Discovery of microglia gonadotropin-releasing hormone receptor and its potential role in polycystic ovarian syndrome

FAN WANG[1*], ZHENGHONG ZHANG[2*], JUNYONG HAN[3], JIANJUN ZHENG[4], XIN WANG[1] and ZHENGCHAO WANG[1,2]

[1]Provincial University Key Laboratory of Sport and Health Science, School of Physical Education and Sport Sciences; [2]Provincial Key Laboratory for Developmental Biology and Neurosciences, College of Life Sciences, Fujian Normal University, Fuzhou, Fujian 350007; [3]Fujian Key Laboratory of Medical Measurement, Fujian Academy of Medical Sciences; [4]Department of Obstetrics and Gynecology, Fujian Provincial Hospital, Shengli Clinical College of Fujian Medical University, Fuzhou, Fujian 350001, P.R. China

Received August 17, 2022; Accepted January 16, 2023

DOI: 10.3892/mmr.2023.12964

Abstract. Hypothalamic inflammation is a pathophysiological basis of polycystic ovarian syndrome (PCOS), while overactivated and/or excess M1 polarized microglia are considered to be the main reason for the occurrence of hypothalamic inflammation. Therefore, *in vitro* and *in vivo* experiments were performed to assess the relationships between microglia-mediated inflammatory reactions and endocrine functions in the PCOS hypothalamus. The expression of gonadotropin-releasing hormone (GnRH) receptor (GnRHR) was demonstrated in hypothalamic microglia, and it was found that low concentration, GnRH agonist, leuprolide acetate accelerated the expression of M2 polarization marker CD206, while high concentration leuprolide acetate increased the expression of M1 polarization marker CD86 *in vitro*. Furthermore, aerobic exercise not only reduced the levels of serum testosterone, luteinizing hormone and GnRH and the amount of overactivated microglia, but also increased the number of M2 microglia in the hypothalamus of letrozole-induced PCOS rats. In combination, these results not only demonstrated the expression of GnRHR in hypothalamic microglia, but also demonstrated that GnRH can induce microglial polarization, while aerobic exercise may improve the microglia-mediated inflammatory reaction by reducing the expression of GnRHR in the hypothalamic microglia of PCOS rats.

Correspondence to: Dr Zhengchao Wang, Provincial Key Laboratory for Developmental Biology and Neurosciences, College of Life Sciences, Fujian Normal University, 8 Shangsan Road, Fuzhou, Fujian 350007, P.R. China
E-mail: zcwang@fjnu.edu.cn

[*]Contributed equally

Key words: gonadotropin-releasing hormone receptor, microglia, hypothalamic inflammation, aerobic exercise, polycystic ovarian syndrome

Introduction

Polycystic ovarian syndrome (PCOS) is a heterogeneous disease that often occurs during the childbearing years; its etiology includes reproductive, endocrine and metabolic aspects (1). Although the specific pathophysiological mechanism of PCOS remains unclear, dysfunction of the hypothalamic-pituitary-gonadal (HPG) axis is considered to be an important component (2,3). The increased secretion of total gonadotropin-releasing hormone (GnRH) is a very important feature of the abnormal endocrine function of the HPG axis in PCOS (3). The hypothalamus is not only a secretory organ of GnRH, but also a regulatory center, which triggers tissue inflammation and metabolic abnormalities, and its dysfunction can lead to a series of metabolic abnormalities and organ tissue dysfunction (4). Hypothalamic function is an important pathophysiological mechanism of metabolic diseases; therefore, changes in the hypothalamic microenvironment may be closely associated with the occurrence and development of metabolic diseases, especially the inflammatory microenvironment.

Previous studies have reported that hypothalamic inflammation may be an underlying pathophysiological mechanism of PCOS heterogeneity (4,5). Currently, microglia-mediated hypothalamic inflammation is a well-recognized marker of central inflammatory responses and is a key process in the pathogenesis of chronic metabolic diseases (6-8). Microglia, as a macrophage of the central nervous system, serve a dual role in various pathophysiological states, mainly due to its two polarized states (pro-inflammatory M1 polarization and neurotrophic-protective M2 polarization) (9). Under normal physiological conditions, microglia can be found in a resting state (M0) which serves a role in immune surveillance. However, microglia are rapidly activated in pathological conditions, which is accompanied by adaptive polarization changes in transcriptional functions, resulting in M1 and/or M2

polarization (6-9). M1-polarized microglia induce an inflammatory response and release a large number of inflammatory factors, such as nitric oxide, IL-6, TNF-α and reactive oxygen species (6-7), which are closely associated with insulin resistance, obesity and the HPG axis in PCOS.

In recent years, aerobic exercise has been reported to be one of the effective treatments for PCOS (10), but its mechanism remains to be elucidated. Regular aerobic exercise not only improves PCOS endocrine disorders, but also helps to reduce the risk of developing metabolic complications (10,11). Obese PCOS patients can restore normal menstrual cycle and pregnancy through diet and exercise intervention (12). Moreover, if aerobic exercise intervention is performed prior to employing assisted reproductive technology, the pregnancy rate can be significantly increased (12). The improved effect of aerobic exercise on hypothalamic inflammation has attracted increasing attention from many scholars. Aerobic exercise can improve whole-body metabolic health and also reduce hypothalamic inflammatory factors in obese mice (13); however, there is little evidence that exercise affects the hypothalamus.

However, few study has reported the relation of microglia and the GnRH/GnRH receptor (GnRHR) system, as well as its roles in the pathophysiology of PCOS. Therefore, we hypothesized that the GnRHR may be expressed in rat hypothalamic microglia, and that excessive GnRH secretion may increase the amount of M1 polarized microglia in the hypothalamus of PCOS, which could lead to the occurrence of inflammation. The present study evaluated the effects of microglia polarization on hypothalamic dysfunction in PCOS, which could provide a new direction for the treatment of hypothalamic inflammation.

Materials and methods

Cell culture and treatment. Hypothalamic microglial cells were isolated from newborn female rats (age, <24 h postpartum) as previously described (14). Briefly, each newborn female animal was injected subcutaneously with 300 mg/kg pentobarbital sodium (15), cardiac arrest was used to confirm death, and then the hypothalamus tissue was excised and digested into a single-cell suspension using DMEM/F12 (Hyclone; Cytiva) containing 0.25% trypsin (Hyclone; Cytiva) for 30 min at 37˚C in a 5% carbon dioxide cell incubator. The hypothalamic cell suspension was then filtrated and gradient centrifugation at 300 x g for 10 min at 37˚C was performed to obtain presumptive microglia. Finally, microglial cells were sorted for anti-CD11b-FITC (1:100; cat. no. FITC-65229; Proteintech Group, Inc.) using a FACScan Flow Cytometer (BD Biosciences). The cells were divided into five groups and treated with 0, 10^{-12}, 10^{-10}, 10^{-8} and 10^{-6} mol/l leuprolide acetate (LA, MedChemExpress) at 37˚C in a 5% carbon dioxide cell incubator for 6, 12 and 24 h.

Animals. Thirty-two Sprague-Dawley rats were purchased from Wushi Experimental Animal Supply Co. Ltd. The animals were maintained under a 14 h light/10 h dark schedule at 24±2˚C with humidity of 50±10% and free access to chow and water. The experimental protocol was in accordance with the Guide for the Care and Use of Laboratory Animals, by the Institutional Animal Care and Use Committee, Fujian Normal University (Fuzhou, China; approval no. IACUC-20180011). Each animal was anesthetized using 0.05 mg/kg atropine (Sigma-Aldrich; Merck KGaA) administered subcutaneously and 2.5 mg/kg diazepam (Sigma-Aldrich; Merck KGaA) administered intraperitoneally for deep anesthesia. The abdomen was opened, then the ovaries were removed for subsequent analysis and blood was drawn for the assessment of serum hormone concentrations. All animals were then sacrificed by cervical dislocation while still anesthetized. All efforts were made to minimize animal discomfort and to reduce the number of animals used.

Letrozole-induced PCOS rats. Six-week-old female rats with two consecutive 4-day estrous cycles were randomly divided into 2 groups as follows: The carboxymethyl cellulose (CMC) group as a vehicle group (n=16) and a PCOS group (n=16). PCOS was induced in the PCOS group by the intragastric administration of 1.0 mg/kg/day letrozole dissolved in 1.0% CMC (2.0 ml/kg) for 21 days, while the CMC group was injected with equal volume of CMC, as previously described (16). The estrous cycles of all rats were assessed using a vaginal smear method as previously reported (1). PCOS rats were diagnosed using the Rotterdam diagnostic criteria (1), which are based on the exclusion other diseases that cause hyperandrogenism and the meeting of two of the following three points: i) Oligo-ovulation or anovulation, ii) clinical hyperandrogenism and/or hyperandrogenism, and iii) polycystic ovary.

Treadmill running intervention. Following modeling, the CMC and PCOS groups were further divided into CMC + quiet (CQ, n=8), CMC + exercise (CE, n=8), PCOS + quiet (PQ, n=8) and PCOS + exercise (PE, n=8) groups. After 1 week of acclimatization, all rats in the CE and PE groups were trained on the treadmill at 75% VO_{2max} for 60 min per day, 6 days a week for 4 consecutive weeks; during that time, all the rats in the CQ and PQ groups were allowed free movement around the cage. The treadmill training was performed between 8:00 and 10:00 daily.

Immunocytochemistry analysis for GnRHR. The microglial cells attached to the cell slides at 37˚C in a 5% carbon dioxide cell incubator for 2 days. Following washing, all slides were fixed in 4% paraformaldehyde at room temperature for 10 min followed by incubation with 0.3% H_2O_2 at room temperature for 15 min and blocked using 10% bovine serum albumin (Sigma-Aldrich; Merck KGaA) at room temperature for 45 min. The slides were incubated with anti-transmembrane protein (TMEM)119 (1:200; cat. no. NBP2-30551; Novus Biologicals, LLC), anti-ionized calcium binding adaptor molecule 1 (Iba1; 1:200; cat. no. ab178846; Abcam) and anti-GnRHR (1:50; cat. no. 19950-1-AP; Proteintech Group, Inc.) primary antibodies separately, overnight at 4˚C. Following washing with PBS, the slides were incubated with CoraLite 594-conjugated goat anti-rabbit IgG secondary antibody (1:200; cat. no. SA00013-4, Proteintech Group, Inc.) at room temperature for 1 h. The nuclei were stained using DAPI at room temperature for 10 min. All slides were imaged using a fluorescence microscope.

RNA extraction and agarose gel electrophoresis analysis for GnRHR. Total mRNA was extracted from the primary cultured rat microglia using TRIzol® solution (Life Technologies; Thermo Fisher Scientific. Inc.). The extracted mRNA samples were then reverse-transcribed using an iScript™ Select cDNA synthesis kit (Bio-Rad Laboratories, Inc.). The reverse-transcribed products were amplified using a FasQuant RT Kit (Tiangen Biotech Co., Ltd.) and the mRNA expression levels of GnRHR were assessed using 1% agarose gel electrophoresis, which was stained with ethidium bromide at room temperature for 30 min and imaged using an ultraviolet lamp. The primer sequences used were as follows: GnRHR forward (F), 5'-AGGACCCACGCAAACTAC AG-3' and reverse (R), 5'-TCCAGCAGATGACAAAGGAG-3'; and β-actin F, 5'-CGTAAAGACCTCTATGCCAACA-3' and R, 5'-AGCCACCAATCCACACAGAG-3'. β-actin was used as a loading control. All procedures were performed according to the manufacturer's protocols.

Ovarian histology. The aforementioned rat ovaries were fixed in 4% paraformaldehyde at 4˚C for 48 h and embedded using paraffin. Sections (5 μm) were cut and mounted on slides. The sections were stained at room temperature with hematoxylin for 8 min and eosin for 10 sec, and then imaged using a BX51 light microscope (Olympus Corporation).

ELISA analysis. Blood was centrifuged at 1,000 x g for 20 min to obtain serum at 4˚C. The serum concentrations of testosterone (T), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and GnRH were assessed using T ELISA kit (H090-1-1, Nanjing Jiancheng Bioengineering Institute), LH ELISA kit (H206-1-2, Nanjing Jiancheng Bioengineering Institute), FSH ELISA kit (H101-1-2, Nanjing Jiancheng Bioengineering Institute) and GnRH ELISA kit (H297, Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's protocols.

Western blot analysis. Total protein from cells and hypothalamic tissue was extracted using RIPA lysis buffer (Beyotime Institute of Biotechnology). A total of 20 μg of protein samples were subjected to 10% SDS-PAGE gel electrophoresis and then transferred onto a PVDF membrane. These membranes were washed with TBST (0.1% Tween-20) and probed with primary antibodies (Table SI) overnight at 4˚C. Following washing with TBST (0.1% Tween-20), the membrane was incubated with the corresponding secondary antibodies at 37˚C for 1 h. The immunoblotting signals were assessed using enhanced chemiluminescence BeyoECL Plus buffer (P0018S, Beyotime Institute of Biotechnology) and semi-quantified by densitometry using ImageJ (version 1.44p; National Institutes of Health).

Statistical analysis. Data were presented as the mean ± SE. Differences in mean values among multiple groups were evaluated using one-way ANOVA, followed by Tukey's post-hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

GnRHR expression in rat microglia. *In vitro*, rat microglia were identified using the marker of microglia, TMEM119 (Fig. 1A). The expression of GnRHR in microglia was demonstrated using immunocytochemistry (Fig. 1B) and reverse transcription PCR (Fig. 1C), which indicated the expression of GnRHR in rat microglia.

Effects of GnRH agonist LA on the polarization of rat microglia. To the best of our knowledge there are no previous reports of the effect of GnRH agonist LA on rat hypothalamic microglia. Furthermore, due to the different concentrations of certain factors, LA may have different effects in different microglial polarization states (17); therefore, different concentrations of LA were used to treat the rat primary cultured microglia to evaluate the effects of GnRH on hypothalamic microglia. The results demonstrated that there were no significant differences in the protein expression levels of TMEM119 and GnRHR among all 5 groups following 6 h of treatment (Fig. 2A and C). The markers of microglia polarization were assessed and a significant increase in CD86 protein expression levels was demonstrated in the 10^{-8} and 10^{-6} mol/l groups compared with the 0, 10^{-12}, 10^{-10} mol/l groups (P<0.05, Fig. 2B and D) and a significant decrease in CD206 expression was identified in the 10^{-10}, 10^{-8} and 10^{-6} mol/l groups compared with the control (0 mol/l) group (P<0.05, Fig. 2B and D) following 6 h of treatment. Moreover, no significant differences in the protein expression levels of TMEM119 and GnRHR were demonstrated among groups following 12 (Fig. S1A and C) and 24 (Fig. S2A and C) h of treatment, which was consistent with the results shown by each group when treated for 6 h. Further analysis also demonstrated that the CD86 and CD206 protein expression level changes among the groups when treated for 12 h (Fig. S1B and D) and 24 h treatment (Fig. S2B and D) were similar to those demonstrated following treatment for 6 h.

Effects of aerobic exercise on ovarian histology and endocrine hormone levels in PCOS rats. A PCOS rat model was diagnosed based on the Rotterdam diagnostic criteria (1). The ovarian histology results demonstrated a normal structure follicle with multi-layered (mostly 8-9 layers) granulosa cells, a normal sized follicular antrum and oocyte-corona cumulus complex in the CQ, CE and PE groups (Fig. 3A), the PQ group presented cystic follicles with fewer layers (mostly 2-4 layers) of granulosa cells, a bigger follicular antrum and a lack of oocyte-corona cumulus complex in the ovaries (Fig. 3A). Further analysis demonstrated that, compared with the CQ group, the levels of serum T (P<0.05, Fig. 3B), LH (P<0.05, Fig. 3C) and GnRH (P<0.05, Fig. 3E) were significantly higher in the PQ group, while the level of FSH (P<0.05, Fig. 3D) was significantly lower. Moreover aerobic exercise significantly decreased the levels of serum T, LH and GnRH, and significantly increased the serum FSH level in the PE group compared with PCOS rats without the aerobic exercise intervention (PQ, P<0.05; Fig. 3).

Effects of aerobic exercise on the hypothalamic inflammation of PCOS rats. As the level of serum C reactive protein (CRP) is one of the most important properties of chronic low-grade inflammation (1,5), the present study assessed CRP protein expression levels and demonstrated it was significantly increased in the hypothalamic tissue of PQ rats compared

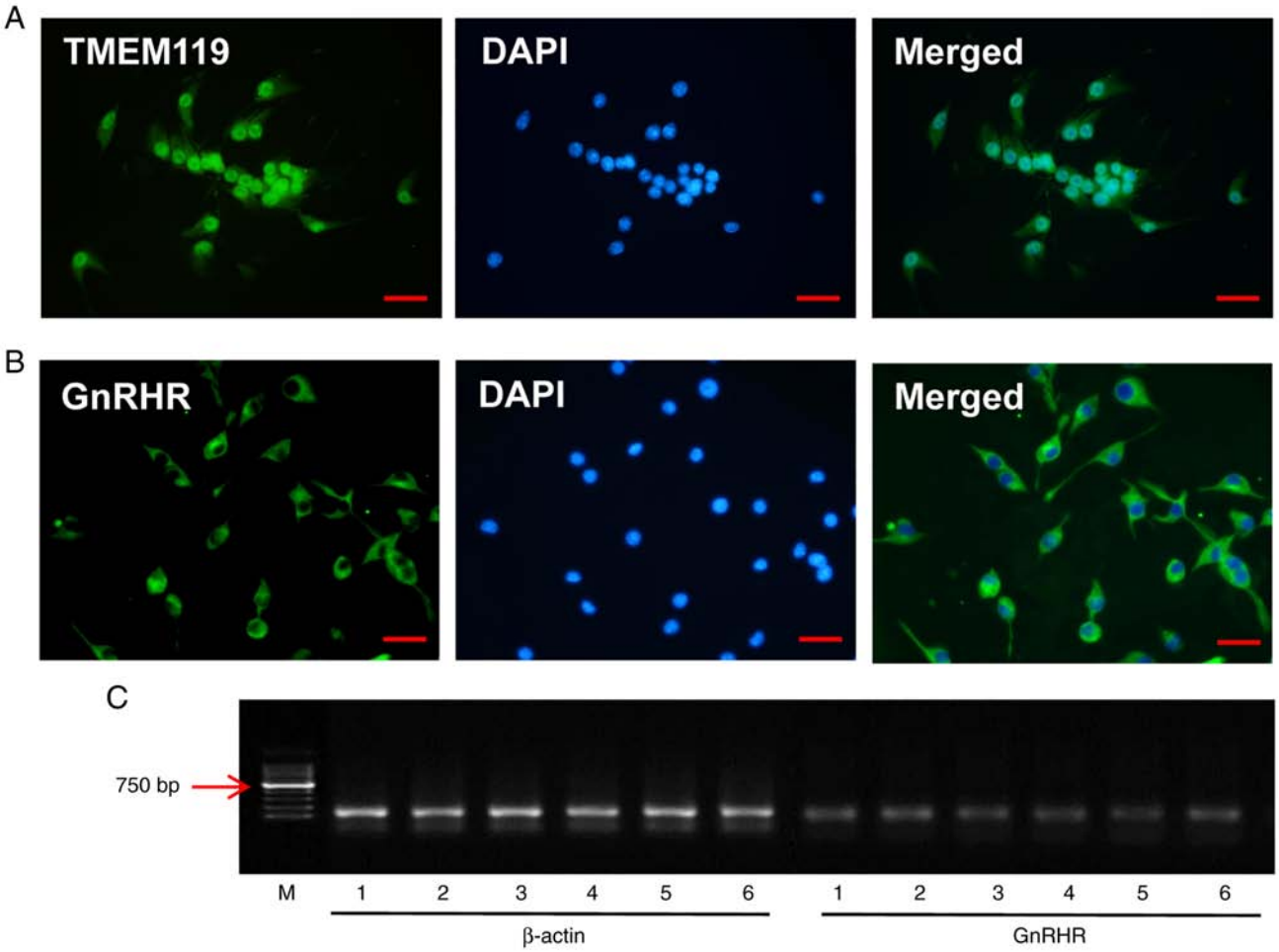

Figure 1. GnRHR expression in hypothalamic microglia. (A) Immunofluorescence signals of TMEM119 appear green and the background counterstaining blue. (B) The immunofluorescence signals of GnRHR appear green and the background counterstaining blue. (C) PCR gel electrophoresis for GnRHR. Scale bar=100 μm. GnRHR, gonadotropin-releasing hormone receptor; TMEM119, transmembrane protein 119.

with the CQ group (P<0.05; Fig. 4A and C). Furthermore, it was significantly decreased following aerobic exercise (PE) compared with the PQ group (P<0.05; Fig. 4A and C). Moreover, the protein expression levels of Iba1, a marker of microglia activity, was also assessed, which demonstrated that the changes in Iba1 protein expression levels were similar to the changes in the protein expression levels of CRP (Fig. 4A and C), which indicated that microglia activity was increased on the hypothalamic inflammation of PCOS rats.

Effects of aerobic exercise on the microglia polarization of PCOS rats. To further evaluate the involvement of microglia-mediated inflammation in the hypothalamic inflammation of PCOS rats, the polarization of hypothalamic microglia was also assessed. The present study demonstrated that CD86 protein expression levels, as a marker of M1 polarized microglia, was significantly increased in the hypothalamic tissue of PQ rats compared with the CQ group (P<0.05; Fig. 4B and D), and then significantly decreased following aerobic exercise (PE) compared with the PQ group (P<0.05; Fig. 4A and C). Furthermore, the protein expression levels of CD206, a marker of M2 polarized microglia, were also examined, which demonstrated that CD206 expression was significantly decreased in the hypothalamic tissue of PQ rats compared with the CQ) group (P<0.05; Fig. 4B and D) and that CD206 protein expression levels were significantly increased following aerobic exercise (PE) compared with the PQ group (P<0.05; Fig. 4B and D). These results demonstrated that microglia polarization was involved in the hypothalamic inflammation of PCOS rats, while aerobic exercise may promote microglia to transform from M1 to M2 polarization.

Discussion

To the best of our knowledge, the present study is the first to have demonstrated the expression of GnRHR in hypothalamus microglia and the effect of GnRH signaling on microglia polarization; it also demonstrated that aerobic exercise improved hypothalamic inflammation by promoting the transformation of microglia from M1 to M2 polarization in PCOS rats.

Due to the heterogeneous clinical characteristics of PCOS, its exact etiology is still to be determined; however, the dysfunction of the HPG axis is an important component of the pathophysiology of PCOS (3,4). In PCOS, GnRH pulse frequency is increased to 50-60 min per pulse, which is similar to the menopausal pulse frequency (3). This pattern of GnRH secretion results in the synthesis and secretion of predominantly LH rather than FSH, which causes further

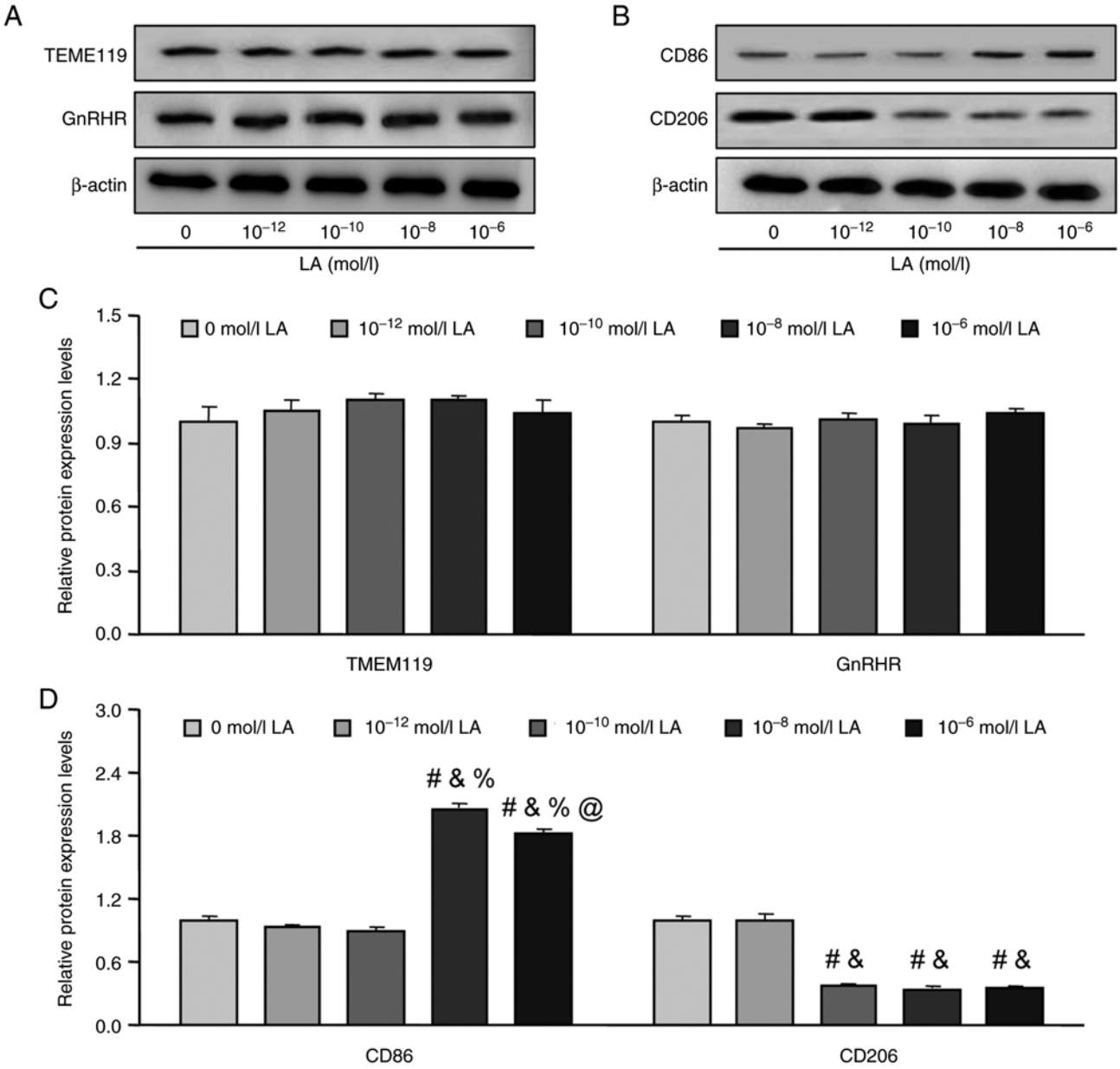

Figure 2. Effects of GnRH agonist LA on the polarization of hypothalamic microglia following treatment for 6 h. Representative western blots of the protein expression levels of (A) TMEM119 and GnRHR and (B) CD86 and CD206. Relative protein expression levels of (C) TMEM119 and GnRHR and (D) CD86 and CD206 normalized to 0 mol/l LA. Data are presented as the mean ± SE. $^{\#}$P<0.05 vs. 0 mol/l LA, $^{\&}$P<0.05 vs. 10^{-12} mol/l LA, $^{\%}$P<0.05 vs. 10^{-10} mol/l LA, $^{@}$P<0.05 vs. 10^{-8} mol/l LA. LA, leuprolide acetate; GnRHR, gonadotropin-releasing hormone receptor; TMEM119, transmembrane protein 119.

excess androgen secretion (3). Furthermore, aerobic exercise, especially moderate intensity exercise, has been reported to be effective in improving the symptoms of PCOS, including endocrine dysfunction and polycystic ovaries (18,19). Early intervention with aerobic exercise can reduce androgen levels in PCOS rats, promoting ovulation and preventing the development of PCOS (17), which is consistent with the results of the present study. In the present study, it was demonstrated that aerobic exercise could significantly reduce serum T, LH and GnRH levels, and significantly increase the serum FSH level in PCOS rats.

Previous studies have reported that hypothalamic inflammation is involved in the pathophysiology of PCOS (2,5,20). Microglia-mediated inflammatory reaction serves an important role in the hypothalamic inflammation (8). Moreover, a recent study focused on the role of microglia in PCOS-like brains, reported that microglia may serve an important role in driving the abnormal neuronal wiring that leads to PCOS-like features in prenatally androgenized mice (21). Furthermore, a main characteristic of endocrine dysfunction in the PCOS hypothalamus is the increased pulsatile secretion of GnRH (2,3). Gao *et al* (22) reported that certain hormones, such as leptin, affected the presence and activity levels of hypothalamic microglia in obesity. Therefore, whether the GnRH/GnRHR system affects microglia activation and/or polarization was evaluated. *In vitro* experiments demonstrated the expression of GnRHR in hypothalamic microglia. LA as a GnRH agonist can bind to GnRHR to activate a series of signaling pathways (4). In the present study, LA did not affect the expression of GnRHR and TMEM119 in rat microglia,

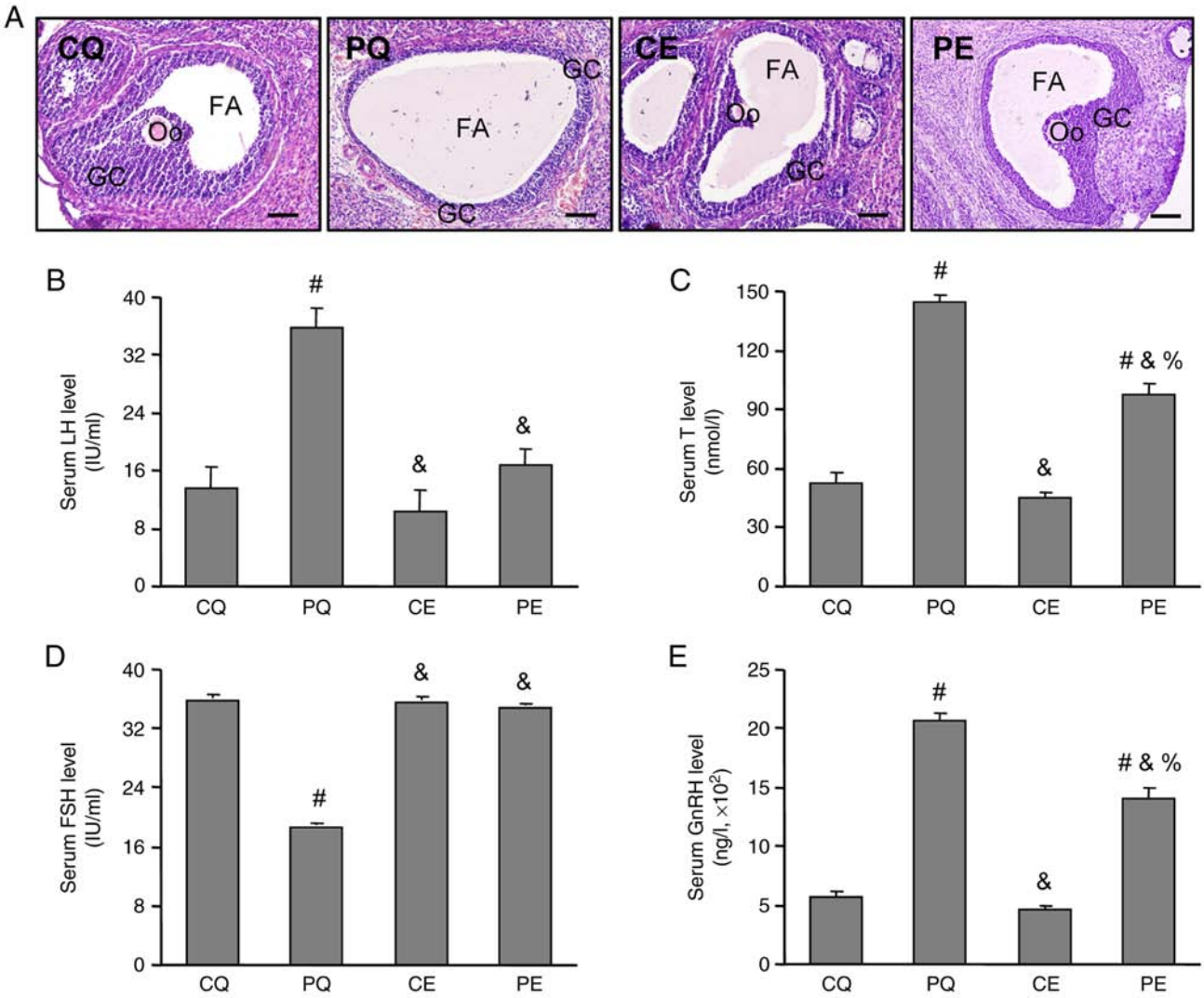

Figure 3. Effects of aerobic exercise on endocrine hormone levels and the ovarian histology of PCOS rats. (A) Effects of aerobic exercise on ovarian histology in PCOS rats. The ovarian sections were stained using hematoxylin and eosin and imaged using a light microscope. Effects of aerobic exercise on serum (B) LH, (C) T, (D) FSH and (E) GnRH levels in PCOS rats. The levels of serum hormones were examined using ELISA kits. Data are presented as the mean ± SE. Scale bar=100 μm. $^{\#}$P<0.05 vs. CQ, $^{\&}$P<0.05 vs. PQ, $^{\%}$P<0.05 vs. CE. PCOS, polycystic ovarian syndrome; GC, granulosa cell; Oo, oocyte; FA, follicular autrum; CMC, carboxymethyl cellulose; CQ, CMC + quiet; CE, CMC + exercise; PQ, PCOS + quiet; PE, PCOS + exercise; LH, luteinizing hormone; T, testosterone; FSH, follicle stimulating hormone; GnRH, gonadotropin-releasing hormone.

which suggested that GnRH was not involved in the regulation of microglia proliferation.

CD86 and CD206 are the markers of M1 and M2 polarized microglia, respectively (23). In the present study, low protein expression levels of CD86 and high protein expression levels of CD206 were demonstrated in microglia treated with 0 and 10^{-12} mol/l LA, which suggested that low LA concentration may contribute to protective M2 microglia polarization. However, the protein expression levels of CD86 were significantly higher under 10^{-8} and 10^{-6} mol/l LA, which indicated that the high LA concentration may cause pro-inflammatory M1 polarization. In the 10^{-10} mol/l LA group, the protein expression level of CD86 was markedly lower compared with the 10^{-8} and 10^{-6} mol/l LA groups, whereas the protein expression level of CD206 in the 10^{-10} mol/l LA group was not significantly different compared with the 0 and 10^{-12} mol/l LA groups. These results suggested that 10^{-10} mol/l LA may contribute to the restoration of the resting state of microglia. These results indicated that the high concentration of LA may contribute to M1 polarization; it was therefore hypothesized that the increased pulsatile release of GnRH may promote M1 polarization to induce hypothalamic inflammation in PCOS.

Studies on local inflammation in PCOS have mainly focused on the surrounding tissues, such as serum, adipose tissue and ovarian tissue, with only a few reports on hypothalamic tissue. Lian *et al* (5) reported a chronic low-grade inflammatory state in the hypothalamus of PCOS rats, but the reasons remain unexplained. A recent study reported that diet-induced hypothalamic inflammation may contribute to the endocrine pathogenesis of PCOS, and modulation of GnRH secretion could be a potential target of hypothalamic inflammation (24). Based on the present *in vitro* results, a letrozole-induced PCOS rat model was used and an aerobic exercise intervention that can effectively reduce GnRH levels was used to explore whether the increased GnRH secretion affected the hypothalamic microenvironment of PCOS. The

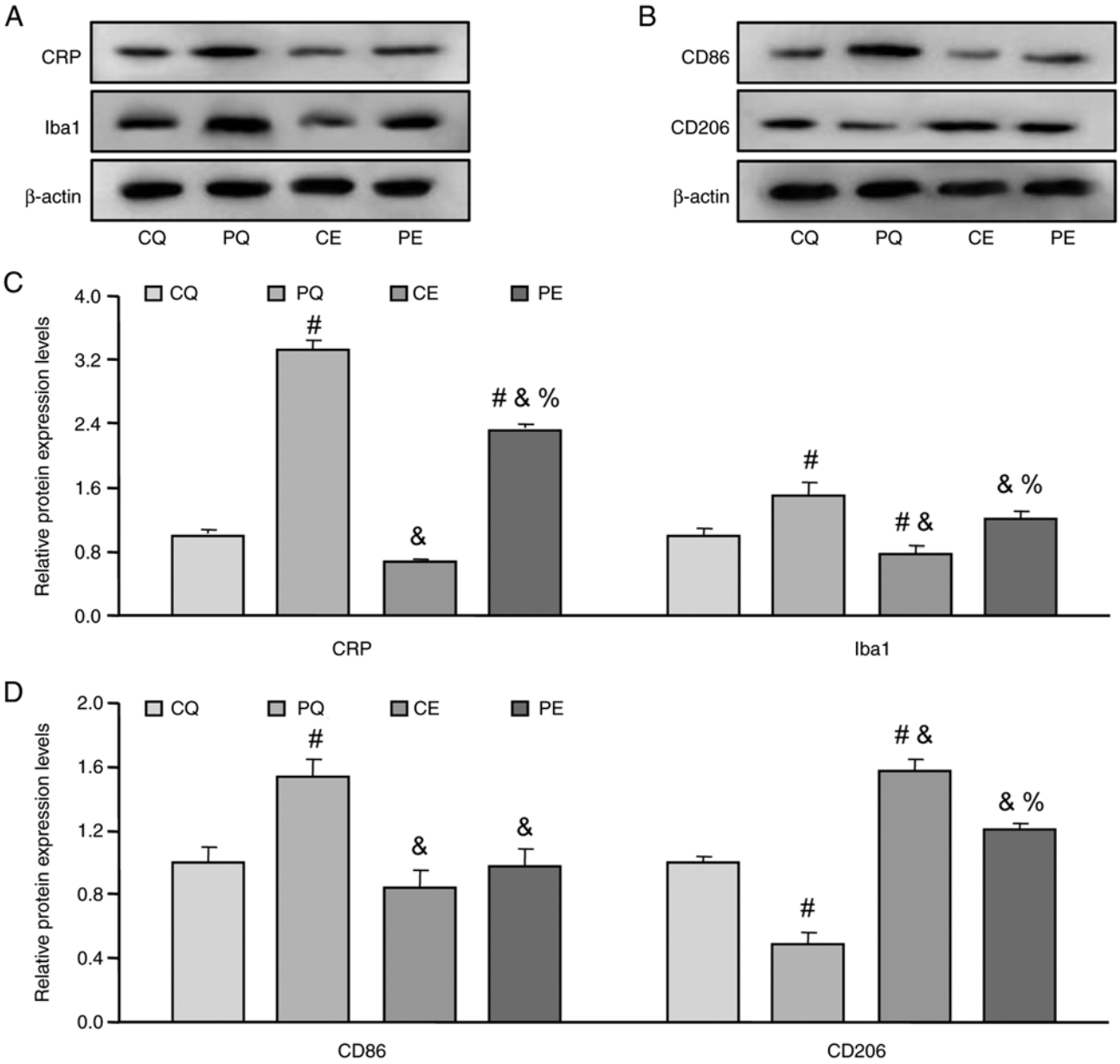

Figure 4. Effects of aerobic exercise on the hypothalamic inflammation and the microglial polarization of PCOS rats. Representative western blots of the protein expression levels of (A) CRP and Iba1 and (B) CD86 and CD206. Protein expression levels of (C) CRP and Iba1 and (D) CD86 and CD206 blots normalized to CQ. Data are presented as the mean ± SE. $^{\#}$P<0.05 vs. CQ, $^{\&}$P<0.05 vs. PQ, $^{\%}$P<0.05 vs. CE. CRP, C reactive protein; PCOS, polycystic ovarian syndrome; CQ, CMC + quiet; CE, CMC + exercise; PQ, PCOS + quiet; PE, PCOS + exercise; CRP, C reactive protein; Iba1, ionized calcium binding adaptor molecule 1.

results demonstrated that hypothalamic inflammation and overactivated microglia existed in PCOS rats, and that aerobic exercise could markedly decrease the degree of inflammation and the number of activated microglia, which suggested that aerobic exercise may improve microglia-mediated inflammation in PCOS, possibly through the reduction of excessive GnRH secretion.

Furthermore, the present results demonstrated that the amount of M1 polarized microglia was increased and the amount of M2 polarized microglia was decreased in PCOS rats, and that aerobic exercise could reverse this phenomenon. This suggested that aerobic exercise could reduce overactivated M1 polarized microglia to release a mass of pro-inflammatory factors, in turn, serving a protective role by promoting M2 polarized transformation through inhibition of the effects of GnRH on microglia polarization. Lee *et al* (23) reported that excessive pro-inflammatory factors released by M1 polarized microglia may activate inflammatory signaling in adjacent neurons, further leading to hypothalamic dysfunctions. Clinical studies have also reported that hyperactivated M1 polarized microglia can cause neuronal incapacitation, damage and degeneration (25). To the best of our knowledge, there are no studies on the effect of aerobic exercise on microglia polarization, but in adipose tissue, aerobic exercise inhibits inflammation through the acceleration of phenotypic switching from M1 to M2 macrophages in high-fat-diet-induced obese mice (26). A limitation of the present study was that the

molecular mechanism of the GnRH signaling pathway was not elucidated using transgenic mice.

In conclusion, the present study demonstrated the expression of GnRHR in rat hypothalamic microglia, and that high concentrations of GnRH agonist LA may promote microglia to M1 polarization *in vitro*, which suggested that GnRH signaling may be involved in the polarization of hypothalamic microglia. Moreover, the results of the *in vivo* experiments demonstrated that aerobic exercise not only decreased the amount of overactivated microglia, but also promoted the switch from M1 to M2 polarization by reducing the increased secretion of GnRH to alleviate hypothalamic inflammation in PCOS rats. These findings provided a new direction for the evaluation of the pathophysiology and therapeutic mechanisms of PCOS.

Acknowledgements

Not applicable.

Funding

This research was funded by the Key Projects of Scientific and Technological Innovation in Fujian Province (grant nos. 2021G02003, 2022G023 and 2022G028), Special Funds of the Central Government Guiding Local Science and Technology Development (grant no. 2020L3008), Fujian Provincial Natural Science Foundation (grant nos. 2019R1011-3, 2020J01176 and 2022J01172), Scientific Research Talent Project of Fujian Provincial Health Commission (grant no. 2018-ZQN-20), and the Innovation and Entrepreneurship Project of Fujian Normal University (grant nos. I202003009 and I202102008).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

FW, ZZ and ZW conceived and designed the study. FW, ZZ, JH, JZ, XW and ZW performed the experiments. FW, ZZ, JH, JZ and XW analyzed the data. FW and ZZ drafted the first version of the manuscript. ZW critically revised the manuscript. FW and ZW confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The animal study protocol was approved by the Ethics Committee of Fujian Normal University (Fuzhou, China; approval no. IACUC-20180011).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Wang F, Wang SB, Zhang ZH, Lin Q, Liu Y, Xiao Y, Xiao K and Wang ZC: Activation of NLRP3 inflammasome in the ovaries during the development and treatment of polycystic ovary syndrome. Int J Clin Exp Patho 10: 5022-5030, 2017.
2. da Costa CS, Oliveira TF, Freitas-Lima LC, Padilha AS, Krause M, Carneiro MTWD, Salgado BS and Graceli JB: Subacute cadmium exposure disrupts the hypothalamic-pituitary-gonadal axis, leading to polycystic ovarian syndrome and premature ovarian failure features in female rats. Environ Pollut 269: 116154, 2021.
3. Wang F, Zhang ZH, Xiao KZ and Wang ZC: Roles of hypothalamic-pituitary-adrenal axis and hypothalamus-pituitary-ovary axis in the abnormal endocrine functions in patients with polycystic ovary syndrome. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 39: 699-704, 2017.
4. Barlampa D, Bompoula MS, Bargiota A, Kalantaridou S, Mastorakos G and Valsamakis G: Hypothalamic inflammation as a potential pathophysiologic basis for the heterogeneity of clinical, hormonal, and metabolic presentation in PCOS. Nutrients 13: 520, 2021.
5. Lian Y, Zhao F and Wang W: Central leptin resistance and hypothalamic inflammation are involved in letrozole-induced polycystic ovary syndrome rats. Biochem Biophys Res Commun 476: 306-312, 2016.
6. Ávalos Y, Kerr B, Maliqueo M and Dorfman M: Cell and molecular mechanisms behind diet-induced hypothalamic inflammation and obesity. J Neuroendocrino 30: e12598, 2018.
7. André C, Guzman-Quevedo O, Rey C, Rémus-Borel J, Clark S, Castellanos-Jankiewicz A, Ladeveze E, Leste-Lasserre T, Nadjar A, Abrous DN, *et al*: Inhibiting microglia expansion prevents diet-induced hypothalamic and peripheral inflammation. Diabetes 66: 908-919, 2017.
8. Tang Y, Purkayastha S and Cai D: Hypothalamic microinflammation: A common basis of metabolic syndrome and aging. Trends Neurosci 38: 36-44, 2015.
9. Deczkowska A, Amit I and Schwartz M: Microglial immune checkpoint mechanisms. Nat Neurosci 21: 779-786, 2018.
10. Kite C, Lahart IM, Afzal I, Broom DR, Randeva H, Kyrou I and Brown JE: Exercise, or exercise and diet for the management of polycystic ovary syndrome: A systematic review and meta-analysis. Syst Rev 8: 51, 2019.
11. Kim CH, Chon SJ and Lee SH: Effects of lifestyle modification in polycystic ovary syndrome compared to metformin only or metformin addition: A systematic review and meta-analysis. Sci Rep 10: 7802, 2020.
12. Lim SS, Hutchison SK, Van Ryswyk E, Norman RJ, Teede HJ and Moran LJ: Lifestyle changes in women with polycystic ovary syndrome. Cochrane Database Syst Rev 3: CD007506, 2019.
13. Park SY, Jeon YK and Choi J: Effects of calorie restriction and aerobic exercise on hypothalamic inflammation factors, appetite suppression neuropeptide and daily feed intake in obese mouse. Korean J Sports Sci 26: 935-945, 2017.
14. Dorfman MD, Krull JE, Douglass JD, Fasnacht R, Lara-Lince F, Meek TH, Shi X, Damian V, Nguyen HT, Matsen ME, *et al*: Sex differences in microglial CX3CR1 signalling determine obesity susceptibility in mice. Nat Commun 8: 14556, 2017.
15. Maslyukov PM, Emanuilov AI and Korzina MB: Development of neurotransmitter specificity in sympathetic ganglionic neurons. Auton Neurosci 135: 66-67, 2007.
16. Kafali H, Iriadam M, Ozardali I and Demir N: Letrozole-induced polycystic ovaries in the rat: A new model for cystic ovarian disease. Arch Med Res 35: 103-108, 2004.
17. Jie W, Xiang JY, Wang Y, Hui-Min L, Zhou S, Hou JP and Wang B: Effects of baicalin and geniposide on polarization of BV2 microglia. Chin J Pharmacol Toxicol 33: 911-912, 2019.
18. Brunk D: Moderate exercise helps insulin sensitivity in PCOS. Fam Prac 35: 22-22, 2005.
19. Cao SF, Hu WL, Wu MM and Jiang LY: Effects of exercise intervention on preventing letrozole-exposed rats from polycystic ovary syndrome. Reprod Sci 24: 456-462, 2017.
20. Nehir Aytan A, Bastu E, Demiral I, Bulut H, Dogan M and Buyru F: Relationship between hyperandrogenism, obesity, inflammation and polycystic ovary syndrome. Gynecol Endocrinol 32: 709-713, 2016.

21. Sati A, Prescott M, Holland S, Jasoni CL, Desroziers E and Campbell RE: Morphological evidence indicates a role for microglia in shaping the PCOS-like brain. J Neuroendocrinol 33: e12999, 2021.
22. Gao Y, Ottaway N, Schriever SC, Legutko B, García-Cáceres C, de la Fuente E, Mergen C, Bour S, Thaler JP, Seeley RJ, et al: Hormones and diet, but not body weight, control hypothalamic microglial activity. Glia 62: 17-25, 2014.
23. Lee CH, Suk K, Yu R and Kim MS: Cellular contributors to hypothalamic inflammation in obesity. Mol Cells 43: 431-437, 2020.
24. Zeng F, Wu Y, Li X, Ge X, Guo Q, Lou X, Cao Z, Hu B, Long NJ, Mao Y and Li C: Custom-made ceria nanoparticles show a neuroprotective effect by modulating phenotypic polarization of the microglia. Angew Chem Int Ed Engl57: 5805-5812, 2018.
25. Zhao YN, Wang F, Fan YX, Ping GF, Yang JY and Wu CF: Activated microglia are implicated in cognitive deficits, neuronal death, and successful recovery following intermittent ethanol exposure. Behav Brain Res 236: 270-282, 2013.
26. Kawanishi N, Yano H, Yokogawa Y and Suzuki K: Exercise training inhibits inflammation in adipose tissue via both suppression of macrophage infiltration and acceleration of phenotypic switching from M1 to M2 macrophages in high-fat-diet induced obese mice. Exerc Immunol Rev 16: 105-118, 2010.