at which exercise is initiated corresponds to the adolescent period (42–44 days) in rats. The animals in the sedentary groups were kept in their cages during this period. The training protocol was carried out between 09:00–15:00 on the small experimental animal treadmill, which was manufactured by the Department of Electrical and Electronic engineering of Ankara University, allowing speed, incline, and electricity control.

Our incremental exercise protocol was based on Wisloff's test model on maximal oxygen uptake in rats [30]. Protocols were performed with maintaining a slope of 25° Before starting the exercise program, incremental load testing was performed to determine the maximum exercise capacity (MEC) of the rats. It starts with 5 m/min and 0° incline for each rat, the speed is increased by 3 m/min, and the slope is increased by 2° every three minutes. The workload that the rat could no longer continue even if mechanically or electrically stimulated was considered that animal's MEC. Rat-specific maximum exercise capacities were determined by applying the incremental load test at the 1st, 2nd, 5th, 8th, and 11th weeks to determine the training protocol to be applied.

In the training program, HIIT exercises were started with a 6-minute warm-up at a 25° incline, at a speed corresponding to 50 % of the MEC, followed by a run for 4 min at a speed of 85–90 % on an incline of 25°, then continued at a speed of 50 % at 25° for 2 min. The defined cycle was repeated 5 times, and the last active rest period was completed for 10 min. The total exercise time was determined as 38 min. The duration of the exercise protocol was modified according to other studies in the literature [31,32].

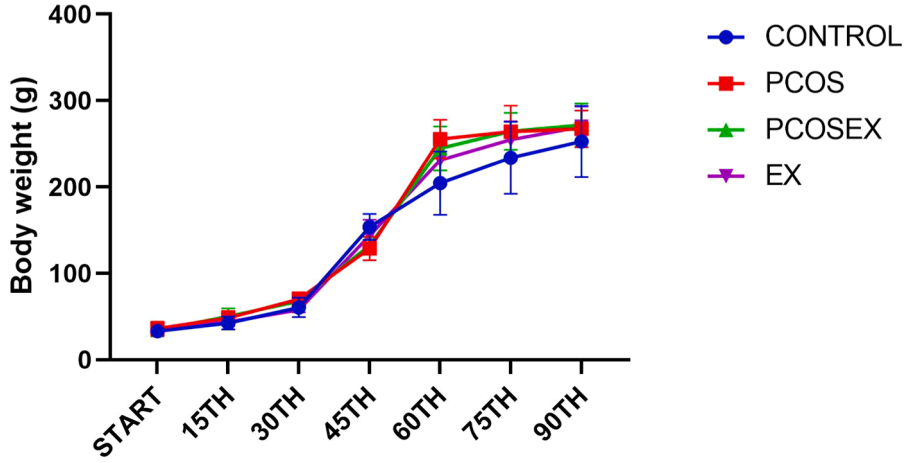

Fig. 1. Body weights of DHEA-induced PCOS rats and other groups. The rats' weights were monitored 15 days interval from beginning of all experiments. Data are presented as mean± standart derivation.

2.4. Muscle function in vivo tests

Neuroscore tests, Kondziela inverted screen tests, and weight tests were performed before, one month after, and at the end of the exercise protocol. The walking track analysis test was evaluated only at the end of the training program to define the tibial nerve function index [33]. Neuroscore tests consist of the forelimb flexion test, hindlimb flexion test, a hind paw grasping reflex test, visually triggered placing test, and contact triggered placing test sections.

The forelimb flexion test was used to evaluate the presence/absence of flexion in the forelimb. The hindlimb flexion test was applied to evaluate the presence/absence of flexion in the hindlimb. The visually triggered placing test was used to evaluate whether the front paws of the rat were extended, while the hind paw grasping reflex test was used to evaluate the grip of the hind paws. The contact-triggered placing test measures the tactile response of the rat's whiskers.

The Kondziela Invert Screen test is a muscle strength test in which rats use their four limbs [34]. The rat is stopped upside down on the wire 40–50 cm above the ground surface on four limbs, and time is kept with a stopwatch. The attachment times of the animals are recorded in seconds.

In the weight test, forefoot muscle strength is measured [34]. When the rat grasped a wire ball with its front paws, the stopwatch was started, and the rat was lifted. The total weight-lifting times of each rat were recorded. The nerve that innervates the soleus muscle is the tibial nerve. The Tibial nerve function index was calculated by walking track analysis, which evaluates the temporal and spatial relationship of one footprint to another during walking. It is evaluated with the data obtained from the numeric values placed in the formula

2.5. Measurement of hemodynamic parameters of rats

As a result of the 12-week exercises of the experimental animals, blood pressure measurement in all groups was carried out by the tail-cuff plethysmography method (MAY NIBP250, Ankara, Turkey). To reduce sensitivity in the rats' tails and help them become accustomed to the experimental environment, our rats underwent daily handling and training for a week for blood pressure measurements. The rats were exposed to heat for 32 °C in cabin related with heater. Once the tail reached a temperature of 32 °C, the cuff was inflated 5 times at 1-minute intervals to help the rat adjust to the cuff pressure. Once the tail temperature reached 32 °C, a pressure of 250 mm Hg was applied to the tail by inflating the cuff, after which the systolic blood pressure was measured. Five measurements were taken from each animal at one-minute intervals. The average of the remaining three values was calculated by subtracting the highest and lowest values. Systolic, diastolic, mean arterial pressure and heart rate parameters were calculated for each rat. Mean Arterial Pressure was obtained using the formula: Mean Arterial Pressure = Diastolic Pressure + (Systolic Pressure - Diastolic Pressure)/3.

2.6. Collection of tissue and blood samples

In vivo experiments were completed 24 h after the rats' last running training. Then, a thoracotomy was performed under deep anesthesia of 50 mg/kg thiopental, the aorta was cut, and samples were taken from the blood filling the thorax. We removed 8–10 ml of blood and put it in a tube with a gold lid to separate serum. Blood samples were centrifuged at 5000 g and +4 °C for 10 min. The pellet was discarded, while the supernatant was preserved. For further examination, the collected serum was aliquoted and kept at −80 °C. The fasting glucose and insulin levels were also measured. HOMA-IR was calculated from the fasting glucose and insulin levels [35].

The muscle and ovary samples used in our study were stored in fixation solutions for histological examination, and the left soleus muscles were stored in a deep freezer at −80 °C for molecular examinations. For in vitro functional studies of the muscles, they were placed in the organ bath system.

2.7. Myokine analysis

Bilateral removals of soleus muscles were performed for functional, histological, and biochemical analysis. Each soleus muscle was weighed, and a digital caliper was used to measure the muscles' length and radius for organ bath system data. We used the right soleus muscle for isolated skeletal muscle function tests. The left pairs of soleus muscles were separated into two pieces. One part was used for histological analysis, while the other part was used for myokine analysis.

Specific enzyme-linked immunosorbent assay (ELISA) kits (Elabscience, Houston, Texas, USA) were utilized for quantifying Interleukin-6 (IL-6), Fibroblast growth factor 21 (FGF21), Angiopoietin like 4 (ANGPTL4), and Meteorin like peptid (METRNL) of soleus muscles following the manufacturer's instructions.

2.8. Oxidative stress parameters analysis

ELISA kits (Rel Assay Diagnostics, Gaziantep, Türkiye) were used to evaluate the levels of Total oxidant status (TOS), Total antioxidant status (TAS), Follicle stimulatig hormone (FSH), Luteinizing hormone (LH), fasting insulin, Native Thiol, Total Thiol, and disulfide in rat serum.

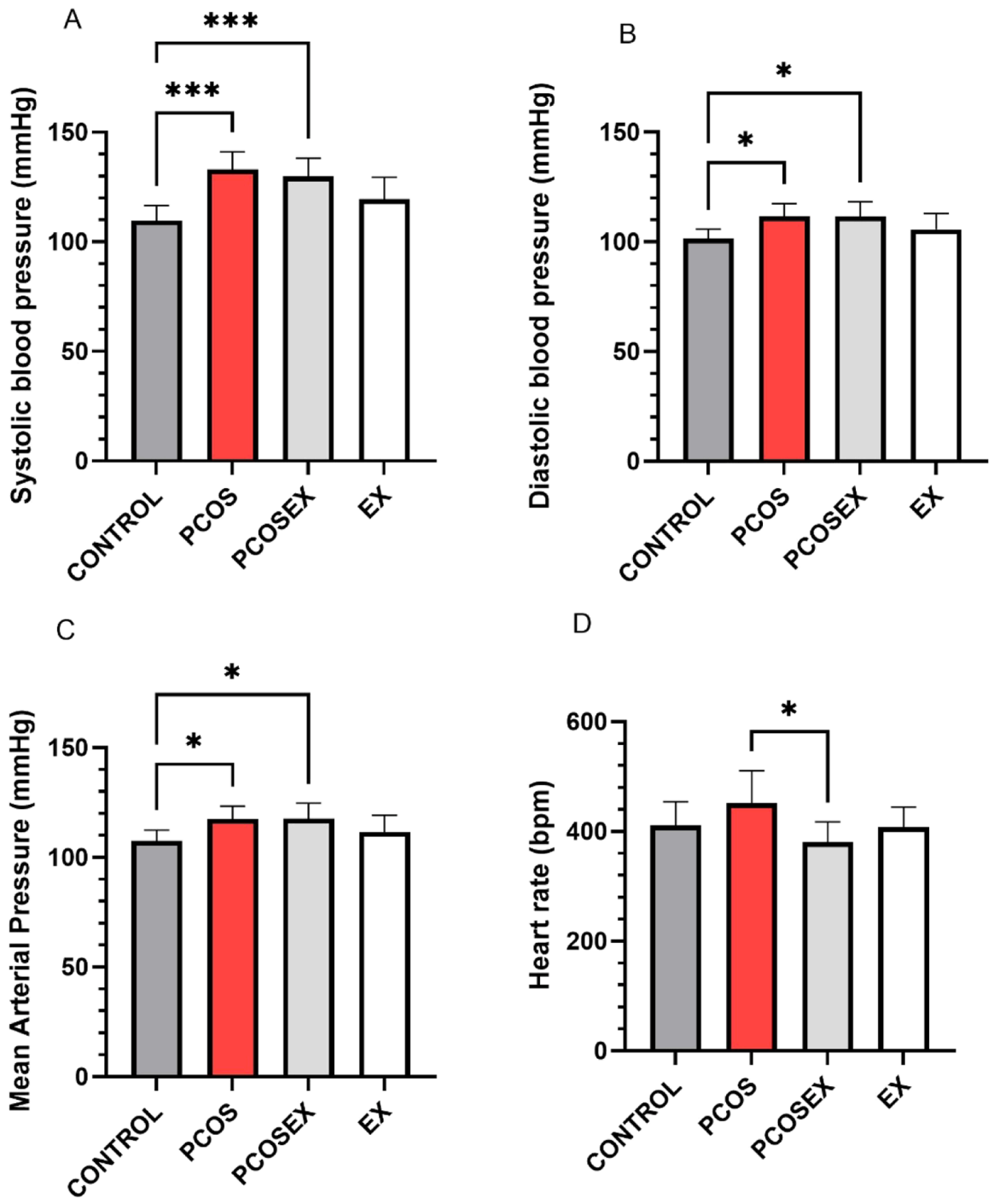

Fig. 2. A: Sistolic blood pressure of all groups B: Diastolic Blood pressure of all groups C: Mean arterial blood pressure of all groups D: Heart rate of all groups. Data are presented as mean± standart derivation. (*$p < 0.05$, *** $p < 0.001$).

2.9. Skeletal muscle function in vitro analysis

2.9.1. Soleus dissection

Cutting the Achilles tendon initiated the dissection of the soleus muscle, which is followed by the proximal lift of the gastrocnemius muscle. Under gastrocnemius, the red soleus muscle was seen. The proximal tendon enters close to the backside of the knee joint, while the distal tendon of soleus is a portion of the Achilles tendon. We cut the tendon at the knee joint after gently separating the distal soleus tendon from the Achilles tendon bundle and removed it.

2.10. Muscle force measurement

The setup for isometric force measurement had a chamber to hold the muscle bathed in physiological solution. The solution is normally bubbled with oxygen or a gas mixture of CO2/O2 (5/95 %) when using a Krebs-Henseleit solution, force transducer, bi-phasic stimulator, A/D converter, and computerized acquisition and analysis of data (Biopac Systems Inc., CA, USA). The muscles were stored in Krebs-Henseleit solution ((mM: 118 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 25 NaHCO3, 2.5 CaCl2, and 11 Glucose; pH: 7.2–7.4) during and after dissection. 4–0 silk suture was used to bind isolated muscles to stainless steel hooks. One tendon of the muscle is connected to the force

Table 1
Oxidative stress parameters between groups.

	CONTROL (Mean ±SD) (n = 15)	PCOS (Mean ±SD) (n = 15)	PCOSEX (Mean ±SD) (n = 15)	EX (Mean ±SD) (n = 15)
TOS (μmol/ml)	7,13 ± 2,54	8,62 ± 5,05	7,70 ± 4,82	6,42 ± 2,47
TAS (mmol/L)	1,94 ± 0,14	1,87 ± 0,16	1,87 ± 0,13	1,93 ± 0,75
OSI	0,36 ± 0,14	0,45 ± 0,23	0,41 ± 0,25	0,35 ± 0,17
Total Thiol (μmol/L)	6.461± 328,11	6.343± 326,82	6.264± 290,65	6.149± 939,68
Native Thiol (μmol/L)	193,66 ± 50,39	297,93 ± 401,64	391,9 3 ± 558,62	206,93 ± 63,76
Disulphide	3.133± 167,81	3.022± 234,71	2.922± 322,06	2.971 ± 473,36

transducer, while the other tendon is connected to an adjustable hook. The muscle is then placed in the chamber. The muscle is electrically stimulated via electrodes made of platinum that are placed along the long axis of the muscle.

Isolated skeletal muscle bath features a jacketed construction that enables the physiological solution's temperature to be adjusted by pumping pre-heated water into the jacket. We optimized the muscle force-length relationship for each muscle such that the muscle length (L0) was set to produce the highest force for a particular stimulus. Maximum tension responses of the muscle were obtained when the stimulus was supramaximal. The mean time to reach peak tension and half relaxation time was calculated by averaging ten consecutive isometric tension responses at maximum isometric tension strength. soleus force-frequency response was determined using the following parameters: 0.5 ms pulses at 5, 10, 15, 20, 30, 50, 75, 100, and 120 Hz. A rest period of one minute was given between each contraction to avoid fatigue. After the last tetanic contraction, the fatigue protocol was applied to the muscles that were kept in a ten-minute rest period. According to this protocol, soleus muscles were stimulated with 0.5 ms square wave pulses at 10 Hz at five-second intervals for fifteen minutes. This stimulation frequency produces a partially coupled tetanic contraction when it reaches ~50 % of the Pt in the test. This protocol was designed to induce significant voltage loss over a relatively long period to allow aerobic metabolism to kick in and, therefore, increase CO2 production [36,37].

The force-frequency response was calculated with the following formula: Force (N)/cm2 = [force (N) × skeletal muscle density (1.06 g/cm3) × muscle length (cm)] / muscle weight (g). Fatigue was calculated in grams.

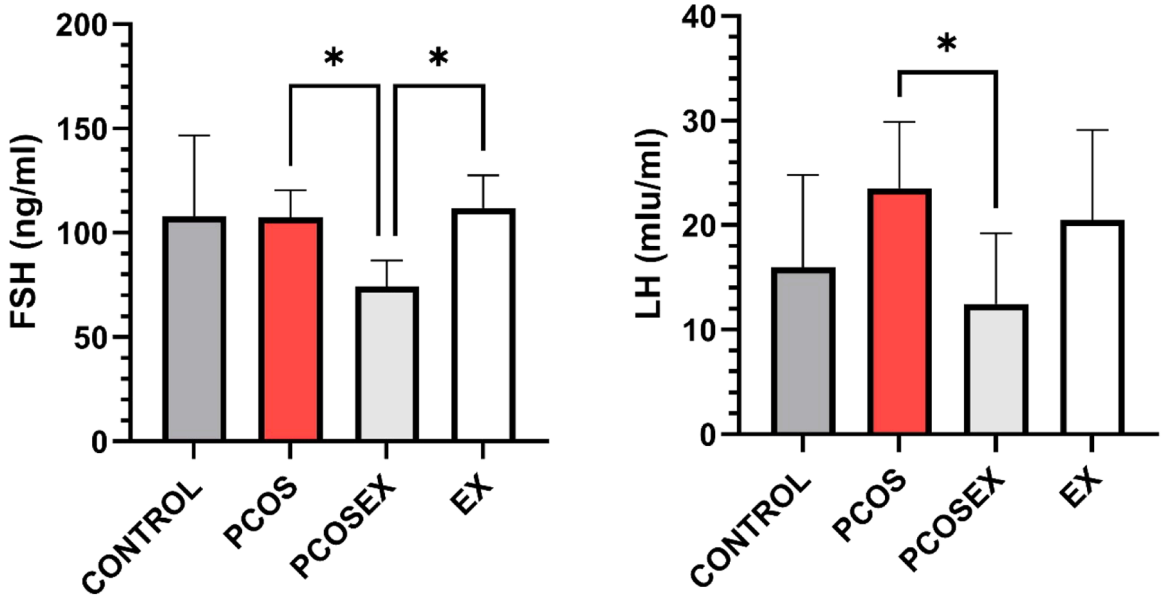

Fig. 3. Serum FSH and LH levels of all groups (* $p < 0.05$). Data are presented as mean± standart derivation.

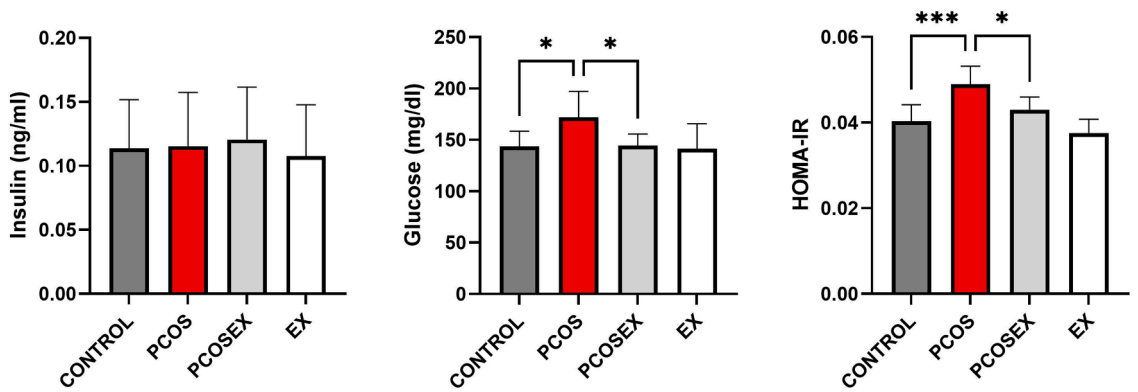

Fig. 4. Serum fasting insulin, fasting glucose and HOMA-IR levels of all groups (* $p < 0.05$, *** $p < 0.001$). Data are presented as mean± standart derivation.

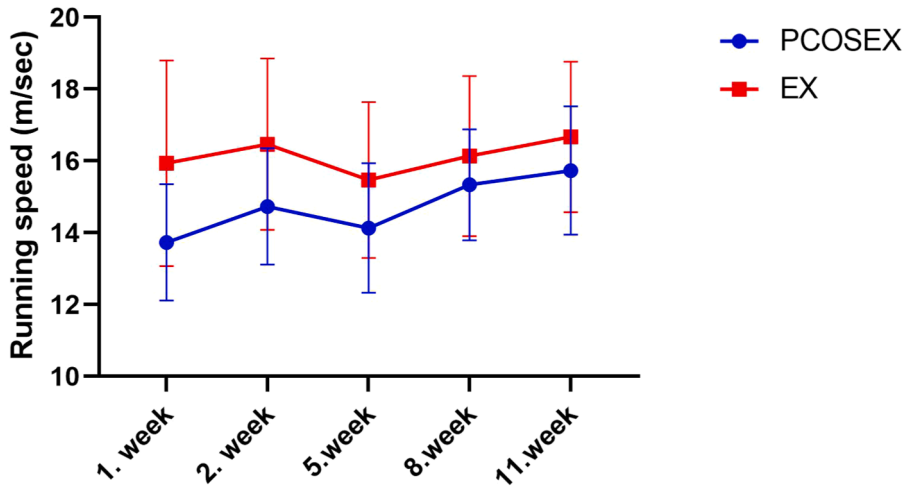

Fig. 5. Maximum exercise capacity levels of PCOS-induced and exercise- treated (PCOSEX) and only exercise- treated (EX) groups. Data are presented as mean± standart derivation.

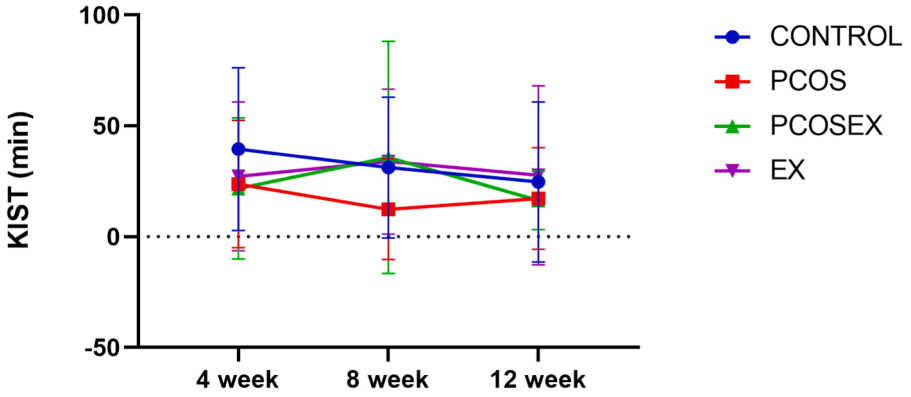

Fig. 6. Kondziela Invert screen test scores of all groups.

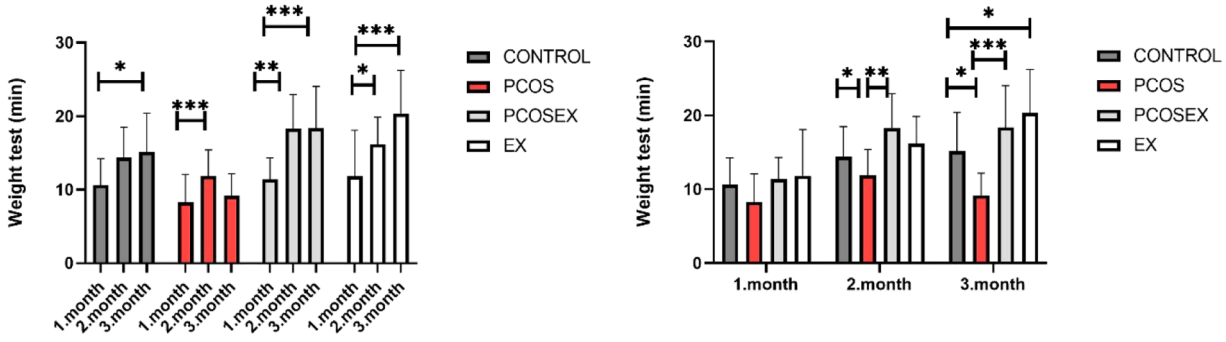

Fig. 7. Time course of weights test of all groups (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Data are presented as mean± standart derivation.

2.11. Histological analysis of ovary and muscle

Soleus muscles and ovarian tissue samples taken from the experimental groups were fixed in 10 % buffered neutral formalin solution, washed in tap water, and dehydrated by passing through 75 %, 96 %, and 100 % alcohol series, respectively. After the procedure, the tissues cleared with xylene were incubated with liquid paraffin in an oven at 60 °C for 3 h and embedded in paraffin blocks.

Once 5 μm thick sections of paraffin blocks were taken on the slide with the Leica RM 2125RT model sliding microtome, the sections were prepared for histological staining following the deparaffinization and rehydration stages and stained with Hematoxylin-Eosin (HE) stain for light microscopy examination. Stained sections of both tissues were examined under the Axio-scope A1 light microscope, and their photographs were taken. The cross-sectional surface measurements made using the Axiovision software program in 5 different areas from the cross-sections obtained from the muscle tissue were evaluated statistically.

2.12. Statistical analysis

Statistical Package for Social Sciences (SPSS) version 26 was used for

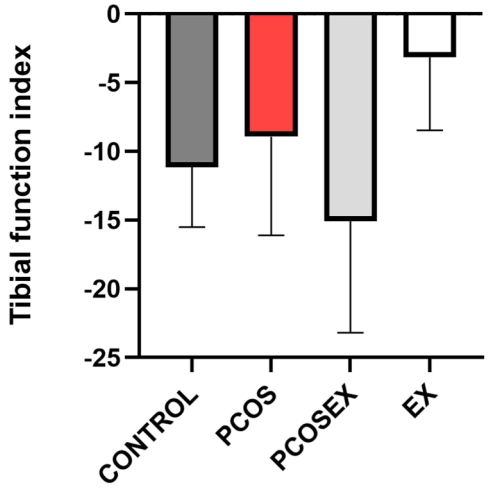

Fig. 8. Tibial Function Index of all groups. Data are presented as mean± standart derivation.

the statistical analysis of research data. GraphPad Prism was preferred for drawing graphics. In our study, the Shapiro-Wilk test was used to test whether the data were normally distributed. Parametric tests were preferred if the data showed a normal distribution. In descriptive statistics, 'mean ± standard deviation (SD)' was used for variables with a normal distribution, and 'median (min; max)' for variables with a non-normal distribution. Since the number of groups was more than two, the 'ANOVA analysis of variance test' was used as the parametric test, and the 'Kruskal-Wallis test' was used as the non-parametric test. The change according to time before and after exercise in continuous variables was evaluated with Two-way ANOVA if the distribution was normal, and with the Mann-Whitney U and Wilcoxon test if the distribution was abnormal. P-values of <0.05 were considered statistically significant.

3. Results

3.1. Changes in weight values of rats

When the rat weight measurement results at 15-day intervals after DHEA administration were examined, the PCOS and PCOS+EX groups were heavier than the control group on the 30th,60th and 75th days of experiments ($p < 0.0001$). Considering the general average of all days, the PCOS and PCOS+EX groups were heavier than the Control group ($p < 0.05$) (Fig. 1).

3.2. Changes in hemodynamic values of rats

The systolic, diastolic, mean arterial pressure and heart rate values of the groups in our study were analyzed at the end of the exercise experiments. Systolic, diastolic, and mean arterial pressures were higher in the PCOS (133,0 ± 8,04; 111,7 ± 5,82;117,5 ± 5,81) and PCOSEX (129,9 ± 8,34; 116,6 ± 6,75; 117,7 ± 6,97) groups compared to the Control group (109,60 ± 6,86;101,5 ± 4,23; 107,6 ± 4,92).When the heart rate is examined, the heart rate of the PCOSEX (380,8 ± 36,25) group is slower than the PCOS (451,2 ± 59,44) group ($p = 0.019$). The differences in systolic, diastolic and mean arterial pressure between PCOS and Control were $p < 0.001$, $p = 0.016$ and $p = 0.023$, respectively. The differences in systolic, diastolic and mean arterial pressure between Control and PCOS+EX were $p < 0.001$, $p = 0.017$ and $p = 0.019$, respectively. (Fig. 2)

3.3. Changes in oxidant and antioxidant parameters in serum of rats

When the TOS, TAS, OSI, Total Thiol, Native Thiol, and disulfide values of the groups in our study were analyzed at the end of the exercise experiements, no significant difference was observed between the groups. Oxidant and antioxidant parameter values are given in the Table 1. Exercise did not affect oxidant and antioxidant parameters ($p > 0.05$).

3.4. Changes in FSH and LH values in serum of rats

When the FSH and LH values in the serum of the groups in our study were analyzed, a significant decrease of FSH was observed in the PCOSEX (74. 29± 12.53) group compared to the PCOS (107.5 ± 12.86) group ($p = 0.033$). In addition, FSH showed a significant increase in the Exercise group compared to the PCOSEX group ($p = 0.013$). LH levels were also decreased in PCOSEX (12.48±6.73) group compared to PCOS group (23.56± 6.33) ($p = 0.036$). Exercise affected FSH and LH values (Fig. 3).

3.5. Fasting insulin, fasting glucose, and HOMA-IR changes in serum of rats

When glucose values in the serum of the groups were analyzed, glucose values were found to be higher in the PCOS group (171.9 ± 25.32) compared to the PCOSEX (144.4 ± 11.25) group ($p = 0.046$). PCOS glucose levels were also higher than Control group (143.7 ± 14.65) ($p = 0.040$). When we analyzed HOMA-IR, PCOS HOMA-IR index was higher than Control group and PCOSEX group ($p< 0,001$ $p = 0.012$). A high HOMA-IR value is an indicator of insulin resistance, and exercise has affected this situation. Insulin levels were similar among groups (Fig. 4).

3.6. Changes in maximum exercise capacity (MEC)

1st, 2nd, 5th, 8th, and 11th-week MEC measurements were examined within the framework of the 12-week HIIT program. A significant decrease was observed in the PCOSEX group values only in the 1st week and 2nd week.($p = 0.010$ $p = 0.011$). There is no significant difference between the groups when all means are examined ($p = 0.478$) (Fig. 5)

3.7. Kondziela invert screen test

When the 3-month Kondziela Invert Screen Test was applied to experimental animals, no significant difference was observed in the time spent between the groups ($p > 0.05$) (Fig. 6)

3.8. Weight test

When the 3-month weight tests of experimental animals were examined, a significant difference was found. At 2 months, a decrease in weightlifting minutes was observed in the PCOS group compared to the Control group ($p < 0.05$) and PCOSEX group ($p < 0.01$). At 3 months, a decrease was observed in the PCOS group compared to the Control ($p < 0.05$) and PCOSEX ($p < 0.001$) groups. In addition, the weightlifting minutes in the EX group were higher than in the Control group ($p < 0.05$) (Fig. 7).

3.9. Tibial function index

When the exercise processes were finished, the tibial function index was examined in the groups. No significant difference was observed between the groups ($p > 0.05$) (Fig. 8).

3.10. Soleus muscle myokine analysis

When the FGF21, METRNL, IL-6, and ANGPTL4 ELISA values of the groups in our study were examined, a difference was observed in IL-6 and ANGPTL4 values. A decrease in the IL-6 level was observed in the

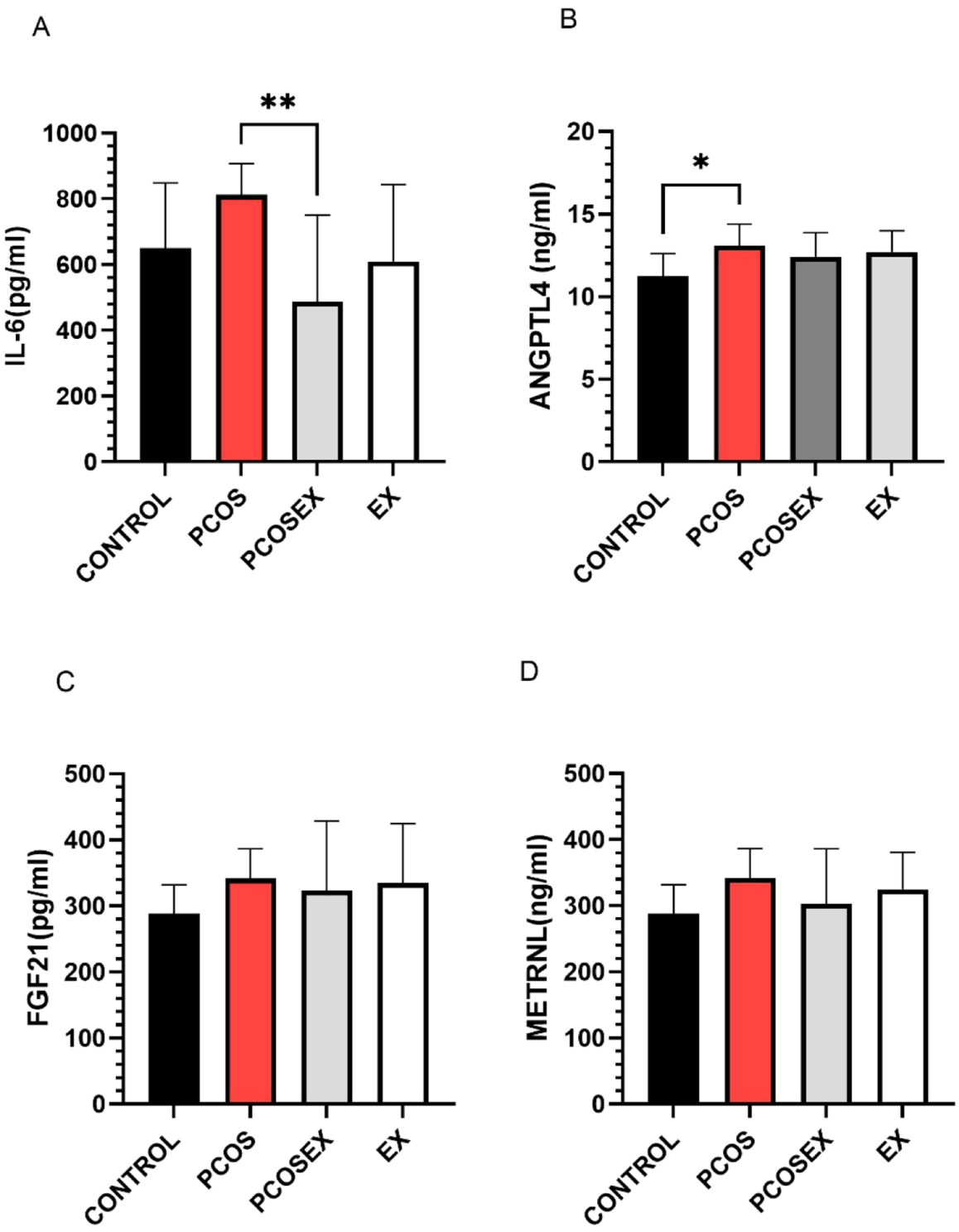

Fig. 9. Soleus muscle myokines of all groups. A: soleus IL-6 levels B: soleus ANGPTL4 levels C: soleus FGF21 levels D: soleus METRNL levels. (* $p < 0.05$, ** $p < 0.01$).

PCOSEX group (488.2 ± 83.04) compared to the PCOS group (812.3 ± 29.87) ($p = 0.007$). There was a significant increase in ANGPTL4 levels in the PCOS group (13.09±1.29) compared to the Control group (11.25 ±1.35) ($p = 0.022$). No difference was observed between the groups in FGF21 and METRNL levels ($p > 0.05$) (Fig. 9)

3.11. Soleus muscle contractility parameters

When the soleus muscle contractility parameters were examined, no difference was observed between the groups in the Time-To-Peak tension, Half Relaxation Time, and Contraction Amplitude values. Considering the fatigue values, the soleus muscle of the PCOS group got tired more quickly in the initial minutes ($p < 0.05$). At the 10th minute, the PCOSEX group got tired later than the Control group ($p < 0.05$). No difference was observed at other times ($p > 0.05$) (Fig. 10)

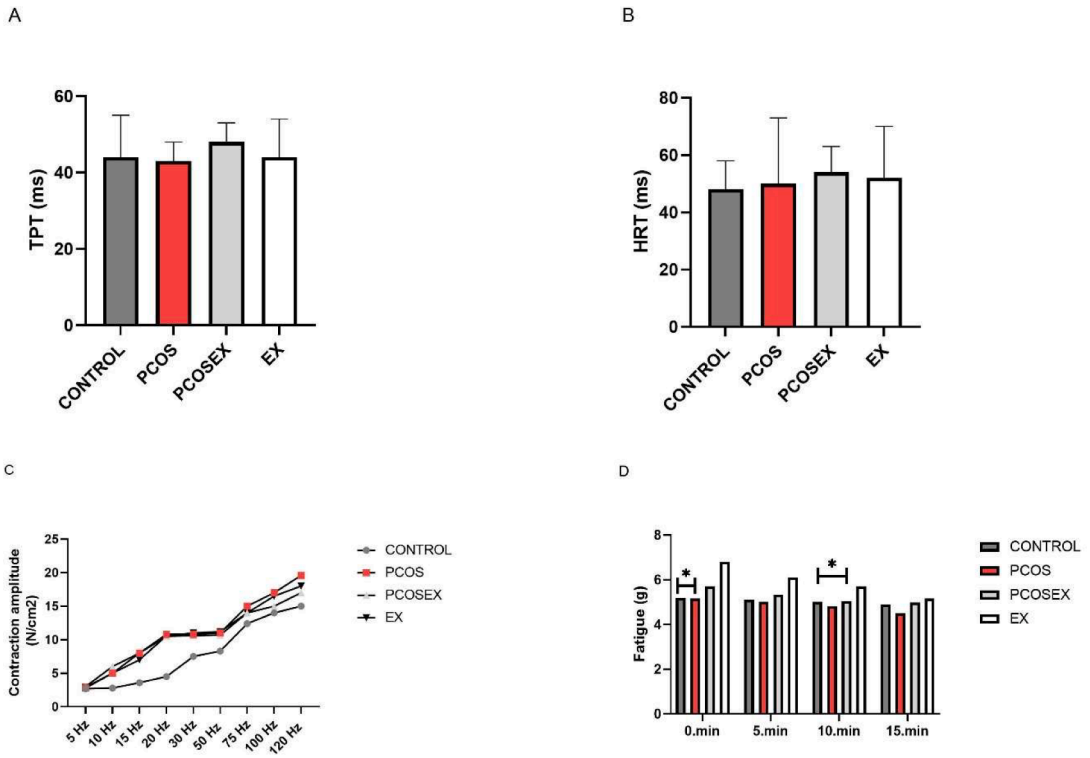

Fig. 10. Soleus muscle contractility results A: Soleus muscle time-to-peak tension (TPT) levels of all groups B: Soleus muscle Half Relaxation Time (HRT) levels of all groups C: Soleus muscle contraction amplitude (N/cm^2) of all groups D: Soleus muscle fatigue of all groups.

3.12. Soleus muscle histological cross-sectional area analysis

Upon examining the histological cross-sectional area values, the PCOS group exhibited the smallest muscle cross-sectional area, indicating muscle atrophy. The cross-sectional area in the PCOS group was significantly lower (2.287±0.92) compared to both the Control (3.272 ±0.70) and PCOSEX (2.982±0.64) groups ($p < 0.001$). In contrast, hypertrophy was observed in the EX group, with a significantly larger cross-sectional area (5.076±0.81) compared to the Control group (3.272 ±0.70) ($p < 0.001$). Histological analysis revealed that both the Control and Exercise groups showed normal muscle structure, with muscle fibers and surrounding connective tissue sheaths appearing intact and healthy. However, in the PCOS group, muscle sections displayed signs of edema and muscle fiber atrophy, indicating muscle degeneration. These findings are illustrated in Fig. 11.

3.13. Histological evaluation of the ovary

In the light microscopy examination of ovarian tissue samples, the control and exercise groups were observed to have a normal appearance. Primordial follicles were seen under the germinal epithelium. Graafian follicles and corpus luteum structures were observed in addition to primary and secondary follicles in the ovarian cortex. The ovarian medulla had a normal appearance and was rich in loose connective tissue and blood vessels.

In contrast, primordial follicles under the germinal epithelium were observed very little in the ovarian tissue belonging to the PCOS groups. Follicles in the form of cystic structures were observed in the cortex. Primary and secondary follicle structures were rare. Corpus luteum formations were seen. Additionally, in the PCOS+exercise groups, besides the cortex appearance similar to the PCOS groups, an increased blood supply and vascular structure in the medulla were noted. Ovarian tissue histology examinations are shown in Figs. 11 and Fig. 12.

3.14. Follicle morpological classification and count

The number of primordial, primary, secondary, graafian, atretic/cystic follicles, and corpora lutea were counted in all groups (Fig. 13). The number of primordial and primary follicles was significantly lower in the PCOS group (11.83±1.94, 7.66±1.83) compared to the Control group (24.67±1.21, 24.83±1.16) ($p < 0.001$). In the PCOS-induced and exercise-treated group, the number of primordial (17.17±1.16) and primary (13.33±0.81) follicles was statistically higher due to exercise. The number of secondary follicles was lower in the PCOS group (2.0 ± 0.89) compared to the Control group (3.0 ± 0.63), but this decrease was not statistically significant. With exercise, the number of secondary follicles was slightly higher in the PCOS+Exercise group (2.16±0.40) compared to the PCOS group ($p > 0.05$). When evaluating the graafian follicle, there was a decrease in the PCOS group (1.33±0.51) compared to the Control group (2.33±0.51) ($p = 0.015$). However, the exercise program did not lead to a positive change in this number. The number of atretic/cystic follicles was significantly higher in the PCOS group (6.66 ±0.81) compared to the Control group (1.33±0.51) ($p < 0.001$). Regarding the corpus luteum, the number was significantly reduced in the PCOS group (2.66±0.51) ($p < 0.001$). The exercise program did not improve this number.

4. Discussion

The current study focused on the effects of HIIT on insulin resistance, oxidative stress, muscle contractility, and myokines in rats with polycystic ovary syndrome. According to the results, HIIT showed a significant improvement in glucose homeostasis, oxidative stress, muscle contractility, and ovarian histopathology. Furthermore, myokines, especially IL-6 and ANGPTL4, seem to have a key role in the underlying mechanism.

We have monitored metabolic parameters such as body weight, blood glucose, insulin level, and blood pressure. Body weight values have fluctuated between days. Although there was no statistical

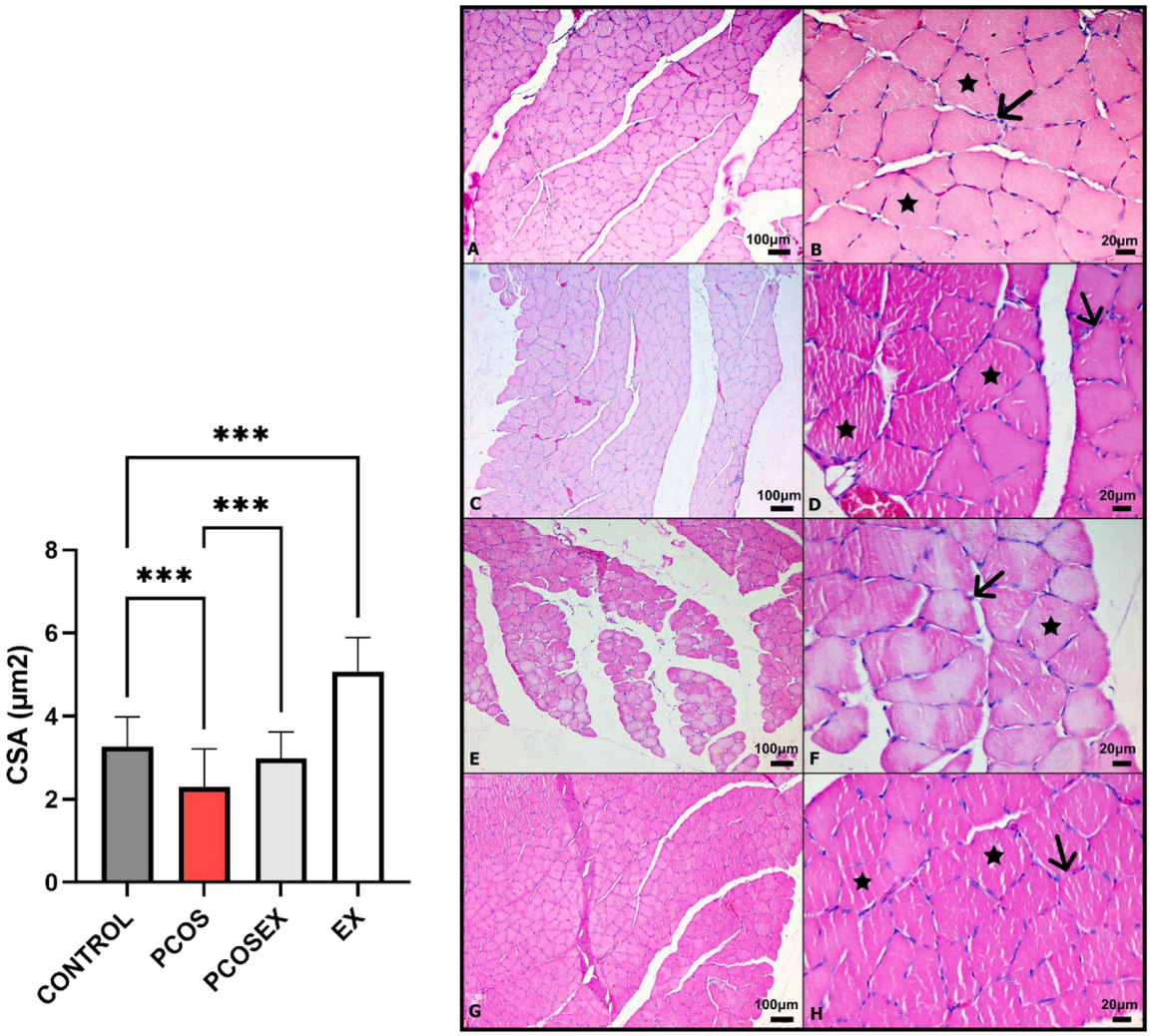

Fig. 11. CSA: Cross-sectional area of soleus muscle (*** $p < 0.001$) A, B: Control C, D: Exercise E, F: PCOS G, H: PCOS+Exercise. In HE-stained sections, light micrographs of the soleus muscle are observed. Arrows indicate skeletal muscle fibers, and the star represents endomysium.

difference between groups at the end, PCOS (both sedentary and exercise groups) animals had significantly higher body weight in the 30th, 60th, and 75th days. Similarly, Lim et al. reported that exercise leads to an improvement in insulin resistance in PCOS patients regardless of body weight [38]. Insulin resistance was prominent in the PCOS group and was reflected in elevations in blood glucose, insulin, and HOMA-IR whereas insulin resistance was significantly alleviated by HIIT application to PCOS groups. The insulin-sensitizing effect of training was reported similarly in different animal studies [39,40].

On the other hand, the overlap of the initiation week of training and the onset of the disease is as important as the training protocol. In the present study, training was started in the adolescence period of animals (42–44 days) immediately after the onset of PCOS. Fischer et al. demonstrated the protective effects of early-life swimming exercise against obesity and glucose metabolism diseases in rats [41]. Current results are consistent with the literature.

Low-grade chronic inflammation, insulin resistance, and cardiovascular complications are present in PCOS patients [42]. Hypertension is one of the cardinal features of the metabolic syndrome ergo PCOS. PCOS patients displayed >40 % likely to have high blood pressure compared to healthy women, regardless of age, body mass index, diabetes, or dyslipidemia [43]. We measured the systolic and diastolic blood pressure of the animals and calculated mean arterial pressure according to these values. All hemodynamic parameters point out a prominent hypertensive phenotype in PCOS groups.

Furthermore, HIIT did not alleviate the aforementioned hypertensive phenotype. Therefore, we may conclude the current protocol did not induce a marked atheroprotection. On the other hand, we could not evaluate the lipid profile of the animals which would give an important insight about vascular health. That was one of the limitations of the current study. Nevertheless, some authors have reported that 12–24 weeks of exercise did not cause any significant alteration in systolic and diastolic blood pressure parameters in PCOS patients [44,45].

As for the heart rate, there was no significant difference between the control and PCOS groups. Kilit et al. showed that the reactivity of the cardiac sympathovagal balance did not change in PCOS, which may explain the current result [46] The bradycardic effect of chronic training is a well-known fact, demonstrated in various studies [44,47]. Parallelly, heart rate was lower in the PCOS+Exercise group compared to the sedentary PCOS animals, whereas we could not see the same tendency in the Control+exercise group.

In addition to insulin resistance, various alterations in gonadotropic hormones are common in PCOS. The normal gonadotropin axis is disrupted in women with PCOS. LH levels increase and FSH levels decrease, resulting in a reversal of the LH/FSH ratio [48]. We observed an increase in LH levels of the PCOS animals that were normalized with HIIT. On the other hand, FSH levels did not differ between control and PCOS animals whereas exercise significantly lowered the FSH levels. Pawar et al. reported that FSH values decreased after exercise in HIIT for 6 weeks [49]. Thus, we may suggest that HIIT may restore hormonal imbalances

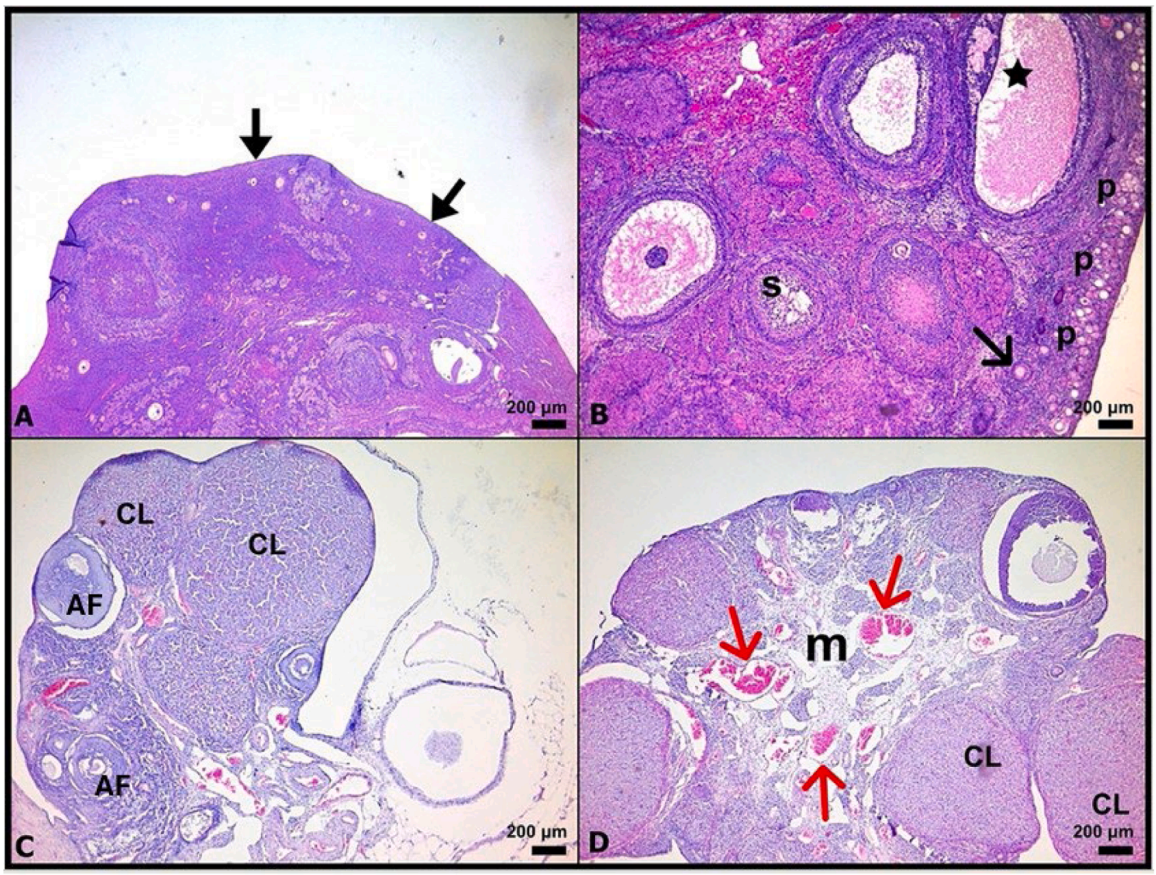

Fig. 12. Light micrographs of ovarian tissue are observed in H-E stained sections. A: Control B: Ex C: PCOS D: PCOSEX ↓: Germinal epithelium; p: primordial follicles; ↓: primary follicle; s: secondary follicle; *: Graafian follicle; CL: Corpus Luteum, m: medulla, AF: atretic follicle red ↓: blood vessel,.

associated with PCOS.

Cystic structures and scarce follicles were observed in the ovaries of the PCOS group that can be characterized as polycystic changes. Additionally, *corpus luteum* formations were observed in the PCOS group. Pathological manifestations associated with PCOS were less prominent in the PCOS+Exercise group. Although the cortical appearance was indifferent, increased blood supply due to strong vascularization was noted in the medullar region of the PCOS+Exercise group. Cao et al. reported that high-intensity exercise can lead to the development of various follicles, including primary, secondary, and antral follicles, in the ovary [50]. In the present study, it can be argued that hormonal imbalance and histopathological alterations in ovarian tissue affect each other reciprocally and HIIT alleviates ovarian pathology by restoring hormonal balance.

In the present study, we examined muscle function both in vivo and in vitro. Our study is the first to examine motor performance with the weight test and Kondziela reverse screen test in the PCOS model. Although Kondziela inverted screen test and tibial nerve function index did not differ among groups, a significant deterioration in motor performance was noted in the weight test in PCOS group, especially at the end of the third month, compared to the controls. In the PCOS+Exercise group, motor performance was significantly improved compared to the PCOS group. On the other hand, we could not observe any appreciable functional differences among groups in isolated soleus muscle samples. There was no difference in contraction and relaxation times and specific power production. The discrepancy between in vivo and in vitro models is apparent. Naturally, isolated muscle functions without the contribution of other bodily systems and it was not limited to antagonist muscles and margins of the body. An antagonist muscle or a muscle with a different dominant fiber type (like extensor digitorum longus) could be isolated and analyzed to gain a different perspective. Unfortunately, only soleus samples were analyzed due to technical restriction which was a major limitation of the current study.

Muscular atrophy was observed in soleus samples of the animals with PCOS and HIIT reversed PCOS-induced muscular atrophy. The histological analysis showed that HIIT increased the soleus muscle's histological cross-sectional area in the PCOS+Exercise group compared to the PCOS group, indicating that it had a beneficial effect on the soleus muscle.

Angiopoietin-like peptides are myokines secreted by skeletal muscle, adipose tissue, intestine, and liver [51]. ANGPTL4 plays a role in insulin sensitivity, lipid metabolism, and adipogenesis [52]. ANGPTL4 inhibits lipoprotein lipase [53] Lipoprotein lipase gene expression is impaired in skeletal muscle in PCOS patients. The impairment of LPL activity in skeletal muscle further disrupts normal lipid metabolism [7,54]. The main function of ANGPTL-4 is to protect the cell from excessive adipogenesis ergo lipotoxicity [55]. In the present study, muscle-derived ANGPTL-4 was significantly higher in the PCOS group and it was normalized by training. We hypothesize that ANGPTL-4-induced metabolic alterations may cause muscular atrophy and functional impairments that were observed in vivo evaluations. A tendency to weaken the thigh muscle was observed in overweight PCOS patients in the evaluations with computed tomography. Furthermore, in PCOS patients who undergo 12 weeks of moderate and vigorous exercise thigh muscle weakening was significantly reduced. Authors have argued that muscle lipid content was lower in PCOS patients but increased with exercise [56]. Stejskal et al. demonstrated a correlation between ANGPTL-4 levels and serum triglyceride levels in metabolic syndrome patients [57].

Although there is no evidence related to the effects of ANGPTL-4 on

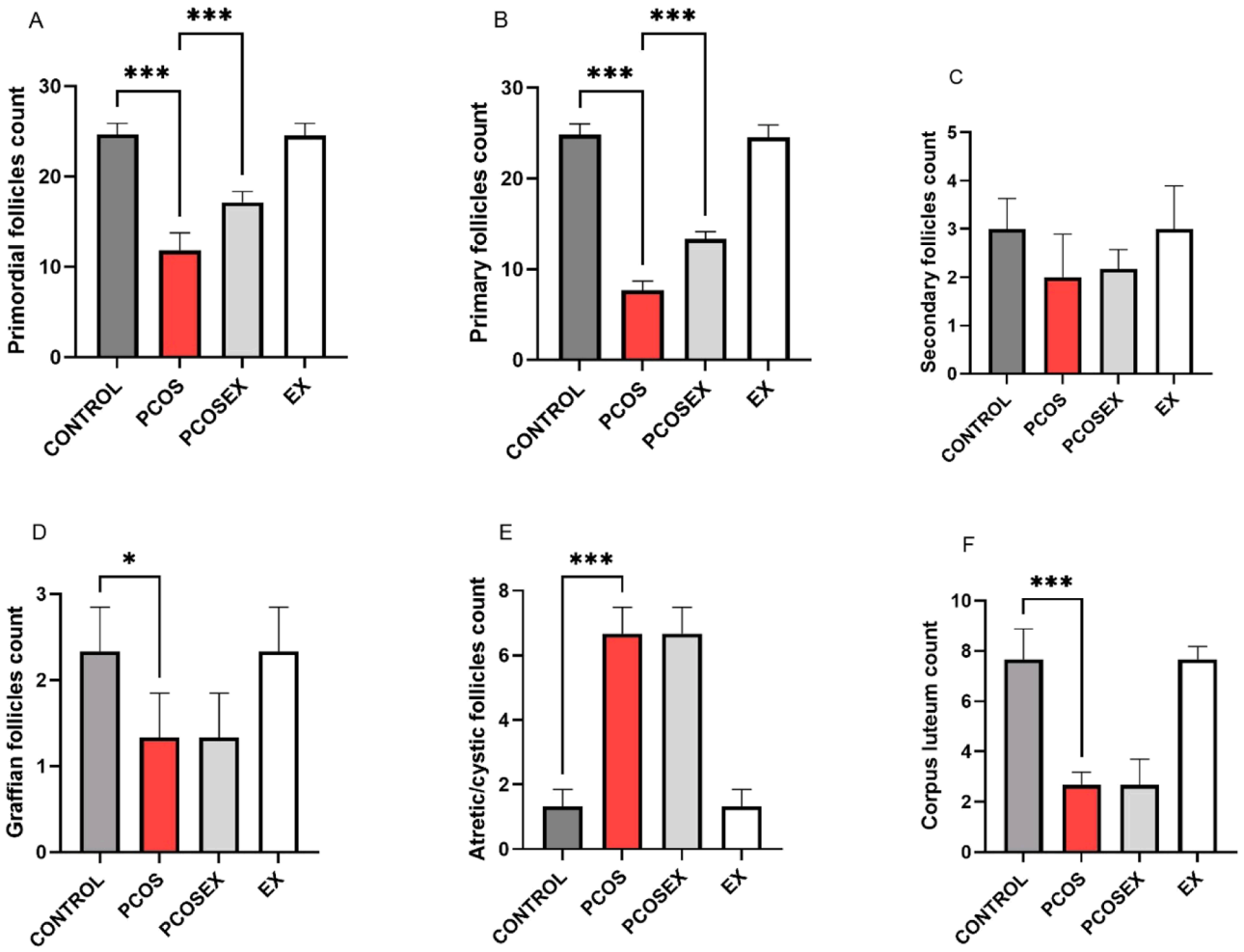

Fig. 13. Folliculogenesis in all groups. A: Primordial follicles number B: Primary follicles number C: Secondary follicles number D: Graffian follicles number E: Atretic/cystic follicles number F: Corpus luteum number. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

the gonadotropin axis, ANGPTL-4 molecules that enter the bloodstream may affect the hormonal imbalance. Further studies are needed to shed light on muscle gonadotropin crosstalk.

IL-6 levels were elevated in the PCOS group,indicating the proinflammatory effect of PCOS on soleus muscle. Furthermore, training lowered IL-6 levels which could be interpreted as the anti-inflammatory effect of HIIT. On the other hand, there are various studies in the literature that report an IL-6 increase after HIIT and argue that this increase has an anti-inflammatory effect [58]. However, there are some pitfalls related to IL-6 homeostasis [59]. The effects of IL-6 are bidirectional and dose-dependent. This peptide can have anti-inflammatory effects but at the same time, proinflammatory effects could be observed in different plasma or tissue levels which is tightly related to the intensity, type, and duration of the training. Moreover, its effect is also modified by accompanying inflammatory cytokines. A detailed analysis of the cytokine panel is needed for a certain conclusion. Fix et al. stated that the level of gp130, which inhibits IL-6 in muscles, increases with exercise [60]. The decrease in IL-6 level in the PCOS+Exercise group may be because gp130 inhibited IL-6 with exercise. Dantas et al. reported an IL-6 decrease both in plasma and skeletal muscle with exercise in a study of PCOS patients [8]. seems to be compatible with the results of our observation.

METRNL is a peptide that activates different intracellular signaling pathways with various physiological effects. It has various functions such as the browning of white adipose tissue, insulin sensitivity, inflammation regulation, skeletal muscle regeneration, and tissue, protection [61]. Fouani et al. reported lower serum METRNL levels in patients with PCOS and the lowest METRNL values were observed in obese infertile women [62]. In another study, obese mice were exercised and METRNL levels were measured in the soleus muscle and blood. There was no change in the soleus muscle METRNL level, but serum METRNL levels increased in the exercised groups [63]. We could not observe any significant alterations in the tissue levels of METRNL in the current study. In the literature, it is seen that METRNL levels vary according to duration, type, and intensity of exercise and also muscle type examined.Therefore, different muscle types (muscles with different types of fibers) should be examined in both HIIT protocol and PCOS animal models in forthcoming studies. Unfortunately, we could not measure the serum levels of myokines which is also a limitation of the current study.

FGF21 is a myokine secreted from the liver, pancreas, adipose tissue, and skeletal muscle [64]. According to preclinical studies, FGF21 has displayed physiological effects such as enhancing insulin sensitivity, decreasing triglyceride concentrations, and causing weight loss in obese rodents and primates [65]. FGF21 protein level was not altered in the presented study. Like METRNL, the change in FGF21 levels may be influenced by the type/intensity of exercise and the tissue examined. Thus, in PCOS animal models and HITT protocols, it can be analyzed in a different muscle and organs.

Oxidative stress is an important factor contributing to the physiopathology of diseases. No change was observed in serum levels in the present study. Rodent studies reported that exercise causes an increase, decrease, and no change in oxidative stress [66]

In our study, the maximum exercise capacity values of the

PCOS+Exercise group were found to be lower than those of the Exercise group at the beginning of the exercise program and in the second week. However, no significant difference was observed in the measurements made in the following weeks. It has been reported that there is endothelial dysfunction in PCOS, which reduces oxygen distribution and use in peripheral tissues, ultimately decreasing exercise capacity This increase in endothelial dysfunction was especially higher in the PCOS group. In a meta-analysis study, it was reported that PeakVO2 values increased after exercise in PCOS patients [67]. Our study's findings also suggest that exercise capacity increased with HIIT.

There are some limitations in our study. The fact that muscle myosin isoforms were not examined with special histological staining techniques did not allow for further elaboration of the results. Additionally, subcutaneous adipose tissue and muscle fat content of the animals were not observed. The examination of adipose tissue in the muscle would have added value to our study.

CRediT authorship contribution statement

Seda Koçak: Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Hasan Çalışkan:** Visualization, Resources, Methodology, Conceptualization. **Göktuğ Ömercioğlu:** Methodology, Investigation. **Fırat Akat:** Methodology, Investigation. **Deniz Billur:** Writing – original draft, Methodology, Investigation. **İrem İnanç:** Visualization, Methodology, Investigation. **Hakan Fıçıcılar:** Methodology, Investigation. **Metin Baştuğ:** Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Conflict of interest statement

The authors declare no conflict of interest.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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