Journal of Ovarian Research

RESEARCH

Open Access

Chiglitazar ameliorates dehydroepiandrosterone-induced polycystic ovary syndrome in rats

Fuzhen Zhao[1,2,4,5], Wei Cui[4,5], Chengmei Fang[4], Yuanyuan Luo[2,3,4,5*] and Cheng Zhang[2,4,5*]

Abstract

Background Polycystic ovary syndrome (PCOS) is an endocrine and metabolic disorder accompanied by ovulatory dysfunction. Insulin resistance (IR) is a key pathogenic mechanism in PCOS, and insulin sensitizers, such as metformin and pioglitazone, can improve PCOS symptoms. Chiglitazar, a pan-peroxisome proliferator-activated receptor (pan-PPAR) agonist, is also an insulin sensitizer; however, its therapeutic effects have not yet been studied in PCOS. We evaluated the therapeutic effects of chiglitazar in a rat model of PCOS.

Methods Sprague–Dawley rats aged 4 weeks were injected subcutaneously with dehydroepiandrosterone (DHEA) (6 mg/100 g/day) to establish PCOS, and a control (CON) group was established. The rats were divided into the CON, PCOS model (DHEA), pioglitazone-treated (DHEA + PIO), and chiglitazar-treated (DHEA + CHI) groups. The DHEA + PIO group received pioglitazone (20 mg/kg/day) and the DHEA + CHI group received chiglitazar (20 mg/kg/day), each for 15 days. Body weight, estrous cycle, and glucose tolerance test (GTT) and insulin resistance test (ITT) results were monitored. Experimental animal energy metabolism systems were utilized to assess metabolic parameters. Enzyme-linked immunosorbent assay was conducted to detect changes in serum hormones, including insulin, adiponectin, sex-related hormones, and lipid metabolism indicators. The ovaries were used for molecular biology experiments to detect changes in Akt/phosphorylated Akt and glucose transporter 4 (GLUT4) expression by Western blotting and quantitative polymerase chain reaction.

Results Chiglitazar and pioglitazone improved PCOS symptoms. However, chiglitazar demonstrated a more pronounced effect on lipid improvement and weight gain than pioglitazone. In the DHEA + PIO and DHEA + CHI groups, there was notable recovery in oxygen consumption and carbon dioxide output; substantial improvement in GTT and ITT results; an increase in adiponectin; and a reduction in serum insulin, androgens, luteinizing hormone (LH), and LH/follicle-stimulating hormone ratio. Compared with the DHEA group, the DHEA + CHI group exhibited notable decreases in triglycerides, free fatty acids, and atherosclerosis index, while the DHEA + PIO group demonstrated no changes. Granulosa cells and healthy follicles increased in ovarian sections. Ovarian steroidogenic enzymes also increased in the DHEA + PIO and DHEA + CHI groups compared with the DHEA group. Mechanistically, chiglitazar increased Akt phosphorylation.

*Correspondence:
Yuanyuan Luo
luoyuanyuan@cqu.edu.cn
Cheng Zhang
zhangcheng0309@cqu.edu.cn
Full list of author information is available at the end of the article

Conclusion Chiglitazar significantly improved ovulation in rats with PCOS and may be a potential novel therapeutic strategy for PCOS.

Keyword Chiglitazar, Polycystic ovary syndrome, Insulin resistance

Background

Polycystic ovary syndrome (PCOS) is a serious endocrine disorder that typically manifests with hyperandrogenemia accompanied by decreased ovarian function, chronic infrequent ovulation, and/or a micro-polycystic-like ovarian morphology [1]. Recently, the incidence of PCOS has increased, affecting 8%–13% of females globally [2]. PCOS can also lead to various other health problems, such as excessive androgen secretion, obesity, glucose intolerance, insulin resistance (IR), diabetes mellitus, dyslipidemia, arterial hypertension, non-alcoholic fatty liver disease, and low-grade chronic inflammation, amongst others [3]. However, due to the varied clinical characteristics and etiologies of PCOS, its pathogenesis remains unclear.

Recently, IR and high androgen concentrations have been regarded as factors influencing the onset and development of PCOS and are considered to play a crucial role in PCOS pathogenesis [1]. According to epidemiological surveys, approximately 50%–70% of patients with PCOS exhibit IR [4]. Therefore, in addition to the lifestyle interventions recommended in most guidelines, including exercise and dietary modifications, as well as the conventional application of estrogen and progesterone, drugs that target IR, such as metformin and pioglitazone, have demonstrated therapeutic effectiveness in PCOS. However, long-term use of estrogen therapy in postmenopausal women is associated with an increased risk of endometrial cancer, which increases with the treatment dose and duration [5]. The side effects of drugs such as pioglitazone are well-documented, including weight gain and potential risks during pregnancy [6], and these side effects limit their clinical potential. Therefore, the search for new therapeutic drugs for PCOS continues.

Chiglitazar, a pan-peroxisome proliferator-activated receptor (pan-PPAR) agonist, stimulates peroxisome proliferator-activated receptors (PPARs), including not only PPAR-γ, but also PPAR-α and PPAR-β/δ. Chiglitazar is widely used for the treatment of type 2 diabetes mellitus [7], which possesses the therapeutic advantages of glycemic control and dyslipidemia management. Furthermore, the pan-PPAR agonist chiglitazar has demonstrated in preclinical trials the capacity to ameliorate hepatic function and insulin sensitivity in a murine model of NAFLD, reverse hepatic steatosis, and mitigate hepatic inflammation and oxidative stress [8]. Similar to pioglitazone, chiglitazar activates the transcription factor PPAR-γ, which promotes the synthesis of glucose transporters, such as glucose transporter 4 (GLUT4), and regulates adipokine secretion, including adiponectin and tumor necrosis factor-α, thereby improving IR [9, 10]. Chiglitazar also improves IR by activating PPAR-α and PPAR-β/δ. PPAR-α increases the binding of insulin receptors to insulin, thereby increasing glucose uptake through GLUT4 via the PI3K system [11]. Furthermore, PPAR-α, which is a key regulator of fatty acid (FA) metabolism, modulates the expression of enzymes related to FA oxidation at the transcriptional level [11], diminishes free fatty acid (FFA) levels, lessens glucose inhibition, and increases affinity receptors, thereby alleviating IR. Illustrating these concepts, a previous study demonstrated that the PPAR-α agonists bezafibrate and fenofibrate ameliorate IR, decrease blood glucose concentration, and improve glucose tolerance, thus averting the onset of diabetes mellitus [12]. Another study showed that PPAR-δ regulates peroxisomal β-oxidation of FFAs by improving insulin sensitivity [13]. Therefore, whether chiglitazar is useful for the treatment of PCOS and whether its therapeutic effect is superior to that of pioglitazone remains to be established.

In this study, we aim to explore whether chiglitazar has a therapeutic effect on PCOS and whether its efficacy is superior to that of the traditional PPAR-γ agonist pioglitazone. In addition, we explored the possible mechanisms by which chiglitazar improves PCOS.

Materials and methods

Animals

Thirty-two 4-week-old female Sprague–Dawley rats whose weight range of 80–116 g were selected. The thirty-two rats were purchased from Sichuan Zhonghong Mingyan Biotechnology Co., Ltd. and housed under constant environmental conditions in the animal room of the Central Laboratory of the Three Gorges Hospital affiliated with Chongqing University. Four rats per cage were fed and watered ad libitum on a 12-h light–dark cycle. All animal experiments were conducted in accordance with the guidelines and regulations set forth by the Animal Ethics Committee of the Chongqing University Three Gorges Hospital. The rats were housed in 25 ± 1 °C with a 12-h light/dark cycle under 45–55% humidity. Four-week-old rats are divided into four groups, with n = 8 in each group. Every day, the female rats are subcutaneously

injected with DHEA dissolved in sesame oil (6 mg / 100 g body weight) continuously for 15 days [14–16]. The rats in the CON group were administered sesame oil as a solvent using the same dosing regimen, and the success of the modeling was determined by vaginal cytology for two sequential cycles (about 8–10 days) to assess the estrous cycle. There are four groups: rats received solvent sesame oil as control (CON); rats given DHEA as a modeling group (DHEA); the remaining two groups were treated with Pioglitazone (DHEA + PIO) and Chiglitazar (DHEA + CHI), respectively. The DHEA + PIO group was treated with a 20mg·kg^{-1}d^{-1} as the positive control group, and the DHEA + CHI group was treated with a 20 mg·kg^{-1}d^{-1} [7, 17, 18]**.** Chiglitazar is a new-generation insulin sensitizer developed by Chipscreen Biosciences (China).

Vaginal smear

The estrous cycle of SD rats was determined by observing the proportion of vaginal exfoliated cells using the vaginal tissue exfoliation cell smear method. Firstly fix the female rats and expose the rat's vagina. Next, a 10ul gun tip was used to aspirate 10ul of sterile saline, which was then gently rinsed in the rats' vagina 3–4 times. Finally, the vaginal fluid from the rat was evenly applied to the prepared slide. Use absolute ethanol to fix at room temperature for 10 min, and then carry out HE staining. The slides underwent a gentle rinse via the use of running water, followed by a shaking process for drying. Unbound hematoxylin was eluted by treatment with 1% hydrochloric acid-alcohol fractionation solution for 3 s, and then gently rinsed off with running water. Unbound hematoxylin was eluted by treatment with 1% hydrochloric acid-alcohol fractionation solution for 3 s, and then gently rinsed off with running water. Dye with eosin for 30 s, gently rinse with running water, air dry, and seal with neutral resin. Then under the microscope, we can observe changes in the cellular composition of vaginal smears and the estrous cycle of rats in each group. The staging judgment is as follows: Unkeratinized nucleated epithelial cells predominate in proestrus; In estrus, epithelial cells are mature and are predominantly anucleate keratinocytes; During the stage of metestrus, estrogen levels decrease and leukocytes begin to infiltrate, mainly consisting of leukocytes; All three types of cells are visible during diestrus [19].

Glucose tolerance test、insulin tolerance test and metabolic cage

Energy expenditure was measured in rats at 22 °C using a Columbus apparatus (Oxymax-clams). Rats were monitored for 72 h and provided unrestricted access to a standard diet and water. For glucose tolerance experiments, female rats were fasted for 12 h, watered ad libitum, and injected intraperitoneally with d -glucose (2g/kg body weight). Blood was taken from the tail, and blood glucose levels were measured in rats at 0, 15, 30, 60, 90, and 120 min after glucose injection, which was examined using an Accu—Chek type glucose monitor (Roche Diagnostics). For insulin resistance experiments, female SD rats were fasted for 4h, watered ad libitum, and injected intraperitoneally with insulin (0.75U / kg body weight). Blood glucose levels were measured before and 15 min, 30 min, 60 min, 90 min, and 120 min after insulin injection, respectively. It is worth noting that the ITT experiment was performed one week after the GTT.

RNA isolation and quantitative real-time PCR

The CDS sequence of the target gene was obtained from the National Center for Biotechnology Information (NCBI). Corresponding primers (Table S1) were designed using Primer6 software and synthesized by Kingsley Biotechnology after passing BLAST. Primer sequences are shown in Supplementary Table 1. RNA was extracted from the tissue using a TRIZOL reagent from Takara Corporation. The PrimeScript RT reagent Kit with gDNA Eraser (Takara Corp.) was used to reverse transcribe 2 μg of total RNA. Real-time quantitative PCR analysis was conducted using TB Green Premix Ex Taq II (Tli RNaseH Plus) (Takara Corporation). Each reaction was repeated three times, and the mean value was normalized by the housekeeping gene β-actin. Finally, the change in expression level was calculated using the 2^(-ΔΔct) method.

Western blot analysis

The tissue was dissolved in RIPA buffer (Beyotime) with a mixture of protease and phosphatase inhibitors (Roche Diagnostics). It was then milled for 30 min at -50 degrees Celsius, and the supernatant was extracted through centrifugation at 4 degrees Celsius and 13,000 rpm. The concentration of total protein was measured using the Quantitative BCA Protein Kit (Beyotime), and samples were boiled in 5X loading buffer (Beyotime). Total protein (30 μg/lane) was separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (yamei biotechnology) and transferred to a PVDF membrane on top of the membrane (sigma-aldrich), Then, everyblot Blocking buffer (Bio-Rad) was used for twenty minutes. The membranes were washed three times with TBST (Sevier TBS powder plus 0.1% Tween20) and then incubated overnight at 4 °C with primary antibodies, following the manufacturer's instructions.Use the following primary antibodies: AKT (1:1000;4685T, CST), p-AKT (Ser473, 1:1000;4060, CST), β-actin(1:20,000, 66,009–1-Ig, proteintech); The following day, the membranes were incubated with secondary antibodies at room

temperature for 1–1.5 h. Afterward, they were washed three times with TBST before developing luminescence using Super-sensitive ECL chemiluminescent substrate (biosharp). The intensity of each protein band was quantified relative to internal reference bands for β-actin.

Blood analysis

The SD rats were weighed and anesthetized with 20% urethane. Whole blood was then collected from the abdominal aorta by puncture, which was left at room temperature for 30 min and then centrifuged at 3,000 rpm for 15 min at 4℃. The serum from each group of female SD rats was stored in a refrigerator at -80 degrees. Subsequently, we tested hormone levels using rat ELAISA kit. Calculated using the formula: homeostatic model assessment of insulin resistance (HOMA-IR) = (FBG × insulin level)/22.5; and insulin sensitivity index (QUICKI) = 1/[lg (fasting insulin (μU/mL)) + lg (fasting glucose (mg/dL))]; Free androgen index (FAI) = (testosterone/SHBG) × 100%; Gonadosomatic index (GSI) = ovarian weight / total body weight × 100%; Atherosclerosis Index (AI) = ((TC − HDL-C) /HDL-C) × 100%.

Hematoxylin–eosin stain

Each rat's left ovary was removed, cleaned of adherent connective tissue, and fixed in 4% formaldehyde buffer for at least 24 h. Thereafter, the samples were dehydrated, embedded in paraffin and sliced from the maximum cross-section of the ovary and then 5 μ m thick sections were stained with hematoxylin–eosin (hematoxylin and eosin, HE) and photographed with an Olympus BX63 type biomicroscope. Follicles and corpora lutea can be recognized by their morphology, diameter, and cellular distribution status.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 9 software. One-way ANOVA and Two-way ANOVA, with Tukey's post hoc test, were used for comparisons between groups. $*P<0.05$, $**P<0.01$, $***P<0.001$. All data are expressed as mean ± SEM. $P<0.05$ was considered statistically significant.

Results

Chiglitazar reversed acyclicity and hormone concentrations in rats with PCOS

To elucidate the role of chiglitazar in PCOS, Sprague–Dawley rats were subjected to dehydroepiandrosterone (DHEA) for 15 days by subcutaneous injection to establish the PCOS model. Irregular menstruation is one of the key criteria for the diagnosis of PCOS [20]; Therefore, we analyzed the estrous cycle of rats by vaginal smear for 10 days, including two sequential cycles, to confirm the establishment of PCOS before subsequent evaluation of the therapeutic effects of chiglitazar and pioglitazone. We performed hematoxylin and eosin staining on vaginal secretion smears and recorded the characteristics of each period. There was a large number of nucleated epithelial cells and few keratinocytes in the proestrus. The estrus showed numerous pink keratinocytes and few nucleated epithelial cells. Keratinized cells and leukocytes were observed in the metestrus, and in the diestrus, leukocytes were abundant, and there was a meager amount of mucus (Supplementary Fig. 1A). The PCOS group showed a disrupted estrous cycle, with extended metestrus and diestrus phases and a reduced estrus phase (Fig. 1A). However, the estrous cycle of rats with PCOS in the treatment group was significantly ameliorated. Estrus began to appear after treatment with chiglitazar or pioglitazone, and in the DHEA + CHI and DHEA + PIO groups, the estrous cycle was much more regular than in DHEA group, with a sharp increase in estrus and percentage estrus (Fig. 1B). These findings suggest alleviation of ovulatory dysfunction with either chiglitazar or pioglitazone.

To confirm the treatment efficacy, we performed serologic tests. The testosterone to estradiol ratio in rats with PCOS increased significantly, while it sharply recovered after treatment with chiglitazar or pioglitazone (Fig. 1C). Moreover, there was a notable increase in the FAI in the DHEA group (Fig. 1I), despite no changes in SHBG (Supplementary Fig. 1B). In parallel, the concentrations of serum androgens in the DHEA group, such as testosterone (T), free testosterone (FT), dehydroepiandrosterone sulphate (DHEA-S), and androstenedione, were significantly higher than those in the healthy CON group,

(See figure on next page.)

Fig. 1 Chiglitazar reversed acyclicity and hormone concentrations in rats with polycystic ovary syndrome (PCOS). **A** Line plots of estrus cycles for each group ($n=8$). **B** The percentage of each stage in the estrus cycle. **C** The free androgens (FT) /estrogen (**E**) ratio. **D**–**I** The serum testosterone, free testosterone, DHEA-S, androstenedione, and estrogen levels in the fasting state. The FAI was calculated by the formula: (testosterone ÷ SHBG) × 100% ($n=6$). **J** Serum LH concentration ($n=6$). **K** LH/FSH ratio. All of the above were subjected to one-way analysis of variance with Tukey's post hoc test. $*P<0.05$, $**P<0.01$, $***P<0.001$. All data are expressed as the mean ± standard error of the mean. D, diestrus; E, estrus; M, metestrus; P, proestrus; E, estrogen; FT, free testosterone; FAI, free androgen index; LH, luteinizing hormone; FSH, follicle-stimulating hormone; DHEA-S, dehydroepiandrosterone sulphate

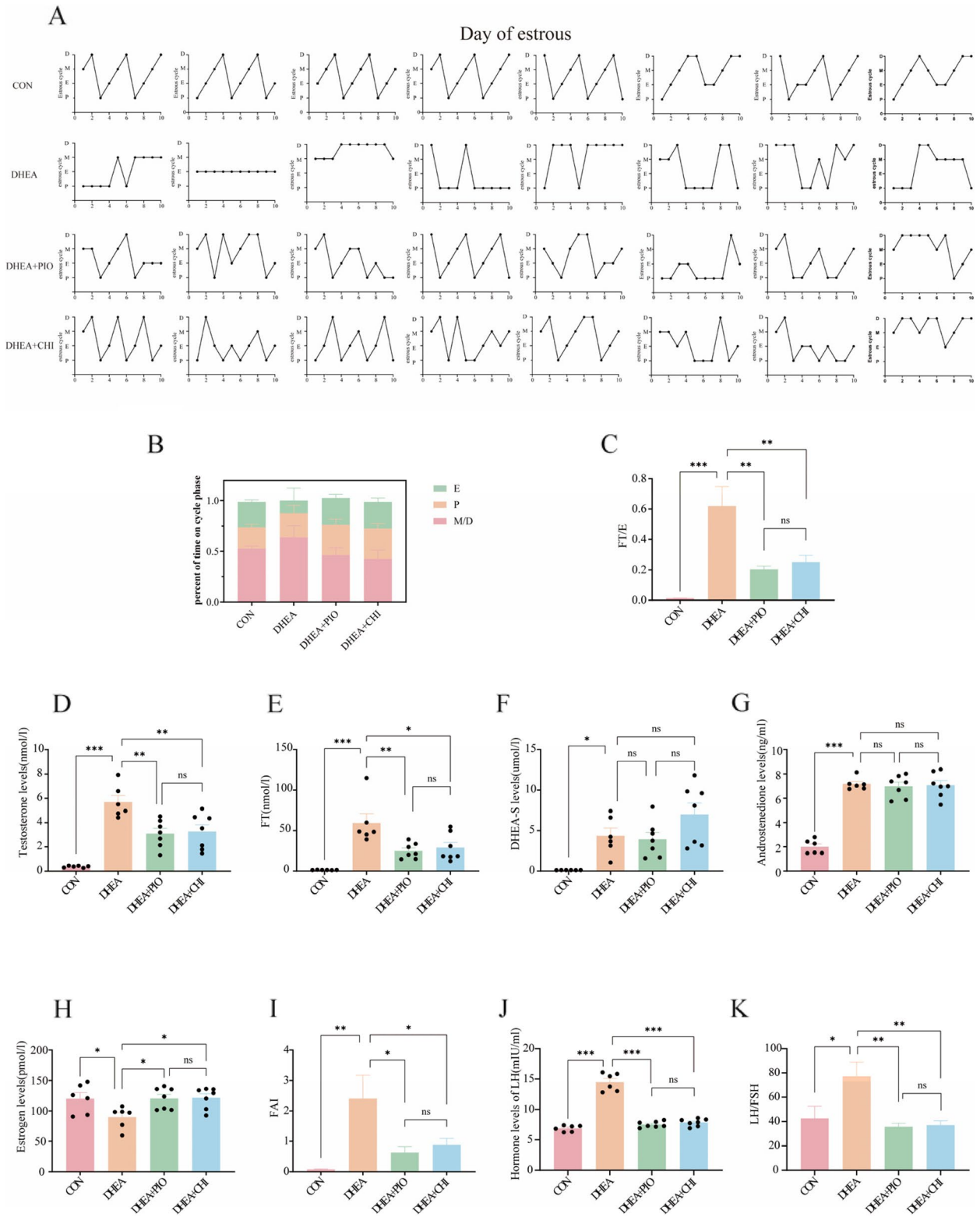

Fig. 1 (See legend on previous page.)

while the estrogen concentration was lower. However, after treatment with chiglitazar or pioglitazone, there was a significant decrease in plasma testosterone and free testosterone, and the estrogen concentration was elevated (Fig. 1D–H). There were no substantial alterations in DHEA-S and androstenedione after treatment, presumably because DHEA-S and androstenedione primarily originate from the adrenal gland [21] and chiglitazar and pioglitazone regulate the ovaries. As such, they have no effect or a weak effect on adrenal gland-derived androgens.

High luteinizing hormone (LH) and an elevated LH to follicle-stimulating hormone (FSH) ratio are recognized as biomarkers for the diagnosis of PCOS in women [22]. An increase in the concentration of LH facilitates the generation of testosterone and DHEA-S, leading to ovarian cyst formation [23]. Therefore, we assessed serum LH and FSH levels, indicated that although there was no FSH changes across groups (Supplementary Fig. 1C), the LH and LH/FSH ratio in the DHEA group were significantly higher than those in the CON group, and, promisingly, they were also higher than those in the DHEA + PIO and DHEA + CHI groups (Fig. 1J–K). These results illustrate that chiglitazar reversed the abnormal estrous cycle and hormone status in rats with PCOS, similar to pioglitazone.

Chiglitazar attenuated ovarian dysfunction in rats with PCOS

The ovaries of patients with PCOS exhibit a typical polycystic state. We analyzed the effects of chiglitazar on ovarian morphology in rats with DHEA-induced PCOS. Grossly, the ovarian polycystic state was evident in the DHEA group (Fig. 2A, Supplementary Fig. 1D). We also calculated the gonadal index, which was significantly reduced in the DHEA group (Fig. 2C). This may be attributed to the abnormally higher androgen concentration coupled with the reduced estrogen concentration, resulting in follicular atresia, ovarian aging, and reduced corpora lutea [24]. After treatment with pioglitazone or chiglitazar, there was discernible augmentation in ovarian volume, which occurred concomitantly with a rebound in ovarian weight (Fig. 2A–2C). Progesterone has a close relationship with the corpora lutea [25], and its biosynthesis is controlled by various enzymes, such as steroidogenic acute regulatory protein (STAR) [26], which transfers cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane, thereby providing substrates for steroid hormone biosynthesis. Notably, there was a decrease in progesterone in the DHEA group (Fig. 2E), and partially recovered in treatment groups which aligned with our hypothesis.

For a deeper understanding of the effects of PCOS treatment in rats, hematoxylin and eosin staining was performed on ovarian sections to analyze the morphological alterations in the ovaries. In the CON group, the ovaries displayed typical follicular membrane cell layers, mature follicles, and corpora lutea, with distinct cell structures and morphologies. While the DHEA group exhibited multiple enlarged cystic follicles with fewer granulosa cells and reduced corpora lutea (★) (Fig. 2D). Treatment with chiglitazar or pioglitazone reduced cystic follicles, increased granulosa cell layers, and normalized follicles in ovaries. Consistent with the histological findings, treatment with chiglitazar or pioglitazone significantly reversed the expression of the steroidogenic enzymes cytochrome P450 family 19 subfamily A (Cyp19a1) member, 3β-hydroxysteroid dehydrogenase, and STAR in the ovaries (Fig. 2F). The results of hormone analysis, hematoxylin and eosin staining, and ovarian steroidogenic enzyme expression all suggest that chiglitazar effectively alleviated PCOS symptoms, as did pioglitazone.

Chiglitazar reversed IR and metabolic abnormalities in rats with PCOS

Extensive evidence has shown that IR and compensatory hyperinsulinemia resulting in hyperandrogenemia and reproductive dysfunction are major factors driving the pathogenesis of PCOS [1]. We explored the related indicators, and the results revealed that the DHEA group exhibited no notable change in the fasting glucose concentration compared with the CON group (Supplemental Fig. 2A). Nonetheless, the glucose tolerance test (GTT) and insulin tolerance test (ITT) showed that glucose tolerance and insulin sensitivity were significantly reduced in the DHEA group which improved with pioglitazone or chiglitazar treatment (Fig. 3A–D). Subsequently, we evaluated the serum insulin concentration in rats. In the DHEA group, the serum insulin concentration was significantly higher than in the CON group and there was marked hyperinsulinemia. However, after treatment with chiglitazar or pioglitazone, the insulin concentration recovered significantly (Fig. 4A). Furthermore, by calculating the IR index (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI), we found that both chiglitazar and pioglitazone improved severe insulin resistance, with over 50% reduction in HOMA-IR and normalization of QUICKI (Fig. 4C, D). Additionally, adiponectin levels (Fig. 4B) were restored, indicating that chiglitazar enhanced glucose tolerance and insulin responsiveness. Therefore, chiglitazar may also be a desirable therapeutic option for metabolic irregularities.

Next, we explored the influence of chiglitazar on energy metabolism. Chiglitazar or pioglitazone treatment

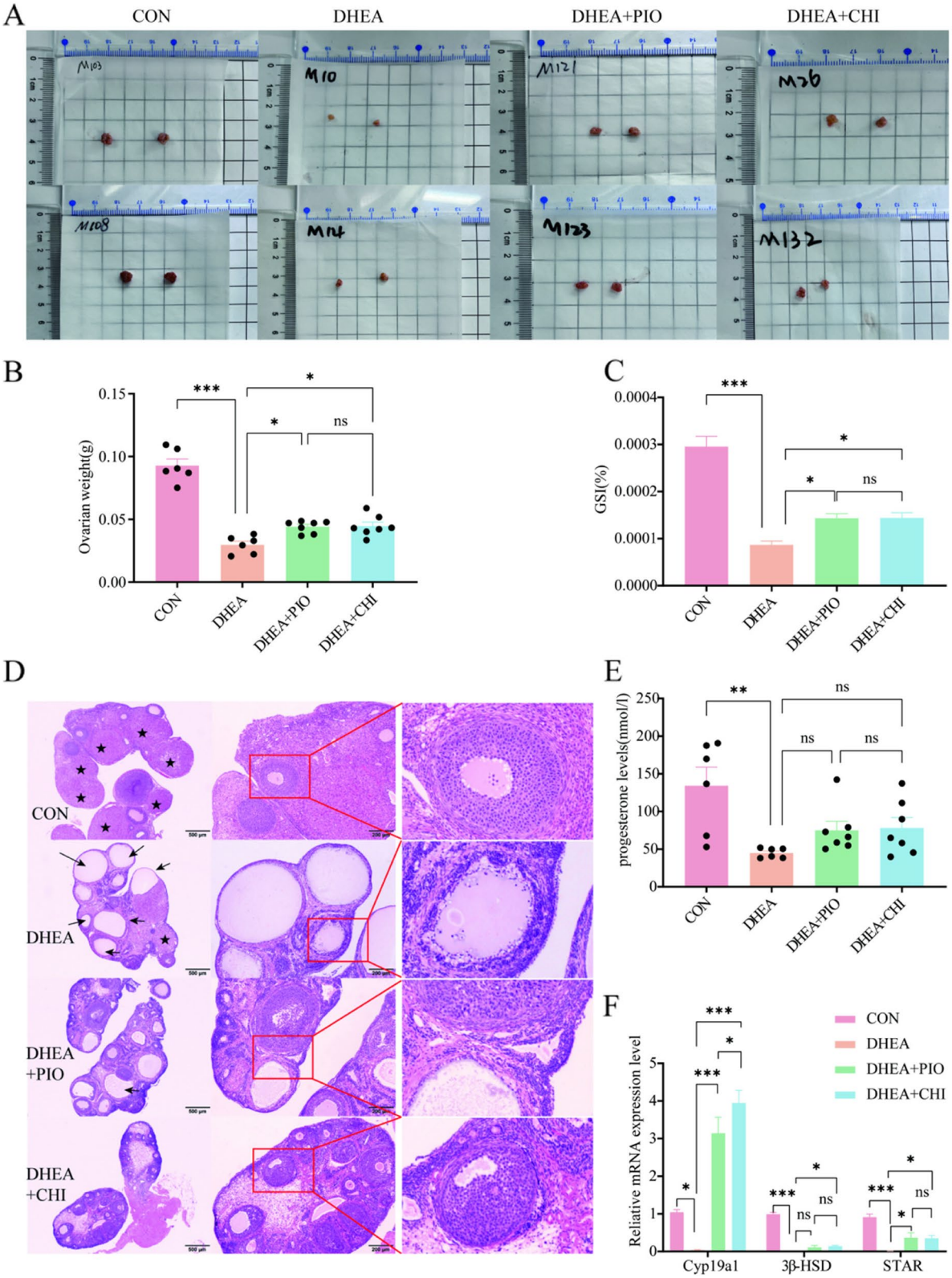

Fig. 2 Chiglitazar attenuated ovarian dysfunction in rats with polycystic ovary syndrome (PCOS). **A** Representative ovarian morphology. **B** Ovarian weight. **C** GSI calculated in rats based on the weight of the ovaries. **D** Ovarian histology using hematoxylin and eosin staining. **E** Serum progesterone. **F** Ovarian steroidogenic enzyme mRNA expression. All of the above were subjected to one-way analysis of variance with Tukey's post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. All data are expressed as the mean ± standard error of the mean. GSI, gonadosomatic index

elevated oxygen consumption and carbon dioxide production (Fig. 3E, F, I) and rebounded DHEA-induced nocturnal energy expenditure in the DHEA group with PCOS. Chiglitazar and pioglitazone enhanced caloric consumption and energy metabolism in DHEA-induced PCOS rats (Fig. 3G, H), accompanied by an increasing trend in activity (Supplementary Fig. 2C), which significantly improved energy metabolism. Notably, weight gain after chiglitazar treatment was significantly lower than in the DHEA + PIO group (Fig. 3J), despite no significant difference in food consumption between the groups (Supplementary Fig. 2B).

We scrutinized pertinent indicators of serum lipid metabolism to assess the therapeutic efficacy of chiglitazar and pioglitazone. Not surprisingly, FFAs were significantly reduced after both treatments (Fig. 4G). Cholesterol and triglycerides exhibited a significant reduction in the DHEA + CHI group compared with the DHEA group. Notably, the downward trend in the DHEA + PIO group was not as pronounced as that in the DHEA + CHI group (Fig. 4E–F). Our findings also unveiled notable abnormalities in both high-density lipoprotein (HDL) and low-density lipoprotein (LDL) concentrations in the DHEA group. After treatment, there was a trend toward an improvement in both HDL and LDL (Fig. 4I, J), the importance of which should be clarified in future research with larger sample sizes. Moreover, studies have indicated that PCOS can lead to atherosclerotic conditions [27]. Therefore, we calculated the atherosclerosis index, which is an indicator of atherosclerosis [28] and observed enhancements after both treatments. However, Chiglitazar therapy showed greater enhancement in atherosclerosis index improvement (Fig. 4H). These findings indicate that chiglitazar reverses IR, energy metabolism, and lipid metabolism and compared with pioglitazone, chiglitazar had a more pronounced influence on metabolism.

Chiglitazar protected from PCOS via Akt phosphorylation

Both chiglitazar and pioglitazone enhanced tissue insulin sensitivity. Akt is the best characterized AGC kinase in the insulin signaling pathway, and its targeted deletion produces IR and glucose intolerance [29]. Hence, we explored whether chiglitazar and pioglitazone could ameliorate PCOS symptoms by activating Akt phosphorylation. We compared the changes in p-Akt and Akt expression between the DHEA group and the treatment groups via Western blotting of Akt expression in muscle, adipose, and liver tissues from rats. The total Akt protein expression in the skeletal muscle, adipose tissue, and liver was not significantly different among the groups. Akt phosphorylation was significantly lower in the DHEA group, while after treatment, p-Akt expression was upregulated, indicating that chiglitazar enhanced Akt phosphorylation and improved insulin sensitivity (Fig. 5A–C). Furthermore, there was no discernible difference compared with the DHEA + PIO group.

In adipocytes, GLUT4 serves as an indicator of systemic insulin sensitivity [30] and it plays a key role in glucose sensing. Several studies have indicated that GLUT4 translocation stimulated by insulin is reliant on the phosphorylation of Akt substrate (AS160) [31]. Some studies have indicated that an increase in GLUT4 mRNA expression is accompanied by a marked improvement in IR, menstrual pattern, and androgen spectrum [32]. Consequently, we analyzed the mRNA expression of GLUT4 in the adipose tissue and found that GLUT4 mRNA expression was reduced in the DHEA group but increased with chiglitazar or pioglitazone treatment in adipose tissue (Supplementary Fig. 3D). This suggests that pioglitazone and chiglitazar may improve tissue insulin sensitivity and alleviate PCOS symptoms by enhancing Akt phosphorylation and increasing GLUT4 expression.

Discussion

PCOS is a significant global health issue, yet existing treatment modalities are limited and primarily focus on managing symptoms, often with noticeable side effects. Previous studies have indicated that oral contraceptives are effective for controlling menstruation and treating acne in patients with PCOS. However, formulations incorporating drospirenone are associated with an elevated incidence of thrombotic events [33]. Anti-androgen medications are another viable option for managing hirsutism and acne in patients with PCOS, though their application is restricted by their teratogenic properties

(See figure on next page.)

Fig. 3 Chiglitazar reversed insulin resistance (IR) and metabolic abnormalities in rats with polycystic ovary syndrome (PCOS). **A**–**D** Glucose concentration and area under the curve in the control group, DHEA group, and treatment groups during the GTT and ITT ($n = 5$). **E**–**I** Metabolic cage experiments were performed using Sprague–Dawley rats, including oxygen consumption rate (VO_2), heat production, and carbon dioxide production (VCO_2) ($n = 4$). **J** Weight gain from baseline of Sprague–Dawley rats in the control, modeling, and treatment groups ($n = 7$). Two-way analysis of variance was performed in A, C, E and G. One-way analysis of variance with Tukey's post hoc test was performed in B, D, F, H, I, and J. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. All data are presented as the mean ± standard error of the mean. GTT, glucose tolerance test; ITT, insulin resistance test

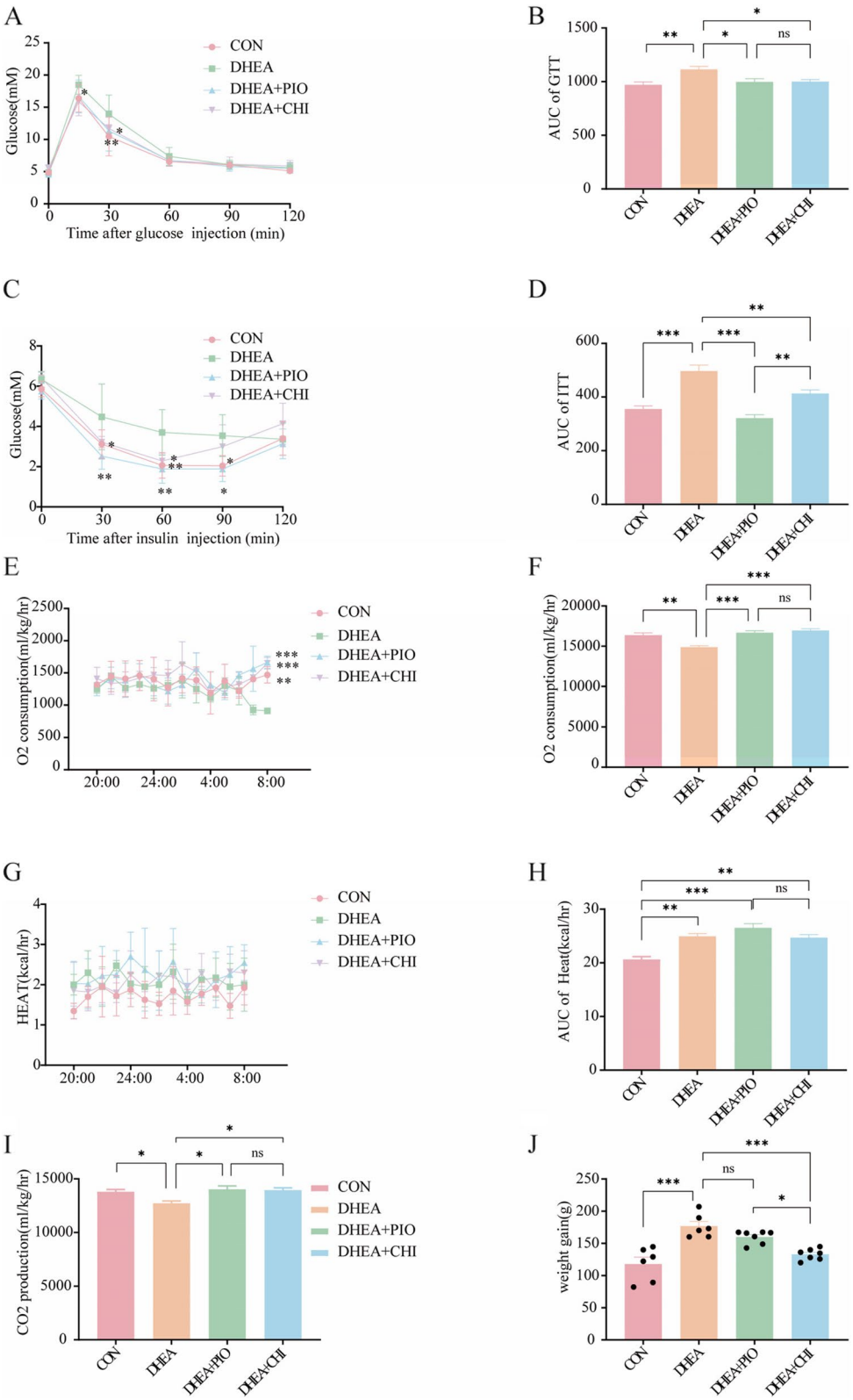

Fig. 3 (See legend on previous page.)

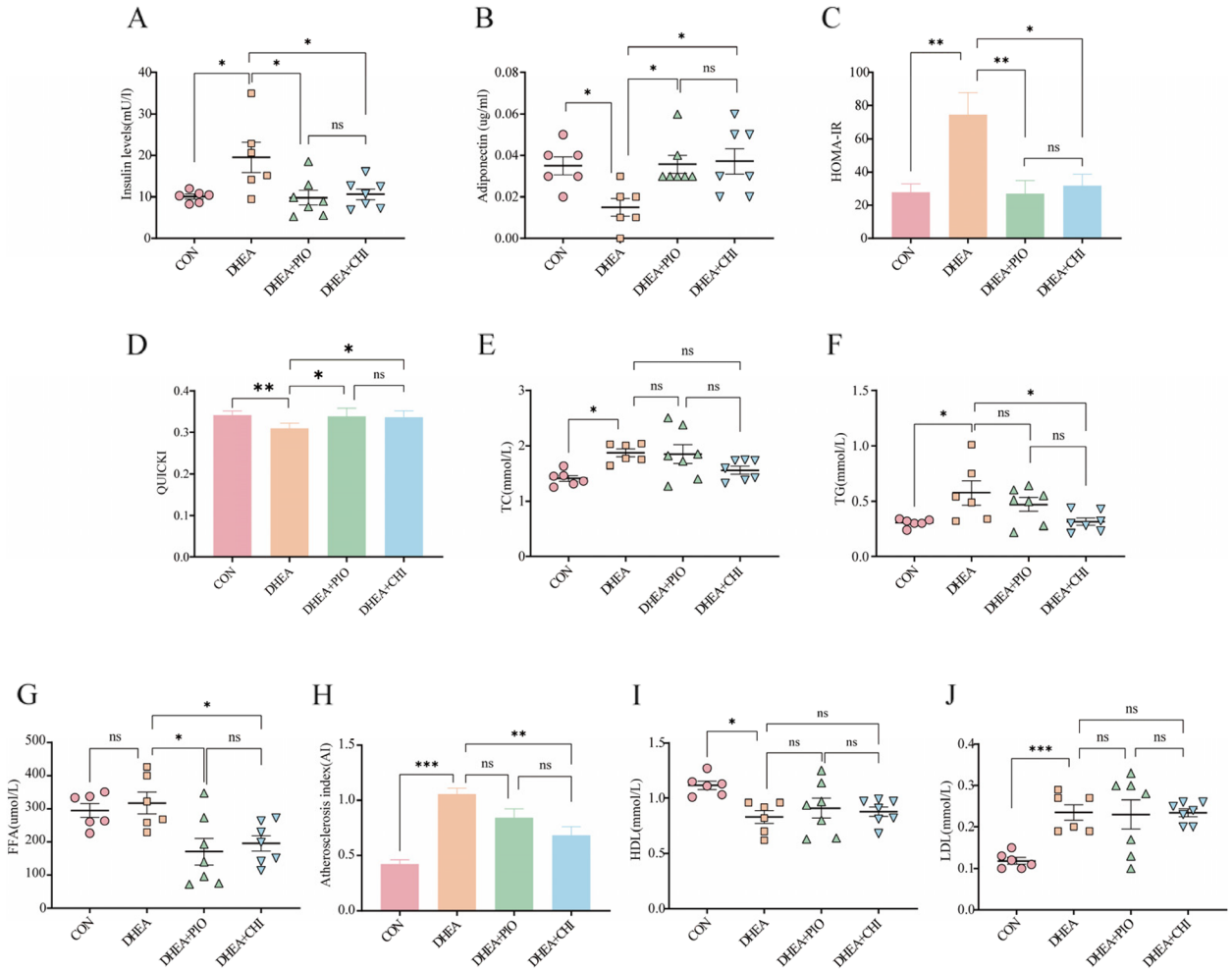

Fig. 4 Chiglitazar reversed insulin resistance (IR) and metabolic abnormalities in rats with polycystic ovary syndrome (PCOS). The serum Insulin (**A**) and adiponectin (**B**) levels. **C** HOMA-IR was calculated according to the formula, HOMA-IR = (FBG × insulin level)/22.5 test. **D** QUICKI was calculated according to the formula, insulin sensitivity index (QUICKI) = 1/[lg(fasting insulin (μU/mL)) + lg(fasting glucose (mg/dL))]. **E**–**G** Serum cholesterol (TC), triglyceride (TG), free fatty acid (FFA) concentration in each group. **H** Atherosclerosis index (AI) was calculated according to the formula, AI = [TC—HDL] ÷ HDL. Serum HDL (**I**) and LDL (**J**) concentrations. All of the above were subjected to one-way analysis of variance with Tukey's post hoc test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. All data are expressed as the mean ± standard error of the mean. HDL, high-density lipoprotein; LDL, low-density lipoprotein

[6]. GnRH antagonists, including cetrorelix and danazol, treat PCOS but may also disrupt estrogen production, risking infertility [34]. Metformin treatment is also limited because it causes mild to moderate changes in the menstrual cycle and hyperandrogenemia as well as nausea and diarrhea [6, 35]. Thiazolidinedione drugs, such as pioglitazone, improve menstruation and reproductive hormone concentrations, but they are associated with weight gain and potential pregnancy risks [6]. While GLP-1 enhances insulin sensitivity, it also intensifies menstrual disturbances and raises FAI, accompanied by gastrointestinal upset. What's more, its impact on reducing insulin resistance is less significant than that of pioglitazone [6]. Statins appear to ameliorate hyperandrogenism by diminishing dihydrotestosterone and testosterone production, and by curbing cancer cell proliferation [6]. Nonetheless, they are associated with significant adverse effects, including myopathy, irregular hepatic function, and congenital abnormalities. Hence, there is an urgent need to develop more effective treatment methods for PCOS. They are associated with significant adverse effects, including myopathy, irregular hepatic function, and congenital abnormalities. Hence, there is an urgent need to develop more effective treatment methods for PCOS.

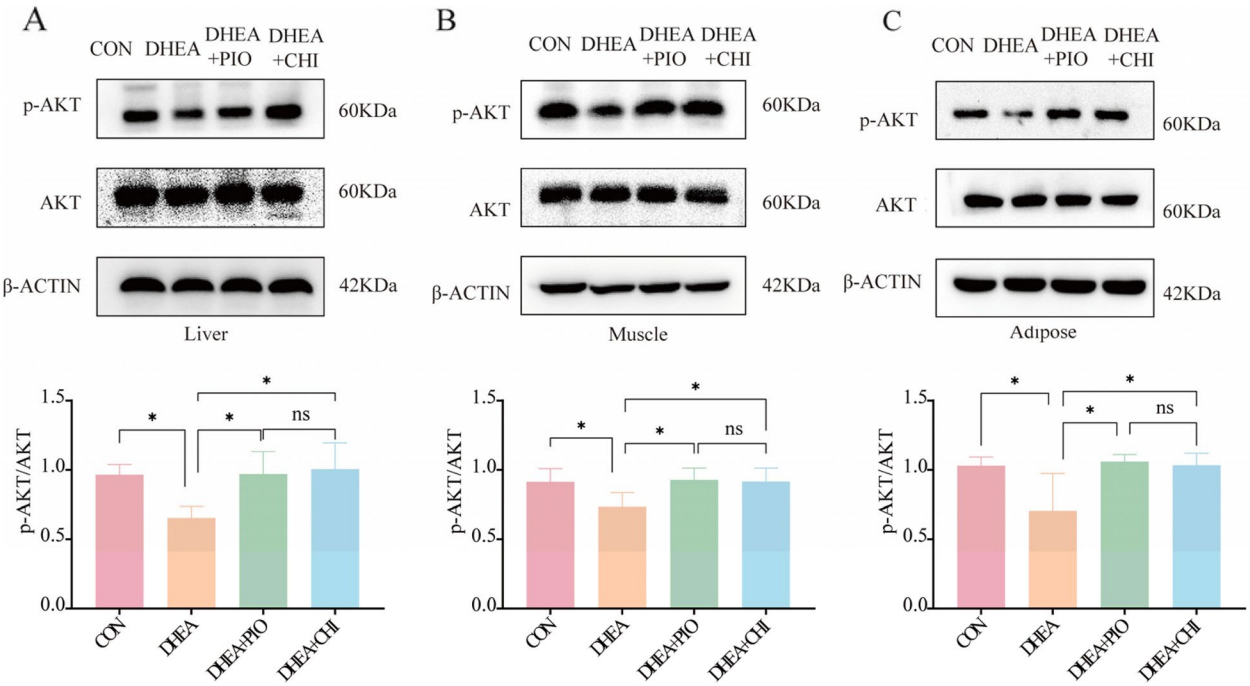

Fig. 5 Chiglitazar protected from polycystic ovary syndrome (PCOS) via Akt phosphorylation. Protein expression of Akt and p-Akt in (**A**) liver tissue, (**B**) muscle tissue, and (**C**) adipose tissue. All of the above were subjected to one-way analysis of variance with Tukey's post hoc test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. All data are expressed as the mean ± standard error of the mean

The PPAR agonist chiglitazar is an excellent insulin sensitizer. It demonstrates efficacy that is commensurate with that of pioglitazone, while showcasing favorable clinical results and safety [36]. Moreover, it significantly reduces the side effects of thiazolidinediones, proving highly effective in the treatment of type 2 diabetes mellitus and dyslipidemia [37]. In the present study, we discovered that chiglitazar ameliorated PCOS symptoms in rats, including endocrine disorders, ovarian dysfunction, and IR. Meanwhile, though there was no significant difference in the treatment effect compared with pioglitazone, the regulatory effect of chiglitazar on lipid metabolism in rats with PCOS was more pronounced, which would benefit from confirmation in future research based on a larger sample size. Furthermore, the present study revealed that chiglitazar substantially enhanced Akt phosphorylation and adiponectin expression, providing a potential mechanistic explanation for the protective effects of chiglitazar in PCOS. To our best knowledge, this study is the first to show that chiglitazar is associated with PCOS in an animal model and that it significantly improves PCOS symptoms.

Previous research has indicated a robust correlation between decreased PPAR function and the manifestation of PCOS symptoms, including polycystic ovaries and irregular estrous cycles [18]. Consequently, PPAR agonists have emerged as promising therapeutic agents for PCOS. Our research indicates that the activation of PPAR targets by chiglitazar or pioglitazone can effectively ameliorate PCOS symptoms. Rats with PCOS treated with the pan-PPAR agonist chiglitazar showed gradual restoration of the estrous cycle from a stagnant state to a regular state, along with an overall enhancement in ovarian health, including an increased number of corpora lutea and granulosa cell layers. These findings illustrate that chiglitazar can regulate follicle development, ovulation, oocyte maturation, and corpus luteum maintenance. At the molecular level, PPAR-γ activation controls the expression of genes that are essential for follicular growth, ovulation, oocyte maturation, and corpus luteum maintenance [38]. Moreover, PPAR-γ activates cytochrome P450 aromatase (CYP19A1) to transform androgens into estrogens, as well as restoring LH through negative feedback, leading to reduced LH concentrations [38] and preserving ovarian energy equilibrium [39]. Consistent with this, the expression of ovarian steroidogenic enzymes increased after treatment with chiglitazar, and the LH/FSH ratio in rats with PCOS returned to normal.

We also explored the mechanism through which chiglitazar displayed beneficial therapeutic effects in PCOS. It has been widely appreciated that women with PCOS show IR and glucose intolerance [40]. IR leads to an increased insulin concentration, which in turn reduces

the hepatic synthesis of SHBG, stimulates excessive androgen production by the ovaries, and disrupts normal follicular development, resulting in ovulation disorders [41]. Hence, enhancing IR can bolster the body's insulin responsiveness, allowing the tissues to more effectively absorb and utilize insulin. This results in a decreased insulin concentration and reduced androgen synthesis, and it aids in the restoration of ovarian function and normal estrogen concentrations. Patients with PCOS often exhibit metabolic abnormalities, including obesity, hyperglycemia, and hyperlipidemia, among other conditions. Enhancing insulin sensitivity can mitigate adipocyte resistance to insulin and facilitate glucose utilization and lipid metabolism, resulting in weight reduction, enhancing glycemic control, improving the lipid profile, and ultimately ameliorating metabolic dysregulation. We observed a significant increase in body weight in the DHEA group in the present study. This may be due to the apparent IR in rats with PCOS. IR makes it difficult for the body to utilize glucose, leading to its accumulation and conversion to fat, in turn resulting in abnormal energy metabolism and weight gain. PPAR agonists regulate gene expression linked to energy metabolism, and they participate in thermogenesis, simultaneously boosting IR, elevating activity rates, and thereby boosting internal energy metabolism. This balances the body's energy intake and expenditure and improves weight gain. It is notable that following chiglitazar treatment, rats with PCOS exhibited a significant reduction in weight gain, whereas rats treated with pioglitazone did not demonstrate any significant change in weight gain. This may be because chiglitazar, as a full PPAR agonist, achieves well-balanced activation of the PPAR-α, -δ, and -γ isoforms. The increased fat oxidation promoted by PPAR-α and PPAR-δ counteracts the negative effects of PPAR-γ, such as obesity or weight gain [37], improves insulin sensitivity, and regulates glucose and lipid metabolism [42]. PPAR-γ is involved in glycolipid metabolism and insulin sensitivity regulation [43]. PPAR-γ agonists indirectly reduce androgen synthesis in the ovaries by improving peripheral IR [44]. PPAR-α, which is an important regulator of lipid metabolism, ameliorates IR by reducing glucose inhibition through lowering peripheral fatty acids. Meanwhile, PPAR-α stimulates insulin secretion by inhibiting calcium signaling [45]. PPAR-δ is widely distributed in somatic cells, and somatic cell activation is involved in elevated insulin sensitivity [46]. This significantly improves pancreatic islet function, increases β-cell quality [47], and reverses metabolic abnormalities. Moreover, PPAR-α and PPAR-δ reduce plasma triglycerides, cholesterol, and FFA concentrations [7], which may have unexpected effects in patients with PCOS with high blood lipids. Consistent with previous studies, after treatment with chiglitazar or pioglitazone, abnormal glucose tolerance and IR significantly improved. Additionally, compared with the DHEA + PIO group, the DHEA + CHI group showed more significant improvements in lipid metabolism, with notable decreases in triglycerides, cholesterol, and atherosclerosis index.

Insulin signal transduction is achieved through the PI3K/Akt signaling pathway, in which Akt serves as the crucial mediator of insulin-stimulated GLUT4 translocation and glucose transport [48]. We therefore examined the alterations in Akt expression in the tissues associated with insulin sensitivity and demonstrated that Akt phosphorylation significantly rebounded, and GLUT4 mRNA expression significantly increased after chiglitazar treatment. Therefore, we suspect that chiglitazar enhances insulin responsiveness, potentially by increasing Akt phosphorylation and GLUT4 expression. Moreover, we postulate that adiponectin may be partially responsible for the ameliorative effects of chiglitazar on IR. Adiponectin is an adipocytokine secreted by the adipose tissue [49]. It has been shown that treatment with adiponectin directly improves hyperglycemia, increases insulin receptor expression [50], and restores ovulation [46]. It has also been demonstrated that the beneficial effects of brown adipose tissue transplantation on PCOS are mediated in part by elevated circulating adiponectin [51]. Moreover, previous studies have shown that adiponectin can significantly increase the expression of ovulation-related genes and enhance the induction of FSH-mediated Areg, Has2, and Ptgs2 [49, 50]. Furthermore, Hazrati et al.reported that the PCOS-induced mice treated with adiponectin represented an increase in CYP19A and promote ovarian development, follicular growth, and ovulation [53]. In vitro experiments have shown that adiponectin inhibits the production of androstenedione and LH receptor expression, as well as inhibiting key enzymatic components of the androgen pathway [54]. Interestingly, PPAR facilitates adipocytes to generate adiponectin [55]. Thus, chiglitazar, as a pan-PPAR agonist, stimulates adiponectin production in the adipose tissue, inhibiting androstenedione production, the expression of key enzymes associated with the LH receptor, and androgen synthesis, providing plausible explanations for the improvement in the PCOS phenotype. In agreement with this, adiponectin was significantly upregulated in the DHEA + CHI and DHEA + PIO groups compared with the DHEA group. Hence, from one perspective, chiglitazar is thought to enhance insulin sensitivity in the liver and peripheral tissues to ameliorate PCOS, while from another perspective, chiglitazar may improve PCOS by increasing adiponectin. However, the precise mechanisms underlying these phenomena still require further exploration.

Conclusions

This research significantly contributes to the understanding of the usefulness of chiglitazar in PCOS, demonstrating that it has a positive impact on productive and reproductive hormones, blood glucose, lipid profile, body weight, estrous cycle, and ovarian morphology, and this effect was thought to be associated with Akt phosphorylation, GLUT4 expression, and adiponectin concentration.

Abbreviations

PCOS	Polycystic ovary syndrome
DHEA	Dehydroepiandrosterone
CON	Control
PIO	Pioglitazone
CHI	Chiglitazar
PPAR	Peroxisome proliferator-activated receptor
GLUT4	Glucose transporter 4
GTT	Glucose tolerance test
ITT	Insulin Tolerance Test
HI	Hyperinsulinemia
T3	Triiodothyronine
T4	Thyroxine
IGF1	Insulin-like growth factor 1 receptor
GSI	Gonadosomatic index
D	Diestrus
E	Estrus
M	Metestrus
P	Proestrus
T	Testosterone
E	Estrogen
FT	Free testosterone
FAI	Free androgen index
SHBG	Sex hormone-binding globulin
LH	Luteinizing hormone
FSH	Follicle-stimulating hormone
DHEA-S	Dehydroepiandrosterone sulphate

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13048-024-01554-6.

Supplementary Material 1.

Acknowledgements

We express our gratitude to the dedicated staff and participants involved in this study for their valuable contributions.

Authors' contributions

Z designed the research program, performed experimental operations, conducted data analysis and interpretation of results, and played an important role in the process of writing the paper. C and F assisted in the animal experiment. L and Z contributed to conceptualization, writing - review and editing, supervision, project administration. All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by grants from National Natural Science Foundation of China (82000794).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]School of Medicine, Chongqing University, Chongqing 400044, China. [2]Department of Endocrinology, Chongqing University Three Gorges Hospital, Chongqing 404000, China. [3]Department of Endocrinology, the Second Affiliated Hospital, Chongqing Medical University, Chongqing 400010, China. [4]Chongqing Municipality Clinical Research Center for Endocrinology and Metabolic Diseases, Chongqing University Three Gorges Hospital, Chongqing 404000, China. [5]Department of Central Laboratory, School of Medicine, Chongqing University Three Gorges Hospital, Chongqing University, Chongqing 40400, China.

Received: 27 June 2024 Accepted: 6 November 2024
Published online: 19 November 2024

References

1. Walters KA, Bertoldo MJ, Handelsman DJ. Evidence from animal models on the pathogenesis of PCOS. Best Pract Res Clin Endocrinol Metab. 2018;32:271–81.
2. Khan MJ, Ullah A, Basit S. Genetic basis of Polycystic Ovary Syndrome (PCOS): current perspectives. Appl Clin Genet. 2019;12:249–60.
3. Fauser BCJM, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, et al. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertil Steril. 2012;97:28-38.e25.
4. Bannigida DM, Nayak BS, Vijayaraghavan R. Insulin resistance and oxidative marker in women with PCOS. Arch Physiol Biochem. 2020;126:183–6.
5. Joshi M, Shankar R, Pathak K, Yadav R. Polycystic ovarian syndrome: a review covering phytoconstituents for its outstrip management. Pharmacol Res - Modern Chin Med. 2021;1:100011.
6. Vitek W, Alur S, Hoeger KM. Off-label drug use in the treatment of polycystic ovary syndrome. Fertil Steril. 2015;103:605–11.
7. Li P-P, Shan S, Chen Y-T, Ning Z-Q, Sun S-J, Liu Q, et al. The PPARalpha/gamma dual agonist chiglitazar improves insulin resistance and dyslipidemia in MSG obese rats. Br J Pharmacol. 2006;148:610–8.
8. Liu L, Sun W, Tang X, Zhen D, Guan C, Fu S, et al. Chiglitazar attenuates high-fat diet-induced nonalcoholic fatty liver disease by modulating multiple pathways in mice. Mol Cell Endocrinol. 2024;593: 112337.
9. Tyagi S, Gupta P, Saini AS, Kaushal C, Sharma S. The peroxisome proliferator-activated receptor: a family of nuclear receptors role in various diseases. J Adv Pharm Technol Res. 2011;2:236–40.
10. Zhang Y, Xu J, Zhou D, Ye T, Zhou P, Liu Z, et al. Swimming exercise ameliorates insulin resistance and nonalcoholic fatty liver by negatively regulating PPARγ transcriptional network in mice fed high fat diet. Mol Med. 2023;29:150.
11. Banke NH, Wende AR, Leone TC, O'Donnell JM, Abel ED, Kelly DP, et al. Preferential oxidation of triacylglyceride-derived fatty acids in heart is augmented by the nuclear receptor PPARalpha. Circ Res. 2010;107:233–41.
12. Koh EH, Kim M-S, Park J-Y, Kim HS, Youn J-Y, Park H-S, et al. Peroxisome proliferator-activated receptor (PPAR)-alpha activation prevents diabetes in OLETF rats: comparison with PPAR-gamma activation. Diabetes. 2003;52:2331–7.
13. Smati S, Régnier M, Fougeray T, Polizzi A, Fougerat A, Lasserre F, et al. Regulation of hepatokine gene expression in response to fasting and feeding: influence of PPAR-α and insulin-dependent signalling in hepatocytes. Diabetes Metab. 2020;46:129–36.
14. Chen X, He H, Long B, Wei B, Yang P, Huang X, et al. Acupuncture regulates the apoptosis of ovarian granulosa cells in polycystic ovarian syndrome-related abnormal follicular development through LncMEG3-mediated inhibition of miR-21-3p. Biol Res. 2023;56:31.

15. Stener-Victorin E, Padmanabhan V, Walters KA, Campbell RE, Benrick A, Giacobini P, et al. Animal models to understand the etiology and pathophysiology of polycystic ovary syndrome. Endocr Rev. 2020;41:bnaa010.
16. Jiang Y-C, Ma J-X. The role of MiR-324-3p in polycystic ovary syndrome (PCOS) via targeting WNT2B. Eur Rev Med Pharmacol Sci. 2018;22:3286–93.
17. Wu Y, Li P, Zhang D, Sun Y. Metformin and pioglitazone combination therapy ameliorate polycystic ovary syndrome through AMPK/PI3K/JNK pathway. Exp Ther Med. 2018;15:2120–7.
18. Suriyakalaa U, Ramachandran R, Doulathunnisa JA, Aseervatham SB, Sankarganesh D, Kamalakkannan S, et al. Upregulation of Cyp19a1 and PPAR-γ in ovarian steroidogenic pathway by Ficus religiosa: a potential cure for polycystic ovary syndrome. J Ethnopharmacol. 2021;267:113540.
19. Ji S, Yang H, Ji Y, Wu W, Dong Y, Fu H, et al. Liraglutide improves PCOS symptoms in rats by Targeting FDX1. Reprod Sci. 2024;31:2049–58.
20. Ibáñez L, de Zegher F. Adolescent PCOS: a postpubertal central obesity syndrome. Trends Mol Med. 2023;29:354–63.
21. Misiak B, Frydecka D, Loska O, Moustafa AA, Samochowiec J, Kasznia J, et al. Testosterone, DHEA and DHEA-S in patients with schizophrenia: a systematic review and meta-analysis. Psychoneuroendocrinology. 2018;89:92–102.
22. Taylor AE, McCourt B, Martin KA, Anderson EJ, Adams JM, Schoenfeld D, et al. Determinants of abnormal gonadotropin secretion in clinically defined women with polycystic ovary syndrome. J Clin Endocrinol Metab. 1997;82:2248–56.
23. Rosenfield RL, Ehrmann DA. The Pathogenesis of Polycystic Ovary Syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocr Rev. 2016;37:467–520.
24. Hsueh AJ, Billig H, Tsafriri A. Ovarian follicle atresia: a hormonally controlled apoptotic process. Endocr Rev. 1994;15:707–24.
25. Moolhuijsen LME, Visser JA. Anti-müllerian hormone and ovarian reserve: update on assessing ovarian function. J Clin Endocrinol Metab. 2020;105:3361–73.
26. Chang H-M, Cheng J-C, Klausen C, Leung PCK. BMP15 suppresses progesterone production by down-regulating StAR via ALK3 in human granulosa cells. Mol Endocrinol. 2013;27:2093–104.
27. González F, Rote NS, Minium J, Kirwan JP. Evidence of proatherogenic inflammation in polycystic ovary syndrome. Metabolism. 2009;58:954–62.
28. Zhou W, Li S, Sun G, Song L, Feng W, Li R, et al. Early Warning of Ischemic Stroke Based on Atherosclerosis Index Combined With Serum Markers. J Clin Endocrinol Metab. 2022;107:1956–64.
29. Saltiel AR. Insulin signaling in health and disease. J Clin Invest. 2021;131(e142241): 142241.
30. Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, et al. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. Nature. 2001;409:729–33.
31. Larance M, Ramm G, Stöckli J, van Dam EM, Winata S, Wasinger V, et al. Characterization of the role of the Rab GTPase-activating protein AS160 in insulin-regulated GLUT4 trafficking. J Biol Chem. 2005;280:37803–13.
32. Jensterle M, Janez A, Mlinar B, Marc J, Prezelj J, Pfeifer M. Impact of metformin and rosiglitazone treatment on glucose transporter 4 mRNA expression in women with polycystic ovary syndrome. Eur J Endocrinol. 2008;158:793–801.
33. Hoeger KM, Dokras A, Piltonen T. Update on PCOS: consequences, challenges, and guiding treatment. J Clin Endocrinol Metab. 2021;106:e1071-83.
34. Kulkarni S, Gupta K, Ratre P, Mishra PK, Singh Y, Biharee A, et al. Polycystic ovary syndrome: current scenario and future insights. Drug Discovery Today. 2023;28:103821.
35. Ma R-L, Deng Y, Wang Y-F, Zhu S-Y, Ding X-S, Sun A-J. Short-term combined treatment with exenatide and metformin for overweight/obese women with polycystic ovary syndrome. Chin Med J (Engl). 2021;134:2882–9.
36. Cheng HS, Tan WR, Low ZS, Marvalim C, Lee JYH, Tan NS. Exploration and development of PPAR modulators in health and disease: an update of clinical evidence. Int J Mol Sci. 2019;20:5055.
37. Gross B, Pawlak M, Lefebvre P, Staels B. PPARs in obesity-induced T2DM, dyslipidaemia and NAFLD. Nat Rev Endocrinol. 2017;13:36–49.
38. Froment P, Gizard F, Defever D, Staels B, Dupont J, Monget P. Peroxisome proliferator-activated receptors in reproductive tissues: from gametogenesis to parturition. J Endocrinol. 2006;189:199–209.
39. Dupont J, Chabrolle C, Ramé C, Tosca L, Coyral-Castel S. Role of the peroxisome proliferator-activated receptors, adenosine monophosphate-activated kinase, and adiponectin in the ovary. PPAR Res. 2008;2008:176275.
40. Legro RS. Polycystic ovary syndrome and cardiovascular disease: a premature association? Endocr Rev. 2003;24:302–12.
41. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev. 2012;33:981–1030.
42. Deeks ED. Chiglitazar: first approval. Drugs. 2022;82:87–92.
43. Tang H, Zeng Q, Tang T, Wei Y, Pu P. Kaempferide improves glycolipid metabolism disorder by activating PPARγ in high-fat-diet-fed mice. Life Sci. 2021;270:119133.
44. Schoppee PD, Garmey JC, Veldhuis JD. Putative activation of the peroxisome proliferator-activated receptor gamma impairs androgen and enhances progesterone biosynthesis in primary cultures of porcine theca cells. Biol Reprod. 2002;66:190–8.
45. Ropero AB, Juan-Picó P, Rafacho A, Fuentes E, Bermúdez-Silva FJ, Roche E, et al. Rapid non-genomic regulation of Ca2+ signals and insulin secretion by PPAR alpha ligands in mouse pancreatic islets of Langerhans. J Endocrinol. 2009;200:127–38.
46. Jung TW, Lee SH, Kim H-C, Bang JS, Abd El-Aty AM, Hacımüftüoğlu A, et al. METRNL attenuates lipid-induced inflammation and insulin resistance via AMPK or PPARδ-dependent pathways in skeletal muscle of mice. Exp Mol Med. 2018;50:1–11.
47. Li L, Li T, Zhang Y, Pan Z, Wu B, Huang X, et al. Peroxisome proliferator-activated receptorβ/δ activation is essential for modulating p-Foxo1/Foxo1 status in functional insulin-positive cell differentiation. Cell Death Dis. 2015;6: e1715.
48. Yang Y-Y, Qi J-J, Jiang S-Y, Ye L. Esculin ameliorates obesity-induced insulin resistance by improving adipose tissue remodeling and activating the IRS1/PI3K/AKT/GLUT4 pathway. J Ethnopharmacol. 2024;319:117251.
49. Nigro E, Scudiero O, Monaco ML, Palmieri A, Mazzarella G, Costagliola C, et al. New insight into adiponectin role in obesity and obesity-related diseases. Biomed Res Int. 2014;2014:658913.
50. Singh A, Bora P, Krishna A. Direct action of adiponectin ameliorates increased androgen synthesis and reduces insulin receptor expression in the polycystic ovary. Biochem Biophys Res Commun. 2017;488:509–15.
51. Yuan X, Hu T, Zhao H, Huang Y, Ye R, Lin J, et al. Brown adipose tissue transplantation ameliorates polycystic ovary syndrome. Proc Natl Acad Sci U S A. 2016;113:2708–13.
52. Campos DB, Palin M-F, Bordignon V, Murphy BD. The "beneficial" adipokines in reproduction and fertility. Int J Obes (Lond). 2008;32:223–31.
53. Hazrati P, Ramezani M, Ahmadimoghaddam D, Asl SS, Artimani T. The effects of AdipoRon on cytochrome P450-related gene expression, acute steroidogenic regulatory protein, and structure of ovary in polycystic ovary syndrome model. J Assist Reprod Genet. 2023;40:2453–61.
54. Morishita M, Endo T, Baba T, Kuno Y, Ikeda K, Kiya T, et al. Pioglitazone is effective for multiple phenotyepes of the Zucker fa/fa rat with polycystc ovary morphology and insulin resistance. J Ovarian Res. 2018;11:24.
55. Oana F, Takeda H, Hayakawa K, Matsuzawa A, Akahane S, Isaji M, et al. Physiological difference between obese (fa/fa) Zucker rats and lean Zucker rats concerning adiponectin. Metabolism. 2005;54:995–1001.

Publisher's Note