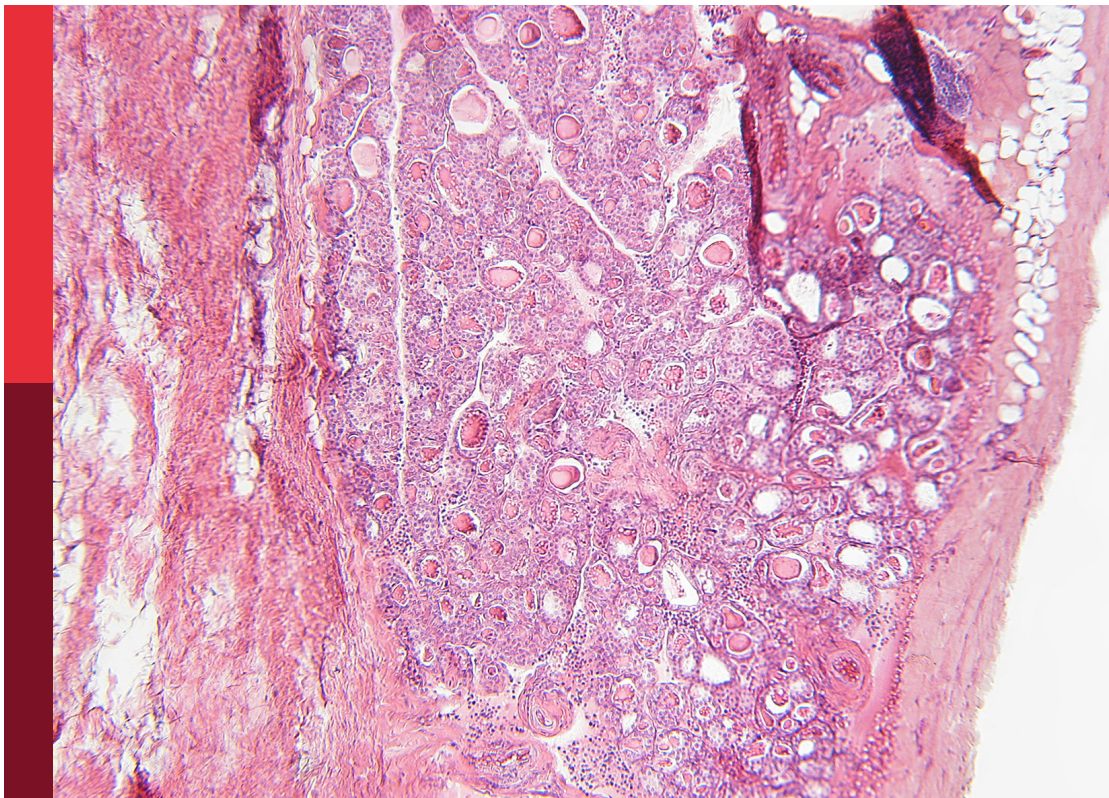
frontiers | Research Topics

Polycystic ovary syndrome (PCOS): Mechanism and management, volume II

Edited by
Yanting Wu and He-Feng Huang

Published in
Frontiers in Endocrinology
Frontiers in Physiology



ISSN 1664-8714
ISBN 978-2-8325-3228-7
DOI 10.3389/978-2-8325-3228-7

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Polycystic ovary syndrome (PCOS): Mechanism and management - volume II

Topic editors

Yanting Wu — Fudan University, China

He-Feng Huang — Fudan University, China

Citation

Wu, Y., Huang, H.-F., eds. (2023). *Polycystic ovary syndrome (PCOS): Mechanism and management - volume II*. Lausanne: Frontiers Media SA.

doi: 10.3389/978-2-8325-3228-7

Table of contents

frontiers | Frontiers in Endocrinology

TYPE Editorial
PUBLISHED 31 July 2023
DOI 10.3389/fendo.2023.1247679

OPEN ACCESS

EDITED AND REVIEWED BY
Richard Ivell,
University of Nottingham, United Kingdom

*CORRESPONDENCE
Yanting Wu
yanting_wu@163.com
Hefeng Huang
huanghefg@hotmail.com

RECEIVED 26 June 2023
ACCEPTED 03 July 2023
PUBLISHED 31 July 2023

CITATION
Wang Y, Leung P, Li R, Wu Y and Huang H (2023) Editorial: Polycystic ovary syndrome: mechanism and management—volume II. *Front. Endocrinol.* 14:1247679. doi: 10.3389/fendo.2023.1247679

Editorial: Polycystic ovary syndrome: mechanism and management—volume II

Yishu Wang[1,2,3], Peter Leung[4], Rong Li[5,6], Yanting Wu[2,3]* and Hefeng Huang[2,3]*

[1]International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China, [2]Research Units of Embryo Original Diseases, Chinese Academy of Medical Sciences, Shanghai, China, [3]Obstetrics and Gynecology Hospital, Institute of Reproduction and Development, Fudan University, Shanghai, China, [4]Department of Obstetrics and Gynaecology, BC Children's Hospital Research Institute, University of British Columbia, Vancouver, BC, Canada, [5]Centre for Reproductive Medicine, Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China, [6]National Clinical Research Centre for Obstetrics and Gynecology, Beijing, China

KEYWORDS

polycystic ovary syndrome, PCOS, management, biomarker, microbiome, complication

Editorial on the Research Topic
Polycystic ovary syndrome: mechanism and management—volume II

Introduction

Polycystic ovary syndrome (PCOS) is a complex endocrine disorder that affects a significant number of women of reproductive age worldwide (1, 2). It is characterized by a combination of clinical and biochemical features, including irregular menstrual cycles, hyperandrogenism, and polycystic ovaries (3). Beyond its reproductive manifestations, PCOS is often associated with various metabolic, reproductive, and psychological disturbances, making it a multifaceted condition with significant implications for women's health.

Driven by the desire to understand its underlying mechanisms, identify diagnostic markers, and develop effective treatment strategies, PCOS has emerged as a topic of considerable interest within the medical and scientific community in recent years. Understanding the intricate mechanisms driving PCOS is vital for devising targeted interventions and improving clinical outcomes. More and more diseases are defined as PCOS-associated disorders, indicating its pathogenesis remains multifactorial. Efforts to manage PCOS encompass a comprehensive approach that addresses both the reproductive and metabolic aspects of the syndrome.

To discuss the challenges and future directions for PCOS, we call for the second round of paper in this Research Topic, providing an overview of the current understanding of PCOS mechanisms and highlight the advancements in its management. We anticipate that this topic will shed light on the complex nature of PCOS and emphasize the importance of a multidisciplinary approach to its diagnosis and treatment.

Biomarkers of PCOS

Accurate and timely diagnosis of PCOS is paramount for effective management and prevention of associated complications. Nevertheless, the diagnostic process remains complex, hinging upon a combination of clinical evaluation, symptomatology, and laboratory investigations. An increasing focus has been placed on identifying and validating biomarkers that can assist in the diagnosis, classification, and prognosis of PCOS.

Guan et al. searched for the significantly different metabolites in follicle fluid and embryo culture medium, defining androsterone sulfate, glycerophosphocholine, and elaidic carnitine as potential biomarkers to predict the abortion rate of the PCOS group. Zhou et al. pointed out that the frequency of mucosal-associated invariant T cells was significantly reduced in the peripheral blood of PCOS patients. Furthermore, they also observed a corresponding higher level of the cytokine IL-17.

In reports of pioneering studies, it has been investigated that vitamin D deficiency may be involved in the pathophysiology of PCOS. Within our Research Topic, two articles specifically delve into the correlation between vitamin D and the syndrome. Białka-Kosiec et al. observed no correlation between the level of vitamin D and AMH, leptin, HOMA-IR and FGF23. Additionally, while the classical and alternate complement cascades were elevated in PCOS women, Moin et al. confirmed that they did not show a correlation with 1,25(OH)2D3. Both studies concluded that vitamin D may not serve as a reliable biomarker for PCOS.

Management of PCOS

Therapeutic strategies for PCOS aim to alleviate symptoms, restore hormonal balance, improve fertility outcomes, and mitigate long-term health risks (4). Lifestyle modifications, including dietary changes, regular exercise, and weight management, form the cornerstone of non-pharmacological interventions. Pharmacological interventions, such as oral contraceptives, anti-androgens, and insulin-sensitizing agents, are often prescribed to manage specific symptoms and metabolic abnormalities.

For optimizing the outcomes of *in vitro* fertilization (IVF), Zeng et al. demonstrated the significance of adjusting the initial Gn dosage based on body weight to prevent ovarian hyperstimulation. Besides, a comparison implemented by Philbois et al. displayed no differences between moderate-intensity continuous training and high-intensity interval training group, thus suggesting both those training protocols were recommended for PCOS.

To gain insights into the updates of pharmacological interventions for PCOS, Xing et al. and Jiang et al. respectively recommended metformin plus liraglutide therapy and Cangfudaotan in improving reproductive abnormalities. Wang et al. provided an experimental study in a mouse model which depicts that the antibiotic cocktail intervention improved glucose metabolic disorders and hyperinsulinemia. The potential role for SGLT2 inhibitors was reviewed by Pruett et al. in treating obesity-associated cardiometabolic complications in PCOS.

PCOS-associated diseases

Increasing evidence has supported a potential correlation between PCOS and asthma, which were previously regarded as diseases originating from two independent systems. A retrospective cross-sectional analysis by Juber et al. collected from February 2016 to April 2022 involving 1334 Emirati females revealed that pediatric asthma was an independent risk factor for adult PCOS. The view is strongly supported in the review by Xu et al., in which the correlation between asthma and PCOS highlighting the internal common pathophysiology and adverse influences on women's health, is interpreted. Furthermore, given the shared common risk factors, Wu et al. presented the latest evidence of the bidirectional association between PCOS and periodontal disease. Wang et al. conducted a meta-analysis involving nine articles with 1,107 subjects and depicted that PCOS is positively associated with the risk of sleep disturbances.

Among the adverse impacts of PCOS, the adverse pregnancy outcomes shouldn't be turned a blind eye on, apart from the impairment to woman herself. Emerging lines of evidence underscore the notion that PCOS has a negative impact on pregnancy outcomes, with an increased risk of gestational diabetes, hypertensive disease during pregnancy, and preterm birth (5, 6). In pregnancies affected by PCOS, the interrelated conditions are characterized by a dynamic interplay between hyperandrogenism and hyperinsulinemia. In the review summarized by Neven et al., the complex endocrine and metabolic interactions in pregnancies complicated by PCOS were elucidated.

Concluding remarks

The growing prevalence of PCOS, along with its significant impact on women's health, has prompted extensive research to unravel its underlying mechanisms and develop effective management strategies. The collection of papers that form part of this Research Topic displays the different facets of PCOS. We hope to provide investigators an extensive overview of cutting-edge issues in PCOS, further enriching their understanding of the mechanism reside with management of this complicated disorder.

Author contributions

YSW drafted this editorial. PL, RL, YTW and HH revised and approved the final submitted version.

Funding

This work is supported by the National Key Research and Development Program of China (2021YFC2700701), the National Natural Science Foundation of China (82088102), CAMS Innovation Fund for Medical Sciences (2019-I2M-5-064), Collaborative Innovation Program of Shanghai Municipal Health Commission (2020CXJQ01), Clinical Research Plan of SHDC (SHDC2020CR1008A), Shanghai Clinical Research Center for Gynecological Diseases (22MC1940200), Shanghai Urogenital System Diseases Research Center (2022ZZ01012) and Shanghai Frontiers Science Research Base of Reproduction and Development.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. *J Clin Endocrinol Metab* (2004) 89:2745–9. doi: 10.1210/jc.2003-032046

2. Asunción M, Calvo RM, San Millán JL, Sancho J, Avila S, Escobar-Morreale HF. A prospective study of the prevalence of the polycystic ovary syndrome in unselected caucasian women from Spain1. *J Clin Endocrinol Metab* (2000) 85:2434–8. doi: 10.1210/jcem.85.7.6682

3. Azziz R, Carmina E, Chen Z, Dunaif A, Laven JSE, Legro RS, et al. Polycystic ovary syndrome. *Nat Rev Dis Primers* (2016) 2:16057. doi: 10.1038/nrdp.2016.57

4. Jayasena CN, Franks S. The management of patients with polycystic ovary syndrome. *Nat Rev Endocrinol* (2014) 10:624–36. doi: 10.1038/nrendo.2014.102

5. Roos N, Kieler H, Sahlin L, Ekman-Ordeberg G, Falconer H, Stephansson O. Risk of adverse pregnancy outcomes in women with polycystic ovary syndrome: population based cohort study. *BMJ* (2011) 343:d6309. doi: 10.1136/bmj.d6309

6. Palomba S, de Wilde MA, Falbo A, Koster MPH, La Sala GB, Fauser BCJM. Pregnancy complications in women with polycystic ovary syndrome. *Hum Reprod Update* (2015) 21:575–92. doi: 10.1093/humupd/dmv029

frontiers | Frontiers in Endocrinology

ORIGINAL RESEARCH
published: 01 July 2022
doi: 10.3389/fendo.2022.922044

The Essential Role of Body Weight in Adjusting Gn Dosage to Prevent High Ovarian Response for Women With PCOS During IVF: A Retrospective Study

Rujun Zeng[1,2†], *Hanxiao Chen*[1,2†], *Xun Zeng*[1,2*] *and Lang Qin*[1,2*]

[1] *Reproductive Centre, Department of Obstetrics and Gynaecology, West China Second University Hospital, Sichuan University, Chengdu, China,* [2] *Key Laboratory of Birth Defects and Related Diseases of Women and Children of the Ministry of Education, West China Second University Hospital, Sichuan University, Chengdu, China*

OPEN ACCESS

Edited by:
Yanting Wu,
Fudan University, China

Reviewed by:
Junhao Yan,
Shandong University, China
Jiming Chen,
Changzhou No.2 People's Hospital, China

***Correspondence:**
Xun Zeng
zx657@sina.com
Lang Qin
cacier@163.com

†*These authors have contributed equally to this work and share first authorship*

Specialty section:
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

Received: *17 April 2022*
Accepted: *31 May 2022*
Published: *01 July 2022*

Citation:
Zeng R, Chen H, Zeng X and Qin L (2022) The Essential Role of Body Weight in Adjusting Gn Dosage to Prevent High Ovarian Response for Women With PCOS During IVF: A Retrospective Study. Front. Endocrinol. 13:922044. doi: 10.3389/fendo.2022.922044

Polycystic ovarian syndrome (PCOS) is the major cause of anovulatory infertility. Since women with PCOS are often accompanied by increased body weight and hyper response to controlled ovarian stimulation, individualized gonadotropin (Gn) dose is required to achieve a therapeutic effect while minimizing the risk of ovarian hyperstimulation simultaneously. We aimed to investigate the essential role of body weight in optimizing initial Gn dosage for PCOS patients during *in vitro* fertilization (IVF). We retrospectively included 409 infertile PCOS patients who used gonadotropin-releasing hormone (GnRH)-antagonist fixed protocol and underwent their first cycle of IVF in West China Second University Hospital from January 2019 to June 2021. Baseline characteristics controlled ovarian stimulation parameters, and reproductive outcomes were compared between patients with different body weights and different ovarian responses. Multivariable linear regression analyses were adopted to investigate the relationship between body weight and initial Gn dosage. Receiver operating characteristic (ROC) curves were drawn to find the optimal cut-off value of body weight in predicting the starting Gn dosage so as to prevent high ovarian response (HOR). We found that luteinizing hormone (LH) level and Anti-Mullerian hormone (AMH) level were lowest in the group with body weight over 70 kg and was highest in the group with body weight less than 50 kg. Increased body weight was significantly correlated to the rise of initial Gn dosage (Beta = 0.399, t = 8.921, $p < 0.001$). Normal ovarian response (NOR) patients had significantly less fresh cycle cancel rate and ovarian hyperstimulation syndrome (OHSS) rate which outweighed the fewer embryos compared with HOR patients. Using ROC curves, 53.25 kg (sensitivity, 84.2%; specificity, 53.8%) and 70.5 kg (sensitivity, 58.8%; specificity, 93.0%) were identified as the optimal cut-off values to predict the initial Gn dosage of no more than 150 IU and 225 IU, respectively. In conclusion, adjusting the initial Gn dosage based on body weight is crucial to preventing ovarian hyperstimulation while not influencing reproductive outcomes for PCOS patients during IVF.

Keywords: polycystic ovarian syndrome, *in vitro* fertilization, body weight, gonadotropin, ovarian response

INTRODUCTION

Polycystic ovarian syndrome (PCOS), a complex and multifaceted disorder characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovaries, has an increasing incidence rate of 4.47% and an age-standardized incidence rate of 1.45% per year globally (1). Approximately half of PCOS patients are overweight or obese and obesity plays an important role in the pathogenesis of PCOS and may aggravate the adverse metabolic outcomes of PCOS (2). About three-quarters of PCOS patients suffered from another common complication, infertility, making PCOS the major cause of anovulatory infertility (3). Assisted reproduction technology (ART), including *in vitro* fertilization (IVF) and embryo transfer (ET) and intracytoplasmic sperm injection (ICSI), is often needed when patients are resistant to ovarian induction or complicated with other infertility factors.

Women with PCOS exhibit higher sensibility and exaggerated response to gonadotropins which could result in an increased risk of ovarian hyperstimulation. Ovarian hyperstimulation syndrome (OHSS) is a serious iatrogenic complication characterized by fluid shifting from intravascular to extravascular spaces due to arteriolar vasodilatation and increased capillary permeability (4). It occurs as mild type in 20%-30% of IVF cycles and may develop to moderate or severe type in 2%-3% of cycles (5). Individualized exogenous gonadotropin (Gn) dose is essential for minimizing OHSS risk and optimizing follicle recruitment at the same time. The strategies to manage OHSS include initial dosage selection and dose adjustment during cycle (6). Several studies have developed a series of algorithms to predict the proper initial dosage of Gn based on age, Anti-Mullerian hormone (AMH), body mass index (BMI), baseline follicle stimulating hormone (FSH) level, or ovarian response of the previous cycle (7). Obesity also takes an essential part in ovarian response and could augment adverse reproductive outcomes (2). However, these studies were lacking in evidence-based on body weight and regimens aimed at PCOS patients were limited.

Body weight is one of the factors associated with pharmacokinetic parameters. Patients with different body wight require dose adjustment to achieve equivalent therapeutic effects. Some had reported that body weight is more important than BMI in determining the dosage of exogenous Gn (8). Studies have found that body weight was negatively associated with exogenous Gn levels (8). One study reported that weight-adjusted rFSH dose could predict follicular growth and retrieval in general women, while the complications and pregnancy outcomes were not reported (9). As a large number of PCOS patients are accompanied by increased body weight, upregulating Gn dose is often needed to achieve the therapeutic effect, however, this in turn may increase the risk of ovarian hyperstimulation. Therefore, it is important to weigh the pros and cons and find the balance when adjusting Gn dosage according to body weight.

In this retrospective cohort study, we aimed to investigate the association between body weight and individualized Gn dosage and the ART outcomes in women with PCOS undergoing IVF cycles. In addition, we tried to find the optimal cut-off value of body weight in predicting the increase of initial Gn dosage in PCOS patients in order to prevent high ovarian response (HOR).

MATERIALS AND METHODS

Study Population

In this study, we retrospectively enrolled infertile patients diagnosed with PCOS who underwent their first cycle of IVF in the Reproductive Center, Department of obstetrics and gynecology, West China Second University Hospital from January 2019 to June 2021. The Ethical Review Board of West China Second University Hospital, Sichuan University, approved the study and waived the need for written informed consent (Approval No. 2021-033).

PCOS was diagnosed according to the European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) (Rotterdam criteria) (10). Only the first controlled ovarian stimulation (COS) cycles treated with gonadotropin-releasing hormone (GnRH)-antagonist fixed protocol were included. Exclusion criteria include: male factor infertility, other COS protocols (e.g., GnRH-antagonist flexible protocol, depot GnRH-agonist protocol, long GnRH-agonist protocol, etc.); second or further COS cycles; female with any known systemic or endocrine diseases, such as Cushing syndrome, androgen secreting ovarian tumors or adrenal tumors, functional hypothalamic amenorrhea (FHA), thyroid diseases, hyperprolactinemia, premature ovarian insufficiency (POI); couples with abnormal chromosome karyotype not including chromosome polymorphisms; female with a history of recurrent spontaneous abortion.

Baseline clinical characteristics including age, type of infertility, oligo/hypomenorrhea, height, weight, and BMI were extracted from the patient records. Laboratory examination data including baseline testosterone (T) level, dehydroepiandrosterone sulfate (DHEAS) level, androstadienone (AND) level, sex hormone-binding globulin (SHBG) level, FSH level, luteinizing hormone (LH) level, polycystic ovarian morphology (PCOM) measured by antral follicle count (AFC) level, and AMH level were also collected. Sex hormones and ultrasonographic examinations were measured on day 2-4 of the menstrual cycle.

Controlled Ovarian Stimulation Protocols

As mentioned above, only patients who were treated with fixed GnRH-antagonist protocol were included in this study. Briefly, exogenous Gn (Gonal-F, Merck Serono, Germany), generally 100-375 IU/day, was administered starting from day 2-3 of menstruation. The doses were determined by experienced (more than 10 years) fertility/infertility specialists based on the patient's age, BMI, PCOM, and follicular response. GnRH-antagonist (Cetrotide, 0.25 mg, Merck Serono, Germany) was daily administrated after 5-7 days usage of Gn and the administration of GnRH-antagonist

continued until triggering. Urine human chorionic gonadotropin (hCG) (Ovidrel, 8000-10000 U, Merck-Serono, Germany) was given to trigger ovulation when two leading follicles reached a mean diameter of 18 mm, or three follicles reached a mean diameter of 17 mm. A decreased dose of urine hCG (5000 IU), recombinant hCG (250 ug), or GnRH agonist (0.2 mg) with urine hCG (2000 IU) was used to trigger ovulation when patients were at high risk of OHSS. Initial Gn dosage, stimulation time, total Gn dosage, number of oocytes with diameter ≥14 mm on trigger day, estradiol (E2), progesterone (P), LH, endometrial thickness on trigger day measured by sonographic examinations, and number of oocytes retrieved were recorded.

Fresh Embryo Transfer Cycle

Oocytes were retrieved transvaginally 36-38 h after the trigger. If the patients showed increased progesterone level or at high risk of OHSS, fresh ET cancellation and freeze-all strategy were applied. All the additional embryos were cryopreserved. The morphology of embryo or blastocyst was assessed to determine its quality (11). The day 3 embryo was defined as good-quality if it presented two pronuclei (PN) when fertilization, had six to 10 blastomeres and no more than 20% fragmentation. The blastocyst was defined as good-quality if it met with the inner cell mass/trophectoderm score of AA, AB, BA, or BB.

In the present study, data including mature (MII) oocyte rate, IVF fertilization rate, IVF normal fertilization rate, ICSI fertilization rate, ICSI normal fertilization rate, cleavage rate, good-quality D3 embryo rate, blastocyst formation rate, good-quality blastocyst rate, fresh ET cancellation rate, severe OHSS rate, clinical pregnancy rate after fresh ET, and cumulative clinical pregnancy rate were collected. Fertilization was defined as the presence of PN 16-18 h post-insemination or post-injection. Normal fertilization was defined as the presence of 2PN on D1 post-insemination or post-injection. In this study, HOR was defined as a patient who had at least one of the following features (12) (1): >15 retrieved oocytes during COS cycle or cycle cancellation due to excessive follicular development (2); > 20 oocytes larger than 12-14mm in diameter during COS cycle (3); moderate or severe OHSS after COS. Poor ovarian response (POR) was defined as patients who had at least two of the following three characteristics (13) (1): maternal age ≥40 years or any other known risk factor for POR (2); previous POR history (i.e., retrieved less than three oocytes under a conventional stimulation protocol) (3); abnormal ovarian reserve test. And the rest was defined as the normal ovarian response (NOR). Serum hCG test was conducted 14 days after ET and transvaginal ultrasound (TVS) was done 28 days after ET. Clinical pregnancy was recorded when the gestational sac was observed by TVS. Patients who were hospitalized because of severe OHSS were recorded.

Statistical Analyses

We first divided the participants into four groups based on body weight: body weight between 40-50 kg (Group A), body weight between 50-60 kg (Group B), body weight between 60-70 kg (Group C), and body weight greater than 70 kg (Group D). Continuous variables were expressed as mean ± standard deviation (SD) and category variables were displayed as frequency (n) and percentage (%). One-way analysis of variance (ANOVA) test was applied to compare normal distribution continuous variables between the four groups and LSD test was used as the *post hoc* test, and median and interquadrant range (IQR) and Kruskal-Wallis H tests were used to compare abnormal distribution variables. Chi-square test and/or Fisher's exact test were used for the comparison between the four groups as appropriate, and Bonferroni correction was used for comparison between every two groups. Multivariable linear regression analyses were also performed to compare the set-up of initial Gn dosage. Age, weight, T, LH, and AMH were used in the regression model. Afterward, we compared the reproductive outcomes between PCOS patients with HOR and NOR. While the student's t-test was adopted for the comparison between two normal distributed continuous variables, Mann-Whitney U test was used to compare two abnormal distributed continuous variables, Chi-square test and/or Fisher's exact test were used to compare category variables between the aforementioned two groups. A receiver operating characteristic (ROC) curve was used to identify a cut-off value for body weight to accurately predict the need of increasing initial Gn dosage. The optimized cut-off value was selected where the ROC curve reached the maximum area under the curve (AUC) with the greatest sum of sensitivity and specificity. For all comparisons, a two-sided p-value less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 22.0 (IBM, Armonk, NY, USA).

RESULTS

Clinical Characteristics and Reproductive Outcomes Between PCOS Patients of Different Body Weight Groups

A total of 409 women diagnosed with PCOS who used GnRH-antagonist fixed protocol as COS protocol were included in the present study. Among the four groups, the group with body weight over 70 kg had the lowest LH and AMH level while the group with body weight less than 50 kg had the highest LH and AMH level. Except for BMI, other baseline characteristics were similar among the four groups (all $p > 0.05$). Details of baseline clinical characteristics of the PCOS participants are shown in **Table 1**.

Initial Gn dosage and total dosage of Gn were significantly higher in the group with higher body weight (Group A = 150 (125-150), Group B = 150 (150-184.4), Group C = 175 (150-225), Group D = 212.5 (175-300), $p < 0.001$, and Group A = 1350 (1125-1512.5), Group B = 1575 (1350-1846.9), Group C = 1775 (1500-2250), Group D = 2137.5 (1800-2587.5), $p < 0.001$, respectively). Notably, more patients presented HOR in the group with body weight less than 50 kg, with 65.08% HOR patients in Group A, 64.89% in Group B, 60.55% in Group C, and 40.82% in Group D ($p = 0.018$). The clinical outcomes between

TABLE 1 | Baseline characteristics of PCOS patients in four body weight groups (n = 409).

Characteristics		Weight 40~50kg Group (n = 63)	Weight 50~60 kg Group (n = 188)	Weight 60~70 kg Group (n = 109)	Weight ≥70 kg Group (n = 49)	P
Age (years)		28 (26-30.5)	29 (27-32)	29 (26.3-31)	29 (26-31)	0.513
Type of infertility	Primary	68.25% (43/63)	63.83% (120/188)	70.64% (77/109)	51.02% (25/49)	0.106
	Secondary	31.75% (20/63)	36.27% (68/188)	29.36% (32/109)	48.98% (24/49)	
Oligo/hypomenorrhea		90.48% (57/63)	84.57% (159/188)	88.99%(97/109)	83.67% (41/49)	0.499
BMI (kg/m2)		18.8 (17.3-19.7)	21.4 (20.3-22.6)[ab]	24.5 (23.4-25.9)[cd]	28.2 (27.3-28.9)[ef]	<0.001*
T (ng/ml)		0.5 (0.3-0.6)	0.4 (0.3-0.5)	0.4 (0.3-0.6)	0.4 (0.3-0.8)	0.266
DHEAS (ug/dl)		0 (0-186)	90.1 (0-204.8)	0 (0-235.3)	135 (0-221.5)	0.725
AND (ng/ml)		0 (0-3.2)	1.7 (0-3.3)	0 (0-3.1)	1.3 (0-4.2)	0.604
SHBG (nmol/L)		0 (0-56)	0 (0-40.7)	0 (0-21.4)	6.8 (0-22.9)	0.237
FSH		6.6 (5.9-7.9)	6.6 (5.7-8.1)	6.3 (5.6-7.4)	6.7 (5.9-7.5)	0.646
LH		12.6 (8.3-17.9)	8.6 (5.6-14.7)	7.9 (4.7-11)[c]	6.1 (3.9-11.2)[e]	0.014*
LH/FSH		2.1 (1.1-2.9)	1.3 (0.8-2.2)	1.3 (0.8-1.8)	1 (0.6-1.8)	0.031
PCOM		80.95% (51/63)	79.26% (149/188)	84.40% (92/109)	83.67% (41/49)	0.706
AMH (ng/ml)		12.4 (8.7-15.5)	10.5 (6.9-15.7)	9.7 (5.8-13.3)	4.8 (3.1-8.3)[ef]	<0.001*

*BMI, body mass index; T, testosterone; DHEAS, dehydroepiandrosterone sulfate; AND, androstadienone; SHBG, sex hormone-binding globulin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PCOM, polycystic ovarian morphology; AMH, Anti-Mullerian hormone. *, P values < 0.05; [a]p<0.05 between Group A and Group B; [b]p<0.05 between Group B and Group C; [c]p<0.05 between Group A and Group C; [d]p<0.05 between Group C and Group D; [e]p<0.05 between Group A and Group D; [f]p<0.05 between Group B and Group D.*

the four weight groups were similar, except for blastocyst formation rate and good-quality blastocyst rate (76.71% vs. 73.17% vs. 72.60% vs. 63.94%, p = 0.003 and 27.21% vs. 35.40% vs. 29.66% vs. 35.95%, p = 0.018, respectively). Details of the COS and clinical outcomes of the four weight groups are displayed in **Table 2**.

Clinical Characteristics and Reproductive Outcomes Between Different Ovarian Response Groups

There were 249 patients of HOR and 160 patients of NOR in our study. And none of our patients expressed POR. Compared with PCOS patients with NOR, those with HOR had lower body weight,

TABLE 2 | COS and clinical outcomes of PCOS patients in four body weight groups (n = 409).

Characteristics		Weight 40~50kg Group (n = 63)	Weight 50~60 kg Group (n =188)	Weight 60~70 kg Group (n = 109)	Weight ≥70 kg Group (n = 49)	P
Initial Gn dosage (IU/day)		150 (125-150)	150 (150-184.4)[ab]	175 (150-225)[cd]	212.5 (175-300)[ef]	<0.001*
Stimulation time (days)		9 (8.5-10)	10 (9-11)	10 (9.3-11)[c]	10 (9-12)[e]	<0.001*
Total dosage of Gn (IU)		1350 (1125-1512.5)	1575 (1350-1846.9)[ab]	1775 (1500-2250)[cd]	2137.5 (1800-2587.5)[ef]	<0.001*
Number of oocytes with diameter ≥14mm on hCG day		12 (8-13.5)	10 (7-13)	9 (8-11.8)[c]	8 (7-9.3)[e]	0.009*
E2 on hCG day (pg/ml)		5200.3 (3470.2-8891.9)	5128.2 (3249.8-7094)	4254.8 (2776.2-6748.7)	2786.5 (1989-3793.9)[ef]	<0.001*
P on hCG day (ng/ml)		1.2 (0.8-1.4)	1 (0.8-1.5)	0.9 (0.6-1.3)	0.7 (0.5-1.1)[ef]	0.009*
LH on hCG day (IU/L)		1.7 (1.1-3.1)	1.7 (0.9-3.5)	2.1 (1.1-3.2)	2.4 (1.2-3.1)[e]	0.012*
Endometrial thickness on hCG day (mm)		5 (4.5-5.6)	5 (4.2-5.6)	5 (4.4-5.5)	5 (4.2-5.4)	0.283
Ovarian response	High	65.08% (41/63)	64.89% (122/188)	60.55% (66/109)	40.82% (20/49)[gh]	0.018*
	Normal	34.92% (22/63)	35.11% (66/188)	39.45% (43/109)	59.18% (29/49)	
Number of oocytes retrieved		13 (9.5-20)	16 (11-21.8)	15 (10-20)	12 (9.8-16.3)	0.06
MII oocyte rate		83.66% (814/973)	84.52% (2729/3229)	84.78% (1521/1794)	86.69% (573/661)	0.405
IVF fertilization rate		74.86% (658/879)	74.14% (2340/3156)	72.73% (1264/1738)	78.23% (485/620)	0.06
IVF normal fertilization rate		60.87% (574/943)	60.15% (1923/3197)	58.94% (1035/1756)	58.85% (389/661)	0.706
ICSI fertilization rate		85.95% (104/121)	91.60% (218/238)	87.10% (162/186)	88.68% (47/53)	0.334
ICSI normal fertilization rate		79.34% (96/121)	87.17% (197/226)	79.03% (147/186)	81.13% (43/53)	0.119
Cleavage rate		97.64% (744/762)	98.28% (2514/2558)	98.60% (1406/1426)	98.50% (524/532)	0.411
Good-quality D3 embryo rate		55.96% (310/554)	52.54% (952/1812)	51.15% (512/1001)	53.74% (201/374)	0.322
Blastocyst formation rate		76.71% (326/425)	73.17% (982/1342)	72.60% (583/803) [i]	63.94% (172/269)[gh]	0.003*
Good-quality blastocyst rate		27.21% (83/305)	35.40% (314/887)[j]	29.66% (159/536)	35.95% (55/153)[g]	0.018*
Fresh ET cancellation rate		76.19% (48/63)	66.49% (125/188)	70.64% (77/109)	53.06% (26/49)	0.06
Severe OHSS rate		4.76% (3/63)	3.19% (6/188)	1.83% (2/109)	2.04% (1/49)	0.753
Clinical pregnancy rate after fresh ET		66.67% (10/15)	50.79% (32/63)	50.00% (16/32)	52.17% (12/23)	0.715
Cumulative clinical pregnancy rate		64.10% (25/39)	53.85% (70/130)	57.58% (38/66)	60.00% (18/30)	0.694

*Gn, gonadotrophin; E2, estradiol; P, progesterone; LH, luteinizing hormone; OSI, ovarian sensitivity index; MII, mature; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; ET, embryo transfer; OHSS, ovarian hyperstimulation syndrome. *P values < 0.05; [a]p<0.05 between Group A and Group B; [b]p < 0.05 between Group B and Group C; [c]p < 0.05 between Group A and Group C; [d]p < 0.05 between Group C and Group D; [e]p < 0.05 between Group A and Group D; [f]p < 0.05 between Group B and Group D; [g]p < 0.0125 between Group A and Group D; [h]p < 0.0125 between Group B and Group D; [i]p < 0.0125 between Group C and Group D; [j]p < 0.0125 between Group A and Group B.*

higher AMH level, higher LH to FSH ratio, and less percentage of PCOM. During COS, lower initial Gn dosage (150 (150-200) vs. 175 (150-225), p = 0.008), lower total Gn dosage (1575 (1275-1850) vs. 1725 (1425-2193.8), p = 0.007), and more oocytes retrieved (19.5 (16-24) vs. 10 (8–12), p < 0.001) were observed in HOR patients. Patients with HOR had higher MII oocyte rate (85.26% vs. 82.77%, p = 0.017) and IVF normal fertilization rate (60.45% vs. 57.65%, p = 0.050), lower good-quality D3 embryo rate (51.27% vs. 58.55%, p < 0.001) than the NOR. Although clinical pregnancy rate after fresh ET and cumulative clinical pregnancy rate did not differ between patients with HOR and those with NOR, HOR patients had significantly higher fresh ET cancellation rate and severe OHSS rate (89.96% vs. 32.50%, p < 0.001 and 4.42% vs. 0.63%, p = 0.033, respectively). Details of the comparison of HOR and NOR PCOS patients are displayed in **Table 3**.

The Role of Body Weight in Adjusting Gn Dosage to Prevent HOR

In our multiple linear regression analysis, we aimed to find the association between body weight and initial Gn dosage, and the model was adjusted for age, body weight, T, LH, and AMH. We found that increased body weight was an independent factor that was significantly associated with the increase in initial Gn dosage (Beta = 0.399, t = 8.921, p < 0.001). In addition, AMH was inversely correlated with initial Gn dosage (Beta = -0.246, t = -5.458, p < 0.001). Patient's age, T, and LH level were not associated with initial Gn dosage in our analysis (**Table 4**).

All patients presenting with NOR received an initial Gn dosage between 100 IU and 300 IU. A ROC curve was drawn to identify the optimal cut-off value of body weight in predicting the increase of initial Gn dosage of over 150 IU and 225 IU. As for initial Gn dose of over 150 IU, the AUC was 0.725 (95% CI: 0.645-0.805, p < 0.001). Body weight of 53.25 kg was selected as the optimal cut-off value with a sensitivity of 84.2% and a specificity of 53.8% (**Figure 1A**). The AUC was 0.843 (95% CI: 0.755-0.932, p < 0.001) for initial Gn dose of over 225 IU, and the cut-off value was 70.5 kg for body weight (sensitivity, 58.8%; specificity, 93.0%) (**Figure 1B**).

TABLE 3 | Comparison of characteristics in PCOS patients with high ovarian response and normal ovarian response (n = 409).

Characteristics		HOR group (n = 249)	NOR group (n = 160)	P
Age (years)		29 (27-32)	29 (27-31)	0.722
Type of infertility	Primary	66.67% (166/249)	61.88% (99/160)	0.322
	Secondary	33.33% (83/249)	38.12% (61/160)	
Oligo/hypomenorrhea (%)		87.95% (219/249)	84.38% (135/160)	0.301
Weight (kg)		55 (51-60)	57 (52-65)	0.015*
BMI (kg/m2)		21.8 (20.3-23.6)	22.9 (20.3-25.6)	0.041*
T (ng/ml)		0.4 (0.3-0.5)	0.4 (0.3-0.6)	0.036*
DHEAS (ug/ml)		99.3 (0-210.5)	0 (0-204.5)	0.831
AND (ng/ml)		1.8 (0-3.4)	0 (0-3)	0.462
SHBG (nmol/l)		0 (0-41.9)	0 (0-22.6)	0.064*
FSH		6.5 (5.6-7.6)	6.5 (5.9-8)	0.022*
LH		9.6 (6.4-14.8)	7 (4.5-11.2)	0.001*
LH/FSH		1.6 (1-2.4)	1.1 (0.7-1.7)	<0.001*
AMH (ng/ml)		12 (9-16)	6.9 (4.1-11.6)	<0.001*
PCOM		85.94% (214/249)	74.38% (119/160)	0.003*
Initial Gn dosage (IU/day)		150 (150-200)	175 (150-225)	0.008*
Stimulation time (days)		10 (9-11)	10 (9-11)	0.467
Total Gn dosage (IU)		1575 (1275-1850)	1725 (1425-2193.8)	0.007*
Number of oocytes retrieved		19.5 (16-24)	10 (8-12)	<0.001*
MII oocyte rate		85.26% (4350/5102)	82.77% (1287/1555)	0.017*
IVF fertilization rate		74.34% (3644/4902)	73.98% (1103/1491)	0.781
IVF normal fertilization rate		60.45% (3039/5027)	57.65% (882/1530)	0.050*
ICSI fertilization rate		89.94% (438/487)	83.78% (93/111)	0.064
ICSI normal fertilization rate		83.37% (406/487)	77.78% (77/99)	0.183
Cleavage rate		98.26% (4011/4082)	98.41% (1177/1196)	0.723
Good-quality D3 embryo rate		51.27% (1516/2957)	58.55% (459/784)	<0.001*
Blastocyst formation rate		72.88% (1701/2334)	71.68% (362/505)	0.585
Good-quality blastocyst rate		32.89% (523/1590)	30.24% (88/291)	0.374
Fresh ET cancellation rate		89.96% (224/249)	32.50% (52/160)	<0.001*
Severe OHSS rate		4.42% (11/249)	0.63% (1/160)	0.033*
Clinical pregnancy rate after fresh ET		56.00% (14/25)	51.85% (56/108)	0.708
Cumulative clinical pregnancy rate		59.71% (83/139)	53.97% (68/126)	0.346

*HOR, high ovarian response; NOR, normal ovarian response; BMI, body mass index; T, testosterone; DHEAS, dehydroepiandrosterone sulfate; AND, androstadienone; SHBG, sex hormone-binding globulin; FAI, free androgen index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PCOM, polycystic ovarian morphology; AMH, Anti-Mullerian hormone; Gn, gonadotrophin; E2, estradiol; P, progesterone; OSI, ovarian sensitivity index; MII, mature; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; ET, embryo transfer; OHSS, ovarian hyperstimulation syndrome. *P values < 0.05.*

TABLE 4 | Multiple linear regression analysis of initial Gn dosage.

	Unstandardized coefficients		Standardized coefficients	t	P
	B	SE	Beta		
Age	-0.56	0.698	-0.035	-0.802	0.423
Weight	2.579	0.289	0.399	8.921	<0.001
T	1.756	4.24	0.018	0.414	0.679
LH	0.059	0.205	0.013	0.287	0.774
AMH	-2.87	0.526	-0.246	-5.458	<0.001

SE, standard error; T, testosterone; LH, luteinizing hormone; AMH, Anti-Mullerian hormone.

DISCUSSION

In this retrospective cohort study, we found that increased body weight was significantly correlated to the increase of initial Gn dosage which was also associated with HOR. Compared with HOR patients, NOR patients had significantly less fresh ET cancellation rate and severe OHSS rate. In this way, we believed that adjusting initial Gn dosage based on body weight may be beneficial to reproductive outcomes in PCOS patients. Therefore, using ROC curves, we found that less than 150 IU initial Gn was appropriate for patients with body weight under 53.25 kg, and less than 225 IU initial Gn was suitable for those under 70.5 kg in order to prevent HOR.

According to our results, we found that there was a relationship between initial Gn dose and body weight and AMH. An appropriate dosage of Gn is necessary during ovarian stimulation to improve synchronization of follicular growth and maturity of oocytes at retrieval and avoid unpredicted POR at the same time (14). For young women with AFC>15, rFSH dose (IU per kg) was related to ovarian response, and the starting dose of rFSH adjusted for body weight had a prediction role on day 5 median follicle size and the proportion of antral follicles recruited, when adjusted by age, AFC, and pre-treatment FSH level (9). Several factors have been put forward to adjust the Gn dose during COS, including ovarian response, AMH, and AFC (6, 15, 16). Involving two or more factors could help improve COS results significantly. Some had individualized rFSH doses based on the consistency in r-FSH starting doses for individualized treatment (CONSORT) dosing algorithm (17). However, patients with more than 25 oocytes retrieved, a history of severe OHSS, or BMI over 30 kg/m^2 were excluded. One study set the optimal number of oocytes retrieval to be 9 in women younger than 40 but they also failed to include women with irregular menstrual cycle or presented PCOM (18). Another two PIVET rFSH algorithms adjusted by AMH, AFC, BMI, and age were conducted to optimize the number of retrieved oocytes of no more than 15 (19). The researchers found that the need for elevating rFSH dose increased with the starting dose reduction. The cancellation rate of all and no more than 75 IU FSH groups were 6.2% and 8.7%, respectively. Unfortunately, the clinical pregnancy and live birth outcomes of hyper-responders were not provided.

A higher incidence of HOR in patients with relatively lower body weight was observed in our study, although they received less initial and total Gn dose during COS. Compared with those who showed NOR, patients with HOR, although retrieved more oocytes, had about eight times the incidence of OHSS and 2.5 times of fresh ET cancellation rate. Limited studies have focused

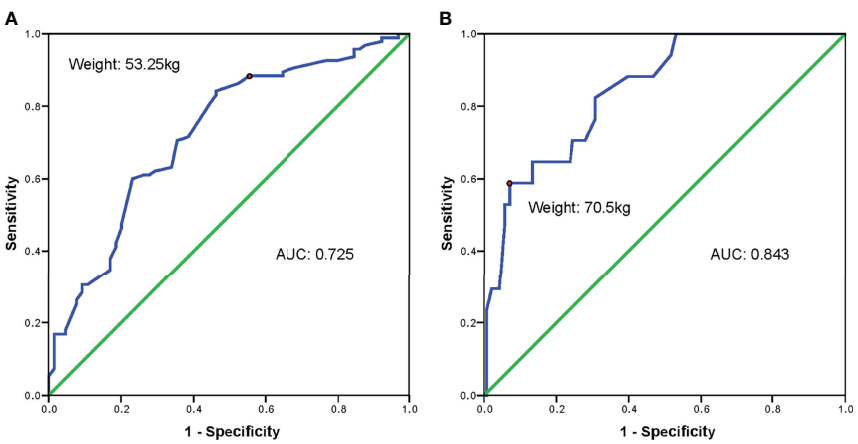


Figure 1 Receiver operating characteristic curves of body weight in predicting initial Gn dosage set up in PCOS patients with normal ovarian response. **(A)** body weight in predicting initial Gn dosage higher than 150 IU/day in PCOS patients with normal ovarian response; **(B)** body weight in predicting initial Gn dosage higher than 225 IU/day in PCOS patients with normal ovarian response. AUC, area under curve.

on the ovarian stimulation of hyper-responders. A meta-analysis summarized the effect of Gn dose grouped by ovarian reserve tests (20). Only two studies were included in predicted hyper-responders' part (AFC>15 or AMH 15-50 pmol/l). The results showed that decreasing the dose from 150IU did not make a difference in clinical pregnancy and live birth rates, while it did reduce the risk of moderate and severe OHSS. Similar results were reported by a previous study that lowered the FSH dose in patients with AMH >32 pmol/L (21). Our results together with these findings showed that the weakening of actual ovarian response reflected by oocytes retrieval and transferable embryos did not outweigh clinical outcomes. As the clinical outcomes would not be improved or even impaired when more than 15-20 oocytes were recovered, together with increased risk of early OHSS or thromboembolic events (22). It is reasonable and vital to control the incidence of HOR. We also found that HOR women expressed relatively higher AMH, LH to FSH ratio, and PCOM, indicating these patients had more serious endocrine dysfunction. AMH and AFC are important indicators of Gn dose. Through regression analysis, we found that body weight is also an independent factor of the starting dose. Since AMH and AFC are generally high in PCOS patients, it is necessary to take body weight into consideration.

According to ESHRE guideline recommendations, a Gn dose of 150 IU in GnRH antagonist protocol was suggested for high responders (23). However, the pharmacokinetics and pharmacodynamics of Gn should be individualized to different patients. The excess weight affects the ovarian response to Gn and exogenous serum FSH level is inversely associated with body weight (24). The volume of extracellular fluid is a key factor for drug distribution. Women with elevated BMI own a larger portion of fat tissue, which contributes to low content of extracellular water than those without (8). In other words, for two patients with the same BMI, the one with a higher body weight owns more extracellular fluid than the other. Therefore, body weight is more predominant in determining FSH distribution than BMI (25). A previous study found that reducing the FSH dose in predicted hyper responders with body weight > 55kg significantly decreased the OHSS occurrence but also decreased the probability of live birth (24). On the contrary, a randomized controlled trial (RCT) found that the reduction of Gn dosage significantly lowered the OHSS incidence, together with no influence on live birth rates (11). However, women involved in this study were free from PCOS. Some researchers came up with a low-dose stimulation with a basement dose of 75 IU and increment/decrement of 25-50 IU according to age, AMH, BMI, and previous onset of OHSS. Patients with PCOS and the control group had comparable clinical pregnancy rates (32.2% vs. 34.4%) and moderated or severe OHSS rates (16.9% vs. 15.7%). Additionally, there was no cancellation because of unexpected poor responses (7). One study made a cut-off value of 60 kg to decrease FSH dose from 150 IU to 112.5 IU in PCOS patients but did not explain the possible reason behind it (26). In our study, as all patients presented HOR or NOR, no patients received a starting dose of over 300 IU as Gn overdose did not lead to increased oocytes and pregnancy outcomes (27). We aimed to clarify the exact body weight to predict the need for lowering the initial Gn dosage from 150 IU or 300 IU, to prevent the risk of HOR. We suggested that patients with body weight below 53.25 kg required a starting dose of Gn of less than 150 IU, and 70.5 kg for Gn dose less than 225 IU, based on the data from NOR patients.

However, our study also has several limitations. Firstly, as a retrospective study, there might be selective bias. Therefore, we collected and compared the baseline characteristics of the enrolled participants. Secondly, the sample size of our study was relatively small, which might cause the insignificance of some findings due to limited power. Thirdly, this study was conducted in a single reproductive center which may limit the external validity of our findings. Also, we were not able to access the pregnancy results and cumulative live birth rate due to the limited following-up time. Further multicenter studies with larger sample sizes and a longer follow-up period are needed.

In summary, our result showed a relationship between body weight and Gn starting dose in ART, which was often overlooked by previous studies. Adjusting initial Gn dosage according to body weight is of great importance for preventing HOR and better reproductive outcomes in PCOS patients and further large scale, randomized, controlled trials should be encouraged in this field.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ethical Review Board of West China Second University Hospital, Sichuan University. The ethics committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

XZ and LQ designed the study. RZ had full access to all the data in the study. HC performed the statistical analyses. RZ, HC, XZ, and LQ contributed to the clinical interpretation of the results. RZ and HC drafted the manuscript. All authors read and approved the final version of the article.

FUNDING

This work was supported by the Sichuan Science and Technology Program [2020YFS0127].

REFERENCES

1. Liu J, Wu Q, Hao Y, Jiao M, Wang X, Jiang S, et al. Measuring the Global Disease Burden of Polycystic Ovary Syndrome in 194 Countries: Global Burden of Disease Study 2017. *Hum Reprod* (2021) 36(4):1108–19. doi: 10.1093/humrep/deaa371
2. Mulders AG, Laven JS, Eijkemans MJ, Hughes EG, Fauser BC. Patient Predictors for Outcome of Gonadotrophin Ovulation Induction in Women With Normogonadotrophic Anovulatory Infertility: A Meta-Analysis. *Hum Reprod Update* (2003) 9(5):429–49. doi: 10.1093/humupd/dmg035
3. Franks S, Hardy K. What Causes Anovulation in Polycystic Ovary Syndrome? *Curr Opin Endocr Metab Res* (2020) 12:59–65. doi: 10.1016/j.coemr.2020.03.001
4. Practice Committee of the American Society for Reproductive Medicine. Prevention and Treatment of Moderate and Severe Ovarian Hyperstimulation Syndrome: A Guideline. *Fertil Steril* (2016) 106(7):1634–47. doi: 10.1016/j.fertnstert.2016.08.048
5. Nelson SM. Prevention and Management of Ovarian Hyperstimulation Syndrome. *Thromb Res* (2017) 151 Suppl 1:S61–s4. doi: 10.1016/S0049-3848(17)30070-1
6. Fatemi H, Bilger W, Denis D, Griesinger G, La Marca A, Longobardi S, et al. Dose Adjustment of Follicle-Stimulating Hormone (FSH) During Ovarian Stimulation as Part of Medically-Assisted Reproduction in Clinical Studies: A Systematic Review Covering 10 Years (2007-2017). *Reprod Biol Endocrinol* (2021) 19(1):68. doi: 10.1186/s12958-021-00744-x
7. Fischer D, Reisenbüchler C, Rösner S, Haussmann J, Wimberger P, Goeckenjan M. Avoiding OHSS: Controlled Ovarian Low-Dose Stimulation in Women With PCOS. *Geburtshilfe Frauenheilkd* (2016) 76(6):718–26. doi: 10.1055/s-0042-100206
8. Ledger WL, Fauser BC, Devroey P, Zandvliet AS, Mannaerts BM. Corifollitropin Alfa Doses Based on Body Weight: Clinical Overview of Drug Exposure and Ovarian Response. *Reprod BioMed Online* (2011) 23 (2):150–9. doi: 10.1016/j.rbmo.2011.04.002
9. Abbara A, Patel A, Hunjan T, Clarke SA, Chia G, Eng PC, et al. FSH Requirements for Follicle Growth During Controlled Ovarian Stimulation. *Front Endocrinol (Lausanne)* (2019) 10:579. doi: 10.3389/fendo.2019.00579
10. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 Consensus on Diagnostic Criteria and Long-Term Health Risks Related to Polycystic Ovary Syndrome. *Fertil Steril* (2004) 81(1):19–25. doi: 10.1016/j.fertnstert.2003.10.004
11. Oudshoorn SC, van Tilborg TC, Eijkemans MJC, Oosterhuis GJE, Friederich J, van Hooff MHA, et al. Individualized Versus Standard FSH Dosing in Women Starting IVF/ICSI: An RCT. Part 2: The Predicted Hyper Responder. *Hum Reprod* (2017) 32(12):2506–14. doi: 10.1093/humrep/dex319
12. Qiao J, Ma C, Liu J, Ma X, Li S, Yang Y, et al. A Consensus of Poor Ovarian Response. *Chin J Reprod Contracept* (2015) 35:211–23. 10.7669/j.issn.0253-357X.2015.04.0211
13. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L. ESHRE Consensus on the Definition of 'Poor Response' to Ovarian Stimulation for *In Vitro* Fertilization: The Bologna Criteria. *Hum Reprod* (2011) 26(7):1616–24. doi: 10.1093/humrep/der092
14. Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst Score Affects Implantation and Pregnancy Outcome: Towards a Single Blastocyst Transfer. *Fertil Steril* (2000) 73(6):1155–8. doi: 10.1016/S0015-0282(00)00518-5
15. van Tilborg TC, Oudshoorn SC, Eijkemans MJC, Mochtar MH, van Golde RJT, Hoek A, et al. Individualized FSH Dosing Based on Ovarian Reserve Testing in Women Starting IVF/ICSI: A Multicentre Trial and Cost-Effectiveness Analysis. *Hum Reprod* (2017) 32(12):2485–95. doi: 10.1093/humrep/dex321
16. Guo Y, Jiang H, Hu S, Liu S, Li F, Jin L. Efficacy of Three COS Protocols and Predictability of AMH and AFC in Women With Discordant Ovarian Reserve Markers: A Retrospective Study on 19,239 Patients. *J Ovarian Res* (2021) 14 (1):1–12. doi: 10.1186/s13048-021-00863-4
17. Humaidan P, Nelson SM, Devroey P, Coddington CC, Schwartz LB, Gordon K, et al. Ovarian Hyperstimulation Syndrome: Review and New Classification Criteria for Reporting in Clinical Trials. *Hum Reprod* (2016) 31(9):1997–2004. doi: 10.1093/humrep/dew149
18. La Marca A, Papaleo E, Grisendi V, Argento C, Giulini S, Volpe A. Development of a Nomogram Based on Markers of Ovarian Reserve for the Individualisation of the Follicle-Stimulating Hormone Starting Dose in *In Vitro* Fertilisation Cycles. *BJOG* (2012) 119(10):1171–9. doi: 10.1111/j.1471-0528.2012.03412.x
19. Yovich JL, Alsbjerg B, Conceicao JL, Hinchliffe PM, Keane KN. PIVET rFSH Dosing Algorithms for Individualized Controlled Ovarian Stimulation Enables Optimized Pregnancy Productivity Rates and Avoidance of Ovarian Hyperstimulation Syndrome. *Drug Des Devel Ther* (2016) 10:2561–73. doi: 10.2147/dddt.S104104
20. Lensen SF, Wilkinson J, Leijdekkers JA, La Marca A, Mol BWJ, Marjoribanks J, et al. Individualised Gonadotropin Dose Selection Using Markers of Ovarian Reserve for Women Undergoing *In Vitro* Fertilisation Plus Intracytoplasmic Sperm Injection (IVF/ICSI). *Cochrane Database Syst Rev* (2018) 2(2): Cd012693. doi: 10.1002/14651858.CD012693.pub2
21. Sopa N, Larsen EC, Westring Hvidman H, Andersen AN. An AMH-Based FSH Dosing Algorithm for OHSS Risk Reduction in First Cycle Antagonist Protocol for IVF/ICSI. *Eur J Obstet Gynecol Reprod Biol* (2019) 237:42–7. doi: 10.1016/j.ejogrb.2019.02.001
22. Steward RG, Lan L, Shah AA, Yeh JS, Price TM, Goldfarb JM, et al. Oocyte Number as a Predictor for Ovarian Hyperstimulation Syndrome and Live Birth: An Analysis of 256,381 *In Vitro* Fertilization Cycles. *Fertil Steril* (2014) 101(4):967–73. doi: 10.1016/j.fertnstert.2013.12.026
23. The ESHRE guideline Group on Ovarian Stimulation, Bosch E, Broer S, Griesinger G, Grynberg M, Humaidan P, et al. ESHRE Guideline: Ovarian Stimulation for IVF/ICSI(†). *Hum Reprod Open* (2020) 2020(2):hoaa009. doi: 10.1093/hropen/hoaa009
24. Leijdekkers JA, van Tilborg TC, Torrance HL, Oudshoorn SC, Brinkhuis EA, Koks CAM, et al. Do Female Age and Body Weight Modify the Effect of Individualized FSH Dosing in IVF/ICSI Treatment? A Secondary Analysis of the OPTIMIST Trial. *Acta Obstet Gynecol Scand* (2019) 98(10):1332–40. doi: 10.1111/aogs.13664
25. Waki M, Kral JG, Mazariegos M, Wang J, Pierson RNJr., Heymsfield SB. Relative Expansion of Extracellular Fluid in Obese vs. Nonobese Women. *Am J Physiol* (1991) 261(2 Pt 1):E199–203. doi: 10.1152/ajpendo.1991.261.2.E199
26. Shi Y, Wei D, Liang X, Sun Y, Liu J, Cao Y, et al. Live Birth After Fresh Embryo Transfer vs Elective Embryo Cryopreservation/Frozen Embryo Transfer in Women With Polycystic Ovary Syndrome Undergoing IVF (FreFro-PCOS): Study Protocol for a Multicenter, Prospective, Randomized Controlled Clinical Trial. *Trials* (2014) 15:154. doi: 10.1186/1745-6215-15-154
27. Rombauts L. Is There a Recommended Maximum Starting Dose of FSH in IVF? *J Assist Reprod Genet* (2007) 24(8):343–9. doi: 10.1007/s10815-007-9134-9

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Endocrinology

REVIEW
published: 05 July 2022
doi: 10.3389/fendo.2022.936948

Associations Between Asthma and Polycystic Ovary Syndrome: Current Perspectives

Yue Xu[1†], *Zhi-Yang Zhou*[1†], *Jie-Xue Pan*[1*] and *He-Feng Huang*[1,2,3*]

[1] Obstetrics & Gynecology Hospital, Institute of Reproduction and Development, Fudan University, Shanghai, China, [2] Shanghai Key Laboratory of Embryo Original Diseases, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China, [3] Research Units of Embryo Original Diseases, Chinese Academy of Medical Sciences, Shanghai, China

OPEN ACCESS

Edited by:
Da Li,
ShengJing Hospital of China Medical University, China

Reviewed by:
Bei Shi,
China Medical University, China
Liangshan Mu,
Zhejiang University School of Medicine, China

***Correspondence:**
He-Feng Huang
huanghefg@fudan.edu.cn
Jie-Xue Pan
panjiexue@fudan.edu.cn

[†]These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

Received: 05 May 2022
Accepted: 16 May 2022
Published: 05 July 2022

Citation:
Xu Y, Zhou Z-Y, Pan J-X and Huang H-F (2022) Associations Between Asthma and Polycystic Ovary Syndrome: Current Perspectives. Front. Endocrinol. 13:936948. doi: 10.3389/fendo.2022.936948

A potential correlation between polycystic ovary syndrome (PCOS) and asthma, used to be identified as diseases originating from two independent systems, has been supported by increasing evidence. From an epidemiological perspective, mounting studies have confirmed that women suffering from PCOS exhibit increased susceptibility to asthma. Meanwhile, PCOS and asthma seem to share several mutual pathological conditions, such as metabolic disorders, hormonal fluctuation, proinflammatory state, etc. Here, we further elucidate the correlation between asthma and PCOS by focusing on the internal common pathophysiology and adverse influences on women's health. Understanding the internal connection between PCOS and asthma may shed light on developing new prevention and control strategies to fight against these conditions.

Keywords: polycystic ovary syndrome, asthma, metabolic syndrome, chronic inflammation, reproductive health

INTRODUCTION

As a common endocrinopathy among women of childbearing age, polycystic ovary syndrome (PCOS) poses a considerable threat to the health of women throughout the whole world with a prevalence of 15%-20% (1, 2). According to the revised 2003 Rotterdam criteria, a final diagnosis of PCOS can be made if two of the following indicators are met: hyperandrogenism, ovulatory dysfunction, and polycystic changes in the ovary (3). In addition to reproductive health issues (4), PCOS carries an important responsibility for female metabolic disorders and mental problems (5, 6). Notably, chronic systemic and local ovarian inflammation has emerged as a vital focus for the study of PCOS pathophysiology (7–9). To be more specific, previous studies have indicated that PCOS women usually exhibit abnormally elevated levels of inflammatory cytokines (e.g. TNF-α,

Abbreviations: PCOS, polycystic ovary syndrome; T2DM, diabetes mellitus type 2; CRP, C-reactive protein; AHR, hyperresponsiveness; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; BMI, body mass index; MS, metabolic syndrome; IR, insulin resistance; IGT, impaired glucose tolerance; GLUT4, glucose transporter 4; IGF-1, insulin-like growth factor 1; FRC, functional residual capacity; ERV, expiratory volume; SPA, surface-active protein A; GC, granulosa cell; TTP, time to pregnancy; PTX3, pentraxin-3; PMA, perimenstrual asthma; MRI, magnetic resonance imaging; HPA, hypothalamic-pituitary-adrenal; CAR, cortisol awakening response; AMH, anti-Müllerian hormone; COVID-19, coronavirus disease 2019.

interleukins, CRP) (9), as well as DNA single-nucleotide polymorphisms of relevant inflammatory mediators (10).

Asthma, a chronic airway inflammatory disease with recurrence and reversibility, is characterized by paroxysmal wheezing, shortness of breath, chest tightness, and cough. To date, approximately 235 million people worldwide suffer from asthma, causing a considerable burden of disease to both families and societies (11). The pathogenesis of asthma involves complex interplay of multiple factors, such as airway hyperresponsiveness (AHR), chronic low-grade inflammation, and airway structural changes (12). In particular, intensive investigations have proposed a potential causal relationship between hormonal fluctuation and the occurrence or severity of asthma, and extensively highlighted the gender disparities that can change with age, which probably accounts for the reproductive disorders in asthmatic women. Intriguingly, as indicated by epidemiological studies, females with metabolic syndrome tend to exhibit an elevation in susceptibility to asthma attacks, whereas the mechanisms underlying the pathophysiology are poorly understood.

Following the similar risk factors as well as the intersection of potential pathogenesis, several lines of evidence have gradually revealed a non-negligible association between asthma and PCOS in metabolic syndrome, impaired fertility, irregular menstruation, and mood disturbances. And chronic local and systemic inflammation may act as one of the common molecular mechanisms behind these phenomena. Therefore, by literature retrieval and inductive analysis, we further expounded the correlation between asthma and PCOS by focusing on the internal common pathophysiology and insults on female health, to provide unique insights for further exploration of disease prevention and therapy (**Figure 1**).

CONCOMITANCE OF ASTHMA AND PCOS

PCOS is comorbid with respiratory diseases with a considerable frequency, and asthma in particular. A retrospective cohort study in 2015, which collected and mined the data from Australian statewide hospitals, reported that the prevalence of respiratory disease in women with PCOS was about 22.8%, compared with 14.2% in the control group; among them, 10.6% of the former were admitted to hospital for asthma, while only 4.5% of the latter, suggesting that women with PCOS were more prone to develop asthma (13). And other epidemiological studies of different populations have reached similar conclusions (4, 12–16). In support of the above findings, Underdal et al. propose a potential link between PCOS and asthma from the perspective of lung function, reporting a combined obstructive (forced expiratory volume in one second (FEV1) % predicted, 93.7 vs 102.0) and restrictive (forced vital capacity (FVC) % predicted, 94.5 vs 103.7) respiratory impairment in PCOS compared with controls (17). Meanwhile, a historical cohort study reported that irregular period was associated with higher odds of atopic asthma (18), which once again verified the potential connection between asthma and ovulatory dysfunction. Recent studies have further explored whether confounding factors alter the association between asthma and PCOS, and indicate that the incidence of asthma remained higher even after adjusting for

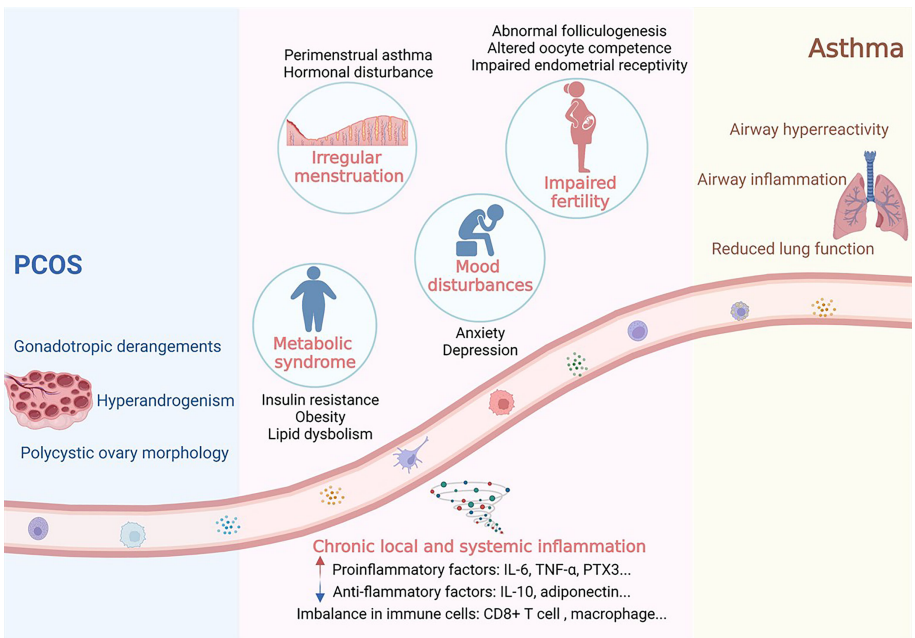


FIGURE 1 | Potential associations between PCOS and asthma. Women with PCOS or asthma are characterized by several common disease phenotypes, including metabolic syndrome, irregular menstruation, impaired fertility and mood disturbances. And chronic local and systemic inflammation may act as one of the common molecular mechanisms behind these phenomena. Image was created with Biorender®.

heterogeneity of body mass index (BMI), smoking status, and dietary intake (12, 16). Furthermore, Sun H et al. (19) performed a meta-analysis that systematically evaluated the prevalence of asthma as related to PCOS, and determined PCOS as an independent risk factor for asthma.

Although the link between PCOS and asthma incidence is well established, results from different studies on asthma severity among women with or without PCOS are conflicting. To be specific, Zierau et al. investigated the severity of asthma based on the use of anti-asthma medication, and indicated that PCOS status is not a contributing factor to exacerbation of asthma (14). However, other studies report opposite results, which are verified by a higher hospital admission rate for asthma and increased doses of anti-asthma medication in PCOS women (13, 15). In combination, the researches mentioned above suggest an important correlation between PCOS and asthma attack and provide a reliable perspective to investigate the corresponding pathogenesis (**Table 1**).

OVERLAP BETWEEN FEATURES OF PCOS AND ASTHMA

Metabolic Syndrome

Metabolic syndrome (MS) is a cluster of complex disorders characterized by abnormal glucose metabolism, dyslipidemia, overweight or obesity, and hypertension, and has been recognized as the most critical long-term health problem associated with PCOS. Both obese and lean women with PCOS are reported to have a much higher risk of MS compared with the control group (20–22). So far, numerous pathologic changes have been identified in exploring the pathogenesis of PCOS, such as hyperandrogenemia, hyperinsulinemia, dysfunction of the hypothalamic-pituitary-ovarian axis, and disturbances of adipokines secretion. These specific alterations interact with each other in fat tissues, liver, muscle, and ovary, resulting in diverse phenotypes of MS.

Remarkably, insulin resistance (IR) and hyperandrogenism stand out as pivotal factors in the development of PCOS. Approximately 50-80% of PCOS females exhibit varying degrees of IR, which possibly accounts for the higher annual conversion rate to impaired glucose tolerance (IGT) or diabetes mellitus type 2 (T2DM) in them (23, 24). Previous studies have identified several abnormal or dysfunctional cells and molecules involved in the IR status of PCOS women, such as glucose transporter 4 (GLUT4), insulin receptor β subunit, pancreatic β-cells, and so on (25, 26). In PCOS, adipose tissue dysfunction, possibly leading to excessive visceral fat depots, is thought to be involved in determining both IR and dysregulated androgen metabolism (27). When exposed to excessive androgen, adipocytes seem to be prone to hypertrophy, releasing superabundant adipokines and inflammatory mediators. TNF-α, for instance, can block the tyrosine kinase phosphorylation of insulin receptors (28), and affect glucose transport by inhibiting GLUT-4 activity (29), thus inducing the obesity-related IR or hyperinsulinemia. Functionally, insulin acts as an essential factor

TABLE 1 | General characteristics of key studies.

Author	Year	Country	Study design	PCOS criteria	Asthma criteria	Characteristics of the subjects		Asthma incidence % (n)		P value
						PCOS	Control	PCOS	Control	
Hart et al. (13)	2015	Australia	Retrospective cohort study	ICD-10	Medical record	Median age: 27.9	Not available	10.56% (271/2566)	4.52% (1160/25660)	<0.01
Underdal et al. (17)	2020	Norway	Prospective cohort study	Rotterdam criteria	Medical record	Mean age: 38 Mean BMI: 28.5	Mean age: 38 Mean BMI: 25.7	19.31% (28/145)	8.74% (38/435)	<0.01
Doherty et al. (4)	2015	Australia	Retrospective cohort study	ICD-10	Medical record	Not available	Not available	13.64% (478/3505)	9.90% (3450/34856)	<0.01
Zierau et al. (14)	2018	Denmark	Prospective cohort study	Rotterdam criteria	Medical record	Mean age: 34 BMI: 25.8 ± 5.3	Mean age: 34 BMI: 24.3 ± 4.9	19.59% (266/1358)	14.48% (786/5430)	<0.001
Glintborg et al. (15)	2015	Denmark	Prospective cohort study	ICD-10	Medical record	Mean age: 30.6	Mean Age: 30.6	3.05% (586/19199)	2.21% (1270/57483)	<0.001
Htet et al. (16)	2017	Australia	Prospective cohort study	Questionnaire	Questionnaire	Age: 30.5 ± 0.1 BMI: 28.0 ± 0.3	Age: 30.6 ± 0.02 BMI: 25.1 ± 0.1	16.11% (77/478)	10.52% (856/8134)	0.004
Grieger et al. (12)	2020	Australia	Prospective cohort study	Questionnaire	Questionnaire	Age: 33.5 ± 1.4 BMI: 28.5 ± 7.4	Age: 33.7 ± 1.5 BMI: 25.6 ± 5.8	13.64% (478/3505)	9.90% (3450/34856)	0.004

in the development of hyperandrogenism (30). Insulin not only directly stimulates follicular theca cells to produce androgen and encourages the development of stromal cells in the ovary, but also induces the secretion of insulin-like growth factor 1 (IGF-1) so as to promote androgen synthesis in adrenal and gonads (31). Thus, adipose tissue dysfunction, inflammation, IR, and hyperandrogenism form a vicious cycle that leads to different phenotypes in PCOS patients.

In recent years, growing evidence also supports MS is a potential risk factor for asthma. Multiple studies have linked MS to asthma attacks but the correlation is still under dispute. Lee et al. (32) put forward that patients with MS are more likely to develop symptoms of wheeze and dyspnea at rest or after exercise. This is consistent with a large retrospective cohort study from Norway showing that excessive waist circumference and diabetes increase the susceptibility to asthma in adults (33). What's more, cohort studies in Japan and China also revealed that overweight or obesity can dramatically aggrandize susceptibility to asthma in females but not in males (21, 34). The sexual heterogeneity makes it reasonable to presume that specific gonadal hormones and metabolic disorders may complement each other to exert promoting effect on the pathogenesis of asthma. However, whether every element of MS can induce female asthma attacks is still controversial. Assad et al. pointed out that the association between abdominal adiposity, elevated blood pressure, impaired fasting glucose or diabetes and asthma incidence is no longer statistically significant after adjustment for BMI (35). This raises the possibility that BMI or obesity is a stronger predictor of asthma than MS. In turn, so far little is known about the influence of asthma on obesity incidence. Several studies have shown that early-life of asthma may result in a higher susceptibility to obesity in later childhood and adolescence (36, 37), but further investigations are needed to verify the promoting effect of asthma on obesity.

The underlying mechanism of overweight or obesity prompting asthma remains uncertain, and attention is mainly focused on the mechanical factors and altered inflammation responses. First, obesity is theorized to encourage drastic changes in normal lung physiology. Excessive accumulation of fat in the chest and abdominal cavity contributes to lung compression and reduced lung volume, resulting in a pronounced decrease in functional residual capacity (FRC) and expiratory volume (ERV) (38, 39). Low lung volume leads to AHR through impaired ability to stretch the airway wall (40). Among patients with late-onset non-allergic asthma, obesity induces excessive collapsibility of airways by reducing distal airway wall stiffness (41). Second, it is not unexpected that obesogenic dietary patterns tend to contain much fatty acids and fructose but little fiber and antioxidants. Growing evidence verifies that food rich in saturated fatty acids can encourage pulmonary lymphocyte infiltration, induce congenital AHR and further allergic airway inflammation through the IL-1β pathway (42, 43). Third, inflammatory effects of obesity on the lung involve dysregulation of proinflammatory adipokines (e.g. excess leptin) and anti-inflammatory adipokines (e.g. adiponectin deficiency). Excessive adipose tissue secrets redundant pro-inflammatory cytokines, such as TNF-α, interleukin, and leptin, which further trigger systemic inflammation and AHR. Meanwhile, the downregulated level of bronchoalveolar surface-active protein A (SPA) in obese subjects with asthma can cause airway eosinophil aggregation (44), thereby increasing the risk of allergic airway inflammation. In combination, MS may then act as a link between PCOS and asthma, with a co-existence of MS and PCOS aggravating asthma.

Impaired Fertility

It is well established that PCOS status threatens fertility in women of childbearing age. Although women with PCOS are typically characterized by an increase in the quantity of preantral follicles, the quality of oocytes is usually diminished, resulting in impaired fertilization, cleavage as well as embryo implantation. Wild et al. (45) demonstrated that 17.5% of PCOS women, 11 times that of the control group, reported fertility problems, of which 66% of PCOS women suffered from infertility and 24% relied on ovulation induction. Consistently, other researchers also observed remarkable growths in the non-fertility rate, gestational age, and the utilization rate of assisted reproductive technology in PCOS women (46, 47). Hormonal imbalance is the most striking feature of PCOS, accounting for diverse reproductive problems. In PCOS, hypersecretion of LH during follicle formation significantly impairs oocyte maturation, fertilization, and embryo quality (48, 49). Part of the cellular and molecular abnormalities caused by excessive LH have been identified, including suppressed FSH function, dysfunctional granulosa cell (GC), damaged oocyte nucleus, and impaired extrusion of the first polar body (50, 51). Excessive insulin can cooperate with LH to stimulate androgen production (52), which thereby retards the development of dominant follicles, impacts endometrial receptivity, and increases the risk of various obstetric complications (53–55).

Recently, much more attention has been paid to the similar association between allergic diseases and infertility. Gade et al. (56) revealed that asthma was responsible for a prolonged time to pregnancy (TTP), with TTP of about 27% of asthmatic women extended beyond one year, and specific treatment for asthma could effectively abolish the extension of TTP (57). Hansen et al. (58) explored the association between asthma and the need for fertility treatment among women with life births, and pointed out that the association remained significant after adjusting for age, BMI, and smoking habits. What's worse, despite fertility treatment, asthma is still associated with unfavorable pregnancy outcomes (59). Rocklin et al. reported that maternal asthma may increase the risk of preterm birth, low birth weight, and perinatal mortality (60). Overall, the vast majority of evidence points toward the possibility that asthma in women has an inextricable link with delayed conception, lowered genitality, and poor pregnancy outcomes.

Obesity and inflammation response are two pathologic conditions closely related to both PCOS and asthma, which

may provide insights into the impaired fertility associated with these two conditions. Obesity is partially responsible for poor oocyte quality through a variety of pathways, including follicular development retardation, oocyte maturation problems, meiosis abnormalities, as well as mitochondrial dynamics disorder (61–64). Excessive free fatty acids are presumed to exert toxic effects on reproductive tissues through oxidative stress, leading to damage or apoptosis of female gametes (64). Recently, studies have demonstrated that several adipokines may carry metabolic responsibilities for female reproductive function, especially in ovarian physiology. Through the interaction with LH and insulin, adiponectin encourages the expression of genes related to periovulatory maturation of ovarian follicles (65). Lower adiponectin levels were reported in the plasma of either PCOS (66) or asthma (67) women, as well as in the follicles of PCOS women (68). These suggest its possible role in the follicular arrest and ovulatory dysfunction. In combination, obesity, independently or jointly with endocrine disruption, impair female fertility in women with PCOS or asthma.

What's more, abundant available evidence supports chronic systematic inflammation as the common cornerstone for the internal pathogenesis of impaired fertility in both PCOS and asthma. The immune system plays a pivotal part in the reproductive process. Concretely speaking, Th2 immunity supports the pregnancy process by reducing the rejection of fetal tissue, while Th1 immunity is thought to be unfavorable to the "foreign" fetus (56). Based on this recognition, researchers speculate that asthma might cause female fertility impairment and poor pregnancy outcomes by inducing an imbalance of the adaptive immune system. The systemic inflammation induced by asthma may increase the infiltration of inflammatory cells and alter cytokine profiles in the ovary and uterus exerting a negative effect on fertility. For example, TNF-α is regarded as a potential regulator of follicular development, ovulation, and oocyte apoptosis, playing an important role in reproductive failure (69–71). Imbalanced IL-6 is associated with recurrent miscarriages and failed implantation by regulating the proliferation, differentiation, and survival of germ cells (56, 72). Intriguingly, growing evidence supports that PCOS is also closely related to chronic systemic inflammation. The amount of immune cells in the peripheral blood of PCOS women dramatically increases with high androgen levels, inducing the secretion of inflammatory factors such as CRP, TNF-α, and IL-6 (73). In addition, previous studies revealed the altered immune cell profiles (e.g. macrophages, dendritic cells, and $CD8^+$ T cells) in the ovary and endometrium of PCOS women (74, 75), which possibly compromise ovarian function, normal implantation, and endometrial health in the long run. For example, pentraxin-3 (PTX3), a crucial humoral innate immunity component secreted by macrophages, which stands out as a structural constituent of the cumulus oophorus extracellular matrix essential for female fertility, was reported abnormally high both in plasma and ovary in PCOS women (76–78). And higher PTX3 is associated with hyperandrogenism (79), which might perturb interactions of the sperm with the matrix during *in vivo* fertilization (80). Low-grade systemic inflammation, therefore, carries pivotal responsibility for impaired female fertility, which may build a bridge connecting asthma and PCOS.

Irregular Menstruation

Irregular menstruation refers to oligomenorrhea, amenorrhea, abnormal uterine bleeding, etc. Numerous studies have revealed that PCOS women tend to exhibit a higher prevalence of irregular menstruation, which is often initially onset at a younger age and is positively correlated to BMI (81, 82). Compared with the age-matched controls, PCOS females reported more frequent hospitalization for menstrual abnormalities (20.3% vs 4.9%), endometriosis (26.4% vs 4.4%), endometrial hyperplasia (1.8% vs 0.1%) and other gynecological diseases (13). Based on the available evidence, lifestyle interventions, insulin sensitizers, and anti-androgen drugs are thought to be able to improve the reproductive health of PCOS women by inhibiting IR or declining androgen levels.

Abnormal regulation of steroidogenesis and ovarian regulation is a pivotal part of the pathophysiology of PCOS. Hypersecretion of LH and IGF-1, anti-Müllerian hormone (AMH) interact with each other to induce hyperandrogenism (83). For one thing, excessive androgen enhances the initial recruitment of primordial follicles into the growth pool, thus regulating the growth of small antral follicles (84). For another, hypersecretion of androgen encourages premature luteinization, thus inhibiting the selection of the dominant follicle and hindering normal ovulation (49, 85). Ovarian follicular arrest results in oligo-ovulation or anovulation, characterized by delayed menstruation, amenorrhea, or abnormal uterine bleeding (85).

Meanwhile, growing evidence demonstrates a strong correlation between menstruation and asthma attacks. Earlier menarche or irregular menstruation seems to predict worse lung function and increased susceptibility to asthma in adulthood (86–88). Women with abnormal or irregular menstrual cycles tend to exhibit significantly lowered FVC and elevated prevalence of asthma attacks (89). Furthermore, the exacerbation of asthma during menstruation, known as perimenstrual asthma (PMA), affects 30-40% of women with asthma. Compared with normal asthma attacks, PMA is characterized by more severity, increased drug use, lower vital capacity and PEF, as well as higher airway responsiveness (90). The association between deviations in levels of sex hormones and PMA has been suggested. Compared to non-PMA asthmatics, PMA subjects exhibit elevated estradiol levels in the luteal phase of the cycle (91). Rubio et al. reported that on the 5th and 21st days of the menstrual cycle, approximately 80% of asthmatic women exhibit abnormal hormonal changes (92). These studies indicate that sex hormone fluctuations may act as a risk factor for severe asthma attacks.

Indeed, more pronounced incidence and severity of asthma starting around puberty usually occur in females but not in males (86), suggesting the important role of sex hormones in the development of female airway pathology. Estrogen is the most widely studied ovarian hormone in airway inflammation. Mouse models have suggested the role of estrogen receptor (ER)

signaling in allergic airway inflammation by increasing the function of dendritic cells, M2 macrophages, and mast cells (93–95). Besides, 17β-estradiol and progesterone promote T_H17 cell differentiation and subsequently enhance IL-17A-mediated airway inflammation (96). From the perspective of neurological factors, as the parasympathetic nerve pathway is an essential determinant of bronchial tension, estradiol can indirectly cause bronchial muscle contraction and bronchial mucosal swelling by regulating the activity of acetylcholine and cholinesterase (97, 98). Generally speaking, given the commonly harbored hormonal disorders in PCOS women, these studies provide a new insight to investigate the correlation between PCOS and asthma by elucidating the effect of sex hormones.

Mood Disturbances

Clear evidence from epidemiological studies corroborates that women with PCOS have an increased incidence of depression, anxiety, sleep disorders, or other psychological disorders (46, 99, 100). Since the presence of overweight or obesity and infertility may exacerbate depression and anxiety reported in the general population, Damone et al. indicated that the associations between PCOS and depression or anxiety remain statistically significant, although weakened after adjusting for BMI, infertility, and sociodemographic variables (101). This confirms the hypothesis that the PCOS state itself may have an independent effect on mental functioning. When shown negative emotional images, PCOS women with IR exhibited increased activation in the left prefrontal cortex and the ventral anterior cingulate region compared with controls. These affected areas are responsible for the integration and regulation of cognitive and emotion-related information (102). In addition, PCOS women with IR were found to have greater marginal activation in emotional tasks than controls, leading to the aggravation of anxiety symptoms (103).

Meanwhile, numerous epidemiological studies have determined mental disorders as one of the important comorbidities of asthma. Adolescents with asthma are more prone to develop anxiety unipolar depressive disorder and bipolar disorder than the control group (104, 105). However, Slattery et al. have reached conflicting conclusions, demonstrating depressive symptoms were not significantly associated with a lifetime history of asthma (106). Different research methodology and diagnostic criteria, as well as a lack of adjustment for other co-existing allergic disorders, may explain the inconsistency. In recent years, magnetic resonance imaging (MRI) has been applied to investigate brain regions that may play a mechanistic role in asthma and mood disorders. Compared with the healthy controls, asthmatic patients showed abnormal structural connectivity in the bilateral frontal gyrus, right temporo-parietal cortex, and limbic regions, as well as decreased globus pallidus volume, suggesting changes in the function of brain regions involved in emotional regulation (107, 108).

Several common pathophysiological alterations may carry responsibility for mood disturbances in both PCOS and asthma, such as hypothalamic-pituitary-adrenal (HPA) dysfunction and inflammatory responses. HPA axis dysfunction is a potential mechanism underlying a variety of mental health conditions, especially anxiety and depression (109, 110). Cortisol, a steroid hormone with diurnal fluctuations, is widely recognized as a highly sensitive biomarker of stress-related changes in the maintenance of metabolic homeostasis (111). Flattening the circadian rhythm of cortisol results in dysregulation of several downstream biological and behavioral systems, including immunity, metabolism, energy, as well as appetite. Interestingly, chronic HPA axis disorders are common in asthmatic children (109). Compared with the healthy controls, asthmatic children had lower cortisol levels in hair samples, reduced cortisol reactivity in saliva samples, and a blunted cortisol awakening response (CAR) (112, 113). Further study indicated that adrenal insufficiency is not simply a consequence caused by inhaled corticosteroid treatments for asthma, it may also be a feature of asthma itself (114). Likewise, abnormal HPA activity in PCOS women has also been widely reported, but results vary. Some studies indicated that women with PCOS or different hyperandrogenic states are characterized by HPA-axis overactivity (115, 116). Nevertheless, Prelevic et al. (117) observed significantly lower cortisol levels at nighttime in PCOS women. Taken together, as abnormal cortisol levels are observed among mental disorders, asthma, and PCOS, HPA dysfunction could be an underlying mechanism that predisposes patients with asthma or PCOS to develop mood disturbances.

Inflammation responses could also explain this co-association between asthma or PCOS and multiple mental disorders. Increasing evidence shows that the levels of pro-inflammatory cytokines (e.g. IL-6, TNF-α, CRP) in peripheral blood of patients with mental problems are dramatically increased (118–121). Given that both asthma and PCOS are characterized by chronic systemic inflammation, these findings provide a basis for such association. The oversecreted pro-inflammatory cytokines can penetrate the blood-brain barrier during allergic reactions (122). On the one hand, it activates abnormal neuroimmune mechanisms in certain neural circuits involved in emotion regulation; On the other hand, it leads to decreased synthesis and increased uptake of 5-hydroxytryptamine, a neurotransmitter associated with various psychiatric disorders (123–125). Meanwhile, elevated levels of peripheral cytokines can activate microglia (126), which are involved in neuronal cell apoptosis, neurogenesis, and synaptic interactions (127). Inflammation-induced hyperactivation of microglia can inhibit neuronal activity through increased phagocytosis of dendritic spines, thus encouraging the development of mood disturbances (128). So far, the role of inflammatory response in the pathogenesis of mood disorders has not been fully elucidated, but it possibly becomes one of the promising targets to protect asthma or PCOS patients from mood disorders.

DISCUSSION

Both PCOS and asthma are common diseases in adult females, causing severe damage to women's physical and mental health as well as their quality of life. Clarifying the full landscape of pathogenesis and development of these two diseases under physiological conditions can elucidate the complex negative effects on female health from a whole-life perspective. Previous

studies mainly focus on inheritance, molecular cytology, epigenetics, or other aspects, and have already made significant breakthroughs. In recent years, growing epidemiological studies have demonstrated that PCOS, to a certain degree, correlates to asthma in several aspects. Specifically, women with PCOS are prone to develop asthma, and in return, asthma patients tend to exhibit increased susceptibility to metabolic syndrome, impaired fertility, irregular menstruation, and other clinical symptoms similar to PCOS. Furthermore, in the context of the coronavirus disease 2019 (COVID-19) pandemic, emerging data link the risk of severe COVID-19 with both PCOS and asthma. Researchers have identified potential overlap between common PCOS features and key risk factors of COVID-19, including cardio-metabolic comorbidity, hyperandrogenism, hyper-inflammation, etc. (129). Studies that have addressed the issue of asthma patients' susceptibility to the disease have come to discrepant conclusions, possibly due to complex interplay between numerous factors such as geographical differences, asthma phenotypes, and asthma medication (130–133). Of note, patients suffering from Th2-low asthma, especially the subjects with concomitant MS, are at a higher risk for progression to severe COVID-19 (134, 135). This provides new insights into the association between PCOS and asthma and requires further research. Interestingly, PCOS and asthma have been confirmed to share common physiological and pathological changes, such as specific hormonal abnormalities, insulin resistance as well as chronic systemic inflammation, whereas the potential mechanisms linking asthma to PCOS remain poorly investigated. It is important to mention that both conditions were recognized to originate in the early stages of life (136, 137).

PCOS is considered to have a strong correlation with autoimmune and chronic systematic inflammation related to IR, T2DM, or other metabolic syndromes, suggesting a possible mechanism that may occur when it comes to airway inflammation or even asthma in PCOS women. Given that numerous studies have reported an increased incidence of asthma-related pathological changes in PCOS females, further longitudinal studies and more precise elucidation of pathogenesis are required to examine the deeper connection between PCOS and asthma. To date, most data support a negative impact of asthma on female reproductive health through inducing irregular menstruation and reduced fertility. Based on the available evidence, it could be speculated that the imbalance of sex hormones and metabolic disorders behind impaired female reproductive health is likely to be one of the potential explanations for this correlation.

Currently, most evidence comes from observational studies, making it hard to establish a causal relationship between PCOS and asthma. The pathophysiology of the two conditions may involve diverse factors, and further prospective studies are of great significance to elucidate the complex cross-influence of inflammatory, metabolic disturbance, as well as endocrine disorders, and to provide broader screening and treatment strategies for women suffering from PCOS, asthma, or related complications.

AUTHOR CONTRIBUTIONS

H-FH and J-XP conceived of the study; YX and Z-YZ wrote the manuscript; J-XP and YX revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by National Key R&D Program of China (2021YFC2700603), the National Nature Science Foundation of China (82088102, 82171688, 82192873), Collaborative Innovation Program of Shanghai Municipal Health Commission (2020CXJQ01), CAMS Innovation Fund for Medical Sciences (2019- I2M-5-064), Clinical Research Plan of SHDC (SHDC2020CR1008A), Shanghai Frontiers Science Center of Reproduction and Development and Research Project of Shanghai Municipal Health Commission (20204Y0234).

ACKNOWLEDGMENTS

Image was created with Biorender®.

REFERENCES

1. Bozdag G, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO. The Prevalence and Phenotypic Features of Polycystic Ovary Syndrome: A Systematic Review and Meta-Analysis. *Hum Reprod Oxf Engl* (2016) 31(12):2841–55. doi: 10.1093/humrep/dew218
2. Sirmans SM, Pate KA. Epidemiology, Diagnosis, and Management of Polycystic Ovary Syndrome. *Clin Epidemiol* (2013) 6:1–13. doi: 10.2147/CLEP.S37559
3. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 Consensus on Diagnostic Criteria and Long-Term Health Risks Related to Polycystic Ovary Syndrome. *Fertil Steril* (2004) 81(1):19–25. doi: 10.1016/j.fertnstert.2003.10.004
4. Doherty DA, Newnham JP, Bower C, Hart R. Implications of Polycystic Ovary Syndrome for Pregnancy and for the Health of Offspring. *Obstet Gynecol* (2015) 125(6):1397–406. doi: 10.1097/AOG.0000000000000852
5. Ollila MME, Piltonen T, Puukka K, Ruokonen A, Järvelin MR, Tapanainen JS, et al. Weight Gain and Dyslipidemia in Early Adulthood Associate With Polycystic Ovary Syndrome: Prospective Cohort Study. *J Clin Endocrinol Metab* (2016) 101(2):739–47. doi: 10.1210/jc.2015-3543
6. Cooney LG, Lee I, Sammel MD, Dokras A. High Prevalence of Moderate and Severe Depressive and Anxiety Symptoms in Polycystic Ovary Syndrome: A Systematic Review and Meta-Analysis. *Hum Reprod Oxf Engl* (2017) 32(5):1075–91. doi: 10.1093/humrep/dex044
7. Rudnicka E, Suchta K, Grymowicz M, Calik-Ksepka A, Smolarczyk K, Duszewska AM, et al. Chronic Low Grade Inflammation in Pathogenesis of PCOS. *Int J Mol Sci* (2021) 22(7):3789. doi: 10.3390/ijms22073789
8. Abraham Gnanadass S, Divakar Prabhu Y, Valsala Gopalakrishnan A. Association of Metabolic and Inflammatory Markers With Polycystic Ovarian Syndrome (PCOS): An Update. *Arch Gynecol Obstet* (2021) 303(3):631–43. doi: 10.1007/s00404-020-05951-2

9. Samy N, Hashim M, Sayed M, Said M. Clinical Significance of Inflammatory Markers in Polycystic Ovary Syndrome: Their Relationship to Insulin Resistance and Body Mass Index. *Dis Markers* (2009) 26(4):163–70. doi: 10.3233/DMA-2009-0627
10. Hesampour F, Namavar Jahromi B, Tahmasebi F, Gharesi-Fard B. Association Between Interleukin-32 and Interleukin-17a Single Nucleotide Polymorphisms and Serum Levels With Polycystic Ovary Syndrome. *Iran J Allergy Asthma Immunol* (2019) 18(1):91–9. doi: 10.18502/ijaai.v18i1.634
11. Moraes TJ, Sears MR, Subbarao P. Epidemiology of Asthma and Influence of Ethnicity. *Semin Respir Crit Care Med* (2018) 39(1):3–11. doi: 10.1055/s-0037-1618568
12. Grieger JA, Hodge A, Mishra G, Joham AE, Moran LJ. The Association Between Dietary Intake, Asthma, and PCOS in Women From the Australian Longitudinal Study on Women's Health. *J Clin Med* (2020) 9(1):E233. doi: 10.3390/jcm9010233
13. Hart R, Doherty DA. The Potential Implications of a PCOS Diagnosis on a Woman's Long-Term Health Using Data Linkage. *J Clin Endocrinol Metab* (2015) 100(3):911–9. doi: 10.1210/jc.2014-3886
14. Zierau L, Cortes R, Thomsen SF, Jimenez-Solem E, Lindenberg S, Backer V. Asthma Severity and Fertility Outcome in Women With Polycystic Ovary Syndrome: A Registry-Based Study. *ERJ Open Res* (2018) 4(4):00138–2017. doi: 10.1183/23120541.00138-2017
15. Glintborg D, Hass Rubin K, Nybo M, Abrahamsen B, Andersen M. Morbidity and Medicine Prescriptions in a Nationwide Danish Population of Patients Diagnosed With Polycystic Ovary Syndrome. *Eur J Endocrinol* (2015) 172(5):627–38. doi: 10.1530/EJE-14-1108
16. Htet TD, Teede HJ, de Courten B, Loxton D, Real FG, Moran LJ, et al. Asthma in Reproductive-Aged Women With Polycystic Ovary Syndrome and Association With Obesity. *Eur Respir J* (2017) 49(5):1601334. doi: 10.1183/13993003.01334-2016
17. Underdal MO, Salvesen Ø, Henriksen AH, Andersen M, Vanky E. Impaired Respiratory Function in Women With PCOS Compared With Matched Controls From a Population-Based Study. *J Clin Endocrinol Metab* (2020) 105(1):dgz053. doi: 10.1210/clinem/dgz053
18. Galobardes B, Patel S, Henderson J, Jeffreys M, Smith GD. The Association Between Irregular Menstruations and Acne With Asthma and Atopy Phenotypes. *Am J Epidemiol* (2012) 176(8):733–7. doi: 10.1093/aje/kws161
19. Sun H, Li D, Jiao J, Liu Q, Bian J, Wang X. A Potential Link Between Polycystic Ovary Syndrome and Asthma: A Meta-Analysis. *Reprod Sci Thousand Oaks Calif* (2022) 29(1):312–9. doi: 10.1007/s43032-021-00662-8
20. Dokras A, Bochner M, Hollinrake E, Markham S, Vanvoorhis B, Jagasia DH. Screening Women With Polycystic Ovary Syndrome for Metabolic Syndrome. *Obstet Gynecol* (2005) 106(1):131–7. doi: 10.1097/01.AOG.0000167408.30893.6b
21. Zhang J, Fan P, Liu H, Bai H, Wang Y, Zhang F. Apolipoprotein A-I and B Levels, Dyslipidemia and Metabolic Syndrome in South-West Chinese Women With PCOS. *Hum Reprod Oxf Engl* (2012) 27(8):2484–93. doi: 10.1093/humrep/des191
22. Sanchez-Garrido MA, Tena-Sempere M. Metabolic Dysfunction in Polycystic Ovary Syndrome: Pathogenic Role of Androgen Excess and Potential Therapeutic Strategies. *Mol Metab* (2020) 35:100937. doi: 10.1016/j.molmet.2020.01.001
23. Legro RS, Gnatuk CL, Kunselman AR, Dunaif A. Changes in Glucose Tolerance Over Time in Women With Polycystic Ovary Syndrome: A Controlled Study. *J Clin Endocrinol Metab* (2005) 90(6):3236–42. doi: 10.1210/jc.2004-1843
24. Celik C, Tasdemir N, Abali R, Bastu E, Yilmaz M. Progression to Impaired Glucose Tolerance or Type 2 Diabetes Mellitus in Polycystic Ovary Syndrome: A Controlled Follow-Up Study. *Fertil Steril* (2014) 101(4):1123–1128.e1. doi: 10.1016/j.fertnstert.2013.12.050
25. Seow KM, Juan CC, Hsu YP, Hwang JL, Huang LW, Ho LT. Amelioration of Insulin Resistance in Women With PCOS *via* Reduced Insulin Receptor Substrate-1 Ser312 Phosphorylation Following Laparoscopic Ovarian Electrocautery. *Hum Reprod Oxf Engl* (2007) 22(4):1003–10. doi: 10.1093/humrep/del466
26. Panidis D, Macut D, Farmakiotis D, Rousso D, Kourtis A, Katsikis I, et al. Indices of Insulin Sensitivity, Beta Cell Function and Serum Proinsulin Levels in the Polycystic Ovary Syndrome. *Eur J Obstet Gynecol Reprod Biol* (2006) 127(1):99–105. doi: 10.1016/j.ejogrb.2005.12.016
27. Spritzer PM, Lecke SB, Satler F, Morsch DM. Adipose Tissue Dysfunction, Adipokines, and Low-Grade Chronic Inflammation in Polycystic Ovary Syndrome. *Reprod Camb Engl* (2015) 149(5):R219–227. doi: 10.1530/REP-14-0435
28. Hotamisligil GS, Budavari A, Murray D, Spiegelman BM. Reduced Tyrosine Kinase Activity of the Insulin Receptor in Obesity-Diabetes. Central Role of Tumor Necrosis Factor-Alpha. *J Clin Invest* (1994) 94(4):1543–9. doi: 10.1172/JCI117495
29. Stephens JM, Pekala PH. Transcriptional Repression of the GLUT4 and C/EBP Genes in 3T3-L1 Adipocytes by Tumor Necrosis Factor-Alpha. *J Biol Chem* (1991) 266(32):21839–45. doi: 10.1016/S0021-9258(18)54714-1
30. Zeng X, Xie YJ, Liu YT, Long SL, Mo ZC. Polycystic Ovarian Syndrome: Correlation Between Hyperandrogenism, Insulin Resistance and Obesity. *Clin Chim Acta Int J Clin Chem* (2020) 502:214–21. doi: 10.1016/j.cca.2019.11.003
31. Delitala AP, Capobianco G, Delitala G, Cherchi PL, Dessole S. Polycystic Ovary Syndrome, Adipose Tissue and Metabolic Syndrome. *Arch Gynecol Obstet* (2017) 296(3):405–19. doi: 10.1007/s00404-017-4429-2
32. Lee EJ, In KH, Ha ES, Lee KJ, Hur GY, Kang EH, et al. Asthma-Like Symptoms are Increased in the Metabolic Syndrome. *J Asthma Off J Assoc Care Asthma* (2009) 46(4):339–42. doi: 10.1080/02770900802660931
33. Brumpton BM, Camargo CA, Romundstad PR, Langhammer A, Chen Y, Mai XM. Metabolic Syndrome and Incidence of Asthma in Adults: The HUNT Study. *Eur Respir J* (2013) 42(6):1495–502. doi: 10.1183/09031936.00046013
34. Tomita Y, Fukutomi Y, Irie M, Azekawa K, Hayashi H, Kamide Y, et al. Obesity, But Not Metabolic Syndrome, as a Risk Factor for Late-Onset Asthma in Japanese Women. *Allergol Int Off J Jpn Soc Allergol* (2019) 68(2):240–6. doi: 10.1016/j.alit.2018.10.003
35. Assad N, Qualls C, Smith LJ, Arynchyn A, Thyagarajan B, Schuyler M, et al. Body Mass Index is a Stronger Predictor Than the Metabolic Syndrome for Future Asthma in Women. The Longitudinal CARDIA Study. *Am J Respir Crit Care Med* (2013) 188(3):319–26. doi: 10.1164/rccm.201303-0457OC
36. Contreras ZA, Chen Z, Roumeliotaki T, Annesi-Maesano I, Baïz N, von Berg A, et al. Does Early Onset Asthma Increase Childhood Obesity Risk? A Pooled Analysis of 16 European Cohorts. *Eur Respir J* (2018) 52(3):1800504. doi: 10.1183/13993003.00504-2018
37. Chen Z, Salam MT, Alderete TL, Habre R, Bastain TM, Berhane K, et al. Effects of Childhood Asthma on the Development of Obesity Among School-Aged Children. *Am J Respir Crit Care Med* (2017) 195(9):1181–8. doi: 10.1164/rccm.201608-1691OC
38. Ding DJ, Martin JG, Macklem PT. Effects of Lung Volume on Maximal Methacholine-Induced Bronchoconstriction in Normal Humans. *J Appl Physiol Bethesda Md 1985* (1987) 62(3):1324–30. doi: 10.1152/jappl.1987.62.3.1324
39. Miethe S, Karsonova A, Karaulov A, Renz H. Obesity and Asthma. *J Allergy Clin Immunol* (2020) 146(4):685–93. doi: 10.1016/j.jaci.2020.08.011
40. Chapman DG, Berend N, Horlyck KR, King GG, Salome CM. Does Increased Baseline Ventilation Heterogeneity Following Chest Wall Strapping Predispose to Airway Hyperresponsiveness? *J Appl Physiol Bethesda Md 1985* (2012) 113(1):25–30. doi: 10.1152/japplphysiol.01582.2011
41. Al-Alwan A, Bates JHT, Chapman DG, Kaminsky DA, DeSarno MJ, Irvin CG, et al. The Nonallergic Asthma of Obesity. A Matter of Distal Lung Compliance. *Am J Respir Crit Care Med* (2014) 189(12):1494–502. doi: 10.1164/rccm.201401-0178OC
42. Wood LG, Garg ML, Gibson PG. A High-Fat Challenge Increases Airway Inflammation and Impairs Bronchodilator Recovery in Asthma. *J Allergy Clin Immunol* (2011) 127(5):1133–40. doi: 10.1016/j.jaci.2011.01.036
43. Everaere L, Ait-Yahia S, Molendi-Coste O, Vorng H, Quemener S, LeVu P, et al. Innate Lymphoid Cells Contribute to Allergic Airway Disease Exacerbation by Obesity. *J Allergy Clin Immunol* (2016) 138(5):1309–1318.e11. doi: 10.1016/j.jaci.2016.03.019
44. Lugogo N, Francisco D, Addison KJ, Manne A, Pederson W, Ingram JL, et al. Obese Asthmatic Patients Have Decreased Surfactant Protein A Levels: Mechanisms and Implications. *J Allergy Clin Immunol* (2018) 141(3):918–926.e3. doi: 10.1016/j.jaci.2017.05.028

45. Lindner PG. Long-Term Health of Offspring of Women With Polycystic Ovarian Syndrome. *Clin Obstet Gynecol* (2021) 64(1):48–54. doi: 10.1097/GRF.0000000000000598
46. Hung JH, Hu LY, Tsai SJ, Yang AC, Huang MW, Chen PM, et al. Risk of Psychiatric Disorders Following Polycystic Ovary Syndrome: A Nationwide Population-Based Cohort Study. *PloS One* (2014) 9(5):e97041. doi: 10.1371/journal.pone.0097041
47. Joham AE, Teede HJ, Ranasinha S, Zoungas S, Boyle J. Prevalence of Infertility and Use of Fertility Treatment in Women With Polycystic Ovary Syndrome: Data From a Large Community-Based Cohort Study. *J Womens Health 2002* (2015) 24(4):299–307. doi: 10.1089/jwh.2014.5000
48. Santos MA, Kuijk EW, Macklon NS. The Impact of Ovarian Stimulation for IVF on the Developing Embryo. *Reprod Camb Engl* (2010) 139(1):23–34. doi: 10.1530/REP-09-0187
49. Franks S, Stark J, Hardy K. Follicle Dynamics and Anovulation in Polycystic Ovary Syndrome. *Hum Reprod Update* (2008) 14(4):367–78. doi: 10.1093/humupd/dmn015
50. Qiao J, Feng HL. Extra- and Intra-Ovarian Factors in Polycystic Ovary Syndrome: Impact on Oocyte Maturation and Embryo Developmental Competence. *Hum Reprod Update* (2011) 17(1):17–33. doi: 10.1093/humupd/dmq032
51. Teissier MP, Chable H, Paulhac S, Aubard Y. Comparison of Follicle Steroidogenesis From Normal and Polycystic Ovaries in Women Undergoing IVF: Relationship Between Steroid Concentrations, Follicle Size, Oocyte Quality and Fecundability. *Hum Reprod Oxf Engl* (2000) 15(12):2471–7. doi: 10.1093/humrep/15.12.2471
52. Nestler JE, Powers LP, Matt DW, Steingold KA, Plymate SR, Rittmaster RS, et al. A Direct Effect of Hyperinsulinemia on Serum Sex Hormone-Binding Globulin Levels in Obese Women With the Polycystic Ovary Syndrome. *J Clin Endocrinol Metab* (1991) 72(1):83–9. doi: 10.1210/jcem-72-1-83
53. Billig H, Furuta I, Hsueh AJ. Estrogens Inhibit and Androgens Enhance Ovarian Granulosa Cell Apoptosis. *Endocrinology* (1993) 133(5):2204–12. doi: 10.1210/endo.133.5.8404672
54. Hillier SG. Current Concepts of the Roles of Follicle Stimulating Hormone and Luteinizing Hormone in Folliculogenesis. *Hum Reprod Oxf Engl* (1994) 9(2):188–91. doi: 10.1093/oxfordjournals.humrep.a138480
55. de Wilde MA, de Ruiter ML, Veltman-Verhulst SM, Kwee A, Laven JS, Lambalk CB, et al. Increased Rates of Complications in Singleton Pregnancies of Women Previously Diagnosed With Polycystic Ovary Syndrome Predominantly in the Hyperandrogenic Phenotype. *Fertil Steril* (2017) 108(2):333–40. doi: 10.1016/j.fertnstert.2017.06.015
56. Juul Gade E, Thomsen SF, Lindenberg S, Backer V. Female Asthma has a Negative Effect on Fertility: What is the Connection? *ISRN Allergy* (2014) 2014:131092. doi: 10.1155/2014/131092
57. Sunyer J, Antó JM, Plana E, Janson C, Jarvis D, Kony S, et al. Maternal Atopy and Changes in Parity. *Clin Exp Allergy J Br Soc Allergy Clin Immunol* (2005) 35(8):1028–32. doi: 10.1111/j.1365-2222.2005.02300.x
58. Vejen Hansen A, Ali Z, Malchau SS, Blafoss J, Pinborg A, Ulrik CS. Fertility Treatment Among Women With Asthma: A Case-Control Study of 3689 Women With Live Births. *Eur Respir J* (2019) 53(2):1800597. doi: 10.1183/13993003.00597-2018
59. Gade EJ, Thomsen SF, Lindenberg S, Backer V. Fertility Outcomes in Asthma: A Clinical Study of 245 Women With Unexplained Infertility. *Eur Respir J* (2016) 47(4):1144–51. doi: 10.1183/13993003.01389-2015
60. Rocklin RE. Asthma, Asthma Medications and Their Effects on Maternal/Fetal Outcomes During Pregnancy. *Reprod Toxicol Elmsford N* (2011) 32(2):189–97. doi: 10.1016/j.reprotox.2011.05.023
61. Metwally M, Li TC, Ledger WL. The Impact of Obesity on Female Reproductive Function. *Obes Rev Off J Int Assoc Study Obes* (2007) 8(6):515–23. doi: 10.1111/j.1467-789X.2007.00406.x
62. Luzzo KM, Wang Q, Purcell SH, Chi M, Jimenez PT, Grindler N, et al. High Fat Diet Induced Developmental Defects in the Mouse: Oocyte Meiotic Aneuploidy and Fetal Growth Retardation/Brain Defects. *PloS One* (2012) 7(11):e49217. doi: 10.1371/journal.pone.0049217
63. Jungheim ES, Schoeller EL, Marquard KL, Louden ED, Schaffer JE, Moley KH. Diet-Induced Obesity Model: Abnormal Oocytes and Persistent Growth Abnormalities in the Offspring. *Endocrinology* (2010) 151(8):4039–46. doi: 10.1210/en.2010-0098
64. Silvestris E, de Pergola G, Rosania R, Loverro G. Obesity as Disruptor of the Female Fertility. *Reprod Biol Endocrinol RBE* (2018) 16(1):22. doi: 10.1186/s12958-018-0336-z
65. Campos DB, Palin MF, Bordignon V, Murphy BD. The 'Beneficial' Adipokines in Reproduction and Fertility. *Int J Obes 2005* (2008) 32(2):223–31. doi: 10.1038/sj.ijo.0803719
66. Li S, Huang X, Zhong H, Peng Q, Chen S, Xie Y, et al. Low Circulating Adiponectin Levels in Women With Polycystic Ovary Syndrome: An Updated Meta-Analysis. *Tumour Biol J Int Soc Oncodevelopmental Biol Med* (2014) 35(5):3961–73. doi: 10.1007/s13277-013-1595-0
67. Zhang MY, Dini AA, Yang XK, Li LJ, Wu GC, Leng RX, et al. Association Between Serum/Plasma Adiponectin Levels and Immune-Mediated Diseases: A Meta-Analysis. *Arch Dermatol Res* (2017) 309(8):625–35. doi: 10.1007/s00403-017-1755-y
68. Bongrani A, Mellouk N, Rame C, Cornuau M, Guérif F, Froment P, et al. Ovarian Expression of Adipokines in Polycystic Ovary Syndrome: A Role for Chemerin, Omentin, and Apelin in Follicular Growth Arrest and Ovulatory Dysfunction? *Int J Mol Sci* (2019) 20(15):E3778. doi: 10.3390/ijms20153778
69. Morrison LJ, Marcinkiewicz JL. Tumor Necrosis Factor Alpha Enhances Oocyte/Follicle Apoptosis in the Neonatal Rat Ovary. *Biol Reprod* (2002) 66(2):450–7. doi: 10.1095/biolreprod66.2.450
70. Nakayama M, Manabe N, Inoue N, Matsui T, Miyamoto H. Changes in the Expression of Tumor Necrosis Factor (TNF) Alpha, TNFalpha Receptor (TNFR) 2, and TNFR-Associated Factor 2 in Granulosa Cells During Atresia in Pig Ovaries. *Biol Reprod* (2003) 68(2):530–5. doi: 10.1095/biolreprod.102.004820
71. lan CL, Yang G, Pan J, Zhang C. Tumor Necrosis Factor α Knockout Increases Fertility of Mice. *Theriogenology* (2011) 75(5):867–76. doi: 10.1016/j.theriogenology.2010.10.029
72. Eddie SL, Childs AJ, Jabbour HN, Anderson RA. Developmentally Regulated IL6-Type Cytokines Signal to Germ Cells in the Human Fetal Ovary. *Mol Hum Reprod* (2012) 18(2):88–95. doi: 10.1093/molehr/gar061
73. Choi YS, Yang HI, Cho S, Jung JA, Jeon YE, Kim HY, et al. Serum Asymmetric Dimethylarginine, Apelin, and Tumor Necrosis Factor-α Levels in non-Obese Women With Polycystic Ovary Syndrome. *Steroids* (2012) 77(13):1352–8. doi: 10.1016/j.steroids.2012.08.005
74. Rostamtabar M, Esmaeilzadeh S, Tourani M, Rahmani A, Baee M, Shirafkan F, et al. Pathophysiological Roles of Chronic Low-Grade Inflammation Mediators in Polycystic Ovary Syndrome. *J Cell Physiol* (2021) 236(2):824–38. doi: 10.1002/jcp.29912
75. Liu S, Hong L, Mo M, Xiao S, Chen C, Li Y, et al. Evaluation of Endometrial Immune Status of Polycystic Ovary Syndrome. *J Reprod Immunol* (2021) 144:103282. doi: 10.1016/j.jri.2021.103282
76. Pan J, Zhou C, Zhou Z, Yang Z, Dai T, Huang H, et al. Elevated Ovarian Pentraxin 3 in Polycystic Ovary Syndrome. *J Assist Reprod Genet* (2021) 38(5):1231–7. doi: 10.1007/s10815-021-02105-4
77. Wyskida K, Franik G, Choręza P, Pohl N, Markuszewski L, Owczarek A, et al. Pentraxin 3 Levels in Young Women With and Without Polycystic Ovary Syndrome (PCOS) in Relation to the Nutritional Status and Systemic Inflammation. *Int J Endocrinol* (2020) 2020:1380176. doi: 10.1155/2020/1380176
78. Tosi F, Di Sarra D, Bonin C, Zambotti F, Dall'Alda M, Fiers T, et al. Plasma Levels of Pentraxin-3, an Inflammatory Protein Involved in Fertility, are Reduced in Women With Polycystic Ovary Syndrome. *Eur J Endocrinol* (2014) 170(3):401–9. doi: 10.1530/EJE-13-0761
79. Jin C, Zou K, Xu Y, Yang H, Pan J. Elevated Plasma Pentraxin-3 in Polycystic Ovary Syndrome is Associated With Hyperandrogenism: A Case-Control Study. *BMC Endocr Disord* (2021) 21(1):240. doi: 10.1186/s12902-021-00886-4
80. Salustri A, Garlanda C, Hirsch E, De Acetis M, Maccagno A, Bottazzi B, et al. PTX3 Plays a Key Role in the Organization of the Cumulus Oophorus Extracellular Matrix and in *In Vivo* Fertilization. *Dev Camb Engl* (2004) 131(7):1577–86. doi: 10.1242/dev.01056
81. Teede HJ, Joham AE, Paul E, Moran LJ, Loxton D, Jolley D, et al. Longitudinal Weight Gain in Women Identified With Polycystic Ovary Syndrome: Results of an Observational Study in Young Women. *Obes Silver Spring Md.* (2013) 21(8):1526–32. doi: 10.1002/oby.20213
82. Balen AH, Conway GS, Kaltsas G, Techatrasak K, Manning PJ, West C, et al. Polycystic Ovary Syndrome: The Spectrum of the Disorder in 1741 Patients.

Hum Reprod Oxf Engl (1995) 10(8):2107–11. doi: 10.1093/oxfordjournals.humrep.a136243
83. Azziz R, Carmina E, Chen Z, Dunaif A, Laven JSE, Legro RS, et al. Polycystic Ovary Syndrome. *Nat Rev Dis Primer* (2016) 2(1):1–18. doi: 10.1038/nrdp.2016.57
84. Jonard S, Dewailly D. The Follicular Excess in Polycystic Ovaries, Due to Intra-Ovarian Hyperandrogenism, may be the Main Culprit for the Follicular Arrest. *Hum Reprod Update* (2004) 10(2):107–17. doi: 10.1093/humupd/dmh010
85. Rosenfield RL, Ehrmann DA. The Pathogenesis of Polycystic Ovary Syndrome (PCOS): The Hypothesis of PCOS as Functional Ovarian Hyperandrogenism Revisited. *Endocr Rev* (2016) 37(5):467–520. doi: 10.1210/er.2015-1104
86. Yung JA, Fuseini H, Newcomb DC. Hormones, Sex, and Asthma. *Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol* (2018) 120(5):488–94. doi: 10.1016/j.anai.2018.01.016
87. Svanes C, Real FG, Gislason T, Jansson C, Jögi R, Norrman E, et al. Association of Asthma and Hay Fever With Irregular Menstruation. *Thorax* (2005) 60(6):445–50. doi: 10.1136/thx.2004.032615
88. Gill D, Sheehan NA, Wielscher M, Shrine N, Amaral AFS, Thompson JR, et al. Age at Menarche and Lung Function: A Mendelian Randomization Study. *Eur J Epidemiol* (2017) 32(8):701–10. doi: 10.1007/s10654-017-0272-9
89. Real FG, Svanes C, Omenaas ER, Antò JM, Plana E, Janson C, et al. Menstrual Irregularity and Asthma and Lung Function. *J Allergy Clin Immunol* (2007) 120(3):557–64. doi: 10.1016/j.jaci.2007.04.041
90. Vrieze A, Postma DS, Kerstjens HA. Perimenstrual Asthma: A Syndrome Without Known Cause or Cure. *J Allergy Clin Immunol* (2003) 112(2):271–82. doi: 10.1067/mai.2003.1676
91. Semik-Orzech A, Skoczyński S, Pierzchała W. Serum Estradiol Concentration, Estradiol-to-Progesterone Ratio and Sputum IL-5 and IL-8 Concentrations are Increased in Luteal Phase of the Menstrual Cycle in Perimenstrual Asthma Patients. *Eur Ann Allergy Clin Immunol* (2017) 49(4):161–70. doi: 10.23822/eurannaci.1764-1489.09
92. Rubio Ravelo L, Gago Rodríguez B, Almirall Collazo JJ, Bell Heredia L, Fernández Fernández L. Comparative Study of Progesterone, Estradiol and Cortisol Concentrations in Asthmatic and non-Asthmatic Women. *Allergol Immunopathol (Madr)* (1988) 16(4):263–6.
93. Carey MA, Card JW, Bradbury JA, Moorman MP, Haykal-Coates N, Gavett SH, et al. Spontaneous Airway Hyperresponsiveness in Estrogen Receptor-Alpha-Deficient Mice. *Am J Respir Crit Care Med* (2007) 175(2):126–35. doi: 10.1164/rccm.200509-1493OC
94. Keselman A, Fang X, White PB, Heller NM. Estrogen Signaling Contributes to Sex Differences in Macrophage Polarization During Asthma. *J Immunol Baltim Md 1950* (2017) 199(5):1573–83. doi: 10.4049/jimmunol.1601975
95. Paharkova-Vatchkova V, Maldonado R, Kovats S. Estrogen Preferentially Promotes the Differentiation of CD11c+ CD11b(intermediate) Dendritic Cells From Bone Marrow Precursors. *J Immunol Baltim Md 1950* (2004) 172(3):1426–36. doi: 10.4049/jimmunol.172.3.1426
96. Newcomb DC, Cephus JY, Boswell MG, Fahrenholz JM, Langley EW, Feldman AS, et al. Estrogen and Progesterone Decrease Let-7f microRNA Expression and Increase IL-23/IL-23 Receptor Signaling and IL-17A Production in Patients With Severe Asthma. *J Allergy Clin Immunol* (2015) 136(4):1025–1034.e11. doi: 10.1016/j.jaci.2015.05.046
97. Abdul-Karim RW, Drucker M, Jacobs RD. The Influence of Estradiol-17 Beta on Cholinesterase Activity in the Lung. *Am J Obstet Gynecol* (1970) 108(7):1098–101. doi: 10.1016/0002-9378(70)90459-x
98. Boulet LP, Boulay MÈ. Asthma-Related Comorbidities. *Expert Rev Respir Med* (2011) 5(3):377–93. doi: 10.1586/ers.11.34
99. Deeks AA, Gibson-Helm ME, Paul E, Teede HJ. Is Having Polycystic Ovary Syndrome a Predictor of Poor Psychological Function Including Anxiety and Depression? *Hum Reprod Oxf Engl* (2011) 26(6):1399–407. doi: 10.1093/humrep/der071
100. Rowlands IJ, Teede H, Lucke J, Dobson AJ, Mishra GD. Young Women's Psychological Distress After a Diagnosis of Polycystic Ovary Syndrome or Endometriosis. *Hum Reprod Oxf Engl* (2016) 31(9):2072–81. doi: 10.1093/humrep/dew174
101. Damone AL, Joham AE, Loxton D, Earnest A, Teede HJ, Moran LJ. Depression, Anxiety and Perceived Stress in Women With and Without PCOS: A Community-Based Study. *Psychol Med* (2019) 49(9):1510–20. doi: 10.1017/S0033291718002076
102. De Silva A, Salem V, Matthews PM, Dhillo WS. The Use of Functional MRI to Study Appetite Control in the CNS. *Exp Diabetes Res* (2012) 2012:764017. doi: 10.1155/2012/764017
103. Marsh CA, Berent-Spillson A, Love T, Persad CC, Pop-Busui R, Zubieta JK, et al. Functional Neuroimaging of Emotional Processing in Women With Polycystic Ovary Syndrome: A Case-Control Pilot Study. *Fertil Steril* (2013) 100(1):200–207.e1. doi: 10.1016/j.fertnstert.2013.02.054
104. Chen MH, Su TP, Chen YS, Hsu JW, Huang KL, Chang WH, et al. Higher Risk of Developing Major Depression and Bipolar Disorder in Later Life Among Adolescents With Asthma: A Nationwide Prospective Study. *J Psychiatr Res* (2014) 49:25–30. doi: 10.1016/j.jpsychires.2013.10.015
105. Dudeney J, Sharpe L, Jaffe A, Jones EB, Hunt C. Anxiety in Youth With Asthma: A Meta-Analysis. *Pediatr Pulmonol* (2017) 52(9):1121–9. doi: 10.1002/ppul.23689
106. Slattery MJ, Essex MJ. Specificity in the Association of Anxiety, Depression, and Atopic Disorders in a Community Sample of Adolescents. *J Psychiatr Res* (2011) 45(6):788–95. doi: 10.1016/j.jpsychires.2010.11.003
107. Gao X, Xiao Y, Lv P, Zhang W, Gong Y, Wang T, et al. Altered Brain Network Integrity in Patients With Asthma: A Structural Connectomic Diffusion Tensor Imaging Study. *Respir Physiol Neurobiol* (2019) 266:89–94. doi: 10.1016/j.resp.2019.05.004
108. Ritz T, Kroll JL, Aslan S, Janssens T, Khan DA, Pinkham AE, et al. Subcortical Gray Matter Volumes in Asthma: Associations With Asthma Duration, Control, and Anxiety. *Brain Imaging Behav* (2020) 14(6):2341–50. doi: 10.1007/s11682-019-00188-3
109. Adam EK, Quinn ME, Tavernier R, McQuillan MT, Dahlke KA, Gilbert KE. Diurnal Cortisol Slopes and Mental and Physical Health Outcomes: A Systematic Review and Meta-Analysis. *Psychoneuroendocrinology* (2017) 83:25–41. doi: 10.1016/j.psyneuen.2017.05.018
110. Dieleman GC, Huizink AC, Tulen JHM, Utens EMWJ, Creemers HE, van der Ende J, et al. Alterations in HPA-Axis and Autonomic Nervous System Functioning in Childhood Anxiety Disorders Point to a Chronic Stress Hypothesis. *Psychoneuroendocrinology* (2015) 51:135–50. doi: 10.1016/j.psyneuen.2014.09.002
111. McEwen BS. Physiology and Neurobiology of Stress and Adaptation: Central Role of the Brain. *Physiol Rev* (2007) 87(3):873–904. doi: 10.1152/physrev.00041.2006
112. Kamps AWA, Molenmaker M, Kemperman R, van der Veen BS, Bocca G, Veeger NJGM. Children With Asthma Have Significantly Lower Long-Term Cortisol Levels in Their Scalp Hair Than Healthy Children. *Acta Paediatr Oslo Nor 1992* (2014) 103(9):957–61. doi: 10.1111/apa.12685
113. Kroll JL, Brown ES, Ritz T. Cortisol Awakening Response and Fractional Exhaled Nitric Oxide in Asthma. *Clin Exp Allergy J Br Soc Allergy Clin Immunol* (2019) 49(8):1150–3. doi: 10.1111/cea.13446
114. Caulfield JI, Cavigelli SA. Individual Differences in Glucocorticoid Regulation: Does it Relate to Disease Risk and Resilience? *Front Neuroendocrinol* (2020) 56:100803. doi: 10.1016/j.yfrne.2019.100803
115. Basu BR, Chowdhury O, Saha SK. Possible Link Between Stress-Related Factors and Altered Body Composition in Women With Polycystic Ovarian Syndrome. *J Hum Reprod Sci* (2018) 11(1):10–8. doi: 10.4103/jhrs.JHRS_78_17
116. Mezzullo M, Fanelli F, Di Dalmazi G, Fazzini A, Ibarra-Gasparini D, Mastroroberto M, et al. Salivary Cortisol and Cortisone Responses to Short-Term Psychological Stress Challenge in Late Adolescent and Young Women With Different Hyperandrogenic States. *Psychoneuroendocrinology* (2018) 91:31–40. doi: 10.1016/j.psyneuen.2018.02.022
117. Prelević GM, Würzburger MI, Balint-Perić L. 24-Hour Serum Cortisol Profiles in Women With Polycystic Ovary Syndrome. *Gynecol Endocrinol Off J Int Soc Gynecol Endocrinol* (1993) 7(3):179–84. doi: 10.3109/09513599309152500
118. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, et al. A Meta-Analysis of Cytokines in Major Depression. *Biol Psychiatry* (2010) 67(5):446–57. doi: 10.1016/j.biopsych.2009.09.033
119. Goldsmith DR, Rapaport MH, Miller BJ. A Meta-Analysis of Blood Cytokine Network Alterations in Psychiatric Patients: Comparisons Between Schizophrenia, Bipolar Disorder and Depression. *Mol Psychiatry* (2016) 21(12):1696–709. doi: 10.1038/mp.2016.3

120. Gao T, Li B, Hou Y, Luo S, Feng L, Nie J, et al. Interleukin-4 Signalling Pathway Underlies the Anxiolytic Effect Induced by 3-Deoxyadenosine. *Psychopharmacol (Berl)* (2019) 236(10):2959–73. doi: 10.1007/s00213-019-5186-7
121. Costello H, Gould RL, Abrol E, Howard R. Systematic Review and Meta-Analysis of the Association Between Peripheral Inflammatory Cytokines and Generalised Anxiety Disorder. *BMJ Open* (2019) 9(7):e027925. doi: 10.1136/bmjopen-2018-027925
122. Yarlagadda A, Alfson E, Clayton AH. The Blood Brain Barrier and the Role of Cytokines in Neuropsychiatry. *Psychiatry Edgmont Pa Townsh* (2009) 6 (11):18–22.
123. Shelton RC, Miller AH. Inflammation in Depression: Is Adiposity a Cause? *Dialogues Clin Neurosci* (2011) 13(1):41–53. doi: 10.31887/DCNS.2011.13.1/rshelton
124. Zhu CB, Lindler KM, Owens AW, Daws LC, Blakely RD, Hewlett WA. Interleukin-1 Receptor Activation by Systemic Lipopolysaccharide Induces Behavioral Despair Linked to MAPK Regulation of CNS Serotonin Transporters. *Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol* (2010) 35 (13):2510–20. doi: 10.1038/npp.2010.116
125. Kolhe JV, Chhipa AS, Butani S, Chavda V, Patel SS. PCOS and Depression: Common Links and Potential Targets. *Reprod Sci Thousand Oaks Calif* (2021). doi: 10.1007/s43032-021-00765-2
126. Caulfield JI, Schopf KJ, Cavigelli SA. Peri-Adolescent Asthma: Acute Impacts on Innate Immune Response, Corticosterone, and Microglia in Mice. *J Neuroimmunol* (2020) 350:577450. doi: 10.1016/j.jneuroim.2020.577450
127. Brites D, Fernandes A. Neuroinflammation and Depression: Microglia Activation, Extracellular Microvesicles and microRNA Dysregulation. *Front Cell Neurosci* (2015) 9:476. doi: 10.3389/fncel.2015.00476
128. Cao P, Chen C, Liu A, Shan Q, Zhu X, Jia C, et al. Early-Life Inflammation Promotes Depressive Symptoms in Adolescence *via* Microglial Engulfment of Dendritic Spines. *Neuron* (2021) 109(16):2573–2589.e9. doi: 10.1016/j.neuron.2021.06.012
129. Kyrou I, Karteris E, Robbins T, Chatha K, Drenos F, Randeva HS. Polycystic Ovary Syndrome (PCOS) and COVID-19: An Overlooked Female Patient Population at Potentially Higher Risk During the COVID-19 Pandemic. *BMC Med* (2020) 18(1):220. doi: 10.1186/s12916-020-01697-5
130. Eger K, Bel EH. Asthma and COVID-19: Do We Finally Have Answers? *Eur Respir J* (2021) 57(3):2004451–4. doi: 10.1183/13993003.04451-2020
131. Johnston SL. Asthma and COVID-19: Is Asthma a Risk Factor for Severe Outcomes? *Allergy* (2020) 75(7):1543–5. doi: 10.1111/all.14348
132. Adir Y, Saliba W, Beurnier A, Humbert M. Asthma and COVID-19: An Update. *Eur Respir Rev* (2021) 30(162):210152. doi: 10.1183/16000617.0152-2021
133. Izquierdo JL, Almonacid C, González Y, Del Rio-Bermudez C, Ancochea J, Cárdenas R, et al. The Impact of COVID-19 on Patients With Asthma. *Eur Respir J* (2021) 57(3):2003142. doi: 10.1183/13993003.03142-2020
134. Skevaki C, Karsonova A, Karaulov A, Xie M, Renz H. Asthma-Associated Risk for COVID-19 Development. *J Allergy Clin Immunol* (2020) 146 (6):1295–301. doi: 10.1016/j.jaci.2020.09.017
135. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical Features of Patients Infected With 2019 Novel Coronavirus in Wuhan, China. *Lancet Lond Engl* (2020) 395(10223):497–506. doi: 10.1016/S0140-6736(20)30183-5
136. Chen X, Lin H, Yang D, Xu W, Liu G, Liu X, et al. Early-Life Undernutrition Reprograms CD4+ T-Cell Glycolysis and Epigenetics to Facilitate Asthma. *J Allergy Clin Immunol* (2019) 143(6):2038–2051.e12. doi: 10.1016/j.jaci.2018.12.999
137. Mimouni NEH, Paiva I, Barbotin AL, Timzoura FE, Plassard D, Le Gras S, et al. Polycystic Ovary Syndrome is Transmitted *via* a Transgenerational Epigenetic Process. *Cell Metab* (2021) 33(3):513–530.e8. doi: 10.1016/j.cmet.2021.01.004

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 17 August 2022
DOI 10.3389/fendo.2022.945609

OPEN ACCESS

EDITED BY
Yanting Wu,
Fudan University, China

REVIEWED BY
Nese Ciar,
Muğla University, Turkey
Dongzi Yang,
Sun Yat-sen Memorial Hospital, China
Antonio Simone Laganà,
University of Palermo, Italy

*CORRESPONDENCE
Bing He
hebing7557@163.com

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

RECEIVED 16 May 2022
ACCEPTED 28 July 2022
PUBLISHED 17 August 2022

CITATION
Xing C, Zhao H, Zhang J and He B (2022) Effect of metformin versus metformin plus liraglutide on gonadal and metabolic profiles in overweight patients with polycystic ovary syndrome. *Front. Endocrinol.* 13:945609. doi: 10.3389/fendo.2022.945609

Effect of metformin versus metformin plus liraglutide on gonadal and metabolic profiles in overweight patients with polycystic ovary syndrome

Chuan Xing, Han Zhao, Jiaqi Zhang and Bing He*

Department of Endocrinology, Shengjing Hospital of China Medical University, Shenyang, China

Objective: To observe the effect of metformin (MET) monotherapy versus MET plus liraglutide (LIRA) on gonadal and metabolic profiles in overweight patients with polycystic ovary syndrome (PCOS).

Methods: Sixty overweight patients with PCOS were recruited from January 2021 to January 2022 in Shengjing Hospital of China Medical University and were randomly assigned to the MET or combination (COM) group to receive 12 weeks of MET monotherapy or MET plus LIRA therapy. Anthropometric measurements, menstrual cycle changes, gonadal profiles, and oral glucose tolerance tests (OGTT) were conducted at baseline and after the 12-week treatment.

Results: Fifty-two subjects completed the trial while eight were lost during the follow-up. Both MET and COM improved menstrual cycles, anthropometric parameters, and glucose metabolism after the 12-week treatment; however, there was no statistical difference between the two groups. MET plus LIRA therapy improved hyperandrogenemia, including TT (total testosterone), SHBG (sex hormone binding globulin) and FAI (free androgen index), whereas MET monotherapy only improved SHBG and FAI when compared with baseline. Furthermore, both MET monotherapy and MET plus LIRA therapy improved E2 (estradiol) while only MET plus LIRA therapy improved LH (luteinizing hormone), FSH (follicle stimulating hormone) and Prog (progesterone) more effectively than baseline. Additionally, MET plus LIRA therapy may improve TT, SHBG, FAI, LH and Prog more effectively than MET monotherapy; however, there were no significant differences on E2, FSH and LH/FSH between the two groups.

Conclusions: In overweight patients with PCOS, both MET monotherapy and MET plus LIRA therapy improved glucose metabolism and relieved insulin resistance (IR). Additionally, MET plus LIRA therapy was more effective than MET monotherapy in improving reproductive abnormalities and hyperandrogenemia, potentially by modulating the hypothalamic-pituitary-ovarian axis.

10.3389/fendo.2022.945609

KEYWORDS

metformin, liraglutide, polycystic ovary syndrome, gonadal profiles, hyperandrogenemia or androgen excess

Introduction

Polycystic ovary syndrome (PCOS) is a common reproductive endocrine disease characterized by ovulatory dysfunction and hyperandrogenemia (HA), affecting 5–15% women of reproductive age (1). Meanwhile, PCOS is considered as one of the main causes of female infertility (2) with a wide range of clinical manifestations, including reproductive disorders, dermatological disorders, and metabolic abnormalities (3). Extensive clinical and epidemiological data indicates that up to 80% of women with PCOS are overweight or obese with the prevalence of abdominal obesity (4). Obesity-related insulin resistance (IR) may induce excessive luteinizing hormone (LH)-stimulated ovarian androgen production and suppress hepatic sex hormone-binding globulin (SHBG) production, thus leading to HA (5). PCOS is also characterized by elevated serum LH levels and an altered ratio between LH and the follicle stimulating hormone (FSH) (6). Women with PCOS not only have higher basal LH levels, but also exhibit an increased number of LH pulses, which together drive the synthesis of androgens by ovarian theca cells, leading to HA (7). The secretion of gonadotropin-releasing hormone (GnRH) from the brain is regulated by several upstream neural and endocrine factors that contribute to both the timing and magnitude of GnRH secretion. Increased androgen signaling in brain may be a potential mechanism in the pathophysiology of PCOS, underlying the hypersecretion of GnRH and LH (8).

Metformin (MET) is a biguanide insulin sensitizer which may reduce hepatic glucose production, stimulate insulin-mediated glucose uptake in the liver and skeletal muscle, and reduce gluconeogenic substrate utilization (9). MET is used as a second-line treatment in PCOS guidelines and has multiple beneficial effects on menstrual disturbances, ovulatory disturbances, HA, metabolic, and cardiovascular abnormalities (10, 11). Glucagon-like peptide-1 (GLP-1) is an intestinal hormone that enhances glucose-stimulated insulin secretion, inhibits glucagon secretion, delays gastric emptying, increases satiety, reduces food intake and appetite, reduces body weight, and exerts other physiological effects (12). Animal and clinical studies have revealed that the effectiveness of GLP-1 receptor agonists (GLP-1 RAs) in treating PCOS and preventing its metabolic consequences (13). Liraglutide (LIRA) is a long-acting GLP-1 analogue with 97% similarity to human GLP-1 that reduces body weight and HA while improving menstrual cycles in patients with PCOS (14). Herein, the aim of the present randomized study was to evaluate the effect of MET monotherapy versus MET plus LIRA therapy on gonadal and metabolic profiles to provide new sights for treatment of overweight patients with PCOS.

Methods

Patients

We recruited 60 patients aged 18 to 40 years, who were diagnosed with PCOS in the outpatient department of Endocrinology, Shengjing Hospital of China Medical University from January 2021 to January 2022. All patients were informed about the study purpose, and signed written informed consent prior to participation. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Shengjing Hospital of China Medical University (registration number: 2020PS624K). The study was registered on Clinical Trials.gov (registration number: NCT04969627 with trial activation on 01/01/2021, first subject enrolled on 01/04/2021, last subject enrolled on 01/04/2022 and database lock on 03/29/2022).

The inclusion criteria for the subjects were as follows: (1) meet the Rotterdam diagnostic criteria of PCOS phenotype B with hyperandrogenism and ovulatory dysfunction (15); (2) body mass index (BMI) $\geq$ 24 kg/m^2; (3) age between 18 and 40 years; (4) no medication that affects insulin sensitivity or ovarian function within the first three months of the trial; (5) use barrier contraception.

The exclusion criteria for the subjects were as follows: (1) allergy to GLP-1 RAs or MET; (2) severe cardiovascular disease; (3) abnormal liver function test results (alanine transaminase (ALT) levels 2.5 times higher than the upper limit of the normal range); (4) renal insufficiency (estimated glomerular filtration rate, eGFR $<$ 60 mL/min/1.73 m^2); (5) thyroid dysfunction; (6) history of cancer; (7) active infection; (8) weekly alcohol intake $>$ 100 g; (9) pregnancy and breastfeeding; (10) 17-hydroxyprogesterone level $>$ 2 ng/mL (to exclude women with hyperandrogenemia due to atypical 21-hydroxylase deficiency).

Study design

This was a prospective, randomized, open-label, parallel-group controlled trial. After informed consent, the following data was obtained to determine eligibility: (1) height, weight, and age; (2) menstrual cycle; and (3) medical history. Eligible patients were assigned to the MET group or MET plus LIRA (COM) group through simple randomization at a 1:1 ratio using computer-generated codes. Patients in MET and COM group were administered MET 1000 mg BID p.o. or LIRA 1.2 mg QD s.c. plus MET 1000 mg BID p.o. for 12 consecutive weeks, respectively. Over the 12-week treatment course, all patients received the same diet and physical exercise. All subjects returned to the hospital for clinical, metabolic, and laboratory evaluations at baseline, the 4th week and the 12th week after treatment initiation (Figure 1).

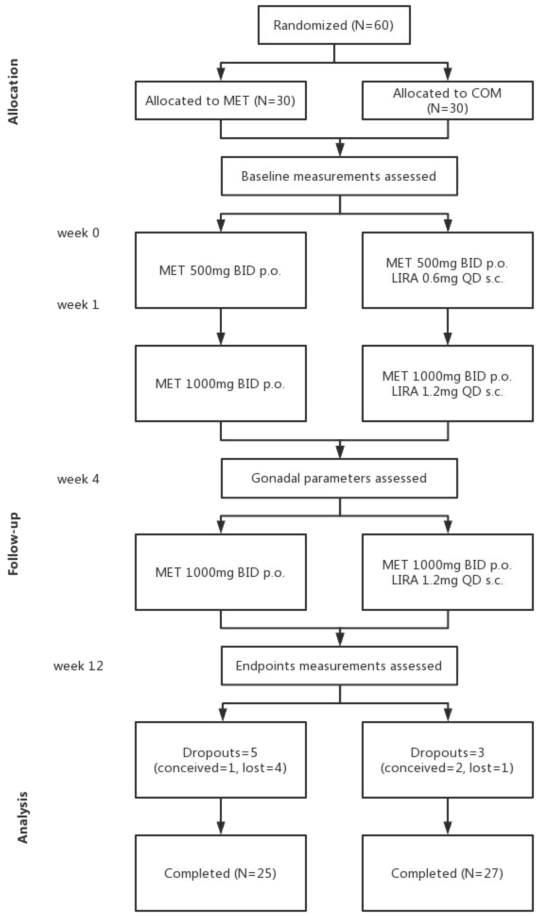


FIGURE 1
Flow chart of the study design.

Assessment of anthropometric measures

At baseline, the 4th week and the 12th week after treatment, trained personnel obtained anthropometric data, including height, weight, and abdominal girth (AG) for all patients using standardized protocols. The height and weight of each subject wearing light clothes were measured to the nearest 0.1 cm and 0.1 kg, respectively. BMI was calculated as weight (kg) divided by the square of height (m). AG was measured to an accuracy of 0.1 cm by placing a tape measure around the body at the level of the navel. Women with a BMI above 24 kg/m^2 were classified as overweight.

Assessment of menstrual cycle

During the 12-week treatment period, patients were asked to use barrier contraception only. Menstrual cycle changes were recorded. Menstrual cycle disorders included oligomenorrhea and amenorrhea. Oligomenorrhea referred to patients with less than six menstrual periods within 12 months while amenorrhea referred to patients who have stopped menstruating for more than 6 months. Each bleeding counts as one menstrual cycle. Menstrual cycle recovery was defined as the recurrence of regular menstrual cycles in patients.

Biochemical assessment

At baseline and the 12th week after treatment, the subjects fasted for 8–12 h overnight. We collected fasting venous blood to determine fasting glucose (FG) and fasting insulin (FINS). Subsequently, we performed a 75 g oral glucose tolerance test (OGTT) and collected venous blood at 0 h, 1 h and 2 h to determine the blood glucose and insulin concentrations. The homeostasis model assessment-insulin resistance (HOMA-IR) score was calculated to evaluate IR. HOMA-IR score = FINS (mU/L) × FG (mmol/L)/22.5. The approximate trapezoidal method was used to estimate the area under the curve of glucose (AUCglu; mmol/L × h) and the area under the curve of insulin (AUCins; mU/L × h). As all patients had irregular menstrual periods at enrollment, gonadal profiles were measured when venous blood samples were collected into BD Vacutainer Tubes (SSTTM II Advance, REF 367953) at baseline, the 4th and the 12th week. We did not perform progestin-induced menstrual bleeding to evaluate the hormones at the follicular phase of the menstrual cycle. The samples were centrifuged at 3600 rpm and 4°C for 10 min. The serum and plasma were aliquoted and stored at –80°C before estimating serum total testosterone (TT), LH, FSH, progesterone (Prog), estradiol (E2), and SHBG levels. LH/FSH = LH (mIU/mL)/FSH (mIU/mL). Free androgen index (FAI) = TT (nmol/L)/SHBG (nmol/L) × 100.

The standard glucose oxidase method was used to determine plasma glucose levels using Abbott CI16200. Radio-immunological assay was used to detect insulin, chemiluminescent immunoassay was used to detect LH, FSH, Prog, E2 and SHBG, while TT was measured by electro-chemiluminescent immunoassay using Beckman Coulter Unicel DXI800. Pre- and post-intervention samples from all subjects were analyzed in the same run, and all samples were measured in the same laboratory using standard laboratory techniques.

Statistical analyses

The sample size was calculated using the average difference formula, assuming that the minimum average difference between the treatment groups was 20%, and the average SE (standard error) was 2.2%. The sample size required for a statistical power of 0.80 and *P*-values on both sides <0.05 was 22 per group. The final sample size was 52. The Anderson–Darling method was used to test the normality of continuous data. Continuous data were presented as mean ± standard deviation (SD) (normal distribution) or median and interquartile range (non-normal distribution). Pre- and post-treatment effects in each group were analyzed using ANOVA or paired t-test (normal distribution) or paired Wilcoxon test (non-normal distribution) if appropriate. Effects between groups at the same time point were analyzed using unpaired t-test (normal distribution) or rank test (non-normal distribution) if appropriate. Categorical variables were expressed as frequencies or percentages and compared using the chi-square test. All *P*-values were two-tailed, and $P < 0.05$ was considered statistically significant. All data analyses were performed using GraphPad Prism 8.0.1 (GraphPad Software, Chicago, IL, USA) and SPSS 23.0 (SPSS Inc., Chicago, IL, USA).

Results

Participants

Sixty participants were randomly allocated to receive MET 1000 mg BID p.o. monotherapy (MET group) or MET 1000 mg BID p.o. plus LIRA 1.2 mg QD s.c. therapy (COM group). For all patients receiving MET, the dose began at 500 mg BID and gradually reached the target dose of 1000 mg BID after 1 week. For all patients receiving LIRA, the dose began at 0.6 mg QD and gradually reached the target dose of 1.2 mg QD after 1 week. Only 52 participants (25 in the MET group and 27 in the COM group) completed the 12-week follow-up; three participants conceived (one in the MET group and two in the COM group), and five participants could not be contacted at the end of the 12^{th} week (four in the MET group and one in the COM group). Mild gastrointestinal side effects, such as nausea, heartburn, vomiting, and diarrhea, occurred in both groups during the first two weeks of treatment with a higher proportion of these adverse reactions in the COM group. Two participants had one episode of hypoglycemia, while one participant in the COM group developed a rash at the injection site. Most adverse reactions were mild and spontaneously resolved after 2 weeks of treatment. No participants dropped out owing to drug intolerance in both groups with the final compliance 83.3% in the MET group and 90% in the COM group. All 52 subjects underwent anthropometric measurements, menstruation monitoring, and gonadal profiles detection at baseline, the 4^{th} and the 12^{th} week; however, only 38 subjects (16 in the MET group and 22 in the COM group) underwent OGTT tests at baseline and the 12^{th} week (Figure 1). All subjects ranged in age from 16 to 32 years (mean ± SD, 24.73 ± 4.65 years) with an average BMI of 29.26 ± 3.84 kg/m^2, and anovulation (menstrual cycle delay of longer than 2 months) at baseline.

Baseline results

The characteristics of the subjects at baseline are presented in Table 1. There was no significant difference between the two groups on Anthropometric parameters (weight, BMI, and AG), menstrual cycle recovery rates, gonadal parameters (E2, LH, FSH, LH/FSH, Prog, TT, SHBG, and FAI), and metabolic parameters (FG, FINS, HOMA-IR, AUCglu, AUCins, and AUCglu/AUCins) at baseline.

Menstruation and anthropometric measurements after treatment

After the 4-week treatment, the recovery rate of menstrual cycle was 52.00% (13/25) in the MET group ($P < 0.05$) and 77.78% (21/27) in the COM group ($P < 0.01$). After the 12-week treatment, the recovery rate of menstrual cycle was 88.00% (22/25) in the MET group ($P < 0.01$) and 92.59% (25/27) in the COM group ($P < 0.01$). There was no difference between the two groups (Figure 2A and Table 2).

After the 4-week and 12-week treatment, body weight was significantly decreased when compared with baseline in both the MET group ($P < 0.01$) and the COM group ($P < 0.01$). After the 4-week and 12-week treatment, BMI was significantly decreased when compared with baseline in both the MET group ($P < 0.01$) and the COM group ($P < 0.01$). AG was also significantly decreased when compared with baseline after the 4-week and 12-week treatment in both the MET group ($P < 0.01$) and the COM group ($P < 0.01$). There was no significant difference between the two groups in improving body weight, BMI, and AG (Figures 2B–D and Table 2).

TABLE 1 Menstruation and anthropometric, gonadal, and metabolic parameters at baseline.

	MET (N = 25)	COM (N = 27)	*P*
Age (years)	23.52 ± 4.65	25.85 ± 4.45	0.071
Regular menstrual cycles (%,n)	0, 0	0, 0	0.999
Weight (kg)	76.50 ± 12.44	79.09 ± 8.46	0.381
BMI (kg/m^2)	28.80 ± 4.25	29.69 ± 3.44	0.411
AG (cm)	91.96 ± 12.09	95.69 ± 8.94	0.210
FG (mmol/L)	5.48 ± 0.52	5.70 ± 0.93	0.373
FINS (μU/mL)	18.13 ± 11.73	18.89 ± 7.15	0.820
HOMA-IR	4.53 ± 3.07	4.85 ± 2.09	0.708
AUCglu (mmol/L*min)	1009.00 ± 109.00	1135.00 ± 144.50	0.264
AUCins (mU/L*min)	13,305.00 ± 4458.00	13,223.00 ± 3817.00	0.960
AUCins/AUCglu	11.31 ± 7.17	11.22 ± 7.11	0.970
E2 (pg/mL)	35.00 (27.49–40.50)	46.00 (35.33–66.50)	0.062
LH (mIU/mL)	11.97 ± 4.20	12.09 ± 5.26	0.925
FSH (mIU/mL)	6.03 ± 1.48	6.22 ± 1.44	0.652
LH/FSH	2.12 ± 0.97	2.04 ± 0.98	0.781
PRL (ng/mL)	10.81 ± 4.21	10.21 ± 3.66	0.583
Prog (ng/mL)	0.55 ± 0.29	0.42 ± 0.21	0.054
TT (ng/mL)	0.84 ± 0.25	0.79 ± 0.20	0.408
SHBG (nmol/L)	18.10 (11.75–24.10)	18.80 (14.90–21.90)	0.713
FAI (%)	15.62 (10.99–26.53)	13.94 (11.93–19.40)	0.537

BMI, body mass index; AG, abdominal girth; FG, fasting glucose; FINS, fasting insulin; HOMA-IR, homeostasis model assessment-insulin resistance; AUCglu, area under the curve (AUC) for glucose; AUCins, AUC for insulin; E2, estradiol; LH, luteinizing hormone; FSH, follicle-stimulating hormone; PRL, prolactin; Prog, progesterone; TT, total testosterone; SHBG, sex hormone-binding globulin; FAI, free androgen index. Results are expressed as mean ± SD or median (25^{th}–75^{th} percentile).

Metabolic parameters after treatment

After the 12-week treatment, FG, FINS, and HOMA-IR were significantly decreased in both the MET group ($P < 0.05$) and the COM group ($P < 0.01$). There was no significant difference between the two groups in improving FG, FINS, and HOMA-IR (Figures 3A–C and Table 2). However, AUCglu, AUCins, and AUCins/AUCglu were not improved in both groups after the 12-week treatment (Figures 3D–H and Table 2).

Gonadal parameters after treatment

E2 increased when compared with baseline in both the MET group ($P < 0.05$) and COM group ($P < 0.01$) (Figure 4A and Table 2). SHBG was higher than baseline only after the 12-week treatment in the MET group ($P < 0.01$) while it was higher than baseline after both the 4-week and the 12-week treatment in the COM group ($P < 0.01$) (Figure 4H and Table 2). After the 12-week treatment, FAI decreased significantly in both the MET group ($P < 0.01$) and the COM group ($P < 0.01$) (Figure 4I and Table 2). After the 12-week treatment, LH ($P < 0.01$), FSH ($P < 0.01$), Prog ($P < 0.01$), and TT ($P < 0.01$) improved significantly only in the COM group (Figures 4B, C, F, G and Table 2). After the 4-week treatment, a significant decrease from baseline in FSH ($P < 0.05$) was observed only in the COM group (Figure 4C and Table 2). However, there was no significant difference in LH/FSH between the two groups after the 4-week and 12-week treatment (Figure 4D and Table 2). After the 12-week treatment, LH [(9.77 ± 5.81) mIU/mL vs. (6.61 ± 4.72) mIU/mL, $P = 0.036$], Prog [0.54 (0.31–1.72) ng/mL vs. 1.08 (0.52–10.61) ng/mL, $P = 0.020$], TT [(0.79 ± 0.31) ng/mL vs. (0.62 ± 0.24) ng/mL, $P = 0.032$], SHBG [22.40 (15.25–34.60) nmol/L vs. 27.00 (22.60–44.90) nmol/L, $P = 0.018$], and FAI [12.72 (6.40–17.71)% vs. 7.06 (3.90–10.19)%, $P = 0.004$] were significantly improved, and more improvement was observed in the COM group. There was no significant difference between the two groups in improving E2, FSH and LH/FSH (Figures 4A–I and Table 2).

Discussion

This study was the first randomized controlled trial designed to compare the effect of MET monotherapy versus MET plus LIRA therapy mainly focusing on gonadal profiles in overweight patients with PCOS. Our results demonstrated that both MET monotherapy and MET plus LIRA therapy improved E2, SHBG and FAI, whereas only MET plus LIRA therapy improved LH, FSH, Prog and TT more effectively than baseline. Additionally, MET plus LIRA therapy was more effective than MET monotherapy in improving serum LH, Prog, TT, SHBG, and

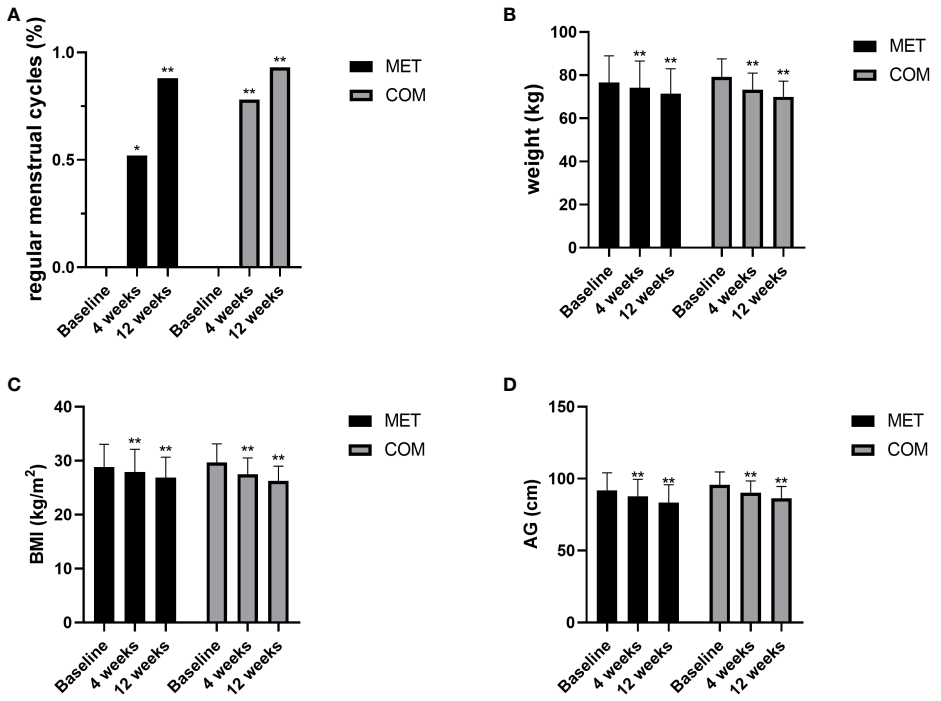


FIGURE 2
Changes in menstruation and anthropometric measurements after MET and COM therapy. **(A)** Changes in menstrual cycles after MET and COM therapy. **(B)** Changes in weight after MET and COM therapy. **(C)** Changes in BMI after MET and COM therapy. **(D)** Changes in AG after MET and COM therapy. MET, metformin; COM, combine; BMI, body mass index; AG, abdominal girth. Results are expressed as mean ± SD; $*P < 0.05$, $**P < 0.01$ (vs. before treatment in each group).

FAI levels after the 12-week treatment. We also found that both MET plus LIRA therapy and MET monotherapy improved menstrual cycles, anthropometric parameters, and glucose metabolism with no significant differences between the two groups.

Approximately 98% of women with PCOS experienced menstrual disorders and infertility (16). Sever et al. found no statistically significant changes after the 12-week intervention in menstruation frequency, neither over time nor when analyzing separately by the type of therapy among LIRA monotherapy, MET monotherapy, or COM therapy groups (17). However, our results showed that menstrual rates were significantly improved in both the MET (88.00%) and COM (92.59%) groups after 12 weeks of treatment with no significant difference between the two groups. The difference between our results and those of Sever et al. may be due to the fact that all of our subjects initially had amenorrhea for more than 3 months and we focused on menstrual recovery, while Sever et al. focused on changes in menstrual frequency. In addition, there was great heterogeneity due to the small number of participants and the short duration of the intervention. So far, few clinical studies have observed the effect of MET and LIRA on menstrual changes in PCOS. Thus, more research is needed in the future to further clarify their effects.

Approximately 60–70% of patients with PCOS are obese, and they have greater AG than women who are simply obese (18). Reductions in BMI, particularly abdominal fat, may play an important role in reducing infertility risk factors, leading to improvements in HA and clinical symptoms (19). In addition to lifestyle intervention and combined oral contraceptives, MET is the most frequent treatment modality in PCOS to relieve IR. However, MET does n0t substantially reduce weight or alter fat distribution in PCOS. In contrast, Frøssing et al. demonstrated that 1.8 mg QD LIRA treatment for 26 weeks could achieve a substantial reduction in liver fat content by 44% and visceral adipose tissue by 18%, with a minor reduction in total body fat, thus leading to weight loss by 5.2 kg (5.6%) and a reduction in the prevalence of the nonalcoholic fatty liver disease by two-thirds when compared with placebo (20). Sever et al. found that after the 12-week treatment, the COM therapy of MET 1000 mg BID and LIRA 1.2 mg QD was superior to either LIRA 1.2 mg QD monotherapy or MET 1000 mg BID monotherapy in reducing weight, BMI, and waist circumference. Subjects in the COM group lost on average of 6.5 ± 2.8 kg compared with a 3.8 ± 3.7 kg loss in the LIRA group and a 1.2 ± 1.4 kg loss in the MET group. BMI decreased by 2.4 ± 1.0 in the COM group compared with 1.3 ± 1.3 in the LIRA group and 0.5 ± 0.5 in the MET group. Waist circumference also decreased by 5.5 ± 3.8 cm in the COM

TABLE 2 Changes of hormonal, metabolic and anthropometric parameters.

Parameters	MET (N=25)			COM (N=27)			^{a}P	^{b}P
	Baseline	4 weeks	12 weeks	Baseline	4 weeks	12 weeks		
Regular menstrual cycles (%,n)	0%,0	52.00%,13*	88.00%,22**	0%,0	77.78%,21**	92.59%,25**	0.08	0.662
Weight (kg)	76.50 ± 12.44	74.10 ± 12.45**	71.44 ± 11.50**	79.09 ± 8.46	73.26 ± 7.71**	69.96 ± 7.23**	0.773	0.586
BMI (kg/m2)	28.80 ± 4.25	27.89 ± 4.21**	26.88 ± 3.76**	29.69 ± 3.44	27.49 ± 3.03**	26.24± 2.75**	0.69	0.485
AG (cm)	91.96 ± 12.09	87.82 ± 11.79**	83.40 ± 12.42**	95.69 ± 8.94	90.28 ± 8.20**	86.30 ± 8.25**	0.384	0.331
FG (mmol/L)	5.48 ± 0.52		5.20 ± 0.32*	5.70 ± 0.93		5.05 ± 0.40**		0.222
FINS (μU/mL)	18.13 ± 11.73		12.87 ± 7.93**	18.89 ± 7.15		11.60 ± 4.22**		0.577
HOMA-IR	4.53 ± 3.07		2.86 ± 2.05**	4.85 ± 2.09		2.62 ± 1.05**		0.677
AUCglu (mmol/L*min)	1009.00 ± 109.00		1007.00 ± 89.43	1135.00 ± 144.50		972.70 ± 135.70		0.687
AUCins (mU/L*min)	13305.00 ± 4458.00		11820.00 ± 3867.00	13223.00 ± 3817.00		8964.00 ± 2919.00		0.253
AUCins/AUCglu	11.31 ± 7.17		10.14 ± 6.84	11.22 ± 7.11		8.39 ± 5.50		0.387
E2 (pg/mL)	35.00 (27.49-40.50)	46.00 (35.33-66.50)*	71.10 (48.37-120.00)**	40.00 (32.00-67.00)	54.00 (41.00-99.00)**	90.00 (58.00-184.00)**	0.053	0.181
LH (mIU/mL)	11.97 ± 4.20	12.08 ± 6.47	9.77 ± 5.81	12.09 ± 5.26	9.54 ± 4.52	6.61 ± 4.72**	0.105	***0.036***
FSH (mIU/mL)	6.03 ± 1.48	5.91 ± 1.72	5.28 ± 2.11	6.22 ± 1.44	5.15 ± 1.93**	4.42 ± 2.59**	0.14	0.197
LH/FSH	2.12 ± 0.97	2.03 ± 1.02	1.91 ± 0.86	2.04 ± 0.98	1.94 ± 0.80	1.59 ± 0.95	0.725	0.215
PRL (ng/mL)	10.81 ± 4.21	10.80 ± 4.52	13.07 ± 5.77*	10.21 ± 3.66	10.65 ± 4.64	12.91 ± 4.97**	0.903	0.914
Prog (ng/mL)	0.50 (0.29-0.75)	0.45 (0.29-1.14)	0.54 (0.31-1.72)	0.42 (0.22-0.56)	0.53 (0.39-1.21)	1.08 (0.52-10.61)**	0.272	***0.020***
TT (ng/mL)	0.84 ± 0.25	0.81 ± 0.35	0.79 ± 0.31	0.79 ± 0.20	0.70 ± 0.24	0.62 ± 0.24**	0.163	***0.032***
SHBG (nmol/L)	18.10 (11.75-24.10)	19.90 (13.90-27.45)	22.40 (15.25-34.60)**	18.80 (14.90-21.90)	22.10 (17.10-26.90)*	27.00 (22.60-44.90)**	0.395	***0.018***
FAI (%)	15.62 (10.99-26.53)	14.22 (6.85-21.85)	12.72 (6.40-17.71)*	13.94 (11.93-19.40)	9.55 (5.78-17.01)	7.06 (3.90-10.19)**	0.104	***0.004***

BMI, body mass index; AG, abdominal girth; FG, fasting glucose; FINS, fasting insulin; HOMA-IR, homeostasis model assessment-insulin resistance; AUCglu, area under the curve (AUC) for glucose; AUCins, AUC for insulin; E2, estradiol; LH, luteinizing hormone; FSH, follicle-stimulating hormone; PRL, prolactin; Prog, progesterone; TT, total testosterone; SHBG, sex hormone-binding globulin; FAI, free androgen index. Results are expressed as mean ± SD or median (25^{th}–75^{th} percentile). *P < 0.05, post-treatment vs. baseline; **P < 0.01, post-treatment vs. baseline; ^{a}P < 0.01, COM vs. MET at the 4^{th} week; ^{b}P < 0.01, COM vs. MET at the 12^{th} week. Statistically significant results are presented in bold.

group compared with 3.2 ± 2.9 cm in the LIRA group and 1.6 ± 2.9 cm in the MET group (17). Jensterle et al. found that BMI and waist circumference reduction in the LIRA 3 mg QD monotherapy group was greater than that in the COM therapy of MET 1000 mg BID and LIRA 1.2 mg QD group (−2.2 ± 1.3 vs −1.3 ± 0.9 kg/m^2 and −4.2 ± 3.4 vs −2.2 ± 6.2 cm) within 12 weeks (21). We found that body weight, BMI, and AG were significantly decreased when compared with baseline in both the MET and the COM groups after 4 and 12 weeks of treatment; however, no statistical differences between the two groups were found. This finding was possibly due to the short duration of the intervention or the different dosage of interventions or the initial BMI of our Chinese participants was lower than that of the European participants included by Sever et al. and Jensterle et al.

MET inhibits the gluconeogenesis of hepatic glycogen, increases glucose uptake and utilization by peripheral tissues, improves hepatic insulin sensitivity and FG in patients with PCOS (22). GLP-1 improves hepatic IR, increases hepatic glucose uptake, and improves adipose IR, thereby reducing free fatty acid production (23). GLP-1 RAs activate GLP-1 receptors on beta cells, mimicking endogenous GLP-1 to stimulate glucose-dependent phase 1 and 2 insulin secretion, thereby reducing fasting and postprandial blood glucose (24). Frøssing et al. demonstrated that the 26-week LIRA treatment caused significant reductions in FG −0.24 [−0.44 to −0.04] mmol/L, HbA1c −1.38 [−2.48 to −0.28] mmol/mol, and AUCglu when compared with placebo, whereas HOMA-IR and AUCins remained unchanged (20). Additionally, Sever et al. found that HOMA-IR tended to be reduced after LIRA monotherapy, MET monotherapy, and COM therapy, but did not significantly decrease in any group while FG and FINS did not consistently improve either after the 12-week intervention. However, patients in the COM group proved the most successful at reducing glucose value after 120 min during OGTT (17). Moreover, Jensterle et al. found that the beneficial effects on glucose metabolism after a 12-week intervention were comparable in both LIRA 3 mg QD monotherapy group and the COM therapy of MET 1000 mg BID and LIRA 1.2 mg QD group (21). Similarly, our results showed that FG, FINS, and HOMA-IR were significantly decreased in both the MET and the COM groups after 12 weeks of treatment with no significant differences between the two groups. However, AUCglu, AUCins, and AUCins/AUCglu were not significantly improved in either group after 12 weeks of treatment. The different results of

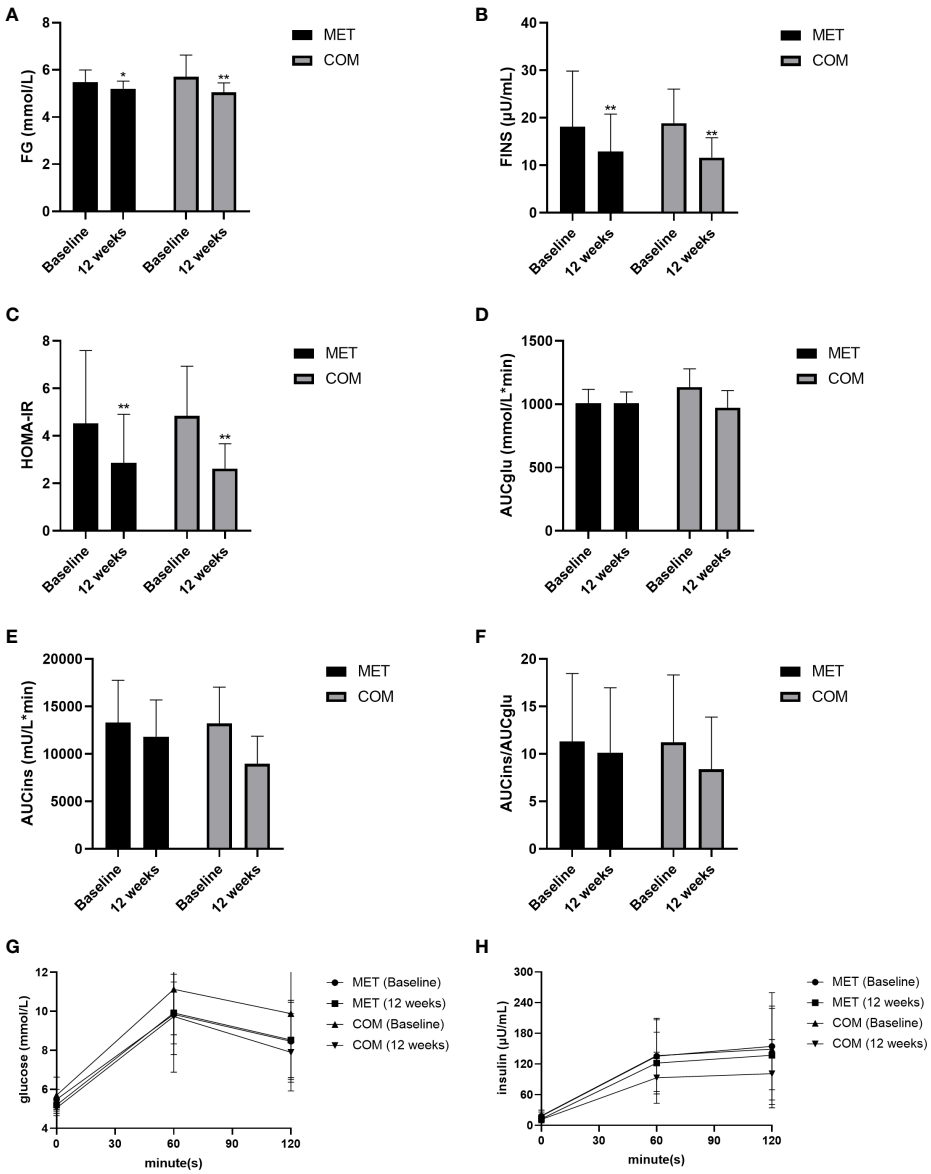


FIGURE 3
Changes in metabolic parameters after MET and COM therapy. **(A)** Changes in FG after MET and COM therapy. **(B)** Changes in FINS after MET and COM therapy. **(C)** Changes in HOMA-IR after MET and COM therapy. **(D)** Changes in AUCglu after MET and COM therapy. **(E)** Changes in AUCins after MET and COM therapy. **(F)** Changes in AUCglu/AUCins after MET and COM therapy. **(G)** Changes in OGTT (glucose) after MET and COM therapy. **(H)** Changes in OGTT (insulin) after MET and COM therapy. MET, metformin; COM, combine; FG, fasting glucose; FINS, fasting insulin; HOMA-IR, homeostasis model assessment-insulin resistance; AUCglu, area under the curve (AUC) for glucose; AUCins, AUC for insulin. Results are expressed as mean ± SD; $^{*}P < 0.05$, $^{**}P < 0.01$ (vs. before treatment in each group).

glucose metabolism changes obtained in different studies may be due to the different drug dosage or intervention duration, which still need to be further confirmed by larger-scale studies in the future.

HA is the most common hormonal change in PCOS, and women with PCOS typically present elevated serum levels of multiple androgens (25). Chen et al. found that women with PCOS have higher LH levels and lower FSH levels when compared with healthy women (26). Although the LH/FSH ratio is not a diagnostic criterion of PCOS, patients with LH/FSH > 2 may have increased adrenal androgen activity and HA, which may lead to the worsening of PCOS symptoms such as hirsutism and acne (27). The ovarian phenotype in PCOS is due to maturation arrest of FSH-sensitive follicles, thus preventing them from reaching full size typically due to IR. This leads to elevated levels of insulin and insulin-related growth factors,

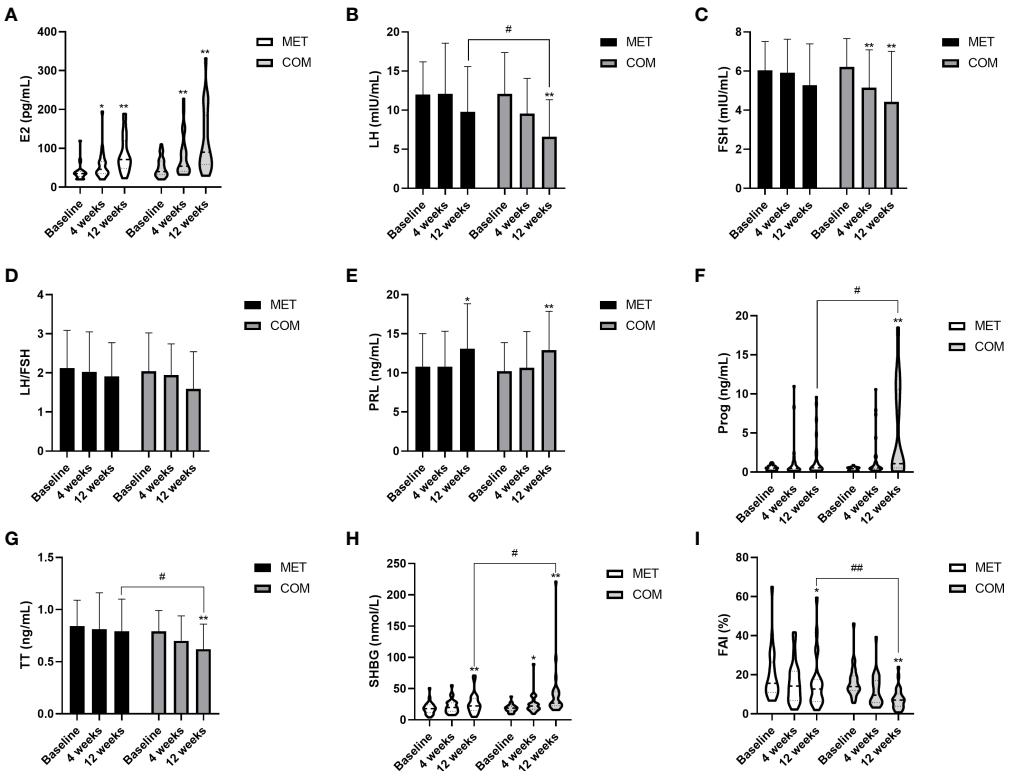


FIGURE 4
Changes in gonadal parameters after MET and COM therapy. **(A)** Changes in E2 after MET and COM therapy. **(B)** Changes in LH after MET and COM therapy. **(C)** Changes in FSH after MET and COM therapy. **(D)** Changes in LH/FSH after MET and COM therapy. **(E)** Changes in PRL after MET and COM therapy. **(F)** Changes in Prog after MET and COM therapy. **(G)** Changes in TT after MET and COM therapy. **(H)** Changes in SHBG after MET and COM therapy. **(I)** Changes in FAI after MET and COM therapy. MET, metformin; COM, combine; E2, estradiol; LH, luteinizing hormone; FSH, follicle-stimulating hormone; PRL, prolactin; Prog, progesterone; TT, total testosterone; SHBG, sex hormone-binding globulin; FAI, free androgen index. Results are expressed as mean ± SD or median (25^{th}–75^{th} percentile); $^{*}P < 0.05$, $^{**}P < 0.01$ (vs. before treatment in each group); $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ (vs. the other treatment).

which stimulate theca cells to produce large amounts of androgens, and in turn interfere with follicle growth, causing abnormal ovulation (28). Patients with PCOS demonstrate increased levels of GnRH leading to a higher frequency of LH pulsation, stimulation of LH-mediated androgen production, and disruption of follicle development. The resulting chronic anovulation is due to the relatively low level of FSH that occurs secondary to the altered GnRH release pattern (29). Recent studies in induced rat models have shown the expression of GLP receptors in the hypothalamus, pituitary, the change of ovary during the ovulatory cycle. Treatment with natural GLP-1 quadrupled the amplitude of the LH surge and resulted in higher progesterone in the luteal phase (30). Based on research regarding the impact of GLP-1RAs on the LH ratio, Bednarz et al. advocated that its modulating abilities can either increase LH surge in hypothalamic-pituitary-ovarian (HPO) axis disturbances due to adipose tissue estrogen aromatization or decrease excessive LH levels that induced ovarian androgen secretion which may also be related to hyperinsulinemia (31). Although trials conducted on GLP-1 RAs in PCOS have all demonstrated improvement in glucose and metabolic parameters, limited clinical research has been performed on gonadal profiles. Our study observed for the first time that COM therapy was more effective than MET monotherapy in reducing serum LH levels [(9.77 ± 5.81) mIU/mL vs. (6.61 ± 4.72) mIU/mL, $P = 0.036$] after 12 weeks of treatment. In addition, COM therapy was more effective than MET monotherapy in increasing serum Prog levels [0.54 (0.31–1.72) ng/mL vs. 1.08 (0.52–10.61) ng/mL, $P = 0.020$] after 12 weeks of treatment, indicating the recovery of local hyperandrogenic state and ovulatory function of PCOS ovaries.

Women with PCOS typically have low serum SHBG levels owing to IR and obesity, thus resulting in high levels of bioavailable active free testosterone (FT), leading to higher rates of menstrual disorders, ovarian cysts, hypo-ovulation, infertility, and hirsutism. Therefore, both the decreased circulating insulin and improved IR status can cause the increase of serum SHBG levels, leading to the decreased serum

FT levels, thereby improving outcomes in patients with PCOS (27). Nylander et al. reported that after 26 weeks of LIRA treatment, SHBG levels increased by 7.4 nmol/L (95% CI 4.1 to 10.7), FT levels decreased by 0.005 nmol/L (95% CI −0.009 to −0.001), while TT levels remained unchanged (32). In a 12-week study, Jensterle et al. found that both LIRA and LIRA plus MET therapy resulted in increased SHBG levels and decreased FT levels with no difference between groups in obese patients with PCOS. However, TT levels decreased only after LIRA plus MET therapy (33). Our 12-week study revealed that SHBG levels were significantly increased and FAI significantly decreased in both MET and COM groups. Moreover, the TT level remained unchanged after 12 weeks of treatment in the MET group but significantly decreased in the COM group, which was also consistent with the findings by Jensterle et al. (33). After 12 weeks of treatment, the COM group had significant differences in the serum TT levels [(0.79 ± 0.31) ng/mL vs. (0.62 ± 0.24) ng/mL, $P = 0.032$], serum SHBG levels [22.40 (15.25–34.60) nmol/L vs. 27.00 (22.60–44.90) nmol/L, $P = 0.018$], and FAI [12.72 (6.40–17.71)% vs. 7.06 (3.90–10.19)%, $P = 0.004$] when compared with those in the MET group, indicating that MET plus LIRA therapy may be more effective than MET monotherapy in improving HA in overweight patients with PCOS.

PCOS is a heterogeneous disease with multiple factors influencing its treatment. To date, several therapeutic strategies have been proposed for the treatment of PCOS, anti-androgens for menstrual disorders and hirsutism/acne; ovulation-stimulating agents for infertility; insulin-sensitizing compounds for hyperinsulinemia. Additionally, lifestyle changes, such as diet and exercise, are considered first-line treatments for women affected by PCOS. Recent evidence also suggests that a low-carbohydrate, ketogenic diet can have beneficial effects on weight loss and improved insulin resistance in 11 women with PCOS with a BMI >27 kg/m^2 following a 24-week low-ketogenic diet. Preliminary studies have reported significant improvements in body weight, free testosterone, LH/FSH ratio, and FINS (34). Different forms of fasting, such as intermittent fasting and regular fasting, can lower glucose and insulin levels, which can have beneficial effects on ovarian function, androgen excess, and infertility in women with PCOS. Fasting may also improve symptoms and signs associated with hyperandrogenemia. In addition, weight loss reduces adipose tissue and may negatively regulate androgen conversion in estrone: in this case, fasting may reduce hypothalamic and pituitary dysregulation, which underlie subfertility in women with PCOS (35). Inositol is a polyol with nine naturally occurring stereoisomers, including D-chiro-inositol (DCI) and inositol (MI), which play important roles in the metabolism of glucose and free fatty acids. Moreover, MI and DCI have been classified as insulin sensitizers and appear to adequately counteract several IR-related metabolic alterations with safe nutritional profiles (36). Laganà et al. administered MI or DCI to two different groups of PCOS patients and found that circulating androgen levels were decreased, LH and LH/FSH ratios were decreased in both groups, through a decrease in the HOMA index, and an increase in SHBG. The results suggest that MI appears to have the most significant effect on the metabolic profile, whereas DCI mainly affects hyperandrogenemia parameters. Furthermore, both groups showed improved menstrual cycle regularity without any significant difference between the two inositol isoforms (37). Unfortunately, our clinical trial only explored the effects of MET and LIRA in overweight PCOS patients, so future longitudinal cohort studies, as well as prospective intervention trials, may help to better elucidate the role of different treatments for PCOS.

The main limitations of our study are the single-center design, relatively small sample size, and short treatment duration. Above all, the 12-week period is very short to compare the sustainability of the benefits of these regimens. Although LIRA plus MET therapy was more effective than MET monotherapy in improving LH, Prog, SHBG, and FAI, longer treatment durations and larger, multicenter, multiethnic group trials are needed to confirm our findings. Additionally, at the end of the 12th week of treatment, some patients did not undergo OGTT tests, resulting in fewer available results for glucose metabolism analysis. The lack of data hindered comprehensive evaluation of the dynamics underlying glucose metabolism. Finally, owing to various reasons, such as the COVID-19 pandemic, the rate of loss of participants during follow-up was relatively high. Although attrition was within the generally accepted threshold of 20%, it might cause potential biases.

Conclusions

In overweight Chinese patients with PCOS, both MET monotherapy and MET plus LIRA therapy improved glucose metabolism and relieved IR. Additionally, MET plus LIRA therapy was more effective than MET monotherapy in improving reproductive abnormalities and hyperandrogenemia, potentially by modulating the hypothalamic-pituitary-ovarian axis.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Shengjing Hospital

of China Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

CX and BH contributed to conception and design of the study. HZ organized the database. JZ performed the statistical analysis. CX wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Funding

The research was funded by Science and Technology Department People's Livelihood Science and Technology Joint Program Funding of Liaoning Province (No. 2021JH2/10300125).

Acknowledgments

Thanks to Editage for assisting with language editing services.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Wolf WM, Wattick RA, Kinkade ON, Olfert MD. Geographical prevalence of polycystic ovary syndrome as determined by region and Race/Ethnicity. *Int J Environ Res Public Health* (2018) 15(11):2589. doi: 10.3390/ijerph15112589

2. De Leo V, Musacchio MC, Cappelli V, Massaro MG, Morgante G, Petraglia F. Genetic, hormonal and metabolic aspects of PCOS: an update. *Reprod Biol Endocrinol* (2016) 14(1):38. doi: 10.1186/s12958-016-0173-x

3. Lentscher JA, Decherney AH. Clinical presentation and diagnosis of polycystic ovarian syndrome. *Clin Obstet Gynecol* (2021) 64(1):3–11. doi: 10.1097/GRF.0000000000000563

4. Barber TM, Franks S. Obesity and polycystic ovary syndrome. *Clin Endocrinol (Oxf).* (2021) 95(4):531–41. doi: 10.1111/cen.14421

5. Arya S, Hansen KR, Wild RA. Metformin, rosiglitazone, or both for obese women with polycystic ovary syndrome? *Fertil Steril* (2020) 113(1):87–8. doi: 10.1016/j.fertnstert.2019.10.006

6. Iervolino M, Lepore E, Forte G, Laganà AS, Buzzaccarini G, Unfer V. Natural molecules in the management of polycystic ovary syndrome (PCOS): An analytical review. *Nutrients* (2021) 13(5):1677. doi: 10.3390/nu13051677

7. Coutinho EA, Kauffman AS. The role of the brain in the pathogenesis and physiology of polycystic ovary syndrome (PCOS). *Med Sci (Basel)* (2019) 7(8):84. doi: 10.3390/medsci7080084

8. Caldwell ASL, Edwards MC, Desai R, Jimenez M, Gilchrist RB, Handelsman DJ, et al. Neuroendocrine androgen action is a key extraovarian mediator in the development of polycystic ovary syndrome. *Proc Natl Acad Sci USA* (2017) 114 (16):E3334–43. doi: 10.1073/pnas.1616467114

9. Bennett WL, Aschmann HE, Puhan MA, Robbins CW, Bayliss EA, Wilson R, et al. A benefit-harm analysis of adding basal insulin vs. sulfonylurea to metformin to manage type II diabetes mellitus in people with multiple chronic conditions. *J Clin Epidemiol* (2019) 113:92–100. doi: 10.1016/j.jclinepi.2019.03.014

10. Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, et al. Diagnosis and treatment of polycystic ovary syndrome: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* (2013) 98(12):4565–92. doi: 10.1210/jc.2013-2350

11. Diamanti-Kandarakis E, Christakou CD, Kandaraki E, Economou FN. Metformin: an old medication of new fashion: Evolving new molecular mechanisms and clinical implications in polycystic ovary syndrome. *Eur J Endocrinol* (2010) 162(2):193–212. doi: 10.1530/EJE-09-0733

12. Andersen A, Lund A, Knop FK, Vilsbøll T. Glucagon-like peptide 1 in health and disease. *Nat Rev Endocrinol* (2018) 14(7):390–403. doi: 10.1038/s41574-018-0016-2

13. Abdalla MA, Deshmukh H, Atkin S, Sathyapalan T. A review of therapeutic options for managing the metabolic aspects of polycystic ovary syndrome. *Ther Adv Endocrinol Metab* (2020) 11:2042018820938305. doi: 10.1177/2042018820938305

14. Sekar R, Singh K, Arokiaraj AW, Chow BK. Pharmacological actions of glucagon-like peptide-1, gastric inhibitory polypeptide, and glucagon. *Int Rev Cell Mol Biol* (2016) 326:279–341. doi: 10.1016/bs.ircmb.2016.05.002

15. Lizneva D, Suturina L, Walker W, Brakta S, Gavrilova-Jordan L, Azziz R. Criteria, prevalence, and phenotypes of polycystic ovary syndrome. *Fertil Steril* (2016) 106(1):6–15. doi: 10.1016/j.fertnstert.2016.05.003

16. Wiwatpanit T, Murphy AR, Lu Z, Urbanek M, Burdette JE, Woodruff TK, et al. Scaffold-free endometrial organoids respond to excess androgens associated with polycystic ovarian syndrome. *J Clin Endocrinol Metab* (2020) 105(3):769–80. doi: 10.1210/clinem/dgz100

17. Jensterle Sever M, Kocjan T, Pfeifer M, Kravos NA, Janez A. Short-term combined treatment with liraglutide and metformin leads to significant weight loss in obese women with polycystic ovary syndrome and previous poor response to metformin. *Eur J Endocrinol* (2014) 170(3):451–9. doi: 10.1530/EJE-13-0797

18. Siamashvili M, Davis SN. Update on the effects of GLP-1 receptor agonists for the treatment of polycystic ovary syndrome. *Expert Rev Clin Pharmacol* (2021) 14(9):1081–9. doi: 10.1080/17512433.2021.1933433

19. Elkind-Hirsch KE, Chappell N, Seidemann E, Storment J, Bellanger D. Exenatide, dapagliflozin, or Phentermine/Topiramate differentially affect metabolic profiles in polycystic ovary syndrome. *J Clin Endocrinol Metab* (2021) 106 (10):3019–33. doi: 10.1210/clinem/dgab408

20. Frøssing S, Nylander M, Chabanova E, Frystyk J, Holst JJ, Kistorp C, et al. Effect of liraglutide on ectopic fat in polycystic ovary syndrome: A randomized clinical trial. *Diabetes Obes Metab* (2018) 20(1):215–8. doi: 10.1111/dom.13053

21. Jensterle M, Kravos NA, Goričar K, Janez A. Short-term effectiveness of low dose liraglutide in combination with metformin versus high dose liraglutide alone in treatment of obese PCOS: randomized trial. *BMC Endocr Disord* (2017) 17(1):5. doi: 10.1186/s12902-017-0155-9

22. LaMoia TE, Shulman GI. Cellular and molecular mechanisms of metformin action. *Endocr Rev* (2021) 42(1):77–96. doi: 10.1210/endrev/bnaa023

23. Alvares D, Hoffman S, Stankovic B, Adeli K. Gut peptide and neuroendocrine regulation of hepatic lipid and lipoprotein metabolism in health and disease. *Biochim Biophys Acta Mol Cell Biol Lipids* (2019) 1864(3):326–34. doi: 10.1016/j.bbalip.2018.12.010

24. Chueire VB, Muscelli E. Effect of free fatty acids on insulin secretion, insulin sensitivity and incretin effect - a narrative review. *Arch Endocrinol Metab* (2021) 65 (1):24–31. doi: 10.20945/2359-3997000000313

25. Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group. revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* (2004) 19(1):41–7. doi: 10.1093/humrep/deh098

26. Chen J, Shen S, Tan Y, Xia D, Xia Y, Cao Y, et al. The correlation of aromatase activity and obesity in women with or without polycystic ovary syndrome. *J Ovarian Res* (2015) 8:11. doi: 10.1186/s13048-015-0139-1

27. Samadi Z, Bambaeichi E, Valiani M, Shahshahan Z. Evaluation of changes in levels of hyperandrogenism, hirsutism and menstrual regulation after a period of aquatic high intensity interval training in women with polycystic ovary syndrome. *Int J Prev Med* (2019) 10:187. doi: 10.4103/ijpvm.IJPVM_360_18

28. Mesinovic J, Teede HJ, Shorakae S, Lambert GW, Lambert EA, Naderpoor N, et al. The relationship between vitamin d metabolites and androgens in women with polycystic ovary syndrome. *Nutrients* (2020) 12(5):1219. doi: 10.3390/nu12051219

29. Szeliga A, Rudnicka E, Maciejewska-Jeske M, Kucharski M, Kostrzak A, Hajbos M, et al. Neuroendocrine determinants of polycystic ovary syndrome. *Int J Environ Res Public Health* (2022) 19(5):3089. doi: 10.3390/ijerph19053089

30. Silva MSB, Desroziers E, Hessler S, Prescott M, Coyle C, Herbison AE, et al. Activation of arcuate nucleus GABA neurons promotes luteinizing hormone secretion and reproductive dysfunction: Implications for polycystic ovary syndrome. *EBioMedicine* (2019) 44:582–96. doi: 10.1016/j.ebiom.2019.05.065

31. Bednarz K, Kowalczyk K, Cwynar M, Czapla D, Czarkowski W, Kmita D, et al. The role of glp-1 receptor agonists in insulin resistance with concomitant obesity treatment in polycystic ovary syndrome. *Int J Mol Sci* (2022) 23(8):4334. doi: 10.3390/ijms23084334

32. Nylander M, Frøssing S, Clausen HV, Kistorp C, Faber J, Skouby SO. Effects of liraglutide on ovarian dysfunction in polycystic ovary syndrome: a randomized clinical trial. *Reprod BioMed Online* (2017) 35(1):121–7. doi: 10.1016/j.rbmo.2017.03.023

33. Jensterle M, Goricar K, Janez A. Metformin as an initial adjunct to low-dose liraglutide enhances the weight-decreasing potential of liraglutide in obese polycystic ovary syndrome: Randomized control study. *Exp Ther Med* (2016) 11(4):1194–200. doi: 10.3892/etm.2016.3081

34. Muscogiuri G, Palomba S, Laganà AS, Orio F. Current insights into inositol isoforms, Mediterranean and ketogenic diets for polycystic ovary syndrome: From bench to bedside. *Curr Pharm Des* (2016) 22(36):5554–7. doi: 10.2174/1381612822666160720160634

35. Chiofalo B, Laganà AS, Palmara V, Granese R, Corrado G, Mancini E, et al. Fasting as possible complementary approach for polycystic ovary syndrome: Hope or hype? *Med Hypotheses* (2017) 105:1–3. doi: 10.1016/j.mehy.2017.06.013

36. Paul C, Laganà AS, Maniglio P, Triolo O, Brady DM. Inositol's and other nutraceuticals' synergistic actions counteract insulin resistance in polycystic ovarian syndrome and metabolic syndrome: state-of-the-art and future perspectives. *Gynecol Endocrinol* (2016) 32(6):431–8. doi: 10.3109/09513590.2016.1144741

37. Laganà AS, Rossetti P, Buscema M, La Vignera S, Condorelli RA, Gullo G, et al. Metabolism and ovarian function in PCOS women: A therapeutic approach with inositols. *Int J Endocrinol* (2016) 2016:6306410. doi: 10.1155/2016/6306410

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 02 September 2022
DOI 10.3389/fendo.2022.982200

OPEN ACCESS

EDITED BY
He-Feng Huang,
Fudan University, China

REVIEWED BY
Yin Tailang,
Wuhan University, China
Zhao-Jia Ge,
Qingdao Agricultural University, China

*CORRESPONDENCE
Hai-xia Jin
jinhx@zzu.edu.cn

†These authors share first authorship

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

RECEIVED 30 June 2022
ACCEPTED 16 August 2022
PUBLISHED 02 September 2022

CITATION
Guan S-y, Liu Y-y, Guo Y-h, Shen X, Liu Y and Jin H-x (2022) Potential biomarkers for clinical outcomes of IVF cycles in women with/without PCOS: Searching with metabolomics. *Front. Endocrinol.* 13:982200. doi: 10.3389/fendo.2022.982200

Potential biomarkers for clinical outcomes of IVF cycles in women with/without PCOS: Searching with metabolomics

Shang-yue Guan[1,2†], Yuan-yuan Liu[1,2†], Yuhan Guo[1,2], Xiao-xue Shen[1], Yan Liu[1,2] and Hai-xia Jin[1,2*]

[1]Center for Reproductive Medicine, Zhengzhou University First Affiliated Hospital, Zhengzhou, China, [2]Henan Key Laboratory of Reproduction and Genetics, Zhengzhou University First Affiliated Hospital, Zhengzhou, China

Background: Polycystic ovary syndrome (PCOS) is a heterogeneous endocrinological and metabolic disorder which is the common cause of female infertility. The dysmetabolism displayed in it has not been completely ascertained. Metabonomics may shed light on understanding many small molecule endogenous metabolites and their associated metabolic pathways.

Objective: To analyze the different metabolites and related metabolic pathways in follicular fluid and embryo culture fluid of PCOS and non-PCOS groups. Finding markers predictable for clinical outcomes of *in vitro* fertilization-embryo transfer (IVF-ET) treatment.

Population and sample: 60 women who underwent IVF-ET were selected, including 30 with PCOS and 30 with the fallopian tubal issues only. We collected the first tube follicular fluid (FF) of all patients at the time of oocyte pick up and the waste embryo culture medium (ECM) after D3 high-quality embryo transplant.

Methods: All samples were performed nontargeted Ultra High Performance Liquid Chromatography-Mass Spectrometry (UHPLC-QE-MS) analysis. Related metabolic pathways were screened by KEGG annotation. To search potential indicators, the logistic regression was made combined with clinical data.

Mean outcome measures: Predictive performance of markers of clinical outcomes (pregnancy rate, delivery rate, live birth rate, miscarriage rate) of assisted reproductive technology (ART).

Results: Comparing the PCOS group against the non-PCOS group, we found 11 significantly different metabolites in the FF and 56 in the ECM. There are a total of 11 kinds of biomarkers associated with clinical outcomes. Androsterone sulfate, Glycerophosphocholine, and Elaidic carnitine seem robust to predict the abortion rate of the PCOS group, with an AUC of 0.941, 0.933, 0.933, respectively. The glycerol phospholipid metabolic pathway is enriched in both the follicular fluid and embryo culture fluid.

Conclusions: The differential metabolites were mainly a variety of lipids. Some of them can predict clinical outcomes to a certain extent.

KEYWORDS

assisted reproductive technology (ART), polycystic ovary syndrome, metabolomics, follicular fluid, embryo culture medium

1 Introduction

Polycystic ovary syndrome (PCOS) is a highly heterogeneous heritable endocrinopathy which affects 5%-10% of women of reproductive age. It is the main cause of female infertility due to oligo- or anovulation. PCOS is characterized by menstrual disorder, hyperandrogenemia, obesity, insulin resistance (IR) and, the elevated LH level in serum (1). PCOS is relevant to an increased risk of metabolic complications, including traditional cardiovascular disease (CVD) risk factors such as obesity, impaired glucose tolerance (IGT), type 2 diabetes (diabetes mellitus, DM), dyslipidemia, and hypertension (2).

Metabolomics is the systematic, top-down quantitative analysis of small molecule metabolites and provides a quantitative understanding of the dynamic response to endogenous and exogenous factors (3). The UHPLC is applied in this research, which can modify the separation situations of a complex sample. Liquid Chromatography-Mass Spectrometry (LC-MS) is one of the most promising techniques for metabolomics research as it can perform both qualitative and quantitative analysis.

Follicular fluid (FF) is a mixture of secretions from granulosa, theca cells, and oocytes and compounds diffusing across the basement membrane from plasma, including proteins, hormones, metabolites, and toxins (4). Certain biochemical characteristics of the follicular fluid surrounding the oocyte may play a key role in determining the quality of oocytes, the potential for fertilization, and subsequent embryonic development. Embryo culture medium (ECM) is essential for the *in vitro* fertilization-embryo transfer (IVF-ET) process. In assisted reproduction techniques, the assessment of embryo cultures may gain different metabolic markers and metabolic profiles, these quantitative parameters allow us to relate them to the ability of transfer or reflect their pathological status (5). As the final product of a cell-modulating procedure, small molecular metabolites in ECM can reflect the biological changes of embryonic metabolism in the early stage (6).

So far, the detailed mechanisms of metabolic disorder in PCOS have not been fully elucidated. The aim of this study was to identify the different metabolites and related metabolic pathways found in follicular fluid and embryo culture fluid of patients with and without PCOS. We performed UHPLC analysis to explore the link between FF and ECM from a metabolic perspective. Furthermore, clinical outcomes after assisted reproduction were combined in order to discover potential markers. This may provide a theoretical basis for further study of the pathogenesis of PCOS.

2 Methods

2.1 Sample collection and preparation

All subjects were recruited from infertility women who underwent IVF-ET in the first affiliated hospital of Zhengzhou University, from September 2019 to October 2019. Including PCOS group(n=30) and non-PCOS group(n=30). For all subjects, we collected the first tube of follicular fluid (FF) at the time of picking up oocytes and the waste embryo culture medium (ECM) after the D3 high-quality embryo transplanted. Women with PCOS were diagnosed based on the Rotterdam 2003 criteria, which require two of the following three features to be met: 1) oligo- or anovulation, 2) clinical and/or biochemical signs of hyperandrogenism, or 3) ultrasound suggestive of polycystic ovarian changes. Patients in the non-PCOS group all underwent IVF-ET purely because of tubal factors. Patients in both groups were younger than 35 years old, undergoing their first IVF-ET, with a body mass index of 18 kg/m^2 < BMI < 24 kg/m^2, and received conventional IVF fertilization.

Exclusion criteria:①Other endocrine disorders such as Cushing's disease, congenital adrenal hyperplasia, etc. ②Women with infertility due to poor ovarian reserve, endometriosis, premature ovarian failure, or other issues may influence follicle development ③Use of donor oocytes or sperm ④infertility due to male factor ⑤patients who underwent preimplantation genetic diagnosis(PGD) or preimplantation genetic screening(PGS)

All subjects underwent controlled ovarian hyperstimulation according to GnRH antagonist protocol, as described elsewhere (7). The mature follicles (diameter≥ 18mm) were aspirated using 17-gauge Cook needles and the first tube of FF was collected at

the same time. The collected FF was centrifuged at 4°C for 10 minutes at 12,000 rpm and approximately 1 ml of the supernatant was collected in an EP tube. Observed under a thermostatic inverted microscope, embryos are judged according to Peter's scoring system (8). After D3 quality embryos (grade I and II) were implanted, approximately 50 μL of discarded ECM were collected in EP tubes. All collected samples were labeled with the patient's information and then frozen at -80°C until further study.

2.2 Metabolites extraction

20 μL of the sample was mixed with the extraction solution (methanol: acetonitrile = 1:1 (V/V), including the isotope-labeled internal standard mixture), sonicated, and left at -40°C for 1 h. The samples were centrifuged and the supernatant was extracted for detection on the machine. All samples were mixed into quality control (QC) samples by taking equal amounts of supernatant.

2.3 Method conditions

A Vanquish UHPLC (Thermo Fisher Scientific) was used for separation in this research, the target compounds were separated by chromatography on a Waters ACQUITY UPLC BEH Amide (2.1 mm × 100 mm, 1.7 μm) liquid chromatography column. The A phase of liquid chromatography was an aqueous phase containing 25 mmol/L ammonium acetate and 25 mmol/L ammonia, and the B phase was acetonitrile. A gradient elution was used: 0~0.5 *min*, 95% B; 0.5~7 *min*, 95%~65% B; 7~8 *min*, 65%~40% B; 8~9 *min*, 40% B; 9~9.1 *min*, 40%~95% B; 9.1~12 *min*, 95% B. The chromatography parameters were as follows: Mobile phase flow rate: 0.5 mL/min, column temperature: 30°C, sample tray temperature: 4°C, injection volume: 3 μL. The Thermo Q Exactive HFX mass spectrometer is capable of primary and secondary mass spectrometry data acquisition under the control of the control software (Xcalibur, Thermo). Detailed parameters are as follows: Sheath gas flow rate: 50 Arb, Aux gas flow rate: 10 Arb, Capillary temperature: 320 °C, Full ms resolution: 60000, MS/MS resolution: 7500, Collision energy: 10/30/60 in

2.4 Date collection, processing and statistical analysis

2.4.1 Raw data processing

Convert raw data to mzXML format using ProteoWizard software, process using internal programs, and then use an internal MS2 database for metabolic annotation. A multivariate statistical analysis was performed to recognize the final date set using SIMCA software version 15.0.2(Sartorius Stedim Date Analytics AB, Umea, Sweden)

2.4.2 Multivariate analysis

To narrow down the results of large-scale untargeted metabolomics assays, principal component analysis (PCA) was used to visualize the sample distribution. For better display and subsequent analysis, orthogonal projections to latent structures discriminant analysis (OPLS-DA) statistical method were used to separate metabolites and classify uncorrelated orthogonal and non-orthogonal variables. PCA and OPLS-DA models were constructed with the reversed-phase liquid chromatography data. The data were logarithmically (LOG) transformed plus UV-formatted using SIMCA software (V15.0.2, Sartorius Stedim Data Analytics AB, Umea, Sweden). The quality of the model was further tested by 7-fold cross-validation, and then the validity of the model was judged by the R^2Y (interpretability of the model for categorical variable Y) and Q^2 (predictability of the model) obtained after cross-validation. Finally, a permutation test was made to confirm the validity of the model.

2.4.3 Univariate statistical analysis

To further screen for differential metabolites, univariate statistical analysis (UVA) was performed, and the cardinality criterion used in this project was a p-value of less than 0.05 for the Student's-test, along with a Variable Importance in the Projection (VIP) of the first principal component of the OPLS-DA model bigger than 1.0. The differential metabolic ions that exerted a major influence on the group membership were selected according to the VIP value. The results are represented as volcano plots.

2.5 Metabolic pathway enrichment and topological analysis

All pathways mapped to the corresponding species (Homo sapiens) were collated by KEGG annotation of the differential metabolites. The major pathways were further screened by comprehensive analysis (including enrichment analysis and topology analysis) of the pathways in which the differential metabolites were located.

2.6 Identify potential biomarkers for clinical outcomes

The differential metabolites obtained from the screening were analyzed by logistic regression with clinical outcomes (pregnancy rate, delivery rate, live birth rate, and miscarriage rate), and the ROC curve was used to find potential markers which could predict clinical outcomes. An area under the ROC

curve (AUC) greater than 0.7 is usually considered to have better predictive performance, so we list results with an AUC greater than 0.7.

3 Results

3.1 Clinical features of enrolled subjects

The comparison of the general condition of two groups was listed in (Table S1).Basal LH was significantly higher in the PCOS group (11.15 ± 6.45 mIU/ml) than in the non-PCOS group (5.62 ± 2.97 mIU/ml), P<0.01; basal T level was higher in the PCOS group (0.47 ± 0.30 ng/ml) than in the non-PCOS group (0.25 ± 0.15 ng/ml), P<0.05 and the difference ware statistically significant; years of infertility in the PCOS group (4.66 ± 3.03 years) was higher than that of the non-PCOS group (3.67 ± 1.97 years), P<0.05. There was no significant difference in other basic information between the two groups.

3.2 Date analysis and screening for differential metabolites in the follicular fluid of women with PCOS

The OPLS-DA model showed a distinct trend of separation between the follicular fluid in the PCOS group(P-FF) and the non-PCOS group(N-FF). Score plots of all metabolites in follicular fluid showed a significant difference in the metabolic fingerprint profile between the PCOS and non-PCOS groups (Table S1). The parameter R^2Y (the interpretability of the model for the categorical variable Y) used to test the validity of the model was close to 1 in both ion models, indicating that the model was consistent with the reality of the sample date (Figure S2).The results of differential metabolite screening are represented by volcano plots (Figure S3).

Eleven main differential metabolites were identified in the follicular fluid of the PCOS group versus non-PCOS patients. Among the differential metabolites in follicular fluid, the levels of DG(15:0/18:3(6Z,9Z,12Z)/0:0), DG(18:2(9Z,12Z)/15:0/0:0), Androsterone sulfate, and L-Erythrulose were significantly higher, whereas the levels of LysoPE(16:0/0:0), L-Palmitoylcarnitine, Linoleyl carnitine, trans-Hexadec-2-enoyl carnitine, 1-Arachidonoylglycerophosphoinositol, 2-propylpentanoic acid, LysoPA(18:1(9Z)/0:0) were significantly lower in the PCOS group than in the non-PCOS control group. The details are shown in the (Table 1).

3.3 Date analysis and screening for differential metabolites in the embryo culture fluid of women with PCOS

The results of the OPLS-DA score plots (Figure S4) show that there is a significant difference in metabolism between the P-ECM and N-ECM groups, with samples largely within the 95% confidence interval. Permutation test for the OPLS-DA model (Figure S5) indicated that the model fits the reality of the sample data. After univariate statistical analysis, 48 significant differential metabolites were screened in the positive ion mode and 17 in the negative ion mode, and the differences in the expression content of differential metabolites in each group in the two modes were represented by volcano plots (Figure S6), respectively.

As known that the abnormalities in follicular fluid metabolism between the PCOS and non-PCOS groups were primarily related to abnormal lipid metabolism. Therefore, of the embryo culture fluid differential metabolites, only lipidic

TABLE 1 Characteristics of the differential metabolites of women with PCOS in follicular fluid.

Metabolites	Parent ion(m/z)	PCOS vs non PCOS	VIP	*P*	Fold Change	Log2-Fold Change
DG(15:0/18:3(6Z,9Z,12Z)/0:0)	576.4910	↑	2.6686	0.0000	2.1622	1.1125
DG(18:2(9Z,12Z)/15:0/0:0)	579.4240	↑	2.0772	0.0165	1.5729	0.6535
Androsterone sulfate	369.1738	↑	2.4548	0.0363	1.6709	0.7406
L-Erythrulose	118.0590	↑	1.6405	0.0395	1.1800	0.2388
LysoPE(16:0/0:0)	454.3276	↓	1.4917	0.0199	0.5978	-0.7423
L-Palmitoylcarnitine	400.2467	↓	3.0841	0.0000	0.0793	-3.6563
Linoleyl carnitine	424.3251	↓	3.2279	0.0000	0.0858	-3.5437
trans-Hexadec-2-enoyl carnitine	399.2434	↓	3.3171	0.0000	0.0670	-3.9007
1-Arachidonoylglycerophosphoinositol	621.2806	↓	1.9488	0.0194	0.7907	-0.3388
2-propylpentanoic acid	143.1077	↓	2.1245	0.0000	0.7864	-0.3467
LysoPA(18:1(9Z)/0:0)	434.2652	↓	3.0200	0.0000	0.0683	-3.8726

↑,up-regulation ↓, down-regulation.

metabolites and the more abundant organic acid and its derivative-like metabolic differentials were selected for analysis. A total of 32 differential metabolites were screened. Respectively L-Alloisoleucine, D-Proline, L-Valin, Taurine, Creatine, beta-Alanine, Ureidopropionic acid,4- Guanidinobutanoic acid, D-Alanine, Allantoic acid, L-Phenylalanine, Glycerophos phocholine, beta-Santalal, L-Asparagine, Elaidic carnitine, Phosphatidylcholine O-34:2, Glycine, Isobutyric acid, Pelargonic acid, Tridecanoic acid, Undecanoic acid, 12-Hydroxydodecanoic acid, L-Serine, Ketoleucine, D- Glutamine, 3-Hydroxycapric acid, 2-Hydroxyethanesulfonate, 2-Hydroxy-3- methylbutyric acid, L-Allothreonine, Undecylenic acid and (R)-3- Hydroxy- tetradecanoic acid. The detailed results are shown in the (Table 2).

3.4 Differential metabolites-related metabolic pathways

Differential metabolites found in follicular fluid were mainly enriched in Glycerolipid metabolism, Glycerophospholipid metabolism, Arginine and proline metabolism and Drug metabolism - cytochrome P450 metabolism (Figure 1). The key pathways most associated with differential metabolites in embryonic cultures were found to be pantothenate and CoA biosynthesis and glycine, serine and threonine metabolism (Figure 2). The differential metabolites in FF and ECM were analyzed separately for relevant pathways (including topological and enrichment analyses). The glycerophospholipid metabolism pathway was identified in both, as shown in the bubble diagram.

TABLE 2 Characteristics of the differential metabolites of women with PCOS in embryo culture fluid.

Metabolites	m/z	PCOSvs non-PCOS	VIP	*P*	Fold Change	Log2-Fold Change
L-Alloisoleucine	132.1019	↑	1.2821	0.0381	1.0806	0.1119
D-Proline	116.0708	↑	1.1747	0.045	1.0787	0.1093
L-Valine	118.0865	↑	1.4155	0.0177	1.0919	0.1269
Taurine	126.022	↑	1.9758	0.0109	1.2286	0.297
Creatine	132.0767	↑	1.2211	0.0468	1.3337	0.4154
beta-Alanine	90.0554	↑	2.1087	0.0005	1.1197	0.1631
Ureidopropionic acid	133.0609	↑	1.1848	0.0259	1.136	0.184
4-Guanidinobutanoic acid	146.0924	↑	1.2246	0.0475	1.1232	0.1676
D-Alanine	90.0553	↑	2.1684	<0.001	1.2194	0.2862
Allantoic acid	177.062	↑	1.3157	0.0401	1.0824	0.1143
L-Phenylalanine	166.0863	↑	1.3482	0.024	1.1028	0.1412
Glycerophosphocholine	258.1099	↑	1.5312	0.0198	1.0877	0.1212
L-Asparagine	133.0607	↑	1.4391	0.008	1.2251	0.2929
Phosphatidylcholine O-34:2	261.0213	↑	1.3214	0.0253	1.1696	0.226
Glycine	76.0399	↑	1.239	0.0381	1.0911	0.1258
Isobutyric acid	89.0601	↑	1.7918	0.0237	1.1343	0.1818
Pelargonic acid	157.1226	↑	2.5068	<0.001	1.3897	0.4748
Tridecanoic acid	213.1857	↑	1.6324	0.0143	1.2805	0.3567
Undecanoic acid	185.1544	↑	2.422	<0.001	1.5071	0.5918
L-Serine	104.0343	↑	1.3685	0.0491	1.1695	0.2259
Ketoleucine	129.0548	↑	1.6732	0.0019	1.4468	0.5328
D-Glutamine	145.0612	↑	1.3007	0.0043	1.3994	0.4848
L-Allothreonine	118.05	↑	1.4751	0.02	1.1894	0.2502
2-Hydroxy-3-methylbutyric Acid	117.0549	↑	1.3789	0.0211	1.202	0.2654
Undecylenic acid	183.1385	↑	1.0536	0.0337	1.1053	0.1444
Cincassiol B	187.094	↓	2.0817	0.0003	0.7569	-0.4018
beta-Santalal	219.1745	↓	1.4369	0.0215	0.7963	-0.3285
Elaidic carnitine	426.3578	↓	1.384	0.0277	0.8273	-0.2736
12-Hydroxydodecanoic acid	215.165	↓	2.8203	<0.001	0.6815	-0.5532
3-Hydroxycapric acid	187.1337	↓	2.1689	0.0018	0.8001	-0.3217
2-Hydroxyethanesulfonate	124.9905	↓	1.9881	0.0224	0.8779	-0.1879
(R)-3-Hydroxy-tetradecanoic acid	243.1964	↓	2.3214	0.003	0.7376	-0.4391

↑, up-regulation ↓, down-regulation.

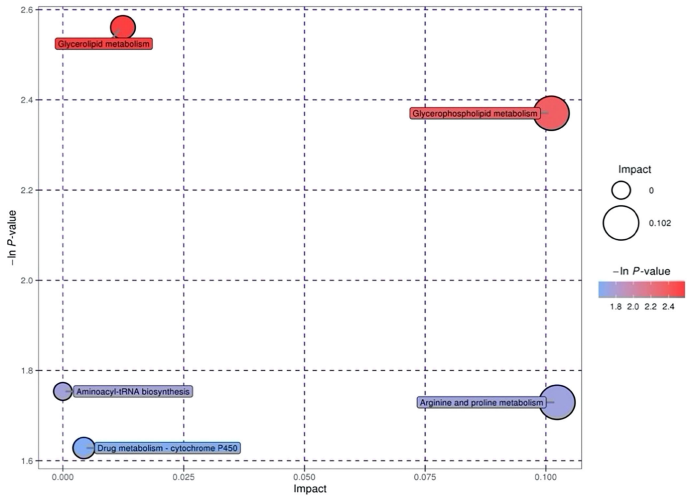


FIGURE 1
Metabolic pathways relevant to the follicular fluid.

3.5 Potential biomarkers for clinical outcomes

As shown by the screening results of the differential metabolite-related metabolic pathways, the differences in follicular fluid and embryo culture fluid metabolism between the PCOS and non-PCOS groups of patients were mainly focused on lipidic metabolism. In order to find potential markers that could predict clinical outcomes, we performed a logistic regression analysis of the screened lipid differential metabolites in combination with clinical outcomes (pregnancy rate, delivery rate, live birth rate, and miscarriage rate). Clinical dates are shown in (Table S2). The results are represented by a Receiver Operating Characteristic Curve (ROC) plot. An area under the ROC curve (AUC) greater than 0.7 is usually considered to have good predictive performance, and greater than 0.9 is considered to have excellent predictive performance, so we list the results with an AUC greater than 0.7.

LysoPE (16:0/0:0), DG (18:2(9Z,12Z)/15:0/0:0), Linoleyl carnitine and Androsterone sulfate in follicular fluid of the PCOS group had better predictive ability for abortion rate, with an AUC of 0.824, 0.706, 0.706 and 0.941, respectively. DG (15:0/18:3(6Z,9Z,12Z)/0:0) and LysoPA (18:1(9Z)/0:0) were good indicators for predicting the live birth rate and delivery rate in PCOS group, with AUC of 0.7 and 0.88. LysoPA (18:1(9Z)/0:0) was a good predictor of pregnancy rate in PCOS group, with an AUC of 0.89. In the follicular fluid of non-PCOS group, LysoPE(16:0/0:0) and DG (18:2(9Z,12Z)/15:0/0:0) had a better ability to predict pregnancy rate, delivery rate and live birth rate, with an AUC of 0.733. Glycerophosphocholine, (R)-3-Hydroxy-

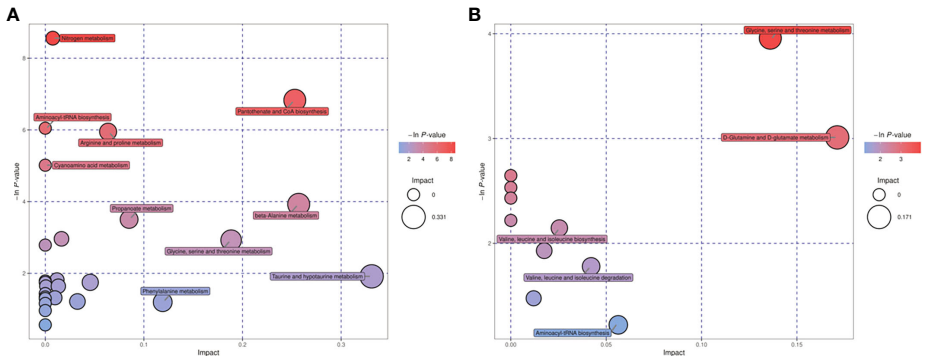


FIGURE 2
Metabolic pathways relevant to the embryo culture fluid. **(A)** positive ion mode, **(B)** negative ion mode.

tetradecanoic acid and Elaidic carnitine in embryo culture medium of PCOS group were important indicators for predicting clinical pregnancy rate, with AUC of 0.933, 0.767 and 0.933, respectively. Pelargonic acid and Elaidic carnitine had better predictive effect on pregnancy rate in PCOS group, with AUC of 0.771 and 0.757, respectively. Pelargonic acid in the PCOS group was better in predicting the birth rate and live birth rate, and the AUC was 0.764. Beta-Santalal and(R)-3- Hydroxy-tetradecanoic acid in embryo culture medium of non-PCOS group were good indicators for predicting live birth rate, delivery rate and pregnancy rate, with AUC of 0.798 and 0.726, respectively. For a better presentation, we listed in Table S3–Table S6 which groups of women these markers were predictive for and in what samples they were found. ROC charts of markers are Figure S7–Figure S10. As can be seen from Figure S10, Androsterone sulfate, Glycerophosphocholine and Glycerophosphocholine had robust predictive capability.,

4 Discussion

4.1 Main findings

After univariate statistical analysis (UVA) screening, there were 11 significantly different metabolites in follicular fluid between PCOS patients and non-PCOS patients. And 56 differential metabolites were found in embryo culture medium, including 32 kinds of lipids and organic acids. The detailed results are listed in chapter 3.3 and 3.4.

KEGG annotation and comprehensive analysis (including enrichment analysis and topology analysis) of the different metabolites showed that the metabolic pathways related to the difference in follicular fluid metabolism between PCOS patients and non-PCOS patients were mainly Glycerolipid metabolism, Glycerophospholipid metabolism, Arginine and proline metabolism, and Drug metabolization-cytochrome P450. Pantothenate and CoA biosynthesis and Glycine, serine and threonine metabolism were the key pathways with the highest correlation with metabolite differences in embryo culture medium. Notably, Glycerophospholipid metabolism was enriched in embryo culture medium, which was the same as that in follicular fluid analysis.

A total of 11 differential metabolites were found to be predictive for clinical outcomes. In particular Androsterone sulfate, Glycerophosphocholine and Elaidic carnitine showed a robust connection with miscarriage rates in PCOS patients. With an AUC of 0.941, 0.933, 0.933, respectively.

4.2 Strengths and limitations

The major strength of this study was the combined analysis of follicular fluid and embryo culture fluid from the perspective of metabolomics. Previous studies have tried to find predictors of PCOS in FF, or metabolites which can reflect embryonic quality in ECM. But so far, no research has combined them together to explore the metabolic connection between them. Besides, we screened 11 markers associated with clinical outcomes of IVF-ET treatment. This may help find an efficient, non-invasive way to predict and ameliorate pregnancy outcomes.

However, the experiment was a single-center, small sample research. For example, due to limited patient numbers, the prediction of pregnancy rate and live birth rate seemed to be the same. To improve prediction accuracy, larger sample, multicenter, repeatability experiments are pending.

4.3 Interpretation

Previous studies have shown that the pathogenesis of PCOS is influenced by a variety of factors, including genetic and environmental factors, which underlie the imbalance of signals in the hypothalamic-pituitary-ovarian axis, which in turn affects the ovaries and adrenal glands to develop hyperandrogenemia. The accumulation of adipose tissue associated with hyperandrogenemia, dysfunctional lipotoxicity and oxidative stress worsen insulin resistance. And abnormalities in lipid metabolism are common (9), with approximately 70% of patients with PCOS having at least one abnormal lipid level. This is consistent with the results of the present study in which multiple lipid differential metabolites were screened in follicular fluid and embryo culture fluid from PCOS patients. The finding of multiple lipid differential metabolites in the ECM sample suggests that for PCOS patients who underwent IVF-ET, endocrine and metabolic disorders are already present in the early stage of embryonic growth. This may explain why many PCOS patients have poor oocytes and embryonic quality and cycle outcomes despite a high rate of follicles retrieval undergoing assisted reproduction techniques (10).

As an emerging, non-invasive and efficient research method, metabolomics has been widely used in the field of reproductive medicine in recent years. In this study, significant differences in carnitine levels were found in both FF and ECM. Combining the screening results of metabolic pathways associated with differential metabolites in FF and ECM, Glycerophospholipid metabolism was found to be the intersection. This finding may help to elucidate the pathogenesis of metabolic abnormalities in polycystic ovary syndrome.

The results of this study revealed differences in the level of various carnitines, such as L-palmitoylcarnitine and linalyl carnitine in PCOS and non-PCOS groups, suggesting that carnitine is very likely to be a potential biomarker for polycystic ovary syndrome. Carnitine is a metabolic compound of essential fatty acids found in the mitochondria, and acylcarnitine is formed by combining carnitine with a fatty acid or an ester (11). Both carnitine and acylcarnitine are

involved in fatty acid oxidation and are important for cellular energy metabolism as well as branched-chain and amino acid metabolism. Characteristic changes in acylcarnitine are present in many disorders of fatty acid oxidation and disorders of branched-chain amino acid metabolism. Acylcarnitine is a biomarker that responds to mitochondrial function and plays an important role in insulin resistance, and mitochondrial dysfunction has previously been identified in metabolomic studies of follicular fluid from PCOS patients (12). Carnitine is involved in the transport of fatty acids across the mitochondrial membrane and is further involved in β-oxidation of mitochondria and assists cells in various normal physiological functions including energy metabolism (13, 14). Patients with PCOS are prone to impaired glycolipid metabolism, and free and total circulating L-carnitine levels are significantly lower in women with PCOS (15, 16). One study found by UPLC that increased concentrations of L-carnitine in the follicular fluid of PCOS patients may lead to decreased embryo utilization (17). In patients with type 2 diabetes, carnitine supplementation may improve insulin sensitivity by increasing the rate of fatty acid oxidation, and glucose metabolism and reducing oxidative stress (18). The addition of L-carnitine improves mitochondrial glucose oxidation by modulating the expression level of glycolytic enzymes and gluconeogenesis (19). In addition, the elevation of various amino acids was found in PCOS group, including branched-chain amino acids (BCAAs) L-Alloisoleucine and L-Valin. BCAAs(leucine, isoleucine and valine) cannot be produced by the body and must be obtained through food (20). It is known that the concentration of BCCAs increase in plasma occurs in metabolic disorders such as diabetes(T2D), obesity and insulin resistance (21, 22). Indicating that BCCAs may be the biomarkers of PCOS (23).

In the current study, we found that LysoPE (16:0/0:0) and LysoPA (18:1(9Z)/0:0) levels in follicular fluid were decreased in the PCOS group, which is consistent with previous research. That study also showed the significant decrease of levels of glycerophospholipids((LysoPC) (16:0), LysoPC (14:0), and LysoPC (18:0)) in the follicular fluid of PCOS women (24). Indicating that glycerophospholipids can be markers of PCOS diagnosis. And KEGG pathway analysis indicated that the glycerolipid metabolic pathway and glycerophospholipid metabolic pathway were altered in PCOS patients. The glycerophospholipid metabolic pathway was also identified in the ECM differential metabolites related pathways. Glycerophospholipids are major components of cell membranes and play an important role in the regulation of transport, signal transduction and protein function (25). Glycerol-3-phosphate is produced *via* a synthetic pathway, followed by the generation of glycerophospholipid acyl chains through the synergistic regulation of phospholipase As (PLAs), acyl-coenzyme a synthases and lysophospholipases (LPLATs) (26, 27), and this remodeling plays a role in the production of glycerophospholipids in a variety of cells. Previous studies have reported reduced PA levels in follicular fluid in PCOS patients, and subsequent studies have found that further reductions in PA, DAG and various GP levels may be associated with altered glycerolipid synthesis pathways and reduced PE remodeling activity due to hyperandrogenemia. Previous studies have shown that LPC is downregulated in obese patients and type 2 diabetics and could be a marker of obesity due to a high-fat diet (28). LPC is an important mediator in the process of fatty acid-induced insulin resistance and plays an important role in several key processes such as glucose transport, uptake and utilization, and it may eventually act as an independent insulin signal to regulate glucose levels *in vivo* (29, 30).

5 Conclusion

1. The metabolism of follicular fluid and embryo culture fluid in PCOS and non-PCOS patients were different, and the metabolites of the difference were mainly a variety of lipids.

2. Different metabolites are related to a variety of metabolic pathways, among which the glycerol phospholipid metabolic pathway is enriched in both the follicular fluid and embryo culture fluid, and abnormal lipid metabolism of PCOS patients has been manifested in early embryo metabolism.

3. Various lipid differential metabolites can predict clinical outcomes to a certain extent. They are LysoPE(16:0/0:0), DG (18:2(9Z,12Z)/15:0/0:0), Elaidic carnitine, DG(15:0/18:3 (6Z,9Z,12Z)/0:0),LysoPA(18:1(9Z)/0:0), Pelargonic acid, beta-Santalal, (R)-3-Hydroxy-tetradecanoic acid, Linoleyl carnitine, Androsterone sulfate and Glycerophosphocholine. Especially Androsterone sulfate, Glycerophosphocholine, and Elaidic carnitine were believed to be connected with the abortion rate of PCOS patients closely.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committees of the first affiliated hospital of Zhengzhou University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

The idea and the overall design of this study were conceived by S-G, Y-YL, YG, SXX, YL, H-XJ. S-YG and Y-YL performed the literature search, data analysis and wrote the manuscript. YG and X-XS constructed the tables and Figures. The sample collection and data analysis were conducted by YL and H-XJ. Critical revision of the manuscript for important intellectual contents were provided by H-XJ. All authors read and approved the final manuscript.

Funding

Natural Science Foundation of He'nan Province of China (General Program) (No.202300410467). Key Projects of Medical Science and Technology in He'nan Province (No.SBGJ202002053)

Acknowledgments

We are thankful to all the participants of the study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.982200/full#supplementary-material

References

1. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* (2004) 81(1):19–25. doi: 10.1016/j.fertnstert.2003.10.004

2. Gibson-Helm M, Teede H, Dunaif A, Dokras A. Delayed diagnosis and a lack of information associated with dissatisfaction in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* (2017) 102(2):604–12. doi: 10.1210/jc.2016-2963

3. Kaddurah-Daouk R, Kristal BS, Weinshilboum RM. Metabolomics: a global biochemical approach to drug response and disease. *Annu Rev Pharmacol Toxicol* (2008) 48:653–83. doi: 10.1146/annurev.pharmtox.48.113006.094715

4. Fortune JE. Ovarian follicular growth and development in mammals. *Biol Reprod* (1994) 50(2):225–32. doi: 10.1095/biolreprod50.2.225

5. Singh R, Sinclair KD. Metabolomics: approaches to assessing oocyte and embryo quality. *Theriogenology* (2007) 68 Suppl 1:S56–62. doi: 10.1016/j.theriogenology.2007.04.007

6. Seli E, Vergouw CG, Morita H, Botros L, Roos P, Lambalk CB, et al. Noninvasive metabolomic profiling as an adjunct to morphology for noninvasive embryo assessment in women undergoing single embryo transfer. *Fertil Steril* (2010) 94(2):535–42. doi: 10.1016/j.fertnstert.2009.03.078

7. Xiao ZN, Peng JL, Yang J, Xu WM. Flexible GnRH antagonist protocol versus progestin-primed ovarian stimulation (PPOS) protocol in patients with polycystic ovary syndrome: Comparison of clinical outcomes and ovarian response. *Curr Med Sci* (2019) 39(3):431–6. doi: 10.1007/s11596-019-2055-x

8. PR Brinsden ed. A textbook of in vitro fertilization and assisted reproduction: The bourn hall guide to clinical and laboratory Practice1992 London, Informa Healthcare. (2005).

9. Wild RA, Painter PC, Coulson PB, Carruth KB, Ranney GB. Lipoprotein lipid concentrations and cardiovascular risk in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* (1985) 61(5):946–51. doi: 10.1210/jcem-61-5-946

10. Qiao J, Feng HL. Extra- and intra-ovarian factors in polycystic ovary syndrome: impact on oocyte maturation and embryo developmental competence. *Hum Reprod Update* (2011) 17(1):17–33. doi: 10.1093/humupd/dmq032

11. Garg U, Dasouki M. Expanded newborn screening of inherited metabolic disorders by tandem mass spectrometry: clinical and laboratory aspects. *Clin Biochem* (2006) 39(4):315–32. doi: 10.1016/j.clinbiochem.2005.12.009

12. Zhao H, Zhao Y, Li T, Li M, Li J, Li R, et al. Metabolism alteration in follicular niche: The nexus among intermediary metabolism, mitochondrial function, and classic polycystic ovary syndrome. *Free Radic Biol Med* (2015) 86:295–307. doi: 10.1016/j.freeradbiomed.2015.05.013

13. Helms RA, Whitington PF, Mauer EC, Catarau EM, Christensen ML, Borum PR. Enhanced lipid utilization in infants receiving oral l-carnitine during long-term parenteral nutrition. *J Pediatr* (1986) 109(6):984–8. doi: 10.1016/s0022-3476(86)80281-5

14. Jia C, Xu H, Xu Y, Xu Y, Shi Q. Serum metabolomics analysis of patients with polycystic ovary syndrome by mass spectrometry. *Mol Reprod Dev* (2019) 86 (3):292–7. doi: 10.1002/mrd.23104

15. Hathcock JN, Shao A. Risk assessment for carnitine. *Regul Toxicol Pharmacol* (2006) 46(1):23–8. doi: 10.1016/j.yrtph.2006.06.007

16. Fenkci SM, Fenkci V, Oztekin O, Rota S, Karagenc N. Serum total l-carnitine levels in non-obese women with polycystic ovary syndrome. *Hum Reprod* (2008) 23(7):1602–6. doi: 10.1093/humrep/den109

17. Chen X, Lu T, Wang X, Sun X, Zhang J, Zhou K, et al. Metabolic alterations associated with polycystic ovary syndrome: A UPLC q-exactive based metabolomic study. *Clin Chim Acta* (2020) 502:280–6. doi: 10.1016/j.cca.2019.11.016

18. Xu Y, Jiang W, Chen G, Zhu W, Ding W, Ge Z, et al. L-carnitine treatment of insulin resistance: A systematic review and meta-analysis. *Adv Clin Exp Med* (2017) 26(2):333–8. doi: 10.17219/acem/61609

19. Berg SM, Beck-Nielsen H, Færgeman NJ, Gaster M. Carnitine acetyltransferase: A new player in skeletal muscle insulin resistance? *Biochem Biophys Rep* (2017) 9:47–50. doi: 10.1016/j.bbrep.2016.11.010

20. Neinast M, Murashige D, Arany Z. Branched chain amino acids. *Annu Rev Physiol* (2019) 81:139–64. doi: 10.1146/annurev-physiol-020518-114455

21. Laferrère B, Reilly D, Arias S, Swerdlow N, Gorroochurn P, Bawa B, et al. Differential metabolic impact of gastric bypass surgery versus dietary intervention in obese diabetic subjects despite identical weight loss. *Sci Transl Med* (2011) 3 (80):80re2. doi: 10.1126/scitranslmed.3002043

22. She P, Van Horn C, Reid T, Hutson SM, Cooney RN, Lynch CJ. Obesity-related elevations in plasma leucine are associated with alterations in enzymes involved in branched-chain amino acid metabolism. *Am J Physiol Endocrinol Metab* (2007) 293(6):E1552–63. doi: 10.1152/ajpendo.00134.2007

23. Siomkajło M, Daroszewski J. Branched chain amino acids: Passive biomarkers or the key to the pathogenesis of cardiometabolic diseases? *Adv Clin Exp Med* (2019) 28(9):1263–9. doi: 10.17219/acem/104542

24. Sun Z, Chang HM, Wang A, Song J, Zhang X, Guo J, et al. Identification of potential metabolic biomarkers of polycystic ovary syndrome in follicular fluid by SWATH mass spectrometry. *Reprod Biol Endocrinol* (2019) 17(1):45. doi: 10.1186/s12958-019-0490-y

25. Jové M, Pradas I, Naudí A, Rovira-Llopis S, Bañuls C, Rocha M, et al. Lipidomics reveals altered biosynthetic pathways of glycerophospholipids and cell signaling as biomarkers of the polycystic ovary syndrome. *Oncotarget* (2017) 9 (4):4522–36. doi: 10.18632/oncotarget.23393

26. Hishikawa D, Hashidate T, Shimizu T, Shindou H. Diversity and function of membrane glycerophospholipids generated by the remodeling pathway in mammalian cells. *J Lipid Res* (2014) 55(5):799–807. doi: 10.1194/jlr.R046094

27. Leventis PA, Grinstein S. The distribution and function of phosphatidylserine in cellular membranes. *Annu Rev Biophys* (2010) 39:407–27. doi: 10.1146/annurev.biophys.093008.131234

28. Li F, Jiang C, Larsen MC, Bushkofsky J, Krausz KW, Wang T, et al. Lipidomics reveals a link between CYP1B1 and SCD1 in promoting obesity. *J Proteome Res* (2014) 13(5):2679–87. doi: 10.1021/pr500145n

29. Han MS, Lim YM, Quan W, Kim JR, Chung KW, Kang M, et al. Lysophosphatidylcholine as an effector of fatty acid-induced insulin resistance. *J Lipid Res* (2011) 52(6):1234–46. doi: 10.1194/jlr.M014787

30. Yea K, Kim J, Yoon JH, Kwon T, Kim JH, Lee BD, et al. Lysophosphatidylcholine activates adipocyte glucose uptake and lowers blood glucose levels in murine models of diabetes. *J Biol Chem* (2009) 284(49):33833–40. doi: 10.1074/jbc.M109.024869

frontiers | Frontiers in Physiology

TYPE Systematic Review
PUBLISHED 29 September 2022
DOI 10.3389/fphys.2022.957112

OPEN ACCESS

EDITED BY
Yanting Wu,
Fudan University, China

REVIEWED BY
Liming Zhong,
Southern Medical University, China
Lianzhou Yang,
Guangdong Agriculture Reclamation Central Hospital, China
Jianrong Na,
General Hospital of Ningxia Medical University, China

*CORRESPONDENCE
Zhenzhen Zheng,
zhengzhenzhen2018@163.com
Kang Wu,
d102_wk@126.com
Riken Chen,
chenriken@126.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Physiology

RECEIVED 30 May 2022
ACCEPTED 12 September 2022
PUBLISHED 29 September 2022

CITATION
Wang C, Huang T, Song W, Zhu J, Liu Y, Chen X, Sun X, Wu Q, Chen H, Liao H, Lin J, Ou X, Zou Z, Wang Z, Zheng Z, Wu K and Chen R (2022), A meta-analysis of the relationship between polycystic ovary syndrome and sleep disturbances risk.
Front. Physiol. 13:957112.
doi: 10.3389/fphys.2022.957112

A meta-analysis of the relationship between polycystic ovary syndrome and sleep disturbances risk

Chaoyu Wang[1,2†], Tao Huang[3†], Wu Song[4†], Jinru Zhu[1†], Yanhong Liu[5†], Xiaojuan Chen[6†], Xishi Sun[3], Qinglan Wu[7], Huimin Chen[1], Huizhao Liao[8], Junyan Lin[8], Xudong Ou[8], Zhihong Zou[8], Zhiwei Wang[8], Zhenzhen Zheng[1*], Kang Wu[8*] and Riken Chen[8*]

[1]Department of Respiratory and Critical Care Medicine, The Second Affiliated Hospital of Guangdong Medical University, Zhanjiang, China, [2]Department of Respiratory and Critical Care Medicine, Taishan Hospital of Traditional Chinese Medicine, Jiangmen, China, [3]Department of Emergency, Affiliated Hospital of Guangdong Medical University, Zhanjiang, Guangdong, China, [4]Department of Laboratory, Dongguan Maternal and Child Health Care Hospital, Dongguan, China, [5]Department of Gynecology, Taishan Hospital of Traditional Chinese Medicine, Jiangmen, China, [6]Medical College, Jiaying University, Meizhou, China, [7]Department of Respiratory and Critical Care Medicine, Central People's Hospital of Zhanjiang, Zhanjiang, China, [8]Guangzhou Medical University, State Key Laboratory of Respiratory Disease, National Clinical Research Center for Respiratory Disease, Guangzhou Institute of Respiratory Health, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China

Objective: A meta-analysis is used to explore the relationship between polycystic ovary syndrome (PCOS) and the risk of Sleep disturbances.

Method: Cochrane Library, PubMed, Embase, and Web of Science databases are searched by computer from their establishment to 1 May 2022. Review Manager 5.4 software is used for the meta-analysis.

Results: A total of nine articles are included, with 1,107 subjects. The results show that PCOS is positively associated with the risk of Sleep disturbances. Comparing with the "PCOS group" (experimental group) with the "NON-PCOS group" (control group), the incidence of Sleep disturbances is higher (OR = 11.24, 95% CI: 2.00–63.10, Z = 2.75, p = 0.006); the Pittsburgh Sleep Quality Index (PSQI) scores of the PCOS group is higher than that of the NON-PCOS group (MD = 0.78, 95% CI: 0.32–1.25, Z = 3.30, p = 0.001); the Epworth Sleepiness Scale (ESS) scores of the PCOS group is higher than that of the NON-PCOS group (MD = 2.49, 95% CI: 0.80–4.18, Z = 2.88, p = 0.004); Apnea hypopnea index (AHIs) in the PCOS group are higher than those in the NON-PCOS group (MD = 2.68, 95% CI: 1.07–4.28, Z = 3.27, p = 0.001); the sleep efficiency of the PCOS group is lower than that of the NON-PCOS group (MD = -5.16, 95% CI: 9.39–-0.93, Z = 2.39, p = 0.02); the sleep onset latency of the PCOS group is higher than that of the NON-PCOS group (MD = 2.45, 95% CI: 1.40–3.50, Z = 4.57, p < 0.001); and the Rapid Eyes Movement (REM) sleep in the PCOS group is higher than that in the NON-PCOS group (MD = 17.19, 95% CI: 11.62–55.76, Z = 6.05, p < 0.001). The studies included in each analysis have publication biases of different sizes. After subgroup analysis and

sensitivity analysis, the heterogeneity of each study in the meta-analysis is reduced, the bias is reduced accordingly, and the stability of the results can be maintained.

Conclusion: PCOS is positively associated with the risk of Sleep disturbances. In order to reduce such risk, attention should be paid to the role of PCOS management, and PCOS prevention and treatment should be actively carried out.

KEYWORDS

polycystic ovary syndrome (PCO), sleep disturbances, relationship, meta-analysis', risk

1 Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age (4–12%) (Azziz et al., 2004), while its prevalence depends on the choice of diagnostic criteria. A community-based prevalence study using the Rotterdam criteria found that approximately 18% of women had PCOS, 70% of whom had not been diagnosed previously (Teede et al., 2010). PCOS usually occurs during puberty with irregular menstrual cycles, signs of hyperandrogenism such as acne and hirsutism, and insulin resistance (Azziz, 2006). PCOS was discovered in the 1930s, but the understanding of its relationship with Sleep disturbances was relatively late. Most studies on this topic were published after 2001, and the research content is relatively small, with some variations in results. Recent studies have linked PCOS to Sleep disturbances, with a prospective case-control study estimating that women with PCOS have a 30-fold higher prevalence of Sleep disturbances than women in the general population (Nandalike et al., 2011). Studies have confirmed that persistent lack of sleep can lead to decreased thinking ability and memory, decreased vigilance and judgment, low immunity, endocrine disorders, anxiety, and irritability, and ultimately lead to the occurrence of diseases such as high blood pressure, cardiovascular and cerebrovascular diseases, and affective psychosis, aggravate the severity of age-related chronic diseases, and increase the risk factors of periodic coronary heart disease in middle-aged and elderly women (Tsay et al., 2003). Sleep itself is an important regulator of endocrine function, and the endocrine system also plays an important role in regulating the sleep-wake cycle (Calandra-Buonaura et al., 2016). There may be a complex relationship between PCOS as an endocrine disorder and sleep. Therefore, this study conducted a meta-analysis of the risk relationship between Sleep disturbances and PCOS in order to provide medical evidence for an exploration of the etiology of PCOS and its preventive treatment.

2 Materials and methods

2.1 Retrieval strategy

According to the meta-analysis of Observational Studies in Epidemiology (MOOSE) statement and standard of preferred reporting items for Systematic Reviews and meta-analyses (PRISMA), and the PRISMA checklist item is depicted in Supplementary File S1. Cochrane Library, PubMed, Embase, and Web of Science databases were searched by computer from their establishment to 1 May 2022. English search terms included "polycystic ovary syndrome", "PCOS", "Stein-leventhal Syndrome", "Sclerocystic Ovarian Degeneration", "Ovary Syndrome, Polycystic", "Syndrome, Polycystic Ovary", "Stein-Leventhal Syndrome", "Stein Leventhal Syndrome", "Syndrome, Stein-Leventhal", "Ovarian Degeneration, Sclerocystic", "Sclerocystic Ovary Syndrome", "Polycystic Ovarian Syndrome", "Sclerocystic Ovaries", "Ovary, Sclerocystic","Sclerocystic Ovary", Sleep Wake Disorders","Disorder, Sleep Wake", "sleep disturbances", "Disorder, Sleep Wake", "Disorders, Sleep Wake", "Sleep Wake Disorder", "Wake Disorder, Sleep", "Wake Disorders, Sleep Disorders", "Disorder, Sleep", "Disorders, Sleep", "Sleep Disorder", "Short Sleeper Syndrome", "Short Sleeper Syndromes", "Sleeper Syndrome, Short", "Sleeper Syndromes, Short", "Syndrome, Short Sleeper", "Syndromes, Short Sleeper", "Short Sleep Phenotype", "Phenotype, Short Sleep", "Phenotypes, Short Sleep", "Short Sleep Phenotypes", "Sleep Phenotypes, Short", "Sleep". The details of the search process for each database

are listed in Supplementary File S2. The type of research design was not limited, and the language was limited to English.

2.2 Literature inclusion and exclusion criteria

Inclusion criteria: ① the subjects of the study were patients with a clinical diagnosis of PCOS in the case group, and healthy women with a normal menstrual cycle or female patients who came to the hospital for other reasons in the control group; ② the types of studies were cohort studies, case-control studies, and cross-sectional studies which were compared with the control group; ③ articles in which the subjects were diagnosed as having PCOS by the criteria of National Institutes of Health (NIH; 1990) (Zawadzki et al., 1992), and Rotterdam criteria (AE-PCOS; 2003) (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004), and Sleep disturbances were diagnosed and investigated by polysomnography, actiwatch, and sleep questionnaires.

Exclusion criteria: ① languages other than English; ② replicated publications (if studies involving the same population were published repeatedly, the latest published studies or those with the larger sample size were selected); ③ studies without a control group; ④ studies for which the effect sizes could not be extracted or calculated; ⑤ studies with little research information or incomplete data and inconsistent outcome indicators; ⑥ studies on the use of contraceptives, metformin and other drugs that affect PCOS; ⑦ studies in which the population is accompanied by hypertension, diabetes, atypical adrenal 21-hydroxylase deficiency, androgen-secreting tumor, Cushing's syndrome, and any other disease that may cause Sleep disturbances; ⑧ the experimental group and control group had significant body mass index (BMI) differences (differences greater than 10 kg/m^2 were excluded) which were likely to cause bias.

2.3 Literature screening, quality assessment, and data extraction

Two researchers independently searched, extracted and screened the literature, then checked each other's work, and provided articles with differences to a third researcher to decide whether they should be included. The methodological quality of the included literature was assessed. The evaluation was performed using the "Risk of Bias Assessment" tool recommended by the Cochrane Collaboration, which is divided into three levels: low risk, unclear, and high risk. The content is: whether it is randomly assigned; whether to perform allocation concealment; whether to use blinding; whether the outcome data is complete; whether the research results are selectively reported; and whether there is other risk of bias. The extracted data included the first author, study area, publication time, sample size, age, BMI, prevalence of Sleep disturbances, AHIs, sleep efficiency, rapid eye movement (REM) sleep, sleep onset latency, Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), outcome measures, and adjustment for confounders. After data extraction, the two datasets were checked, and inconsistent data was extracted again. After checking, the data was analyzed.

2.4 Ending and exposure

The PCOS of the research subjects had to meet the NIH or Rotterdam diagnostic criteria, and Sleep disturbances were diagnosed by polysomnography, actiwatch, and sleep questionnaires. The prevalence of Sleep disturbances (including sleep apnea or obstructive sleep apnea) in the PCOS group and control group, AHIs, sleep efficiency, REM sleep, sleep onset latency, PSQI, and ESS were used as outcome indicators. The incidence of Sleep disturbances between the PCOS group and control group was different, indicating a correlation between PCOS and Sleep disturbances; comparing with the PCOS group and control group, the differences in levels of AHIs, sleep efficiency, REM sleep, sleep onset latency, PSQI, and ESS were included, illustrating the effects of PCOS on Sleep disturbances.

2.5 Statistical methods

Statistical analysis was performed using Review Manager 5.4 software. Quantitative data was expressed as mean ± standard deviation (x ± s), MD and OR values were used for effect evaluation, and 95% CI was calculated. The I^2 statistical value test and Q test were used to analyze the heterogeneity of the studies. If the heterogeneity among the studies was not statistically significant ($I^2 \leq 50\%$ and $p \geq 0.1$), a fixed effects model was used; and if there was heterogeneity ($I^2 > 50\%$ and $p < 0.1$), a random effects model was used. Sensitivity analysis was used to judge the stability and reliability of the combined results. $p < 0.05$ was considered statistically significant.

3 Results

3.1 Literature screening results

A total of 768 articles were retrieved, of which 442 papers were obtained after deduplication, and 237 papers were

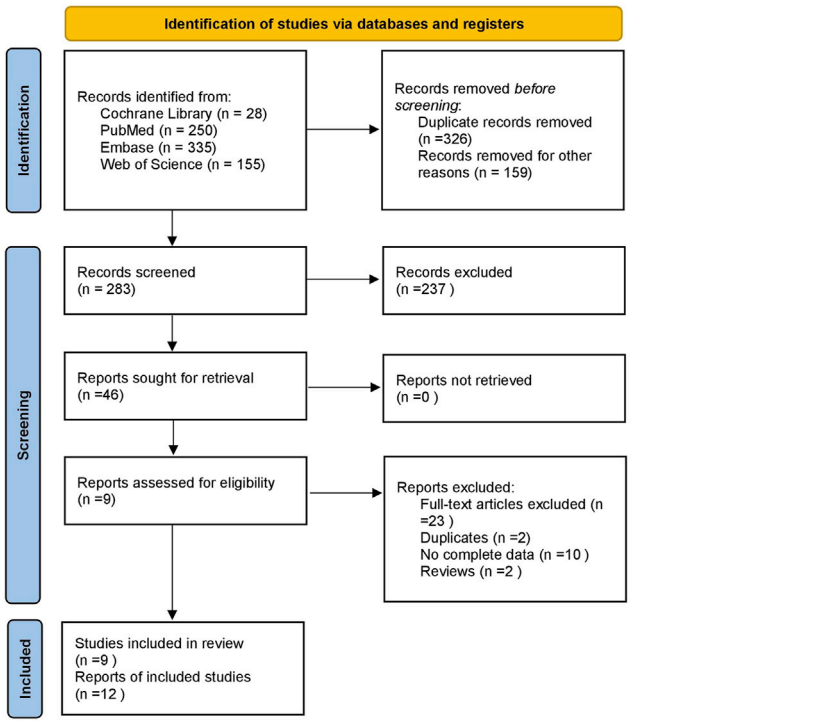


FIGURE 1
Flow chart of literature screening.

excluded by reading the titles and abstracts. After reading the full texts, nine papers (Fogel et al., 2001; Tasali et al., 2008a; Yang et al., 2009; de Sousa et al., 2010; de Sousa et al., 2011; Shreeve et al., 2013; Suri et al., 2016; Su et al., 2017; Azizi et al., 2020) were finally included (Figure 1), with a total of 1,107 research subjects. The study populations were from United States; China; Taiwan, China; Germany; Britain; India; and Iran. The basic characteristics of the literature included in the study are shown in Table 1.

3.2 Quality evaluation of included studies

The Newcastle–Ottawa (NOS) scale was used to evaluate the quality of the included observational studies. The specific evaluation is shown in Table 2. The lowest overall evaluation was 6★ and the highest was 8★, all of which were of high quality. All studies had a low to moderate risk of bias and no studies were excluded due to poor quality (<5★).2.3 Statistical Analysis Results.

3.3.1 Association between PCOS and Incidence of Sleep disturbances

A total of four studies were included (Fogel et al., 2001; Tasali et al., 2008a; Shreeve et al., 2013; Suri et al., 2016), all of which were used in the analysis. There was moderate heterogeneity among the included studies ($p = 0.01$, $I^2 = 72\%$), so a random effects model was used for analysis (Figure 2). The results showed that the incidence of Sleep disturbances in the PCOS group was higher than in the control group (OR = 11.24, 95% CI: 2.00–63.10, Z = 2.75, $p = 0.006$), and the results were statistically significant. In order to reduce the clinical heterogeneity of the research subjects, the sensitivity analysis of the incidence of Sleep disturbances showed that there was no heterogeneity after excluding one study: Shreeve 2013 (Shreeve et al., 2013) ($p = 0.5$, $I^2 = 0\%$). Furthermore, the fixed effects model analysis showed (Figure 3) that the incidence of Sleep disturbances in the PCOS group was higher than in the control group (OR = 28.91, 95% CI: 10.44–80.07, Z = 6.47, $p < 0.001$), further verifying that Sleep disturbances in the PCOS group had a higher prevalence and statistical significance. Looking back at the original text, it was found that the sleep disorder assessment method of Shreeve 2013 (Shreeve et al., 2013) was actiwatch, while the

TABLE 1 Basic features of the included studies.

Study	Country	Ages (Years)	Sample size n (T/C)	Criteria for PCOS	Criteria for SDB	BMI (kg/m2)	Research object characteristics
Fogel, 2001 (Fogel et al., 2001)	United States	T: 31.1 ± 1.3, C: 32.3 ± 1.3	36 (18/18)	NIH	Polysomnography/Sleep questionnaire	T: 36.96 ± 1.3, C: 36.96 ± 1.4	no concomitant disease
Tasali 2008 (Tasali et al., 2008a)	United States	18–40	73 (52/21)	NIH	Polysomnography	>25 kg/m2	no concomitant disease
Yang 2009 (Yang et al., 2009)	Taiwan, China	T: 29.1 ± 1.43	28 (18/10)	Rotterdam	Polysomnography/Sleep questionnaire	T: 21.7 ± 0.57	non-obese
		C: 31.6 ± 3.87				C: 20.9 ± 0.58	no concomitant disease
Sousa 2010 (de Sousa et al., 2010)	Germany	T: 15.2 ± 1.3	33 (22/11)	NIH	Polysomnography	T: 31.7 ± 6.2	no concomitant disease
		C: 15.0 ± 1.0				C: 34.8 ± 8.7	
Sousa 2011 (de Sousa et al., 2011)	Germany	T: 15.0 ± 1.0	50 (31/19)	NIH	Polysomnography	T: 32.7 ± 6.2	no concomitant disease
		C: 15.2 ± 1.1				C: 32.4 ± 4.0	
Shreeve 2013 (Shreeve et al., 2013)	Britain	T: 29.8 ± 3.7	52 (26/26)	Rotterdam	actiwatch/Sleep questionnaire	T: 29.3 ± 8.2	no concomitant disease
		C: 26.3 ± 5.6				C: 24.6 ± 3.3	
Suri 2016 (Suri et al., 2016)	India	T: 27.9 ± 6.44	150 (50/100)	Rotterdam	Polysomnography/Sleep questionnaire	T: 28.0 ± 4.01	no concomitant disease
		C: 28.3 ± 6.05				C: 25.3 ± 2.93	
Su 2017 (Su et al., 2017)	China	T: 29.03 ± 3.26	285 (129/156)	Rotterdam	Sleep questionnaire	Unlimited	no concomitant disease
		C: 31.72 ± 3.86					
Kutenaee 2019 (Azizi et al., 2020)	Iran	T: 27.86 ± 5.84	400 (201/199)	Rotterdam	Sleep questionnaire	T: 22.73 ± 9.62	no concomitant disease
		C: 28.06 ± 6.51				C: 23.95 ± 4.96	

Abbreviations: PCOS, polycystic ovary syndrome; SDB, sleep-disordered breathing; BMI, body mass index; NIH, national institutes of health.

TABLE 2 Newcastle–Ottawa Scale of the included studies.

Study	Year	Selection	Comparablity	Exposure	Quality scoress
Fogel	2001	★★★★	★★	★★	8
Tasali	2008	★★★★	★★	★	7
Yang	2009	★★★★	★★	★★	8
Sousa	2010	★★★★	★	★★	7
Sousa	2011	★★★★	★★	★	7
Shreeve	2013	★★★	★	★★★	7
Suri	2016	★★★	★	★★	6
Su	2017	★★★	★★	★★★	8
Kutenaee	2019	★★★	★★	★★	7

assessment method of the other studies was polysomnography. Considering that the generation of heterogeneity may be related to the method of sleep disorder assessment, there is a certain degree of heterogeneity, suggesting that future research can specifically explore the effects of sleep disorder assessment methods on the study population.

3.3.2 Correlation between PCOS and PSQI scores

A total of two studies were included (de Sousa et al., 2011; Shreeve et al., 2013), both of which were used in the analysis (Figure 4). The heterogeneity among the included studies was mild (p = 0.16, I^2 = 49%), so a fixed effects model was used for

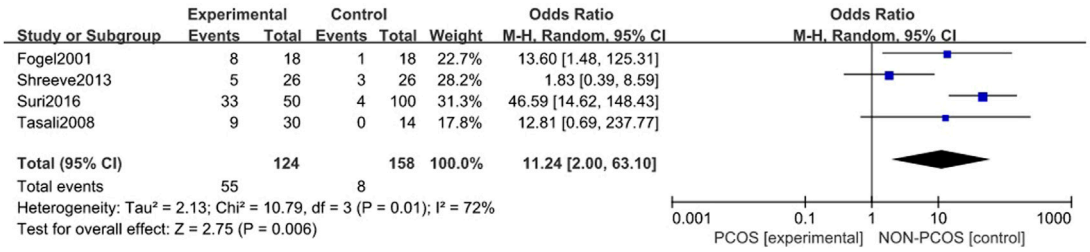


FIGURE 2
Forest plot of the incidence of sleep disturbance in the PCOS group and the control group (before heterogeneity was excluded).

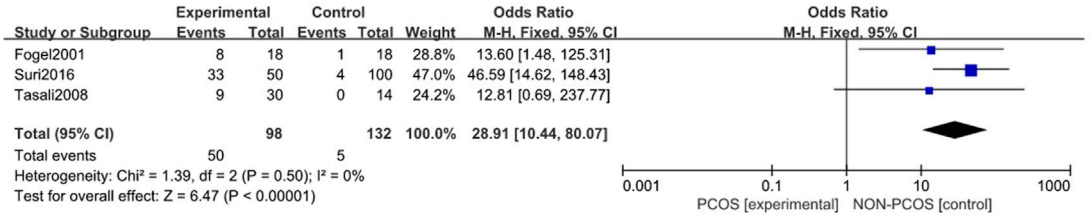


FIGURE 3
Forest plot of incidence of sleep disturbance in PCOS group and control group (after removing heterogeneity studies).

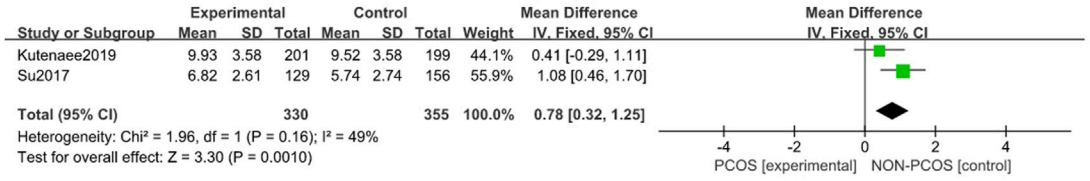


FIGURE 4
Forest plot of PSQI scores of sleep disturbance in PCOS group and control group.

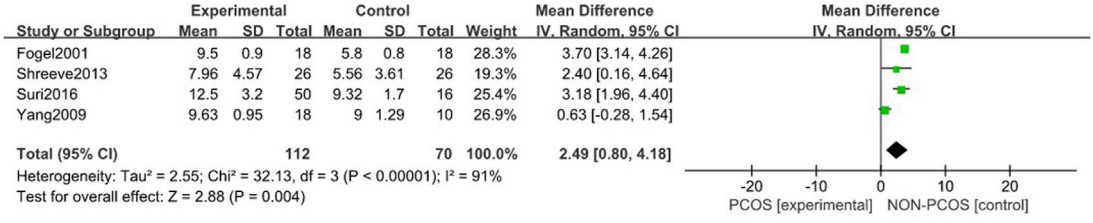


FIGURE 5
Forest plot of ESS scoress in PCOS and control groups (before heterogeneity was excluded).

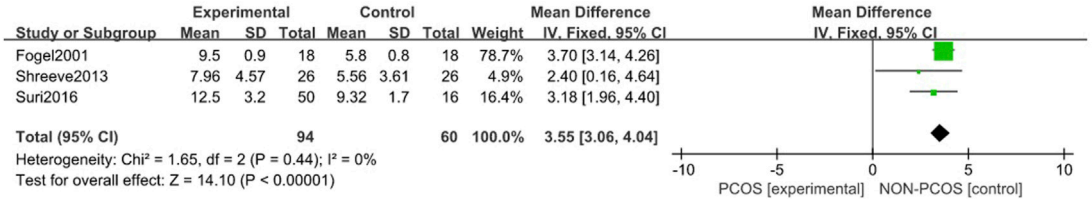


FIGURE 6
Forest plot of ESS scoress in PCOS group and control group (after removing heterogeneity studies).

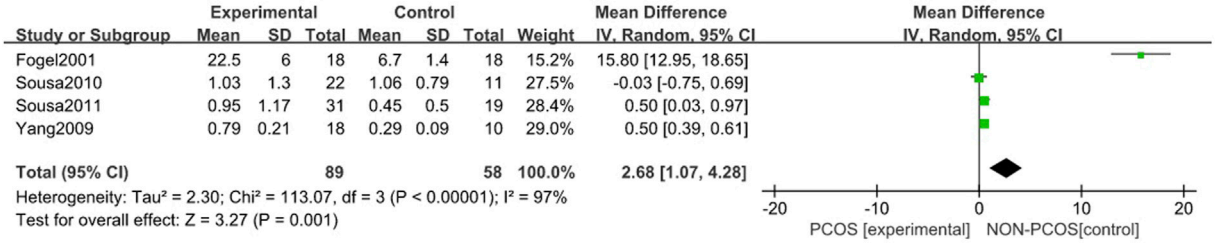


FIGURE 7
Forest plot of AHIs in PCOS group and control group.

analysis. To enter the forest plot of the PSQI scores of Sleep disturbances in the control group. The results showed that the PSQI scores of PCOS group was higher than that of control group (MD = 0.78,95% CI: 0.32–1.25, z = 3.30, $p = 0.001$); In general, the prevalence of sleep disorders in PCOS group was higher, which was statistically significant.

3.3.3 Correlation of PCOS and ESS scoress

A total of four studies were included (Fogel et al., 2001; Yang et al., 2009; Shreeve et al., 2013; Suri et al., 2016), all of which were used in the analysis. There was large heterogeneity among the included studies ($p < 0.001$, $I^2 = 91\%$), so a random effects model was used for analysis (Figure 5). The results showed that the ESS scores in the PCOS group was higher than in the control group (MD = 2.49, 95% CI: 0.80–4.18, Z = 2.88, $p = 0.004$), with statistical significance. In order to reduce the clinical heterogeneity of the research subjects, the sensitivity analysis for the ESS scoress showed that there was no heterogeneity after excluding one study, Yang 2009 (Yang et al., 2009) ($p = 0.44$, $I^2 = 0\%$); further fixed effects model analysis was performed, showing (Figure 6) that the ESS scoress of the PCOS group was higher than that of the control group (MD = 3.55, 95% CI: 3.06–4.04, Z = 14.1, $p < 0.001$), with statistical significance. Looking back at the original text, it was found that the mean BMI of the study population in Yang 2009 (Yang et al., 2009) was lower than 24 kg/m^2, while the mean BMI of the other study populations was higher than 28 kg/m^2, so it was considered that the generation of heterogeneity may be related to the BMI value of the study population. Therefore, there was a certain heterogeneity, suggesting that the effects of BMI on PCOS and Sleep disturbances in the study population should be explored in future studies.

3.3.4 Correlation of PCOS with AHIs

A total of four studies were included (Fogel et al., 2001; Tasali et al., 2008a; Suri et al., 2016; Su et al., 2017), all of which were used in the analysis (Figure 7). There was great heterogeneity among the included studies ($p < 0.001$, $I^2 = 97\%$), so a random effects model was used for analysis. The results showed that AHIs in the PCOS group were higher than those in the control group (MD = 2.68, 95% CI: 1.07–4.28, Z = 3.27, $p = 0.001$), with statistical significance.

3.3.5 Correlation between PCOS and sleep efficiency

A total of six studies were included (Fogel et al., 2001; Yang et al., 2009; de Sousa et al., 2010; Suri et al., 2016; Su et al., 2017; Azizi et al., 2020), all of which were used in the analysis (Figure 8). There was great heterogeneity among the included studies ($p < 0.001$, $I^2 = 93\%$), so a random effects model was used for analysis. The results showed that the sleep efficiency of the PCOS group was lower than that of the control

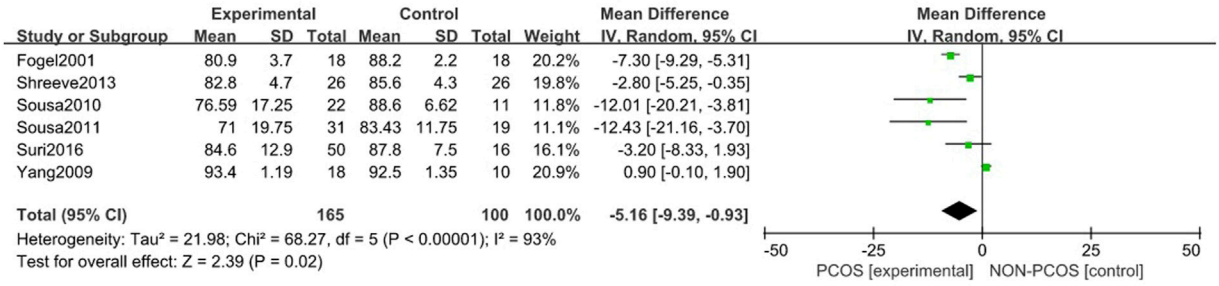


FIGURE 8
Forest plot of sleep efficiency correlation between PCOS group and control group.

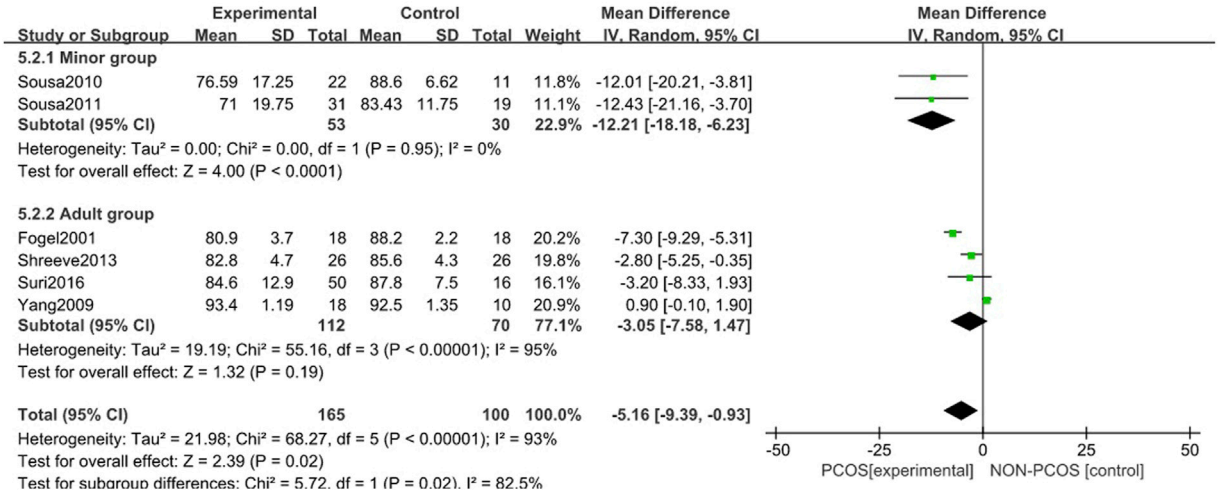


FIGURE 9
Forest plot of sleep efficiency correlation between PCOS group and control group (age subgroup).

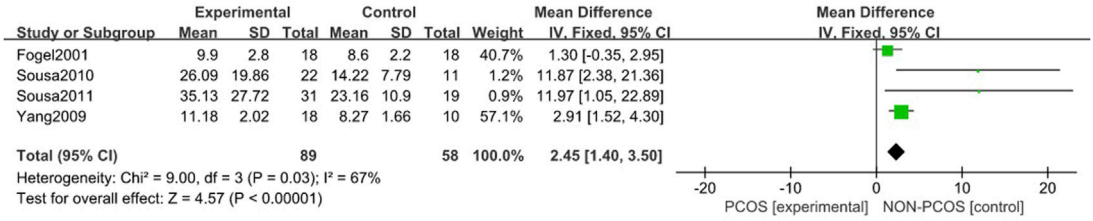


FIGURE 10
Forest plot of correlation between sleep onset latency in PCOS group and control group.

group (MD = -5.16, 95% CI: 9.39–-0.93, Z = 2.39, p = 0.02), which was statistically significant, but the included studies were heterogeneous. To further verify the relationship between PCOS and sleep efficiency, and further reduce the clinical heterogeneity of the study subjects, a subgroup analysis of the study population was performed (Figure 9) in which the

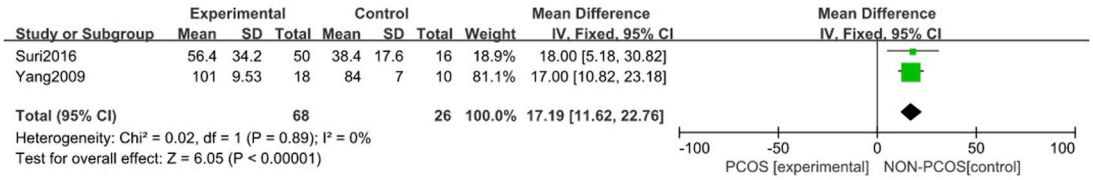


FIGURE 11
Forest plot of REM sleep correlation between PCOS group and control group.

heterogeneity of the minor group was (p = 0.95, I^2 = 0%), and the heterogeneity of the adult group was (p < 0.001, I^2 = 95%). These results showed that compared with the control group, the PCOS group was divided into minors (MD = -12.21, 95% CI: -18.18–-6.23, Z = 4, p < 0.001) and adults (MD = - 0.08, 95% CI: -0.19–0.02, Z = 1.51, p = 0.13), there was no heterogeneity in the subgroup study of minors, the sleep efficiency of the PCOS population in the minor subgroup was lower, and the difference was statistically significant. However, there was large heterogeneity in the adult subgroup study, and the sleep efficiency of the PCOS population in the adult subgroup was low, but the difference was not statistically significant. Considering that the generation of heterogeneity may be related to age, the influencing factor of age needs to be further confirmed by future large-sample, multi-center clinical studies.

3.3.6 Correlation between PCOS and Sleep Onset Latency

A total of four studies were included (Fogel et al., 2001; Suri et al., 2016; Su et al., 2017; Azizi et al., 2020), all of which were used in the analysis (Figure 10). There was moderate heterogeneity among the included studies (p = 0.03, I^2 = 67%), so a random effects model was used. The results showed that the sleep onset latency in the PCOS group was longer than that in the control group (MD = 2.45, 95% CI: 1.40–3.50, Z = 4.57, p < 0.001), which was statistically significant.

3.3.7 Correlation between PCOS and REM sleep

A total of two studies were included (de Sousa et al., 2010; Suri et al., 2016), both of which were included in the analysis (Figure 11). There was no heterogeneity among the included studies (p = 0.89, I^2 = 0%), so a fixed effects model was used for analysis. The results showed that the REM sleep in the PCOS group was higher than that in the control group (MD = 17.19, 95% CI: 11.62–55.76, Z = 6.05, p < 0.001); overall, the REM sleep in the PCOS group was higher, with statistical significance.

3.4 Sensitivity analysis

In this study, sensitivity analysis was conducted for the results with high heterogeneity and statistical significance. In the sensitivity analysis of the incidence of sleep disorders in the PCOS group and the control group, there was no significant change in the combined results after excluding any literature, and the results were stable; After the Shreeve 2013 (Shreeve et al., 2013) study was excluded, there was no heterogeneity among the studies. Tracing back to the original text, it was found that the sleep disorder evaluation method of this study was actiwatch, while the evaluation method of other studies was polysomnography. Considering that the generation of heterogeneity may be related to the sleep disorder evaluation method, there was some heterogeneity, suggesting that the effect of sleep disorder evaluation methods on the study population can be explored in future studies. In the sensitivity analysis of the ESS score in the PCOS group and the control group, there was no significant change in the combined results after excluding any literature, and the results were stable; After the Yang 2009 (Yang et al., 2009) study was excluded, the heterogeneity of the study decreased significantly. It was found that the average BMI of the study population was lower than 24 kg/m^2, while the average BMI of other study populations was higher than 28 kg/m^2. Considering that the generation of heterogeneity may be related to the BMI value of the study population, there was a certain heterogeneity, suggesting that the effect of BMI of the study population on PCOS and sleep disorders can be explored in future studies. In the sensitivity analysis of the AHI in the PCOS group and the control group, there was no significant change in the combined results after excluding any literature, and the results were stable. After the fogel 2001 (Fogel et al., 2001) study was excluded, the heterogeneity of the study decreased significantly. In the sensitivity analysis of thesleep onset latency in the PCOS group and the control group, there was no significant change in the combined results and inter study heterogeneity after excluding any literature.

3.5 Publication bias

The presence of publication bias was assessed using Egger's method. There were four studies on the incidence of Sleep disturbances in the PCOS group and control group. The result of Egger's method was $p = 0.664 > 0.05$, indicating no significant publication bias. There were four studies on the ESS score in the PCOS group and control group. The result of Egger's method was $p = 0.596 > 0.05$, indicating no significant publication bias. There were four studies on the AHI in the PCOS group and control group. The result of Egger's method was $p = 0.427 > 0.05$, indicating no significant publication bias. There were six studies on the Sleep Efficiency in the PCOS group and control group. The result of Egger's method was $p = 0.124 > 0.05$, indicating no significant publication bias. There were four studies on the Sleep Onset Latency in the PCOS group and control group. The result of Egger's method was $p = 0.183 > 0.05$, indicating no significant publication bias.

4 Discussion

PCOS is a complex endocrine disorder that affects reproductive, metabolic, and mental health in women. Clinically-based studies have shown that Sleep disturbances, including obstructive sleep apnea and excessive daytime sleepiness, occur more frequently in women with PCOS compared with control groups of women without PCOS (Fernandez et al., 2018). Sleep is an important part of normal physiology, and Sleep disturbances are common in contemporary society. Abnormal sleep is associated with health conditions and comorbidities such as obesity, hypertension, diabetes, depression, and poor quality of life. Little is known about the relationship between female sleepiness and PCOS, and several limited studies investigating sleep in women of reproductive age suggest that PCOS is associated with Sleep disturbances (Ehrmann, 2005). Women with PCOS also have an increased incidence of depression (Cooney and Dokras, 2018) and metabolic dysfunction (Dunaif et al., 1989), both of which are associated with Sleep disturbances.

Sleep disturbances and disorders can affect many aspects of daytime mood, cognition, and psychomotor function, so Sleep disturbances is a serious health problem. Recognized Sleep disturbances include features of sleep deprivation and insomnia (difficulty falling asleep, difficulty maintaining sleep, waking up too early, or unrefreshing sleep), which may be problematic for the individual, even below the clinically defined 1-month duration time (Moran et al., 2015). Hallmark sequelae include daytime fatigue, lethargy, and irritability. Sleep disturbances can occur in the absence of OSA (in which breathing is disturbed during sleep). Notably, women's subjective experience of Sleep disturbances differs from men's, with insomnia and subsequent depression more characteristic than men's typical snoring, apnea, and daytime sleepiness (Redline et al., 1994; Pillar and Lavie, 1998). At present, there are various ways to evaluate sleep quality, including objective monitoring methods such as polysomnography and various subjective questionnaires such as the PSQI (Buysse et al., 1989), ESS (Johns, 1991), and Leeds sleep evaluation questionnaire (LSEQ) (Skrobik et al., 2018).

A total of nine studies were included in this meta-analysis. The sleep quality of patients was further explored through the analysis of multiple indicators. The results showed that PCOS is closely related to Sleep disturbances, and may increase the risk of Sleep disturbances; among them, the incidence of Sleep disturbances is higher in PCOS patients; PSQI scoress, ESS scoress, AHIs, sleep onset latency, and REM sleep were higher in PCOS patients than those in the control group, the sleep efficiency of PCOS patients was lower than that of the control group, and PCOS patients all had more serious sleep problems.

Specifically, the results of the meta-analysis of the relation between PCOS and Sleep disturbances risk showed that the incidence of Sleep disturbances in PCOS patients was high; after excluding one study, Shreeve 2013 (Shreeve et al., 2013), there was no heterogeneity and no obvious research bias, indicating that this study was the source of the heterogeneity and bias, and that the research conclusions of the other articles were more reliable. However, only four studies were included, so analyzing a larger number of studies can help to further stabilize the research results. When PCOS patients were analyzed and evaluated in terms of PSQI scores, ESS scores, AHIs, sleep onset latency, REM sleep, etc., the degree of Sleep disturbances in PCOS patients was more serious. Prolonged sleep efficiency decreased and REM sleep increased, but excluding any studies had no significant effect on the combined effect size of the remaining literature, confirming that the results were relatively stable, but the sleep efficiency, PSQI, and ESS studies had no statistical significance after deleting any studies, and the number of included studies was not statistically significant. Thus, the amount of data was insufficient, and further confirmation by large-sample, multi-center clinical studies is required.

The pathophysiological mechanism leading to the high incidence of Sleep disturbances in PCOS has not yet been determined, and some studies simply attribute it to the presence of excessive BMI or obesity in women with PCOS, but this is only part of the reason for their sleep problems, as this association persisted after adjusting for the index (de Sousa et al., 2010), and PCOS patients with normal BMI also experience sleep problems. SDB is an independent risk factor for metabolic dysfunction in women with PCOS (Vgontzas et al., 2001). This meta-analysis also showed that sleep parameters were abnormal in both the overweight PCOS population and normal weight PCOS population. At present, it is believed that there are several pathways in the relation of PCOS to Sleep disturbances, and they may be bidirectional: (1) Insulin resistance is an important pathological feature of PCOS, and studies have shown that Sleep disturbances can exacerbate insulin resistance

(Rao et al., 2015), while insulin increases the sympathetic nervous system; this, this may in turn affect sleep architecture, increasing the risk of sleep-disordered breathing and daytime sleepiness (Greco and Spallone, 2015). (2) Women with PCOS have elevated hypothalamic-pituitary-adrenal axis reactivity (Benson et al., 2009), which is associated with Sleep disturbances (Vgontzas, 2008); the circadian sleep-wake cycle also involves peripheral regulation by neurotransmitters and neuromodulators, including melatonin and cytokines. (3) Women with PCOS have an altered cytokine profile (Xiong et al., 2011) and increased diurnal urine levels of the melatonin metabolite 6-sulfamoyloxymelatonin (Luboshitzky et al., 2001), which in turn can affect sleep. (4) Many women with PCOS have bad living habits such as smoking, alcoholism, and lack of physical activity, which can lead to Sleep disturbances (Thakkar et al., 2015; Zomers et al., 2017). (5) The disorders of the stress system in women with PCOS leads to the disorders of the sleep cycle (Vgontzas et al., 2001). (6) Alterations in body fat composition due to excess androgen levels and/or the effects of metabolic syndrome (Tasali et al., 2008b) have been previously associated with an increased risk of Sleep disturbances in patients without PCOS.

Limitations of this study: ① The number of included studies on the correlation between PCOS and Sleep disturbances was small, and the results were not stable, so further research is required to stabilize the research results; ② the included studies were case-control studies carried out to demonstrate the weak strength of the causal relationship, and their overall evidence-based medicine level was low; ③ the diagnostic methods of PCOS and sleep disorder assessment methods in each study were slightly different, and the study populations were from different age groups, which may have caused a certain level of heterogeneity in the results; ④ Sleep disturbances are more common in obese people (Koren and Taveras, 2018), and the populations of the studies included in our meta-analysis mainly consisted of obese patients and few patients with normal BMI, so there may be selection bias. Therefore, the conclusions of this meta-analysis should be interpreted with caution.

5 Conclusion

The results of this study suggest that the incidence of Sleep disturbances in patients with PCOS is high. PSQI scores, ESS scores, AHIs, sleep efficiency, sleep onset latency, and REM sleep may be related to PCOS. In order to understand the incidence and prevention of Sleep disturbances in patients with PCOS Treatment provides evidence-based medical evidence. Effective PCOS prevention and treatment can effectively reduce the risk of Sleep disturbances. Limited by the quantity and quality of included studies, the above conclusions need to be further confirmed by more large-sample, multi-center clinical studies.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

RC, CW, TH, WS, YL, ZZH, XC, and JZ are the guarantor of the manuscript and take responsibility for the content of this manuscript. RC, ZZO, CW, and WS contributed to the design of the study. XS, HC, HL, JL, and XO were involved in the data analysis. ZZO, XO, and ZW contributed to the acquisition of primary data. ZZH, CW, and WS wrote the initial draft of the manuscript. RC and KW contributed significantly to the revision of the manuscript. All authors read and approved the final manuscript.

Funding

This study was funded by the Young Scientist Fund of National Natural Science Foundation of China (No. 8210010336), National Clinical Research Center for Respiratory Disease Independent Project (2020B1111340018) and the Project of The State Key Laboratory of Respiratory Disease (No. SKLRD-Z-202005).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.957112/full#supplementary-material

References

Azizi, K. M., Amirjani, S., Asemi, Z., Taghavi, S. A., Allan, H., Kamalnadian, S. N., et al. (2020). The impact of depression, self-esteem, and body image on sleep quality in patients with PCOS: A cross-sectional study. *Sleep. Breath.* 24, 1027–1034. doi:10.1007/s11325-019-01946-9

Azziz, R. (2006). Controversy in clinical endocrinology: Diagnosis of polycystic ovarian syndrome: The Rotterdam criteria are premature. *J. Clin. Endocrinol. Metab.* 91, 781–785. doi:10.1210/jc.2005-2153

Azziz, R., Woods, K. S., Reyna, R., Key, T. J., Knochenhauer, E. S., and Yildiz, B. O. (2004). The prevalence and features of the polycystic ovary syndrome in an unselected population. *J. Clin. Endocrinol. Metab.* 89, 2745–2749. doi:10.1210/jc.2003-032046

Benson, S., Arck, P. C., Tan, S., Hahn, S., Mann, K., Rifaie, N., et al. (2009). Disturbed stress responses in women with polycystic ovary syndrome. *Psychoneuroendocrinology* 34, 727–735. doi:10.1016/j.psyneuen.2008.12.001

Buysse, D. J., Reynolds, C. R., Monk, T. H., Berman, S. R., and Kupfer, D. J. (1989). The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research. *Psychiatry Res.* 28, 193–213. doi:10.1016/0165-1781(89)90047-4

Calandra-Buonaura, G., Provini, F., Guaraldi, P., Plazzi, G., and Cortelli, P. (2016). Cardiovascular autonomic dysfunctions and sleep disorders. *Sleep. Med. Rev.* 26, 43–56. doi:10.1016/j.smrv.2015.05.005

Cooney, L. G., and Dokras, A. (2018). Beyond fertility: Polycystic ovary syndrome and long-term health. *Fertil. Steril.* 110, 794–809. doi:10.1016/j.fertnstert.2018.08.021

de Sousa, G., Schluter, B., Buschatz, D., Menke, T., Trowitzsch, E., Andler, W., et al. (2010). A comparison of polysomnographic variables between obese adolescents with polycystic ovarian syndrome and healthy, normal-weight and obese adolescents. *Sleep. Breath.* 14, 33–38. doi:10.1007/s11325-009-0276-0

de Sousa, G., Schluter, B., Menke, T., Trowitzsch, E., Andler, W., and Reinehr, T. (2011). Relationships between polysomnographic variables, parameters of glucose metabolism, and serum androgens in obese adolescents with polycystic ovarian syndrome. *J. Sleep. Res.* 20, 472–478. doi:10.1111/j.1365-2869.2010.00902.x

Dunaif, A., Segal, K. R., Futterweit, W., and Dobrjansky, A. (1989). Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *DIABETES* 38, 1165–1174. doi:10.2337/diab.38.9.1165

Ehrmann, D. A. (2005). Polycystic ovary syndrome. *N. Engl. J. Med.* 352, 1223–1236. doi:10.1056/NEJMra041536

Fernandez, R. C., Moore, V. M., Van Ryswyk, E. M., Varcoe, T. J., Rodgers, R. J., March, W. A., et al. (2018). Sleep disturbances in women with polycystic ovary syndrome: Prevalence, pathophysiology, impact and management strategies. *Nat. Sci. Sleep.* 10, 45–64. doi:10.2147/NSS.S127475

Fogel, R. B., Malhotra, A., Pillar, G., Pittman, S. D., Dunaif, A., and White, D. P. (2001). Increased prevalence of obstructive sleep apnea syndrome in obese women with polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.* 86, 1175–1180. doi:10.1210/jcem.86.3.7316

Greco, C., and Spallone, V. (2015). Obstructive sleep apnoea syndrome and diabetes. Fortuitous association or interaction? *Curr. Diabetes Rev.* 12, 129–155. doi:10.2174/1573399811666150319112611

Johns, M. W. (1991). A new method for measuring daytime sleepiness: The Epworth sleepiness scale. *SLEEP* 14, 540–545. doi:10.1093/sleep/14.6.540

Koren, D., and Taveras, E. M. (2018). Association of sleep disturbances with obesity, insulin resistance and the metabolic syndrome. *Metabolism.* 84, 67–75. doi:10.1016/j.metabol.2018.04.001

Luboshitzky, R., Qupti, G., Ishay, A., Shen-Orr, Z., Futerman, B., and Linn, S. (2001). Increased 6-sulfatoxymelatonin excretion in women with polycystic ovary syndrome. *Fertil. Steril.* 76, 506–510. doi:10.1016/s0015-0282(01)01930-6

Moran, L. J., March, W. A., Whitrow, M. J., Giles, L. C., Davies, M. J., and Moore, V. M. (2015). Sleep disturbances in a community-based sample of women with polycystic ovary syndrome. *Hum. Reprod.* 30, 466–472. doi:10.1093/humrep/deu318

Nandalike, K., Strauss, T., Agarwal, C., Coupey, S. M., Sin, S., Rajpathak, S., et al. (2011). Screening for sleep-disordered breathing and excessive daytime sleepiness in adolescent girls with polycystic ovarian syndrome. *J. Pediatr.* 159, 591–596. doi:10.1016/j.jpeds.2011.04.027

Pillar, G., and Lavie, P. (1998). Psychiatric symptoms in sleep apnea syndrome: Effects of gender and respiratory disturbance index. *CHEST* 114, 697–703. doi:10.1378/chest.114.3.697

Rao, M. N., Neylan, T. C., Grunfeld, C., Mulligan, K., Schambelan, M., and Schwarz, J. M. (2015). Subchronic sleep restriction causes tissue-specific insulin resistance. *J. Clin. Endocrinol. Metab.* 100, 1664–1671. doi:10.1210/jc.2014-3911

Redline, S., Kump, K., Tishler, P. V., Browner, I., and Ferrette, V. (1994). Gender differences in sleep disordered breathing in a community-based sample. *Am. J. Respir. Crit. Care Med.* 149, 722–726. doi:10.1164/ajrccm.149.3.8118642

Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group (2004). Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum. Reprod.* 19, 41–47. doi:10.1093/humrep/deh098

Shreeve, N., Cagampang, F., Sadek, K., Tolhurst, M., Houldey, A., Hill, C. M., et al. (2013). Poor sleep in PCOS; is melatonin the culprit? *Hum. Reprod.* 28, 1348–1353. doi:10.1093/humrep/det013

Skrobik, Y., Duprey, M. S., Hill, N. S., and Devlin, J. W. (2018). Low-dose nocturnal dexmedetomidine prevents ICU delirium. A randomized, placebo-controlled trial. *Am. J. Respir. Crit. Care Med.* 197, 1147–1156. doi:10.1164/rccm.201710-1995OC

Su, N., Du, C., Zhang, Y., Lian, D., Tang, T., Zhuang, B., et al. (2017). Retrospective investigation and analysis of sleep disorders on occurrence of polycystic ovary syndrome. *Biomed. Res.* 28, 583–587.

Suri, J., Suri, J. C., Chatterjee, B., Mittal, P., and Adhikari, T. (2016). Obesity may be the common pathway for sleep-disordered breathing in women with polycystic ovary syndrome. *Sleep. Med.* 24, 32–39. doi:10.1016/j.sleep.2016.02.014

Tasali, E., Van Cauter, E., and Ehrmann, D. A. (2008). Polycystic ovary syndrome and obstructive sleep apnea. *Sleep. Med. Clin.* 3, 37–46. doi:10.1016/j.jsmc.2007.11.001

Tasali, E., Van Cauter, E., Hoffman, L., and Ehrmann, D. A. (2008). Impact of obstructive sleep apnea on insulin resistance and glucose tolerance in women with polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.* 93, 3878–3884. doi:10.1210/jc.2008-0925

Teede, H., Deeks, A., and Moran, L. (2010). Polycystic ovary syndrome: A complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. *BMC Med.* 8, 41. doi:10.1186/1741-7015-8-41

Thakkar, M. M., Sharma, R., and Sahota, P. (2015). Alcohol disrupts sleep homeostasis. *ALCOHOL* 49, 299–310. doi:10.1016/j.alcohol.2014.07.019

Tsay, S. L., Rong, J. R., and Lin, P. F. (2003). Acupoints massage in improving the quality of sleep and quality of life in patients with end-stage renal disease. *J. Adv. Nurs.* 42, 134–142. doi:10.1046/j.1365-2648.2003.02596.x

Vgontzas, A. N. (2008). Does obesity play a major role in the pathogenesis of sleep apnoea and its associated manifestations via inflammation, visceral adiposity, and insulin resistance? *Arch. Physiol. Biochem.* 114, 211–223. doi:10.1080/13813450802364627

Vgontzas, A. N., Legro, R. S., Bixler, E. O., Grayev, A., Kales, A., and Chrousos, G. P. (2001). Polycystic ovary syndrome is associated with obstructive sleep apnea and daytime sleepiness: Role of insulin resistance. *J. Clin. Endocrinol. Metab.* 86, 517–520. doi:10.1210/jcem.86.2.7185

Xiong, Y. L., Liang, X. Y., Yang, X., Li, Y., and Wei, L. N. (2011). Low-grade chronic inflammation in the peripheral blood and ovaries of women with polycystic ovarian syndrome. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 159, 148–150. doi:10.1016/j.ejogrb.2011.07.012

Yang, H. P., Kang, J. H., Su, H. Y., Tzeng, C. R., Liu, W. M., and Huang, S. Y. (2009). Apnea-hypopnea index in nonobese women with polycystic ovary syndrome. *Int. J. Gynaecol. Obstet.* 105, 226–229. doi:10.1016/j.ijgo.2009.02.004

Zawadzki, J. K., and Dunaif, A. (1992). "Diagnostic criteria for polycystic ovary syndrome: Towards a rational approach," in *Polycystic ovary syndrome*. Editors A. Dunaif, J. R. Givens, F. P. Haseltine, and G. R. Merriam (Boston: Blackwell Scientific Publications), 377–384.

Zomers, M. L., Hulsegge, G., van Oostrom, S. H., Proper, K. I., Verschuren, W., and Picavet, H. (2017). Characterizing adult sleep behavior over 20 Years-the population-based doetinchem cohort study. *Sleep* 40, zsx085. doi:10.1093/sleep/zsx085

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 30 September 2022
DOI 10.3389/fendo.2022.1000261

OPEN ACCESS

EDITED BY
Andrea Giusti,
Ospedale La Colletta, Italy

REVIEWED BY
Antonino Catalano,
University of Messina, Italy
Emilio Piccione,
University of Roma Tor Vergata, Italy

*CORRESPONDENCE
Agnieszka Białka-Kosiec
agnbialka@gmail.com

SPECIALTY SECTION
This article was submitted to Adrenal Endocrinology, a section of the journal Frontiers in Endocrinology

RECEIVED 21 July 2022
ACCEPTED 06 September 2022
PUBLISHED 30 September 2022

CITATION
Białka-Kosiec A, Orszulak D, Gawlik A and Drosdzol–Cop A (2022) The relationship between the level of vitamin D, leptin and FGF23 in girls and young women with polycystic ovary syndrome.
Front. Endocrinol. 13:1000261.
doi: 10.3389/fendo.2022.1000261

The relationship between the level of vitamin D, leptin and FGF23 in girls and young women with polycystic ovary syndrome

Agnieszka Białka-Kosiec[1]*, Dominika Orszulak[2], Aneta Gawlik[3] and Agnieszka Drosdzol–Cop[2]

[1]Joannitas Hospital in Pszczyna, Pszczyna, Poland, [2]Department of Gynaecology, Obstetrics and Oncological Gynaecology, Faculty of Health Sciences in Katowice, Medical University of Silesia, Katowice, Poland, [3]Department of Pediatrics and Pediatric Endocrinology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland

Polycystic ovary syndrome is an endocrinopathy that mainly affects adolescent girls and young women of childbearing age. In girls, the presence of clinical and biochemical symptoms of hyperandrogenism should be particularly considered. The role of vitamin D deficiency in insulin resistance, inflammation, dyslipidemia, and obesity, i.e. in diseases associated with PCOS, has been investigated, which may suggest its involvement in the pathophysiology of the syndrome. Leptin has been shown to stimulate the formation of FGF23 in bones. There is a relationship between the incidence of dyslipidemia, adipose tissue mass and the concentration of fibroblast growth factor 23. The main aim of the presented research project is to assess the concentration of vitamin D, calcium, and selected hormones as well as the concentration of adipokines (leptin) in girls diagnosed with polycystic ovary syndrome.

Materials and methods: The study included a population of 85 girls and young women aged 14 to 22 years. The study group included 37 girls who were diagnosed with polycystic ovary syndrome according to the modified Rotterdam's criteria. The control group consisted of 48 completely healthy girls. In the first stage of the study participants were required to answer background questions. Next, anthropometric measurements were performed. The laboratory tests assessed: leptin, FGF23, FSH, SHGB, total testosterone, DHEA-S, 25-OH-D3, PTH, calcium, androstadiene, AMH, glucose, insulin.

Results: The vitamin D level in the group with polycystic ovary syndrome was lower than in the control group, but there was no statistically significant difference. The level of anti-Müllerian hormone was significantly higher in the group of girls diagnosed with PCOS compared to the control group. Statistically significant differences between both groups were also noted in the HOMA-IR value. The concentration of calcium, parathyroid hormone,

10.3389/fendo.2022.1000261

FGF23 and leptin in the study and control groups showed no statistically significant difference.

Conclusions: In the studied group of girls with PCOS, no correlation between the level of vitamin D and selected parameters such as: AMH leptin, HOMA-IR and FGF23 was confirmed. On this basis, it can be assumed that additional vitamin D supplementation would not reduce the symptoms of polycystic ovary syndrome.

KEYWORDS

PCOS, young females, vitamin D, leptin, FGF23

Introduction

Polycystic ovary syndrome (PCOS) is an endocrinopathy that mainly affects adolescent girls and young women of childbearing age. The frequency of PCOS depends on the diagnostic criteria used. In girls it has not been thoroughly investigated, and in a recent study carried out on a group of women aged 15–19 it was estimated at 1.14% (1–3).

Determining the diagnosis of PCOS is difficult mainly in the group of adolescents and women in the perimenopausal period. In girls, the presence of clinical and biochemical symptoms of hyperandrogenism should be particularly considered. Some of the symptoms of polycystic ovary syndrome may be also the result of physiological changes in the body of an adolescent woman. Therefore, in this age group, the diagnosis of the syndrome is based on the modified Rotterdam's criteria, i.e. all three Rotterdam criteria must be met (1, 4–7).

Obesity, hyperlipidemia, hyperinsulinemia, and insulin resistance are the major metabolic disorders accompanying PCOS. Hyperinsulinemia and insulin resistance occur in 20-40% of slim women with PCOS and in as many as 80% of obese patients. The coexistence of polycystic ovary syndrome and obesity shows a synergistic, unfavorable effect on the insulin. In adolescents, ovarian volume is correlated with levels of free testosterone and insulin, as well as with insulin resistance. Insulin also stimulates hyperkeratosis and the proliferation of skin fibroblasts. Thus, in PCOS patients, acanthosis nigricans can be observed more often (1, 2, 8).

The pleiotropic effect of vitamin D is evidenced by the fact that VDR (Vitamin D Receptor) is present in cells of various tissues, not only those related to calcium and phosphorus homeostasis, but also in 1-alpha hydroxylase (the enzyme necessary for the formation of vitamin's active form in kidneys) which can be found in keratocytes, macrophages, enterocytes or cells of the human placenta. VDR regulates the expression of approximately 3% of human genetic material (9–12).

Vitamin D has been shown to work in the uterus, ovary, vagina, and placenta. It affects the implantation of the embryo, the course of pregnancy and the health of the progeny. It must be acknowledged that the evidence supporting its contribution to human reproductive health is inconsistent and based mainly on observational studies. The role of vitamin D deficiency in insulin resistance, inflammation, dyslipidemia, and obesity, i.e. in diseases associated with PCOS, has been investigated which may suggest its involvement in the pathophysiology of the syndrome. In their studies, Keshavarz et al. found vitamin D deficiency <20ng/ml in 78.7% of patients with PCO syndrome, and its normal level was only in 2.7% of patients (5, 12, 13)

Fibroblast growth factor 23 (FGF23) is a part of newly identified regulators system that forms the FGF23-bone-kidney axis, with a function comparable to the PTH-vitamin D axis. It is mainly synthesized by osteocytes and osteoblasts, qualifying bone tissue as an endocrine organ (14, 15).

The kidney is the target organ of FGF23 and the 1,25 $(OH)_2D_3$ is its main regulating factor. Excessive secretion of fibroblast growth factor 23 leads to hypophosphatemia, a decrease in $1,25(OH)_2D_3$ concentration and to rachitis or osteomalacia. In turn, FGF23 deficiency results in hyperphosphatemia, an increase in the $1,25(OH)_2D_3$ concentration and the formation of calcifications in soft tissues. The cofactor of FGF23 is the Klotho protein. The PTH, by stimulating the activity of 1α-hydroxylase, increases the synthesis of $1,25(OH)_2D_3$, which in turn enhances the synthesis of the Klotho protein (14, 15).

Leptin has been shown to stimulate the formation of FGF23 in bones. There is a relationship between the incidence of dyslipidemia, adipose tissue mass and the concentration of fibroblast growth factor 23. It has also been shown that estrogens, deficiency of which is the main risk factor for osteoporosis, stimulate the secretion of FGF23 (14, 15).

Changes in the secretion of adipokines are an important link in the development of insulin resistance in peripheral tissues. In this mechanism adipokines may indirectly participate in the pathogenesis of PCO syndrome. Earlier studies showed that the concentration of leptin is significantly higher in PCOS patients who have irregular menstrual cycles compared to the group with regular cycles. The level of fertility in women with PCOS has been shown to be inversely related to the concentration of circulating leptin. In turn, in adolescents with polycystic ovary syndrome, leptin concentration depends on BMI, adipose tissue content, waist circumference and HOMA-IR value (16, 17).

So far, the published literature data assess the level of vitamin D, without explanation of the changes taking place, etiology, and pathogenesis of PCOS. The possible relationship between fibroblast growth factor 23 and leptin in the pathogenesis of PCOS has not been investigated yet. The conducted studies focused almost exclusively on the group of adult patients. The results of the studies published so far are not unequivocal.

The main aim of the presented research project is to assess the concentration of vitamin D, calcium, and selected hormones as well as the concentration of adipokines (leptin) in girls diagnosed with polycystic ovary syndrome. The first symptoms of PCOS, such as hyperandrogenism and anovulatory cycles, usually appear in adolescence, but the moment when the patient visits the doctor, and the diagnosis is made is most often delayed. In this situation, conducting research is particularly important not only for the development of diagnostics and treatment, but also for examining the pathophysiology of the syndrome. Years later, the patients show such multi-system symptoms that it is difficult to recognize the initiating factors.

The possible relationship between fibroblast growth factor 23 and leptin in the pathogenesis of PCOS has not been investigated so far.

The specific aims are:

1. Assessment of the variability of vitamin D levels depending on the occurrence of the syndrome polycystic ovaries.
2. Assessment of the variability of AMH concentrations depending on the presence of polycystic syndrome ovaries.
3. Determination of the relationship between AMH, FGF23, leptin, HOMA-IR and the level vitamin D in the test and control group.

Materials and methods

Materials

The study included a population of 85 girls and young women aged 14 to 22 years. Patients were recruited for the study at the Department of Pediatrics and Pediatric Endocrinology of the John Paul II Upper Silesian Child Health Centre in Katowice and at the children's gynecology sub-department in the Department of Gynaecology, Obstetrics and Oncological Gynaecology in Katowice. The study was conducted between December and March.

The study was conducted between December and March. The study group included 37 girls who were at least two years after the menarche and were diagnosed with polycystic ovary syndrome according to the modified Roterdam's criteria. The mean length of menstrual cycles in the study group was 59.03 days (± 45.17 days). The values of the performed hormonal tests (androstenedione, testosterone, DHEAS and FAI) were increased and on their basis the hyperandrogenism was diagnosed. The exact inclusion criteria for the study group are summarized in Table 1.

The study group was recruited from the patients' population of the hospital outpatient clinic at Department of Pediatrics and Pediatric Endocrinology of the John Paul II Upper Silesian Child Health Centre in Katowice and the Department of Gynaecology, Obstetrics and Oncological Gynaecology in Katowice, during follow-up visits. The control group consisted of 48 completely healthy girls aged between 14 and 22 years old, at least two years after the menarche, menstruating regularly (28 ± 7 days) for at last six months. In the control group the presence of

TABLE 1 The inclusion and exclusion criteria for the study group.

Inclusion Criteria:	Exclusion Criteria:
-age between 14 and 22 years	-pharmacotherapy used in the last 6 months (including hormonal drugs, contraceptives, NSAIDs)
-time over 2 years from the menarche	-additional systemic diseases (e.g., cardiovascular diseases, diabetes, gastric/duodenal ulcer disease, autoimmune diseases)
-consent of a woman or a girl and her legal guardian to participate in the study	-use of dietary supplements, especially those containing vitamin D or calcium, within the last 12 months
-oligo- or anovulation	-sunbathing in the last 3 months
-biochemical indicators of hyperandrogenism or hirsutism	-applying a restrictive diet in the last 12 months
-volume of one of the ovaries >12 ml or >24 follicles in the ovary in pelvic ultrasound examination	- endocrinopathies (congenital adrenal hyperplasia, Cushing's syndrome, hyperprolactinemia, thyroid dysfunction, acromegaly, androgen-secreting tumors)

hyperandrogenism was not confirmed in laboratory tests. The exclusion criteria for this group remained the same as for the study group (Table 1).

All participants of the study were informed in detail about its purpose and method. The consent to take part in the study was obtained from all participants (in case of patients under 18 years of age the consent was obtained from both the respondent and her parents/legal guardians). The consent of the Bioethical Committee of the Medical University of Silesia in Katowice was obtained to conduct the study (consent number - KNW/0022/KB1/136/III/13/14/16).

Methodology

In the first stage of the study participants were asked to answer background questions about medical history (regarding internal diseases, use of dietary supplements, exposure to the sun, addictions) and gynecological history with the assessment of the menstrual cycle.

The normal menstrual cycle in girls was defined as:

- cycles lasting 21-45 days;
- menstrual bleeding 3-7 days;
- blood loss during menstruation 5-80 ml.

Secondary amenorrhea was diagnosed in the event of a lack of period for six months after the previous period of normal menstruation.

In the next stage the anthropometric measurements (height, weight, BMI) were performed. The severity of hirsutism was assessed according to the Ferriman-Gallwey score (diagnosed hirsutism ≥ 8 points). The ultrasound examination of the pelvis was performed. The laboratory tests assessed: leptin, FGF23, FSH, SHGB, total testosterone, DHEA-S, 25-OH-D_3, PTH, calcium, androstadiene, AMH, glucose, insulin. The characteristics of the studied biochemical parameters are presented in *Additional data section*.

Insulin resistance was assessed indirectly based on the obtained results after calculating the value of the HOMA-IR indicator. The formula was as follows: HOMA-IR (Homeostasis Model Assessment) = fasting serum insulin concentration (mU/ml) x fasting serum glucose concentration (mmol/L)/22.5.

Insulin resistance was diagnosed at HOMA-IR values ≥ 2.5.

Free Androgen Index (FAI) was calculated using the following formula: FAI = [Total Testosteron/SHBG] x 100%. Normal values <5%.

Laboratory research was made in the Biochemical Laboratory of the Department of Health Promotion and Obesity Management, Department of Pathophysiology, Faculty of Medical Sciences, Medical University of Silesia in Katowice.

Statistical analysis

The Excel 2007 and STATISTICA v.12PL applications were used for the statistical analysis. The result of the statistical analysis was considered statistically significant if the obtained significance level "p" was less than or equal to 0.05.

The statistical research used:

- Shapiro-Wolf test;
- Mann-Whitney U test;
- CHI2 test with Yates correction;
- Spearman and Kendall correlation test;
- analysis of discrimination - a one-time, multi-parameter comparison of two groups.

Results

General characteristics of the studied groups of girls

The mean age of the participants from the study group was 19.4 ± 2.4 years, while in the control group it was 20.0 ± 2.2 years. The difference between the two groups in this parameter was not statistically significant.

There were no statistically significant differences between the two groups also in terms of body weight, height, and body mass index (BMI). The above data are presented in Table 2.

TABLE 2 Basic characteristics of anthropometric data in groups.

Statistical parameter	Study group		Control group		The Mann-Whitney U Test
	[mean value ± SD]	Median	[mean value ± SD]	Median	
Height [cm]	165,9 ± 6,5	167	165,5 ± 6,1	165,0	p=0,49
Body weight [kg]	64,3 ± 12,4	62,5	61,8 ± 9,9	60,0	p=0,31
BMI [kg/m2]	23,3 ± 4,1	22,6	22,6 ± 3,5	22,3	p=0,55

10.3389/fendo.2022.1000261

Comparison of biochemical parameters in the test and control group

The vitamin D level in the group with polycystic ovary syndrome was lower than in the control group (Table 3). The mean concentration in the study group was 24.88 ng/ml. However, no statistically significant difference was found in these measurements (p=0.80). In both groups the mean level of vitamin D was below the reference value.

The level of anti-Müllerian hormone was significantly higher in the group of girls diagnosed with PCOS compared to the control group (p=0.001). The mean concentration of AMH in the study group was 9.21 ng/ml. A relationship between the level of the anti-Müllerian hormone and the occurrence of polycystic ovary syndrome was demonstrated. Statistically significant differences between both groups were also noted in the HOMA-IR value (2.62 ± 1.92 in the study group and 1.80 ± 1.82 in the control group; p=0.003) (Table 3).

No statistical significance between the control group and the study group in terms of the concentration of calcium, parathyroid hormone, FGF23 and leptin was noticed. The results are detailed in Table 4.

Correlations of selected parameters in the test and control group

In the next stage of statistical analyzes, relationships between the concentrations of selected markers in the study group and the control group were assessed. In the control group, using the Spearman's correlation, a relationship between the concentration of vitamin D and the concentration of FGF23 (p=0.01) (Figure 1) was found. There was no correlation between:

- vitamin D and leptin levels;
- vitamin D and AMH levels;
- leptin and FGF23 concentration (Table 5).

Discussion

According to previously published studies, the serum levels of vitamin D in women with PCO syndrome may be higher, lower, or not significantly different than in healthy controls. This leads to the conclusion that the role of vitamin D in the pathogenesis of PCOS is unclear (18). In the conducted study, lower levels of vitamin D were found in patients with PCOS, but the difference was not statistically significant.

In the study by Ghadimi et al. (19) the mean values of vitamin D were significantly lower in the group of girls (192 girls aged 16-20) with the PCO syndrome in comparison to the healthy control group. Similarly, to the present study they did not show any difference in the level of calcium between the groups, even though animal studies have proven its effect on oocyte maturation and impairment of its regulation system on follicle arrest (8). There was also no correlation between the severity of vitamin D deficiency and the severity of acne, hirsutism, and obesity. Moreover, there was no correlation between the level of vitamin D and the lipid and hormonal profile of both groups (19). On the other hand, in a British study on a group of adult women, no significant difference between the level of vitamin D in patients with and without PCOS was found, although severe deficiency was more common in the study group. However, this study showed an inverse correlation between BMI and serum 25 (OH) D concentration in women with PCOS (18).

While reviewing the literature, one can meet the hypothesis that the level of vitamin D correlates with the BMI, so there is a relationship between the level of vitamin and obesity, and it is not dependent on the occurrence of PCOS (19). However, it should be considered that obese people spend less time outdoors when exposed to sunlight, which leads to insufficient vitamin D biosynthesis in the skin and is the main reason for the difference in 25 (OH) D levels in this group. In the conducted studies, it was noticed that in obese subjects 24 hours after exposure to UV light, the increase in 25 (OH) D level was by 57% lower than in healthy subjects. This may be due to the reduction of vitamin D bioavailability through its sequestration in excessive amounts of adipose tissue. Of course, food preferences should also be

TABLE 3 Vitamin D3, AMH, HOMA-IR level in the study and control group.

Parameter	Group	Group size	Mean	Standard deviation	Median	Min value	Max value	Normality test	The Mann-Whitney U Test
Vitamin D	Study	37	24,88	11,54	22,12	9,31	52,64	p=0,02	NS (p=0,80)
	Control	48	26,29	14,15	22,63	4,62	69,26	p=0,01	
AMH	Study	35	9,21	4,57	8,16	1,84	20,37	p=0,18	p=0,001
	Control	48	6,25	3,67	5,31	1,05	17,19	p=0,002	
HOMA-IR	Study	33	2,62	1,92	2,34	0,58	11,04	p<0,000001	p=0,003
	Control	48	1,80	1,82	1,47	0,13	12,39	p<0,000001	

10.3389/fendo.2022.1000261

TABLE 4 Ca, PTH, FGF23, leptin values in the study and control group.

Parameter	Group	Group size	Mean	Standard deviation	Median	Min value	Max value	Normality test	The Mann-Whitney U Test
FGF23	Study	30	59,67	29,95	52,59	21,86	168,19	p=0,001	NS (p=0,99)
	Control	48	58,57	26,20	52,19	23,25	168,25	p<0,000001	
Ca	Study	30	2,25	0,13	2,26	1,75	2,48	p=0,0002	NS (p=0,45)
	Control	48	2,22	0,18	2,25	1,46	2,47	p=<0,000001	
PTH	Study	30	19,10	7,81	17,68	5,07	46,21	p=0,009	NS (p=0,59
	Control	48	19,46	9,68	19,67	3,51	38,20	p=0,14	
Leptin	Study	37	15,32	10,10	12,07	2,92	48,56	p=0,001	NS (p=0,15)
	Control	48	13,86	13,56	9,82	2,68	80,35	p<0,000001	

considered, as well as vitamin D metabolism in individual groups and their possible impact on the results of the analyzes (18). On the other hand, it has been suggested that the relationship between vitamin D levels and FAI levels results from the reduction of SHBG because of obesity (insulin inhibits SHBG synthesis in the liver), which would support the hypothesis that D hypovitaminosis is only the result of obesity in PCO syndrome (20). Tsakova et al. (21) showed that obese women with PCOS have lower 25 (OH) D levels compared to slim PCOS patients.

In the study by Keshavarz et al. no relationship between the concentration of vitamin D and total testosterone, DHEAS, SHBG and the index of free androgens was found (13). Similarly, in the American study from 2016, no relationship between the level of vitamin D and the concentration of total testosterone, FAI, and hirsutism was found (5).

Analyzing the articles about polycystic ovary syndrome, it was noticed that some seemingly unrelated elements could form a causal relationship and be involved in etiopathogenesis and explain some of the symptoms of PCOS. FGF23 turned out to be the central element in this hypothesis. Its formation is stimulated by leptin and estrogens, and FGF23 itself reduces the concentration of $1,25(OH)_2D_3$ reducing thereby the activity of 1α-hydroxylase (Figure 2) (14, 15).

Despite attempts to fully understand the mechanisms that regulate the FGF23 production and secretion it should be unequivocally stated that this is an extremely complex and complicated issue that has not been fully described yet. Leptin has a major influence on the increased level of FGF23. It was proven that the secretion of FGF23 in the osteocytes is directly stimulated by leptin and can have an important impact on several mineral metabolism parameters. Furthermore, as with FGF23, leptin also inhibits the synthesis of 1,25(OH)2D3 (22).

Another hormone that has a major impact on the bone metabolism by regulating bone markers including FGF23, vitamin D, DBP, especially in women, is estrogen. Estrogen therapy can decrease the FGF23 level, due to the phosphaturia effect of estrogen. Treatment with exogenous estradiol leads to an increase in 25(OH)D3, which increases the active form 1,25 (OH)2D3 in women (22).

Baldani et al. found higher levels of leptin in PCOS patients compared to healthy controls. The same relationship was obtained

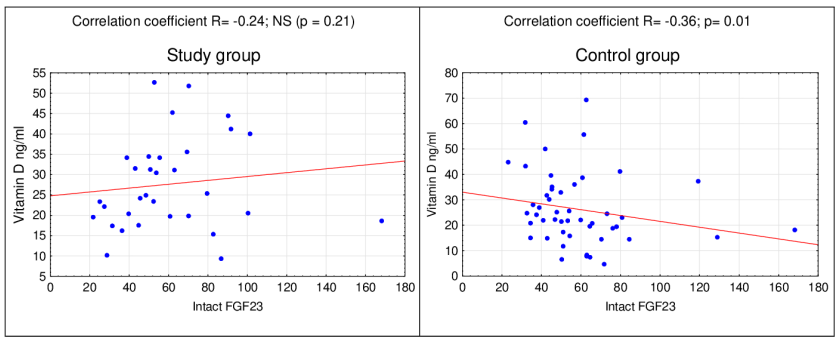


FIGURE 1
Correlation of FGF23 and vitamin D in the study and control group.

10.3389/fendo.2022.1000261

TABLE 5 Spearman's correlations in the study and control group.

Correlation	Study group	Control group
Vitamin D - FGF23	R=0,24; NS (p=0,21)	**R=-0,36; p=0,01**
FGF23 - Leptin	R=-0,18; NS (p=0,34)	R=0,11; NS (p=0,47)
Vitamin D - Leptin	R=-0,05; NS (p=0,75)	R=0,05; NS (p=0,73)
Vitamin D - AMH	R=0,06; NS (p=074)	R=0,11; NS (p=0,44)
Leptin - FGF23	R=-0,18; NS (p=0,34)	R=0,11; NS(p=0,47)
HOMA-IR - AMH	R=-0,08; NS (p=0,65)	R=0,03; NS (p=0,87)
HOMA-IR - BMI	R=0,22; NS (p=0,22)	**R=0,32; p=0,03**
AMH - BMI	R=-0,15; NS (p=0,38)	R=0,10; NS (p=0,49)

after the groups were verified in terms of BMI, which suggests that the relationship is not only the result of higher body weight of patients in the study group. However, they did not notice any correlation between the level of leptin and the concentration of total testosterone and HOMA-IR in the analyzed groups. It was suggested that obesity and the diagnosis of PCOS itself affect the serum leptin level almost equally (23). In our study, we did not find a statistically significant difference in the level of leptin between the control group and the study group. According to other publications, in adolescent patients the concentration of leptin was correlated with the HOMA-IR value (16, 17).

It was noted that in rodents, insulin deficiency was an independent factor increasing the serum levels of FGF23. The administration of insulin reversed this effect. Also, a negative correlation between FGF23 and insulin was observed in humans (24). In our research, we noticed a relationship between the concentration of vitamin D and FGF23, but only in the control group. There was also no correlation between FGF23 and leptin or vitamin D and leptin in any of the groups.

In the studied group of girls with PCOS, the existence of a relationship between the level of vitamin D and selected parameters such as leptin and FGF23 was not confirmed. On this basis, it can be assumed that additional vitamin D supplementation would not reduce the symptoms of polycystic ovary syndrome. On the other hand, in the control group there was a correlation between the vitamin D concentration and the concentration of FGF23. However, the presented relationship is not clinically significant. Further studies of these relationships in a larger group of patients are necessary.

The results of this study do not explain the pathogenesis of PCOS but are another element of research aimed at trying to determine the processes that affect the appearance of the clinical picture presented by patients. The presented results show that the average vitamin D concentration in the study and control group is below the reference value. Moreover, it is suggested that girls diagnosed with PCOS have higher levels of AMH and HOMA-IR. Considering the declining diagnostic value of ultrasound scan in girls in PCOS diagnosis, further studies leading to a discovery of new markers enabling an early diagnosis of the disease are definitely needed.

Limitations of the study

The main limitation of the study is the small number of participants in both study and control groups. This is due to the

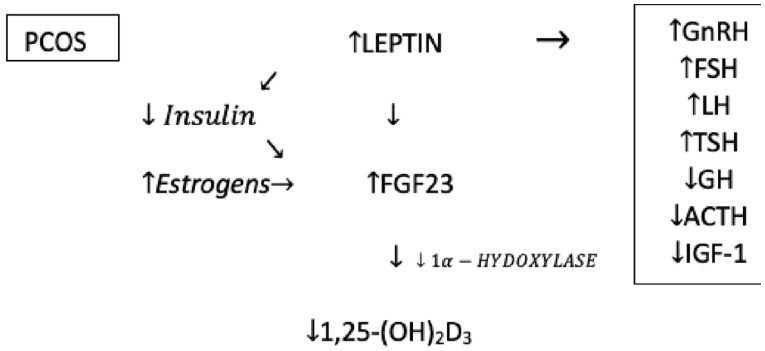


FIGURE 2
Potential relationship between leptin, FGF23 and vitamin D levels in patients with PCOS.

limited ability to qualify girls, caused by the infrequent prevalence and diagnosis of polycystic ovary syndrome in this age group. The use of HOMA-IR for the assessment of insulin resistance, i.e. an indirect method instead of the metabolic clamp being the "gold standard", should also be considered as a limitation of the study. Such a choice was made due to the safety of the adolescent patient and the ambulatory conditions in which the tests were carried out. Moreover, the reference value for the HOMA-IR and AMH index (marked in the conducted study) in the group of adolescent patients has not been established so far.

Additional data section

The characteristics of the studied biochemical parameters:

1. Androstendion

Method sensitivity: 0.40 ng/ml

The coefficient of variation within and between laboratories was 5.2%, 8.7%,

Normal: 0.7-3.1ng/ml

2. AMH (Anti-Mullerian Hormone)

Method sensitivity: 0.08 mg/ml

The coefficient of variation within and between laboratories is as follows: 6.75%, 5.5%

Normal: 0-10.6 ng/mL

3. FSH

Method sensitivity: 0.1 mIU/ml

The coefficient of variation within and between laboratories was 1.7%, 1.7%

Normal: 3.5-12.5 mlU/ml

4. SHBG

Method sensitivity: 0.35 nmol/l

The coefficient of variation within and between laboratories was: 2.4%, 2.2%,

Norm: 26.1 -110 nmol/l

5. Total testosterone

Method sensitivity: 0.0250 nmol/L

The coefficient of variation within and between laboratories was 2.5%, 1.6%

Norm: 0.290-1.67 nmol/l

6. DHEA-S

Method sensitivity: 0.1 µg/dL

43

The coefficients of variation within and between laboratories were 2.3%, 2.5%

Normal: 65.1 - 368 µg/dL

7. Insulin

Method sensitivity: 0.2 µIU/ml

The coefficient of variation within and between laboratories was 1.9%, 1.6%

Normal: 2.6 - 20 µIU/ml

8. Parathyroid hormone

Method sensitivity: 1.20 pg/mL, 0.127 pmol/L

The coefficient of variation within and between laboratories was 1.6% and 2.2%

Standard: 15-65pg/mL, 1.6-6.9 pmol/L

9. 25- Hydroxyvitamin D

Method sensitivity: 9.0 ng/mL

The coefficient of variation within and between laboratories was 5.1% and 2.7%

Normal:> 30 ng/mL

10. Calcium

Method sensitivity: 0.20mmol/L, 0.8 mg/dL

The coefficient of variation within and between laboratories was 1.9% and 1.9%

Normal: 2.10-2.55 mmol/L, 8.4-1.2 mg/dL

11. Leptin

Method sensitivity: 0.2 ng/ml,

The coefficient of variation within and between laboratories was 5.9%, 5.5%

12. FGF23

Method sensitivity: 0.04pg/mL

The coefficients of variation within and between laboratories were: 8.9% and 12.4%

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethic Committee of Medical University of Silesia Katowice 40-055 ul. Poniatowskiego 15. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

AB-K - study conception and design, analysis and interpretation of data, drafting the article, revising the article critically for important intellectual content. DO - analysis and

interpretation of data, revising the article critically for important intellectual content. AG - study conception and design, revising the article critically for important intellectual content, final approval of the version to be published. AD-C - study conception and design, analysis and interpretation of data, drafting the article, revising the article critically for important intellectual content, final approval of the version to be published. All authors contributed to the article and approved the submitted version.

Funding

This study was funded by Medical University of Silesia in Katowice.

Acknowledgments

The Authors wish to thank Andrzej Widera for his support in statistical processing of the results and Michał Skorupa- Kopczyński for proofreading of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Mahde A, Shaker M, Al-Mushhadani Z. Study of Omentin1 and other adipokines and hormones in PCOS patients. *Oman Med J* (2009) 24:108–18. doi: 10.5001/omj.2009.25

2. Kale-Gurbuz T. Adiponectin, leptin and ghrelin levels in obese adolescent girls with polycystic ovary syndrome. *J Pediatr Adolesc Gynecol* (2013) 26:27–30. doi: 10.1016/j.jpag.2012.09.002

3. Kim JJ, et al. Vitamin d deficiency in women with polycystic ovary syndrome. *Clin Exp Reprod Med* (2014) 41(2):80–5. doi: 10.5653/cerm.2014.41.2.80

4. Caspian J. Does vitamin d status correlate with clinical and biochemical features of polycystic ovary syndrome in high school girls? *Caspian J Intern Med* (2014) 5(4):202–8.

5. Tehrani HG, Mostajeran F, Shahsavari S. The effect of calcium and vitamin d supplementation on menstrual cycle, body mass index and hyperandrogenism state of women with poly cystic ovarian syndrome. *J Res Med Sci* (2014) 19(9):875–80.

6. Hahn S, Haselhorst U, Tan S, Quadbeck B, Schmidt M, Roesler S. Low serum 25- hydroxyvitamin d concentrations are associated with insulin resistance and obesity in women with polycystic ovary syndrome. *Exp Clin Endocrinol Diabetes* (2006) 114(10):577–83. doi: 10.1055/s-2006-948308

7. Tsakova A, Gateva A, Kamenov Z. 25(OH) vitamin d levels in premenopausal women with polycystic ovary syndrome and/or obesity. *Int J Vitamin Nutr Res* (2012) 82:399–404. doi: 10.1024/0300-9831/a000137

8. Witkowska-Sędek E, Kądziela K, Miszkurka G, Pyrżak B (2014). Czynnik wzrostu fibroblastów 23 (FGF23) – nowy ważny czynnik w regulacji gospodarki wapniowo- fosforanowej. *Postępy Nauk Medycznych*. 27(10); 14–8.

9. Baldani DP, Skrgatic L, Kasum M, Zlopasa G, Oguic SK, Herman M (2019). Altered leptin, adiponectin, resistin and ghrelin secretion may represent an intrinsicpolycystic ovary. *Gynecol Endocrinol* 35(5):401–5. doi: 10.1080/09513590.2018.1534096

10. Ramanjaneya M, Bensila M., Bettahi I., Jerobin J., Samra T.A., Aye M., et al. (2020) Dynamic changes in circulating endocrine FGF19 subfamily and fetuin-a in response to intralipid and insulin infusions in healthy and PCOS women. *Front Endocrinol (Lausanne)* 11:568500. doi: 10.3389/fendo.2020.568500

11. Skrzypulec-Plinta V, Drosdzol-Cop A. *Ginekologia dziecięca i dziewczęca*. Warszawa: PZWL (2017).

12. Skrzypulec-Plinta V, Radowicki S. *Wybrane zagadnienia z ginekologii dziecięcej i dziewczęcej*. Bielsko-Biała: Medical Project Poland Sp. z o.o (2011).

13. Chung PW, Chan SS, Yiu KW, Lao TT, Chung TT. Menstrual disorders in a paediatric and adolescent gynaecology clinic: patient presentations and longitudinal outcomes. *Hong Kong Med J* (2011) 17(5):391–7.

14. Drosdzol-Cop A, Sidło-Stawowy A, Sajdak A, Skrzypulec- Plinta V. Diagnosing polycystic ovary syndrome in adolescent girls. *Ginekol Pol* (2014) 85:145–8. doi: 10.17772/gp/1705

15. Rackow BW. Polycystic ovary syndrome in adolescents. *Curr Opin Obstet Gynecol* (2012) 24:281–7. doi: 10.1097/GCO.0b013e32835669ff

16. Diamanti-Kandarakis E. PCOS in adolescents. *Best Pract Res Clin ObstetGynaecol* (2010) 24:173–83. doi: 10.1016/j.bpobgyn.2009.09.005

17. Witchel SF, Oberfield SE, Peña AS. Polycystic ovary syndrome: Pathophysiology, presentation, and treatment with emphasis on adolescent girls. *J Endocr Soc* (2019) 3(8):1545–73. doi: 10.1210/js.2019-00078

18. Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, et al. Diagnosis and treatment of polycystic ovary syndrome: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* (2013) 98:4565–92. doi: 10.1210/jc.2013-2350

19. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Fertil Steril* (2018) 110(3):364–79. doi: 10.1016/j.fertnstert.2018.05.004

20. Olędzka R. Witamina d w świetle badań ostatnich lat. *Bromat Chem Toksykol* (2013) 2:121–31.

21. Muscogiuri G, et al. Mechanisms in endocrinology: vitamin d as a potential contributor in endocrine health and disease. *Eur J Endocrinol* (2014) 171(3):101–10. doi: 10.1530/EJE-14-0158

22. Lopez I Pineda C, Rodriquez- Ortiz ME, Diaz- Tocados JM, Rios R (2017). Leptin directly stimulates parathyroid hormone secretion. *Endocrine* 56(3):675–8. doi: 10.1007/s12020-016-1207-z

23. Charzewska J, Chlebna-Sokół D, Chybicka A, Czech- Kowalska J, Dobrzańska A, Helwich E, et al. Aktualne (2009) polskie zalecenia dotyczące profilaktyki niedoboru witaminy D. *Med Prakt Pediatr* (2010) 01.

24. Herian M, Grzesiak M. Rola witaminy D3 i jej receptora w żeńskim układzie rozrodczym. *KOSMOS Problemy nauk biologicznych* (2017) 66 (316):389–99.

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 09 November 2022
DOI 10.3389/fendo.2022.962154

OPEN ACCESS

EDITED BY
Lisa Owens,
St. James's Hospital, Ireland

REVIEWED BY
Pranay Punj Pankaj,
Nagaland University, India
Kehinde Olaniyi,
Afe Babalola University, Nigeria

*CORRESPONDENCE
Shun-min Li
shunminli1955@163.com
Xian-sheng Meng
mxsvvv@163.com
Jin-song Kuang
270174194@qq.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

RECEIVED 12 July 2022
ACCEPTED 24 October 2022
PUBLISHED 09 November 2022

CITATION
Jiang X-l, Tai H, Xiao X-s, Zhang S-y, Cui S-c, Qi S-b, Hu D-d, Zhang L-n, Kuang J-s, Meng X-s and Li S-m (2022) Cangfudaotan decoction inhibits mitochondria-dependent apoptosis of granulosa cells in rats with polycystic ovarian syndrome. *Front. Endocrinol.* 13:962154. doi: 10.3389/fendo.2022.962154

Cangfudaotan decoction inhibits mitochondria-dependent apoptosis of granulosa cells in rats with polycystic ovarian syndrome

Xiao-lin Jiang[1,2†], He Tai[3,4†], Xuan-si Xiao[5†], Shi-yu Zhang[2], Shi-chao Cui[6], Shu-bo Qi[2], Dan-dan Hu[7], Li-na Zhang[2], Jin-song Kuang[8*], Xian-sheng Meng[3*] and Shun-min Li[1*]

[1]Department of Nephrology, The Fourth of Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine (Shenzhen Traditional Chinese Medicine Hospital), Guangzhou University of Traditional Chinese Medicine, Shenzhen, China, [2]Key Laboratory of Ministry of Education for Traditional Chinese Medicine Viscera-State Theory and Applications, Liaoning University of Traditional Chinese Medicine, Shenyang, China, [3]College of Pharmacy, Liaoning University of Traditional Chinese Medicine, Dalian, China, [4]Department of Internal Medicine, Liaoning Provincial Corps Hospital of Chinese People's Armed Police Forces, Shenyang, China, [5]Science and Technology Branch, Liaoning University of Traditional Chinese Medicine, Shenyang, China, [6]NHC Key Laboratory of Male Reproduction and Genetics, Guangdong Provincial Reproductive Science Institute (Guangdong Provincial Fertility Hospital), Guangzhou, China, [7]Department of Internal Medicine, Fujian Provincial Corps Hospital of Chinese People's Armed Police Forces, Fuzhou, China, [8]Department of Endocrinology and Metabolism, The Fourth People's Hospital of Shenyang, Shenyang, China

Polycystic ovary syndrome (PCOS) is a universal endocrine and metabolic disorder prevalent in reproductive aged women. PCOS is often accompanied with insulin resistance (IR) which is an essential pathological factor. Although there is no known cure for PCOS, cangfudaotan (CFDT) decoction is widely used for the treatment of PCOS; nevertheless, the underlying mechanism is not clear. In this study, 40 Sprague-Dawley (SD) rats (female) were randomized to 4 groups, namely the control group, PCOS group, PCOS+CFDT group, and PCOS+metformin group. The rats in the control group were fed a normal-fat diet, intraperitoneally injected with 0.5% carboxymethyl cellulose (CMC, 1 mL/kg/d) for 21 days and orally given saline (1 mL/kg/d) for the next 4 weeks. The rats in the PCOS group, PCOS+CFDT group, and PCOS+Metformin group were fed a high-fat diet (HFD) and intraperitoneally injected with letrozole (1.0 mg/kg) for 21 days. During this period, we recorded the body weight, estrous cycles, and rate of pregnancy in all rats. We also observed the ovarian ultrastructure. Blood glucose indices, serum hormones, and inflammatory factors were also recorded. Then, we detected apoptotic and mitochondrial function, and observed mitochondria in ovarian granular cells by transmission electron microscopy. We also detected genes of ASK1/JNK pathway at mRNA and protein levels. The results showed that CFDT alleviated pathohistological damnification and apoptosis in PCOS rat model. In addition, CFDT improved ovarian function, reduced inflammatory response, inhibited apoptosis of granular cells, and inhibited the operation of ASK1/JNK pathway. These

findings demonstrate the occurrence of ovary mitochondrial dysfunction and granular cell apoptosis in PCOS. CFDT can relieve mitochondria-dependent apoptosis by inhibiting the ASK1/JNK pathway in PCOS rats.

KEYWORDS

polycystic ovary syndrome, granulosa cell, insulin resistance, mitochondrial dysfunction, cangfu daotan decoction, apoptosis

Introduction

Polycystic ovarian syndrome (PCOS) is a widespread endocrine and metabolic disorder, prevalent in reproductive aged women. In general, PCOS is characterized by hyperandrogenism, irregular menstrual cycle, abnormal ovarian function, follicular dysplasia (with multiple cystic ovarian follicles), and insulin resistance (IR) (1). PCOS is associated with IR in approximately 75% of cases (2). Hyperandrogenism, IR, hyperinsulinemia, and a variety of endocrine signals in the follicle can disturb follicular activation and growth in women with PCOS. These effects lead to the accumulation of small follicles around the ovary, polycystic morphology, and injury of follicular maturation, thereby leading to anovulation and infertility. As the main feature, IR refers to reduced insulin sensitivity. IR is a hallmark of metabolic dysfunction in patients with PCOS, and is considered a promoter of hyperandrogenism and chronic oligo- or anovulation (3, 4). Additionally, obesity-related inflammation may have potential implications for ovarian physiology due to the dysregulated adipokine secretion, thereby affecting insulin sensitivity. In adolescents with obesity, increased visceral adiposity is also associated with hormonal changes that impair the hypothalamus and the pituitary function and directly affect ovarian function (5, 6).

Metabolic abnormalities, chronic inflammation, and oxidative stress (OS) have been reported to be associated with IR in PCOS, and those features are related to mitochondrial dysfunction in PCOS (7, 8). Mitochondria are the critical energy controller and the fundamental source of cellular reactive oxygen species (ROS). Mitochondrial abnormalities have organism-wide manifestations that can lead to different metabolic disorders (9). Mitochondria are critical organelles for modulating OS. The abnormal mitochondrial genes and mitochondrial abnormalities in PCOS have been studied (10). Thus, it is essential to understand the mechanism of mitochondrial abnormalities in the pathogenesis of PCOS. However, the regulatory mechanism between mitochondrial abnormalities and PCOS is still unclear. Although there is no known cure for PCOS, traditional Chinese medicine (TCM) may offer a specific therapeutic effects for treating PCOS (11).

The Cang-fu-dao-tan (CFDT) decoction is a classic prescription of TCM for the treatment of PCOS. CFDT decoction is one of the most common prescriptions for PCOS patients in East Asian countries (12). According to TCM theories, the leading causes of PCOS-IR include turbid phlegm, blood stasis, stagnation of liver Qi, deficiency of kidney essence, and deficiency of spleen and kidney. The CFDT decoction comprises 16 medicinal herbs, aiming to solve the pathogenesis of phlegm dampness. A clinical study has reported that the CFDT decoction, used alone or in combination with other western medications, can be used in the treatment of women with PCOS (12), however the mechanism of action of this treatment remains unknown (11–13).

Abbreviations: PCOS, Polycystic ovary syndrome; IR, Insulin resistance; TCM, Traditional Chinese medicine; CFDT, Cang-fu-dao-tan; HFD, high-fat diet; CMC, carboxymethyl cellulose; FINS, fasting insulin; FBG, fasting blood glucose; EDTA, ethylene diamine tetraacetic acid; FSH, follicle stimulating hormone; LH, Luteinizing hormone; E_2, oestradiol; T, testosterone; IL-1β, Interleukin-1β; IL-6, interleukin-6; TNF-α, Tumor necrosis factors-α; CRP, C-reactive protein; ASK1, Apoptosis signal-regulating kinase 1; MAP3K, Mitogen-activated kinase kinase kinase; Mfn1/2, Mitofusin1/2; Drp1, Dynamin-related protein 1; OPA1, Optic atrophy 1; PGC-1α, PPARγ coactivator-1-α; RT-qPCR, Real-time quantitative Polymerase Chain Reaction; Cyt-c, cytochrome C; DAPI, 4',6-diamidino-2-phenylindole; DCFH-DA, 2',7'-Dichlorodihydrofluorescein diacetate; ATP, adenosine triphosphate; RCR, respiratory control rate; MMP, mitochondrial membrane potential; mPTP, mitochondrial permeability transition pore; HPLC, high-performance liquid chromatography; SD, Sprague-Dawley; ANOVA, One-way analysis of variance.

Materials and methods

Chemicals and reagents

The CFDT decoction (Beijing Kang-Ren-Tang Pharmaceutical Co., Ltd., Chengdu, China) was dissolved in heated (60°C) deionized water to obtain a 3.0 g/mL stock solution and stored at 4°C. Metformin and letrozole were purchased from Solarbio Life Sciences Co., Ltd. (China).

Chemical component analysis of the CFDT decoction

The CFDT decoction contained Cyperus rotundus (Dried radix of *Cyperus rotundus L*, Xiangfu in Chinese, 10 g), Citrus × aurantium L (Dried peel of *Citrus*, Chenpi in Chinese, 6 g), Atractylodes lancea (Thunb.) DC (Dried stem of *Atractylodes lancea*, Cangzhu in Chinese, 15 g), Pinellia ternata (Dried radix of *Pinellia ternata* (Thunb.) Breit., Banxia in Chinese, 9 g), Wolfiporia cocos (Dried sclerotium of Syagrus romanzoffiana (Cham.) Glassman, Fuling in Chinese, 12 g), Arisaema erubescens (Dried radix of *Arisaema erubescens* (Wall.) Schott, Tiannanxing in Chinese, 6 g), Astragalus membranaceus (Dried radix of *Astragalus membranaceus* (Fisch.) Bunge., Huangqi in Chinese, 15 g), and Fructus aurantii (Dried fructus of Gleditsia sinensis lam (Dried spine of *Gleditsia sinensis Lam*, Zaojiaoci in Chinese, 10 g). The main components of the CFDT decoction were analyzed through quadrupole-time-of-flight mass spectrometry (UPLC-QQQ-MS) combined with high-performance liquid chromatography (Chromatographic column: Agilent ZORBAX SB-C18 column (100 × 2.1 mm, 1.9 μm); Injection quantity: 5 μL; Flow rate: 0.3 ml/min; Temperature: 20 ℃; Authenticated with standards). The process was guided by positive and negative ionization modes (Gas Temp: 325 ℃; Gas Flow: 7L/Min; Sheath Gas Temp: 350 ℃; Sheath Gas Flow: 11L/Min; Scan: 100–1500 Da; Fragment: 80–185 V; eV: 4–80 eV). The data were analyzed using SCIEX OS software. The compounds were identified according to the mass spectrometry data and matched to the TCM MS/MS Library.

Experimental animals

40 female and 16 male Sprague-Dawley (SD) rats (age, 8 weeks; body weight, 180–220 g) were acquired (Liaoning Changsheng Biotechnology Co., Ltd.; production license: SCXK (Liao) 2015–0001) and housed in cages at 20 ± 3°C temperature and 45–65% humidity, with cycles of 12-h light/12-h dark (lights off, 18:00 h). All of the rats were given food and water ad libitum.

Animal processing procedures and the experimental design were authorized by the Ethical Committee of Animal Handling (2021-210, March 8, 2021) of Liaoning University of Traditional Chinese Medicine, Shenyang, China. We complied with the guidelines of Use and Care of Laboratory Animals published by the US National Institutes of Health and tried our best to decrease the number of rats and alleviate their suffering. At the same time, we tried to provide a

better environment for rats in this study.

40 female rats were randomly divided into four groups, namely, control group, PCOS group, PCOS+CFDT group, and PCOS +Metformin group. The rats in the control group were fed a normal-fat diet, intraperitoneally injected with 0.5% carboxymethyl cellulose (CMC, 1 mL/kg/d) for 21 days, and orally given saline (1 mL/kg/d) for the next 4 weeks. To build the PCOS rat model: the rats were fed a high-fat diet (HFD) and intraperitoneally injected with 1.0 mg/kg letrozole (1 mL/kg/d, dissolved in 0.5% CMC) for 21 days (13, 14). During this period, we recorded all rats' body weights and estrous cycles. Vaginal smears were used to evaluate the onset of PCOS. The PCOS rats were further randomly divided into three groups, namely, PCOS group (intragastrically administered saline), PCOS+CFDT group (intragastrically administered the CFDT decoction, 15 g/kg/d), and PCOS+Metformin group (intragastrically administered metformin hydrochloride, 50 mg/kg/d) for 4 weeks. The dose of CFDT in our study is similar to that in Wang' study (13). The constituents of the HFD included 13% fiber, 44% carbohydrates, 11% unsaturated fat, 25% total fat, and 18% protein, ash and other ingredients.

Vaginal smears were analyzed every morning to observe the estrous cycle (Estrous interval period was mainly characterized by white blood cells; the proestrus period was mainly characterized by nuclear epithelial cells; the estrous period was mainly characterized by keratinized epithelial cells; the late estrous period was characterized by keratinized epithelial cells and white blood cells) (Figure 1). After the last irrigation, blood from the abdominal aorta of each group was collected on the non-estrus period with anesthesia (isoflurane *via* inhalation anesthesia) and oophorectomy. The rats were euthanized with the blood collected. Samples were permitted to coagulate for approximately 1 h at room temperature; then, serum was collected *via* centrifugation (2000 rpm/min) for 20 min and stored at -80°C for use. The ovarian tissues were fixed with 4% paraformaldehyde with the rest preserved at -80°C for expression analysis at genes and protein levels. The other four female rats of each group were mated with male rats at a ratio of 1:1 for 12 h (15). A mixture of sperm and vaginal smears on the following morning led to successful pregnancy, which was considered the 0.5th day of gestation. The pregnant rats were executed on the 15.5th day of gestation (15).

Blood glucose detection and IR calculation

After fasting for 12 h, the rats were weighed. Tail vein blood was collected to measure fasting insulin (FINS) and fasting blood glucose (FBG) levels. HOMA-IR was formulated as IR = FBG (mmol/L) × FINS (mU/L)/22.5.

The rats were euthanized. Then, blood from the abdominal aorta was collected. To obtain serum, blood was centrifuged at 3000 rpm (15 min) and then stored at –80°C. The ovaries were harvested, weighed, and then measured with a calliper.

Ovarian index

The rats were euthanized. The ovaries were harvested and placed in a prechilled medium containing 1 mM EDTA, 10 mM

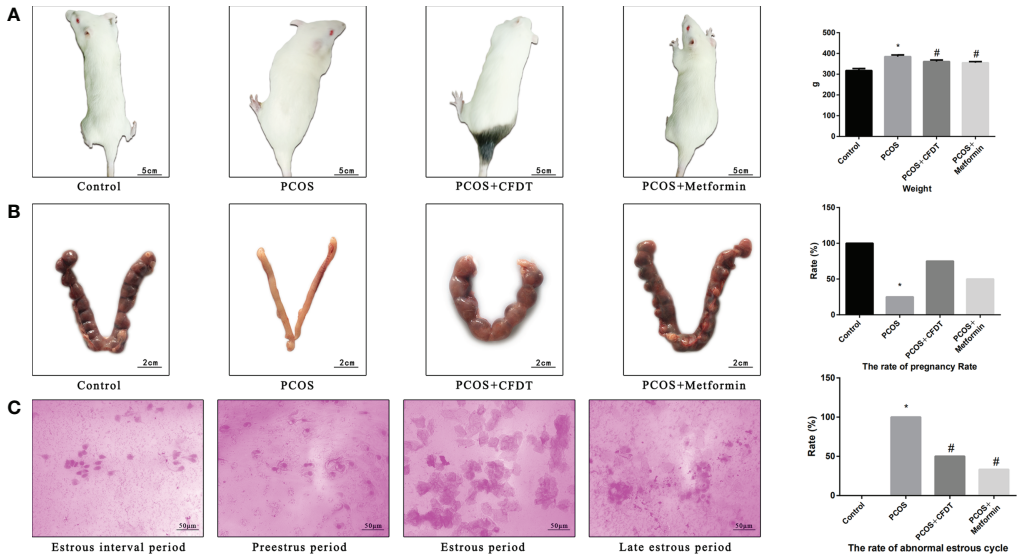


FIGURE 1
CFDT decoction improved the weight, rate of pregnancy, and the rate of abnormal estrous cycle in PCOS rats model. Rats were treated with CFDT decoction (15.0 g/kg.d) and metformin (50 mg/kg) followed by feeding with HFD and intraperitoneal injection with of letrozole (1 ml/kg/d) for 21 days. **(A)** Weight; **(B)** The rate of pregnancy (Female rats of each groups were sent to mate with male rats with a ratio of 1:1 for 12 h. The mixture of sperm and vaginal smears were seen on the next morning indicated the success of pregnancy, and this was considered as the 0.5th day of gestation. The pregnant rats were euthanized on the 13.5th day of the gestation) (n=4); **(C)** The rate of abnormal estrous cycle, estrous cycle of the rats (Estrous interval period: Vagina smear with white blood cells mainly; Preestrus period: With nuclear epithelial cells mainly; Estrous period: With Keratinized epithelial cells mainly; Late estrous period: See Keratinized epithelial cells and white blood cells) (n=6). Data are shown as mean ± SD. $^{*}p < 0.05$ versus control group, $^{\#}p < 0.05$ versus PCOS group, $^{\triangle}p < 0.05$ versus PCOS+ CFDT group. δ The groups between the PCOS+ CFDT and PCOS+metformin do not have statistical difference

Tris-HCl, and 250 mM sucrose, with pH of 7.4. Fatty tissues around the ovaries were removed. Then, the ovaries were weighed. The ovarian index was calculated as the ratio between the wet weight of bilateral ovaries (mg) and body weight (g) (16).

Histological assessment of ovarian tissues

The ovarian tissues were fixed with paraformaldehyde (4%) for 24 h, dehydrated with different concentrations of ethanol, and then embedded in paraffin. Ovarian tissue blocks were cut to generate sections (5-μm-thick) (17). The cross-sections were stained in hematoxylin-eosin and then viewed with a light microscope (Olympus, CX33, Japan). All follicular phases (primary follicles, secondary follicles, and atretic follicles) and the corpora lutea were determined.

Enzyme-linked immunosorbent assays

Arterial blood samples were used in enzyme-linked immunosorbent assays (ELISA) to measure follicle stimulating hormone [FSH, Sangon Biotech Co., Ltd. (D731057-0096)], luteinizing hormone [LH, Sangon Biotech Co., Ltd. (D731015-0096)], estradiol [E_2, Biotech Co., Ltd. (EK7003)], testosterone [T, Biotech Co., Ltd. (EK7014)], interleukin-1β [IL-1β, Solarbio Life Sciences Co., Ltd. (SEKR-0002)], tumor necrosis factors-α [TNF-α, Solarbio Life Sciences Co., Ltd. (SEKR-0009)], C-reactive protein [CRP, Solarbio Life Sciences Co., Ltd. (SEKR-0017)], and interleukin-6 [IL-6, Solarbio Life Sciences Co., Ltd. (SEKR-0005)].

Electron microscopy to observe mitochondria and cell apoptosis

Bilateral ovaries were harvested, minced into 1 mm^3 fragments, fixed in 4% glutaraldehyde at 4°C for 2 h, and washed in 0.1 M sodium dimethylarsenate three times. The samples were postfixed with osmium tetroxide (1%) at 4°C for 90 min and washed with distilled water for three times, followed by dehydration in ethanol and acetone series. Finally, the samples were dehydrated in 100% propionaldehyde for two times. The samples were soaked in embedding agents at 1:3, 1:1, and 3:1. The samples were polymerized at 35°C (24 h), 45°C (24 h), and 60°C (24 h). The samples were trimmed with an ultrathin slicer (70–90 nm) to create a smooth surface. Double staining was carried out with lead citrate and uranyl acetate. The sections were observed using an electron microscope (H-7650; Hitachi, Tokyo, Japan).

Apoptosis assay

The terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay was used to assess apoptosis. For TUNEL staining, 5-μm-thick sections were employed. After deparaffinization and rehydration, the sections were treated with proteinase K (10 μg/mL) for 15 min and incubated with a reaction mixture at 37°C for 60 min in the dark. Following washing, the nuclei were stained with 4',6-diamidino-2-phenylindole. The sections were observed with a fluorescence microscope (Canon, Japan). Eight random visual fields per sample were observed in a blinded manner to assess the number of TUNEL-positive cells.

Caspase-3/9 activity

The AcDEVD-7-pNA substrate (Solarbio Co., Ltd.) was used to detect the caspase-9/3 activity. A 10 mg fragment of ovarian tissue was combined with the reaction buffer and then was incubated at 37°C for 2 h. A fluorimeter was used to quantify the enzyme-catalyzed release at a wavelength of 405 nm.

Preparation of mitochondria suspension and detection of mitochondrial function

The rats were euthanized. Ovaries were harvested, placed in a prechilled medium containing Tris-HCl (10 mM), sucrose (250 mM), and EDTA (1 mM), with pH of 7.4, and homogenized on ice. The samples were consecutively centrifuged at 700 × g (10 min) and 7000 × g (10 min). Then, the mitochondria pellets were resuspended in 5 mL buffer mentioned above and then centrifuged (7000 × g) for 10 min twice. The highly pure mitochondrial pellets were resuspended in sucrose (20 mM), KH_2PO_4 (10 mM), $MgCl_2$ (2 mM), KCl (100 mM), 5 mM HEPES (5 mM), and EDTA (1 mM). The BCA Protein Assay Kit was employed to measure the concentration of protein with the concentration of protein adjusted to 100–1000 μg/mL. The mitochondrial suspensions were used for measuring the MMP (18), opening of mitochondrial permeability transition pore (mPTP) (19), generation of ROS, degree of damaged mtDNA (20), mitochondrial oxygen consumption rate (21), respiratory control rate (RCR) (21), mitochondrial respiratory chain complex enzyme (I, II, III, IV, and V) levels (22), and the adenosine triphosphate (ATP) level (22).

RNA extraction, cDNA dynthesis, and real-time qPCR

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was employed to separate the total RNA. A spectrophotometer was used to assess the RNA quality at a wavelength of 260 nm. The RNA was reverse-transcribed to cDNA using the M-MLV Reverse Transcriptase Kit (Promega, Madison, WI, USA) following to the manufacturer's instructions. After that, real-time qPCR was performed on a Rotor-Gene Q Sequence Detection System (QIAGEN, Germany) using SYBR Premix Ex Taq II (TakaraBio) in line with the manufacturer's instructions (23). The PCR requirements were as follows: 95°C (10 min) and 95°C (10 s), followed by 40 cycles at 60°C (15 s); 72°C (20 s); and 72°C (10 min). The relative mRNA expression of each gene was calculated using the GAPDH (24). Table 1 listed the primer sequences.

Protein detection

RIPA Buffer was used to extract total proteins from ovaries. The BCA Protein Assay Kit was employed to detect the concentration of protein. Equivalent amounts of total protein were subjected to 8–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteins were transferred to PVDF membranes. After blocking with skim milk, the membranes were incubated overnight with an anti-GAPDH, anti-ASK1, anti-p-ASK1, anti-JNK, anti-p-JNK, anti-Bcl-2, anti-Bax, anti-caspase-9/3, anti-Cyt-c, anti-OPA1, anti-Mfn1, anti-Mfn2, anti-Drp1, anti-Fis1, or anti-PGC1a antibody (Table 2). After that, the membranes were incubated with a secondary HRP-conjugated goat anti-rabbit antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The Enhanced Chemiluminescence Kit (Thermo Fisher Scientific) was used to visualize the proteins. Alpha View Software (Cell Biosciences, Preston VIC, Australia) was used for densitometric analysis.

Statistical analysis

Statistical analysis was performed using SPSS 17.0 Software (SPSS Inc. Chicago, IL, USA). The data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to compare four independent groups. Two-to-two comparison among groups was employed to analyze the variance. The LSD-t test was employed to compare multiple comparisons among four groups. We defined $p < 0.05$ as having a statistically significant difference.

Results

Chemical components of the CFDT decoction

The chemical components of the CFDT decoction were measured *via* UPLC-QQQ-MS. They were as follows: narirutin (content: 24.7403 μg/g; CAS: 14259-46-2), chrysin-7beta-monoglucoside (content: 0.74 μg/g; CAS: 31025-53-3), liquiritin (content: 99.97 μg/g; CAS: 551-15-5), nobiletin (content: 39.93 μg/g; CAS: 478-01-3), hesperetin (content: 96.28 μg/g; CAS: 520-33-2), tanshinone IIA (content: 4.92 μg/g; CAS: 568-72-9), calycosin (content: 1.30 μg/g; CAS: 20575-57-9), formononetin (content: 1.83 μg/g; CAS: 485-72-3), stachydrine (content: 4.36 μg/g; CAS: 471-87-4), betaine (content: 61.97 μg/g; CAS: 107-43-

10.3389/fendo.2022.962154

TABLE 1 Sequence of primers for RT-PCR and long PCR.

Target Gene	Primer Sequence	Size (bp)	Tm (°C)
OPA1	Forward: 5'-TGGTTCGAGAGTCGGTTGAA-3'	189	56
	Reverse: 5'- CCTCCCAGTGCTTTGGAGTA -3'		56
Mfn1	Forward: 5'-GGGAAGACCAAATCGACAGA-3'	152	57
	Reverse: 5'-CAAAACAGACAGGCGACAAA-3'		57
Mfn2	Forward: 5'-GAGAGGCGATTTGAGGAGTG-3'	165	58
	Reverse: 5'-CTCTTCCCGCATTTCAAGAC-3'		56
Drp1	Forward: 5'-GCCCGTGGATGATAAAAGTG-3'	215	56
	Reverse: 5'-TGGCGGTCAAGATGTCAATA-3'		56
Fis1	Forward: 5'-AGATGGACTGGTAGGCATGG-3'	84	56
	Reverse: 5'-GACACAGCCAGTCCAATGAG-3'		56
PGC-1α	Forward: 5'-GGACGAATACCGCAGAGAGT-3'	201	59
	Reverse: 5'-CCATCATCCCGCAGATTTAC-3'		56
Tfam	Forward: 5'-TCACCTCAAGGGAAATTGAAG-3'	241	55
	Reverse: 5'-CCCAATCCCAATGACAACTC-3'		56
Long Fragment	Forward:5'-AAAATCCCCGCAAACAATGACCACCC-3'	13400	72
	Reverse: 5'-GGCAATTAAGAGTGGGATGGAGCCAA-3'		72
Shrot Fragment	Forward: 5'-CCTCCCATTCATTATCGCCGCCCTGC-3'	235	60
	Reverse: 5'-GTCTGGGTCTCCTAGTAGGTCTGGGAA-3'		60
Bax	Forward: 5'-GCGATGAACTGGACAACAAC-3'	200	57
	Reverse: 5'-GATCAGCTCGGGCACTTTAG-3'		58
Bcl-2	Forward: 5'-CGAGTGGGATACTGGAGATGA-3'	236	58
	Reverse: 5'- GACGGTAGCGACGAGAGAAG-3'		59
Caspase-3	Forward: 5'-CCCATCACAATCTCACGGTAT-3'	195	57
	Reverse: 5'-GGACGGAAACAGAACGAACA-3'		58
Caspase-9	Forward: 5'-GCCTCTGCTTTGTCATGGAG-3'	181	56
	Reverse: 5'-AGCATGAGGTTCTCCAGCTT-3'		56
ASK1	Forward: 5'-ACAATGAGCAGACGATTGGC-3'	168	56
	Reverse: 5'-CAGCAAGCCTCTTGGATGTC-3'		56
JNK	Forward: 5'-TGGATTTGGAGGAGCGAACT-3'	69	56
	Reverse: 5'-TCACTGCTGCACCTAAAGGA-3'		56
Cyc-c	Forward: 5'-GGACAGCCCCGATTTAAGTA-3'	121	57
	Forward: 5'-TCAATAGGTTTGAGGCGACAC-3'		58
GAPDH	Forward: 5'- AGGTCGGTGTGAACGGATTTG -3'	20	58
	Reverse: 5'- GGGGTCGTTGATGGCAACA-3'		58

7), astragaloside IV (content: 80.79 μg/g; CAS: 84687-43-4), acteoside (content: 26.25 μg/g; CAS: 61276-17-3), rosmarinic acid (content: 6.00 μg/g; CAS: 20283-92-5), and rhein (content: 1.64 μg/g; CAS: 478-43-3). Standard curve of compounds were showed in Table 3.

The CFDT decoction improved the weight, rate of pregnancy, and the rate of the abnormal estrous cycle in the PCOS rat model

To assess the severity of PCOS and the protective effects of the CFDT decoction, we examined the body weight (Figure 1), rate of pregnancy (Figure 1), and rate of the abnormal estrous cycle (Figure 1). The body weight increased in PCOS group compared to control group ($p < 0.05$), but decreased after administration of the CFDT decoction and metformin ($p < 0.05$) (Figure 1). The rate of pregnancy decreased in the PCOS group compared to the control group ($p < 0.05$) but increased after administration of the CFDT decoction and metformin ($p < 0.05$) (Figure 1). Compared to the control group, the rate of abnormal estrous cycles increased in the PCOS group ($p < 0.05$), but decreased after administration of the CFDT decoction and metformin ($p < 0.05$). Daily vaginal smears displayed that the rats in the control group had regular estrous cycles (4–5 days) that included proestrus, estrus, metestrus, and diestrus phases, whereas the rats in the PCOS group showed irregular estrous

10.3389/fendo.2022.962154

TABLE 2 Antibodies used in the study.

Antibodies	Manufacturer	Catalogue No.	Observed MW	Dilution
Anti-ASK1	Proteintech	67072-1-1g	110 KDa	1:2000
Anti-p-ASK1	Proteintech	28846-1-AP	120 KDa	1:1000
Anti-JNK	Proteintech	10176-2-AP	46KDa	1:2000
Anti-p-JNK	Proteintech	80024-1-RR	46 KDa	1:2000
Anti-Bcl-2	Proteintech	26593-1-AP	26 KDa	1:1000
Anti-Bax	Proteintech	50599-2-1g	26 KDa	1:6000
Anti-Caspase-3	Proteintech	19677-1-AP	32 KDa	1:1000
Anti-Caspase-9	Proteintech	10380-1-AP	47 KDa	1: 500
Anti-Cyt-c	Proteintech	12245-1-AP	13 KDa	1:3000
Anti-OPA1	Proteintech	66583-1-Ig	100 KDa	1:1000
Anti-Mfn1	Proteintech	13798-1-AP	86 KDa	1:500
Anti-Mfn2	Proteintech	12186-1-AP	86 KDa	1:3000
Anti-Drp1	Proteintech	10656-1-AP	27 KDa	1:1000
Anti-Fis1	Proteintech	66635-1-Ig	15 KDa	1:3000
Anti-PGC1a	Proteintech	66369-1-1g	100 KDa	1:5000
Anti-GAPDH	Proteintech	60004-1-1g	36 KDa	1:20000

TABLE 3 Standard curve of compounds.

Compounds	LLOQ (μg·mL-1)	linear ranger (μg·mL-1)	Linear regression equation	Correlation coefficient (r)
Narirutin	0.115	0.1148-58.8	Y=0.078X+0.0027	0.9998
Calycosin-7-O-β-D-glucoside	0.011	0.011-14.71	Y=6.1821X-0.7872	0.9990
Liquiritin	0.011	0.011-7.35	Y=0.4695X- 0.0299	0.9995
Nobiletin	0.011	0.011-3.68	Y=106.77X-5.8926	0.9970
Hesperetin	0.011	0.011-7.35	Y=0.3739X-0.1658	0.9988
Tanshinone IIA	0.011	0.011-58.8	Y=0.228X-0.0075	0.9989
Calycosin	0.011	0.011-3.68	Y=7.4149X-0.0274	0.9979
Formononetin	0.011	0.011-0.92	Y=3.3123X-0.0241	0.9997
Stachydrine	0.011	0.011-7.35	Y=0.1754X+0.0506	0.9998
Betaine	0.114	0.114-58.8	Y=0.2471X+1.0458	0.9983
Dioscin	0.011	0.011-58.8	Y=0.0088X+0.0114	0.9996
Astragaloside IV	0.115	0.115-58.8	Y=0.0003X+0.0006	0.9951
Acteoside	0.92	0.92-58.8	Y=0.0051X-0.0027	0.9980
Rosmarinic acid	0.011	0.0114-58.8	Y=0.0266X-0.0211	0.9990
Rhein	0.011	0.0114-58.8	Y=0.036X+0.0319	0.9988

cycles, in diestrus period, showing predominantly leukocytes (Figure 1).

The CFDT decoction improved the ovarian index, ovarian diameter, follicular phases, serum hormones, blood glucose indices, and inflammatory cytokine levels in the PCOS rat model

We examined the ovarian diameter and index (Figure 2), follicular phases (Figure 2), serum hormone levels (Figure 2), blood glucose indices (Figure 2), and inflammatory cytokine levels (Figure 2). The ovarian diameter and index increased in the PCOS group compared to control group ($p < 0.05$), but decreased after administration of the CFDT decoction and metformin ($p < 0.05$) (Figure 2). Light microscopy results showed the primary, secondary, and atretic follicles, the corpora lutea, and the normal granulosa cells in the ovaries of control rats. By contrast, early-stage and atretic follicles were observed in rats with PCOS, in addition to many large cysts and few granulosa cells. The corpora lutea and granulosa cells were visible after the administration of the CFDT decoction and metformin (Figure 2). Serum LH, T, and E_2 levels increased in

10.3389/fendo.2022.962154

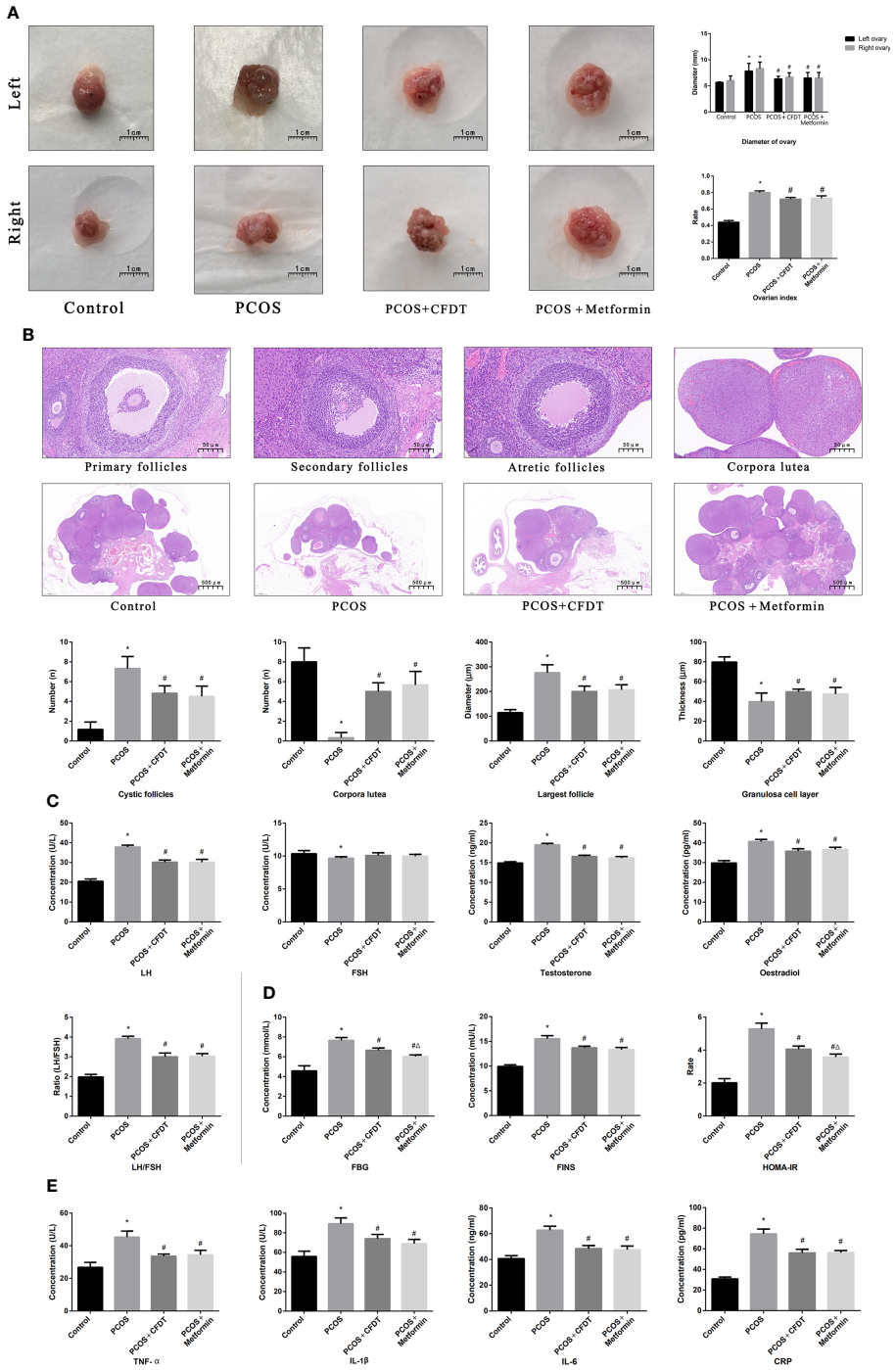


FIGURE 2
CFDT decoction improved the ovarian index, ovarian diameter, the number of all phases of follicles, serum hormones, blood glucose indices, and inflammatory factors in PCOS rats model. Rats were treated with CFDT decoction (15.0 g/kg.d) and metformin (50 mg/kg) followed by feeding with HFD and intraperitoneal injection with of letrozole (1 ml/kg/d) for 21 days. **(A)** Ovarian index (Ovarian index= wet weight of bilateral ovaries (mg)/body weight (g)×100%, the size of the ovary in PCOS group was significantly reduced compared with the control group) and ovarian diameter, the scale bars represent a length of 1 cm on histology; **(B)** Histological assessment of the ovarian tissue using hematoxylin-eosin (HE) staining [All phases of follicles (primary follicles, secondary follicles, and atretic follicles) and corpora lutea were counted], the scale bars represents a length of 20 μm on histology; **(C)** Serum hormones (LH, FSH, T, and E_2); **(D)** Blood glucose indices (FBG, FINS, and HOMA-IR); **(E)** Serum inflammatory factors (TNF-α, IL-1β, IL-6, and CRP). Data are shown as mean ± SD. *$p < 0.05$ versus control group, $^{\#}p < 0.05$ versus PCOS group, $^{\triangle}p < 0.05$ versus PCOS+ CFDT group. (n=6).

the PCOS group compared to the control group ($p < 0.05$), but decreased after the administration of the CFDT decoction and metformin ($p < 0.05$). No difference in the FSH level was detected among the four groups (Figure 2) ($p > 0.05$). FBG, FINS, and HOMA-IR increased in PCOS group ($p < 0.05$), but decreased after the administration of the CFDT decoction and metformin ($p < 0.05$), and metformin was more effective than the CFDT decoction ($p < 0.05$) (Figure 2). TNF-α, IL-1β, IL-6, and CRP levels increased in the PCOS group ($p < 0.05$), but decreased after the administration of the CFDT decoction and metformin ($p < 0.05$) (Figure 2).

The CFDT decoction improved mitochondrial function in the PCOS rat model

The mitochondrial function was assessed by electron microscopy. The results showed intact mitochondria but damaged mitochondria, with membrane swelling and rupture, in the PCOS group. The percentage of damaged mitochondria of ovaries was higher in the PCOS group compared to the control group ($p < 0.05$); however, it decreased after the administration of the CFDT decoction and metformin (Figure 3).

We measured the MMP (ratio of red/green), the opening of mPTP (%), ROS production, degree of mtDNA damage, oxygen consumption rate, RCR, the ATP level, and the activity of mitochondrial respiratory chain complex enzymes (I, II, III, IV, and V) to further evaluate the mitochondrial function of ovaries. ROS production and the opening of mPTP (%) increasedin the PCOS group compared to the control group ($p < 0.05$). Again, both parameters decreased after the administration of the CFDT decoction and metformin ($p < 0.05$). MMP (ratio of red/green), mitochondrial oxygen consumption rate, and RCR decreased in the PCOS group compared to the control group ($p < 0.05$), but increased after administration of CFDT decoction and metformin ($p < 0.05$). The ratio of long-to-short fragments of mtDNA was measured by Real-time qPCR. The ratio of long/short fragments decreased in the PCOS group compared to the control group ($p < 0.05$). However, administration of CFDT decoction and metformin increased the ratio ($p < 0.05$). The activity of mitochondrial respiratory chain complex enzymes (I, II, III, IV, and V) and the ATP level decreased in the PCOS group compared to the control group ($p < 0.05$). However, administration of CFDT decoction and metformin increased these indices ($p < 0.05$) (Figure 3B).

The CFDT decoction affected the mitochondrial function of ovarian in the PCOS rat model

The genes expression was examined by qPCR (mRNA) and Western blotting (protein). OPA1, Mfn1, and Mfn2 were selected as markers of mitochondrial biogenesis, with PGC-1α as a marker of mitochondrial fusion and Drp1 and Fis1 as markers of mitochondrial fission, respectively. OPA1, Mfn1, Mfn2, and PGC-1α expression decreased in the PCOS group compared to the control group ($p < 0.05$), but increased after administration of CFDT decoction and metformin ($p < 0.05$). Drp1 and Fis1 expression increased in PCOS group compared to the control group ($p < 0.05$), but decreased after the administration of the CFDT decoction and metformin ($p < 0.05$) (Figures 4A, B).

CFDT decoction modulated the ASK1/JNK pathway in the PCOS rat model

The levels of target genes in the ASK1/JNK pathway were examined by qPCR (mRNA) and Western blotting (protein). Caspase-9/3, Bax, ASK1, JNK, and Cty-C mRNA expression increased in the PCOS group compared to the control group ($p < 0.05$), whereas the mRNA level expression of these indices decreased after treatment with the CFDT decoction and metformin ($p < 0.05$). PCOS reduced the mRNA level expression of Bcl-2 compared with the control group ($p < 0.05$). The mRNA level expression of Bcl-2 increased after administration of CFDT decoction and metformin ($p < 0.05$). CFDT decoction and metformin inhibited ASK1 and JNK phosphorylation (decreased p-ASK1/ASK1 and p-JNK/JNK) (Figure 5B). The number of apoptotic granulosa cells in the PCOS group increased compared to the control group ($p < 0.05$). However, treatment with the CFDT decoction and metformin decreased the number of apoptotic cells (Figure 5D). Caspase-9/3 activity in ovaries of the PCOS group was higher than that of the control group ($p < 0.05$). However, administration of CFDT decoction and metformin decreased the activity of caspase-9/3 (Figure 5).

Discussion

Our main finding is that CFDT decoction can reduce granulosa cell apoptosis in the PCOS rat model. We also defined the potential protective effects and the mechanism of action of CFDT decoction. Mitochondrial dysfunction may induce granulosa cell apoptosis, whereas the CFDT decoction can reduce apoptosis by improving mitochondrial function *via* inhibition of the ASK1/JNK pathway. To the best of our knowledge, this is the first study to investigate the treatment mechanism of CFDT decoction. To examine the protective mechanism of CFDT decoction, we used HFD and letrozole to establish a PCOS rat model.

The incidence of PCOS continues to increase in females at reproductive age, but the pathogenesis of PCOS remains complex and unclear. There is increasing evidence that IR is related to the pathogenesis of PCOS (25–27). In this study, in addition to the obviously increased HOMA-IR in PCOS rat

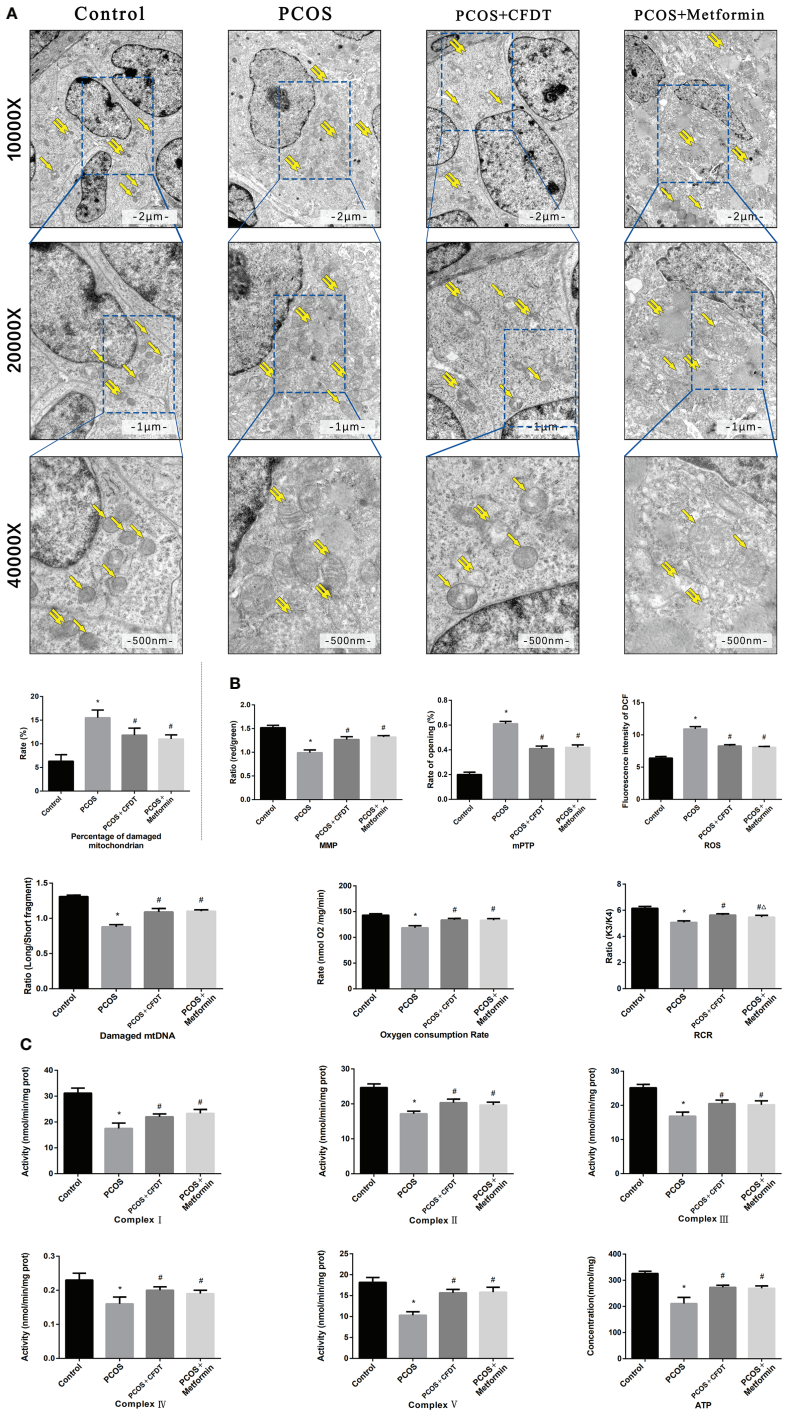


FIGURE 3
CFDT decoction improved mitochondrial function in PCOS rats model. Rats were treated with CFDT decoction (15.0 g/kg.d) and metformin (50 mg/kg) followed by feeding with HFD and intraperitoneal injection with of letrozole (1 ml/kg/d) for 21 days. **(A)** Electron microscope pictures (10,000×; 20,000×; 40 000×) of the ovary in PCOS rats, the scale bars represents a length of 2 μm, 1 μm, and 500 nm on histology respectively. Abnormal mitochondrial (paired yellow arrow) morphology showed mitochondrial membrane rupture or swellings, and normal mitochondrial (single yellow arrow) morphology type showed smooth mitochondrial membrane and distinct inner carinulae and percentage of damaged mitochondria; **(B)** The MMP (ratio of red/green), the opening of mPTP (%), the mitochondrial ROS, the mtDNA damage (ratio of long/short fragments), the mitochondrial RCR, mitochondrial oxygen consumption rate; **(C)** The mitochondrial respiratory chain complex enzymes (I, II, III, IV, and V) and ATP were recorded above. Data are shown as mean ± SD. $^{*}p < 0.05$ versus control group, $^{\#}p < 0.05$ versus PCOS group, $^{\triangle}p < 0.05$ versus PCOS+ CFDT group. (n=6).

10.3389/fendo.2022.962154

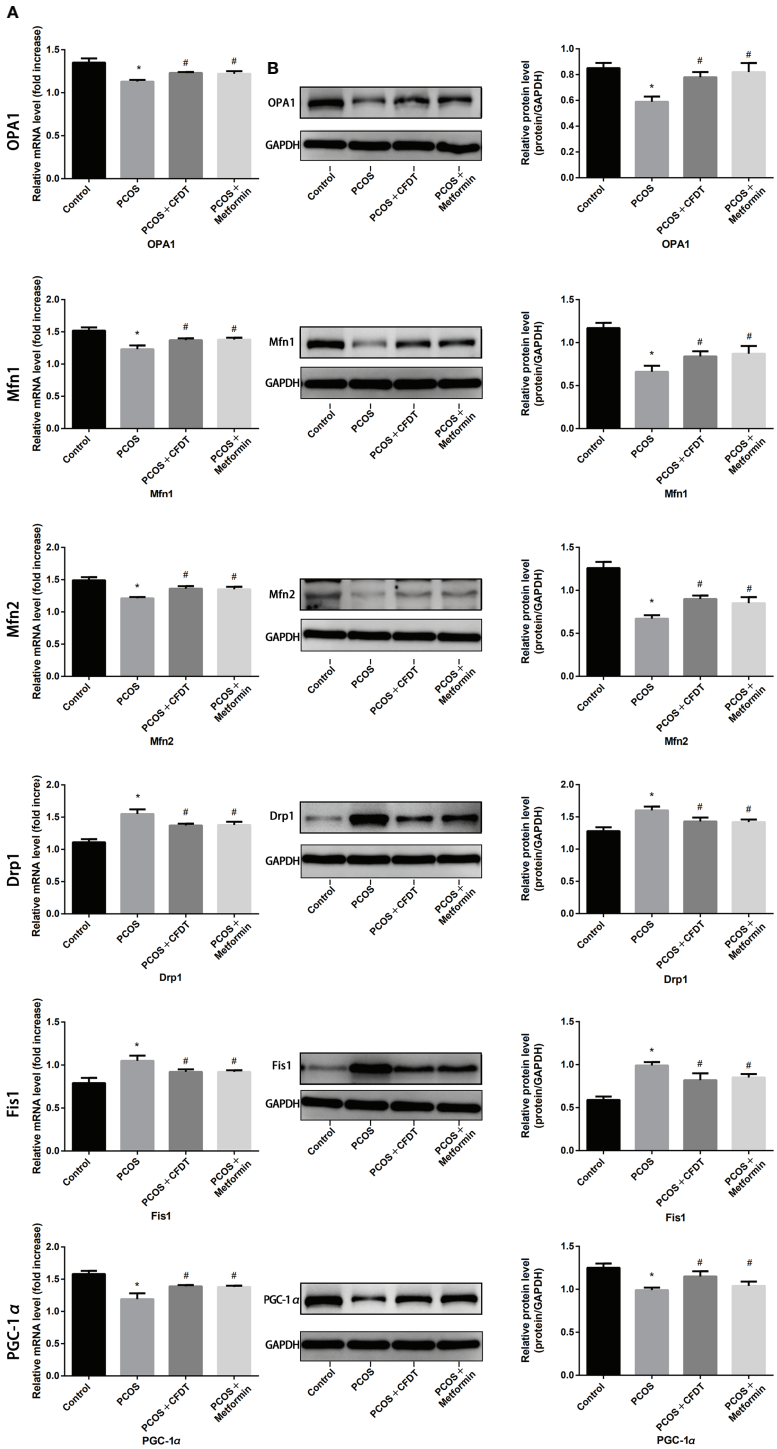


FIGURE 4
CFDT decoction improved mitochondrial biogenesis and dynamics in PCOS rats model. Rats were treated with CFDT decoction (15.0 g/kg.d) and metformin (50 mg/kg) followed by feeding with HFD and intraperitoneal injection with of letrozole (1 ml/kg/d) for 21 days. We used real-time qPCR and western blot to detect mitochondrial biogenesis and dynamics. We chose OPA1, Mfn1, and Mfn2 to represent mitochondrial biogenesis function, PGC-1α to represent the dynamic mitochondrial fusion, and Drp1 and Fis1 to represent mitochondrial fission. The expression of OPA1, Mfn1, Mfn2, PGC-1α, Drp1, and Fis1 at mRNA **(A)** and protein **(B)** levels. Data are shown as mean ± SD. *$p < 0.05$ versus control group, $^{\#}p < 0.05$ versus PCOS group, $^{\triangle}p < 0.05$ versus PCOS+ CFDT group. (n=6).

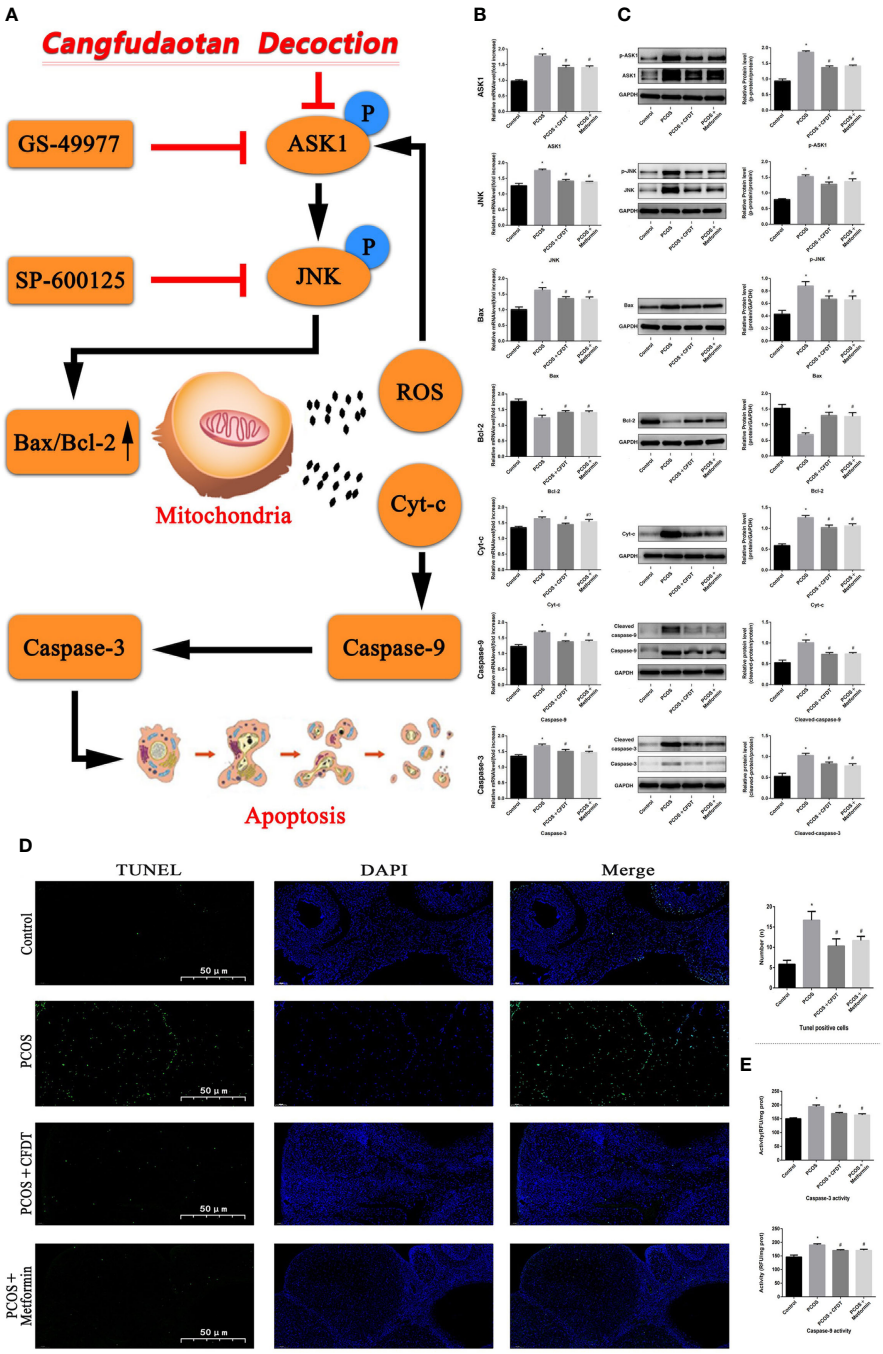


FIGURE 5
CFDT decoction improved mitochondrial function and inhibited apoptosis *via* inhibiting the ASK1/JNK pathway. Rats were treated with CFDT decoction (15.0 g/kg.d) and metformin (50 mg/kg) followed by feeding with HFD and intraperitoneal injection with of letrozole (1 ml/kg/d) for 21 days. **(A)** Graphical abstract: Mitochondrial dysfunction of the ovarian granular cells occurs following PCOS and can lead to granular cells apoptosis. CFDT decoction can relieve granular cell apoptosis by improving mitochondrial function *via* inhibiting the ASK1/JNK pathway *in vivo*. We suggested that CFDT decoction be used as a potential therapeutic agent for PCOS and mitochondria are seen as a potential therapeutic target. We used real-time qPCR and western blot to detect the target genes of the ASK1/JNK pathway at mRNA **(B)** and protein **(C)** levels. **(D)** TUNEL positive cells, the scale bars represent a length of 50 μm on histology; **(E)** The activity of caspase-9/3. Data are shown as mean ± SD. $^{*}p < 0.05$ versus control group, $^{\#}p < 0.05$ versus PCOS group, $^{\triangle}p < 0.05$ versus PCOS+ CFDT group. (n=6).

model, the serum T level, and LH/FSH ratio, significantly increased, which is consistent with results from an earlier study (27). The increase in insulin level can up-regulate androgen levels, thereby inducing hyperandrogenism and up-regulating androgen levels in fatty tissues of PCOS patients *via* increased AKR1C3 activity (26). An irregular estrous cycle (prolonged diestrus period) of the PCOS group was detected based on vaginal smears. Histological analysis showed that the morphology of ovaries was characterized by fewer granulosa cells and oocytes and cystic dilatation of the follicles in the PCOS group, compared with the control group. Hyperandrogenism can induce early luteinization of granulosa cells, inhibit follicular development and growth, and trigger follicle atresia, thereby leading to poor ovulation or anovulation, which is all consistent with the earlier study (28). However, we observed an increased E_2 level in the PCOS rat model. The reason for this phenomenon may be that PCOS causes ovarian injury such that the negative feedback regulates the pituitary gland to increase the secretion of E_2. In addition, we believe that the created PCOS rat model reflects the early stage of PCOS, while clinical patients often suffer from the disease for a long time, where negative feedback regulation is weakened.

After the administration of the CFDT decoction, FPG and HOMA-IR decreased, indicating that the CFDT decoction improved IR in the PCOS rat model. In the PCOS+ CFDT group, serum LH and T levels decreased and the damage of ovarian morphology was alleviated. The effect was similar to that of metformin, which is consistent with the earlier study (27).

As a low-grade chronic inflammatory disease, PCOS often associated with permanently elevated levels of inflammatory markers (TNF-α, CRP, IL-6, IL-8, and IL-18) in patients, and inflammatory markers can induce mitochondrial dysfunction (29, 30). Multiple inflammatory factors, such as CRP, IL-6, TNF-α, IL-8, IL-1, MCP, and CCL_2, increase OS, and inflammatory immune cell infiltration can lead to a low-grade inflammatory response (31). Thus, relieving inflammation may be necessary for treating PCOS. In this study, we showed that the serum levels of IL-1, TNF-α, CRP, and IL-6 increased in the PCOS group, but decreased after the CFDT decoction and metformin administration.

The granulosa cells bring growth factors and hormones, interact with oocytes, build a highly specialized microenvironment, and perform essential roles in follicle development and maturation (32). The hallmark of PCOS is follicular dysplasia, which has been reported in various studies to be associated with granulosa cell apoptosis (33–35). In the PCOS group, the ovaries demonstrated atretic follicles and small follicles at an early stage of development. In addition, there were many large cysts with few granulosa cells. These morphological changes were reversed by administering the CFDT decoction or metformin.

There is no specific Chinese medical term "PCOS" in Chinese medicine books. According to the clinical characteristics, PCOS belongs to the categories of infertility, irregular menstruation, and obesity. TCM has been used to treat menstrual disorders, infertility, and obesity (36, 37). A previous study showed that herbal medicines could alleviate some symptoms of PCOS (38). Analysis of network pharmacology, which focused on seeking the related pathways and chemical compositions of the CFDT decoction for treating PCOS, showed that IR, as well as PI3K-Akt, MAPK, HIF-1 signaling pathways, and Toll-like receptor, was involved in the treatment of PCOS (39). The CFDT decoction is a herbal medicine that has extensive clinical application prospects for PCOS patients (12, 40). Chemical profiling analysis and quality control of the CFDT decoction have been performed (27).

The production of ROS and release of Cyt-c can induce apoptosis, while the opening of mPTP is key to the production of ROS and release of Cyt-c from mitochondria to the cytoplasm; thus, inhibiting the mPTP opening can improve the mitochondrial function and decrease apoptosis (41). It is believed that mitochondrial dysfunction (especially mPTP opening) is targeted in treating ovarian cancer (42). The opening of mPTP induces a decreased MMP, mitochondrial swelling, and inhibition of oxidative phosphorylation. The opening of mPTP operates *via* binding to CyP-D on the inner mitochondrial membrane. Cyclosporine A (CsA) restrains the mPTP opening *via* combining with CyP-D and decreasing injury (43). We hypothesize that the CFDT decoction might play a protective function in granulosa cells *via* increasing mitochondrial function (inhibiting the mPTP opening).

Apoptosis is an ATP-dependent cell death induced by a variety of extracellular and intracellular signals (26, 35, 44). The apoptosis of ovarian oocytes and granulosa cells can induce follicular atresia. A previous study has reported that follicular atresia is a feature of PCOS (45) and that granulosa cell apoptosis is an initiating factor of PCOS (46). Although many apoptotic pathways have been studied, the mitochondrial pathway is the most common apoptotic cascade (47). Previous study showed that apoptosis was increased in PCOS rats and that treatment with the CFDT decoction and metformin could reduce apoptosis, indicating that the CFDT decoction and metformin might ameliorate follicular development in rats with PCOS by modulating apoptosis (13). To further explore the anti-apoptotic mechanism of the CFDT decoction, TUNEL staining, caspase-9/3 activity, and apoptotic genes (Cyt-c, Bcl-2, and Bax) expression were assessed. Our results demonstrated that the number of apoptotic cells in ovaries in the PCOS group increased compared to the control group, and that treatment with the CFDT decoction and metformin could reduce apoptosis. The decreased Bax shifted to mitochondria, integrating with the increased Bcl-2 to form the Bax/Bcl-2 heterodimer on the mitochondrial membrane, and as such promoted the anti-apoptosis effect. As a result, the MMP was steady with little Cyt-c released into the cytoplasm. The Bcl-2 family contains pro-apoptosis molecules (Bad, Bax, and Bid) and anti-apoptosis genes (Bcl-xL, Bcl-2, and Bel-w) (48). Bcl-2, a proto-oncogene, can inhibit cancer cell apoptosis, while Bax is a pro-apoptotic

gene. A previous study demonstrated that the Bcl-2/Bcl-2 homodimer and the Bax/Bcl-2 heterodimer could promote mitochondrial membrane permeability and that the Bax/Bax homodimer could decrease the MMP. The ratio of Bax/Bcl-2 may affect apoptosis (48).

The senescence and apoptosis of granulosa cells are responsible for the decline in ovarian reserve in PCOS (49). Granulosa cell injury is generally associated with mitochondrial dysfunction in PCOS (50). Mitochondrial apoptotic signaling pathways (NF-jB/p53/PUMA and PI3K/Akt/Bad) play an essential role in organ injury, which in turn can induce mitochondrial dysfunction (51–53). In this study, PCOS was associated with morphological changes in mitochondria (swelling or membrane rupture) and increase in the percentage of damaged mitochondria, but CFDT decoction was able to protect the mitochondria. An imbalance of mitochondrial homeostasis plays an essential function in the pathophysiology of PCOS (54). Furthermore, impaired ovarian mitochondrial function and increased OS are critical elements in PCOS (50).

In this study, we firstly established a PCOS rat model. Then, we used CFDT decoction to investigate the MMP, opening of mPTP (%), Cty-c release, ROS production, degree of mtDNA damage, oxygen consumption rate, ATP level, RCR, and activity of mitochondrial respiratory chain complex enzymes (I, II, III, IV, and V). RCR, MMP, oxygen consumption rate, mitochondrial respiratory chain complex enzymes (I, II, III, IV, and V), and ATP levels were reduced in the PCOS group. The ROS production and the opening of mPTP (%) increased in the PCOS group, whereas mitochondrial function indices were improved after treatment with the CFDT decoction. As the copy number of mtDNA in every mitochondrion was steady, the total copy number of mtDNA was applied to evaluate the number of mitochondria (20). Although the mechanism of restoring mtDNA is ambiguous, mtDNA is close to the respiratory chain complex enzymes, which, therefore, is more brittle when exposed to oxidative reactions. Furthermore, we detected damaged mtDNA by calculating the ratio of long/short fragments. The ratio of long/short fragments was reduced in the PCOS group, but increased after treatment with the CFDT decoction.

Mitochondrial biogenesis involves the function of many genes, such as OPA1, Mfn1, and Mfn2. As a regulatory gene, optic atrophy 1 (OPA1) plays an essential role in managing the mitochondrial dynamics and other related roles. In addition, the overexpression of L-OPA1 can reduce neuronal apoptosis *via* increasing the ratio of Bcl-2/Bax and decreasing the caspase-3 level. The overexpression of L-OPA1 can modulate mitochondrial dysfunction by reducing oxidative stress and mitochondrial bioenergetics deficits, preserving mitochondrial integrity, and promoting mitochondrial biogenesis in brain tissues (55). Mitochondrial function is also under the control of mitofusins (Mfns) and dynamin-related protein 1 (Drp1) (56). Mitofusin2 (Mfn2), a conserved dynamin-like GTPase situated in the outer membrane of mitochondria, affects mitochondrial structure and function by modulating fission and fusion (57). Furthermore, Mfn2 preserves cell function by modulating the respiratory chain, MMP, metabolic processes, and apoptosis (58). Mfn2 plays a crucial role in maintaining the integrity of mtDNA (59). As shown in this study, OPA1, Mfn1, Mfn2, mRNA and protein levels decreased in the PCOS group, but increased after treatment with the CFDT decoction. These results further corroborated previous research that Mfn2 can maintain the integrity of mtDNA (59). Mitochondria undergo fission and fusion, and these dynamic processes are essential for maintaining size and shape. PPARγ coactivator-1-α (PGC-1α) is a crucial transcriptional co-activator that modulates critical factors containing Tfam and Nrf1 which facilitate mitochondrial biogenesis (60). Drp1, the significant regulation of mitochondrial fission, is a cytosolic member of the dynamin family of GTPases. Drp1 binds to mitochondrial membranes, and its level increases during apoptosis (61). FiS1 induces apoptosis *via* the interaction with endoplasmic reticulum-localized Bap31, leading to Cyt-c release and ultimately cell death (62). In addition, FIS1 has been implicated in reducing the GTPase activity of Mfn1, Mfn2, and OPA1 (63). Our results showed that the expression of Drp1, FiS1, and PGC-1α changed in PCOS, showing a chaotic fission-fusion balance in mitochondria. We observed an increase in Drp1 and FiS1 levels and a decrease in the PGC-1α level in PCOS. The CFDT decoction could decrease the PCOS-induced imbalance by increasing mitochondrial biogenesis and renewing the balance between fusion and fission.

Apoptosis signal-regulating kinase 1 (ASK1), a kind of universally expressed mitogen-activated kinase kinase kinase (MAP3K), can be activated by all kinds of stimuli, and then activate downstream kinases (JNK and p38) (64). As such, it can play an important role in neurodegenerative disorders, cancer, and inflammatory diseases (65). The ASK1 inhibitor selonsertib (GS-49977) is a possible treatment drug during the early treatment of ALF through reducing JNK-mediated Drp1 mitochondrial translocation and then remedying mitochondrial injury (66). The ASK1/JNK signalling pathway plays a crucial function in inducing mitochondrial-induced apoptosis (67). In this study, CFDT decoction affected the expression of ASK1/JNK pathway-related proteins, inhibited ASK1 and JNK phosphorylation, and downregulated Cyt-c expression and cleaved caspase-9/3, indicating that the CFDT decoction could improve mitochondrial function and inhibit apoptosis through the ASK1/JNK pathway.

Conclusions

In this study, we used isolated mitochondria and showed that PCOS promoted injury of the mitochondria in the ovary and damaged mtDNA and mitochondrial function, including respiratory, biogenesis, and the ROS balance. The opening of mPTP caused mitochondrial swelling and induced flow back of protons from the mitochondrial membrane space to the matrix,

thereby reducing ATP synthesis and the MMP and inducing metabolic abnormalities or even apoptosis. The CFDT decoction could inhibit apoptosis *via* improving mitochondrial function through inhibition of ASK1/JNK pathway in PCOS rats.

Data availability statement

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

Ethics statement

The animal study was reviewed and approved by Ethical Committee of Animal Handling (2021-210, March 8 2021) of Liaoning University of Traditional Chinese Medicine.

Author contributions

XLJ and HT wrote the manuscript and researched data. X-SX, S-BQ, S-YZ, and L-NZ selected rats and extracted blood. HT dealed with the figures. S-ML corrected the discussion. X-SM and J-SK contributed to the discussion and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

Funding

The study was supported by Shenzhen Science and Technology Project (JSGG20191129102216637); Shenzhen Basic Research Project (2021300690); Science and Technology Research Project of Liaoning Provincial Department of Education (L202038); Sanming Project of Medicine in Shenzhen (SZZYSM202111002), Shenzhen Science and Technology Program (JCYJ20210324111404012), and Liaoning Provincial Department of Education Project (LJKMZ20221306).

Acknowledgments

The authors would like to thank all of the rats, the team of investigators, research partners, operations staff involved in this study, and the Key Laboratory of Ministry of Education for Traditional Chinese Medicine Viscera-State Theory and Applications. We want to thank professor Shun-min Li, Xian-sheng Meng, and Jin-song Kuang for scientific and my husband He Tai for help. We thank LetPub (www.letpub.com) and Lisa Owens (St. James's Hospital) for its linguistic assistance during the preparation of this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Goodman NF, Cobin RH, Futterweit W, Glueck JS, Legro RS, Carmina E, et al. American Association of clinical endocrinologists, American college of endocrinology, and androgen excess and pcos society disease state clinical review: Guide to the best practices in the evaluation and treatment of polycystic ovary syndrome–part 1. *Endocr Pract* (2015) 21(11):1291–300. doi: 10.4158/EP15748.DSC

2. Moghetti P, Tosi F. Insulin resistance and PCOS: chicken or egg? *J Endocrinol Invest.* (2021) 44(2):233–44. doi: 10.1007/s40618-020-01351-0

3. González F. Inflammation in polycystic ovary syndrome: underpinning of insulin resistance and ovarian dysfunction. *Steroids.* (2012) 77(4):300–5. doi: 10.1016/j.steroids.2011.12.003

4. Huang Y, Zhang X. Luteolin alleviates polycystic ovary syndrome in rats by resolving insulin resistance and oxidative stress. *Am J Physiol Endocrinol Metab* (2021) 320(6):E1085–92. doi: 10.1152/ajpendo.00034.2021

5. Calcaterra V, Verduci E, Cena H, Magenes VC, Todisco CF, Tenuta E, et al. Polycystic ovary syndrome in insulin-resistant adolescents with obesity: The role of nutrition therapy and food supplements as a strategy to protect fertility. *Nutrients.* (2021) 13(6):1848. doi: 10.3390/nu13061848

6. Ibáñez L, Oberfield SE, Witchel S, Auchus RJ, Chang RJ, Codner E, et al. An international consortium update: Pathophysiology, diagnosis, and treatment of polycystic ovarian syndrome in adolescence. *Horm Res Paediatr* (2017) 88(6):371–95. doi: 10.1159/000479371

7. Murri M, Luque-Ramírez M, Insenser M, Ojeda-Ojeda M, Escobar-Morreale HF. Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis. *Hum Reprod Update.* (2013) 19(3):268–88. doi: 10.1093/humupd/dms059

8. Zeng X, Huang Q, Long SL, Zhong Q, Mo Z. Mitochondrial dysfunction in polycystic ovary syndrome. *DNA Cell Biol* (2020) 39(8):1401–9. doi: 10.1089/dna.2019.5172

9. Wang H, Ruan X, Li Y, Cheng J, Mueck AO. Oxidative stress indicators in Chinese women with PCOS and correlation with features of metabolic syndrome and dependency on lipid patterns. *Arch Gynecol Obstet.* (2019) 300(5):1413–21. doi: 10.1007/s00404-019-05305-7

10. Mohammadi M. Oxidative stress and polycystic ovary syndrome: A brief review. *Int J Prev Med* (2019) 10:86. doi: 10.4103/ijpvm.IJPVM_576_17

11. Shen W, Jin B, Pan Y, Han Y, You T, Zhang Z, et al. The effects of traditional Chinese medicine-associated complementary and alternative medicine on women with polycystic ovary syndrome. *Evid Based Complement Alternat Med* (2021) 2021:6619597. doi: 10.1155/2021/6619597

12. Ding CF, Wang CY, Yang X, Zheng RH, Yan ZZ, Chen WQ. Effect of modified cangfu daotan decoction in improving endometrial receptivity in infertility patients with polycystic ovarian syndrome. *Zhongguo Zhong Xi Yi Jie He Za Zhi.* (2014) 34(11):1297–301.

13. Wang C, Ding C, Hua Z, Chen C, Yu J. Cangfudaotan decoction alleviates insulin resistance and improves follicular development in rats with polycystic ovary syndrome *via* IGF-1-PI3K/Akt-Bax/Bcl-2 pathway. *Mediators Inflamm* (2020) 2020:8865647. doi: 10.1155/2020/8865647

14. Sun J, Jin C, Wu H, Zhao J, Cui Y, Liu H, et al. Effects of electro-acupuncture on ovarian P450arom, P450c17α and mRNA expression induced by letrozole in PCOS rats. *PloS One* (2013) 8(11):e79382. doi: 10.1371/journal.pone.0079382

15. Peng H, Zeng L, Zhu L, Luo S, Xu L, Zeng L, et al. Zuogui pills inhibit mitochondria-dependent apoptosis of follicles in a rat model of premature ovarian failure. *J Ethnopharmacol* (2019) 238:111855. doi: 10.1016/j.jep.2019.111855

16. Han X, Bai L, Wang Y, Li Y, Zhao D, Hu G, et al. Ovarian index of KM mice influenced by longer term consumption of microwave-heated milk. *J Food Prot* (2020) 83(6):1066–71. doi: 10.4315/JFP-19-572

17. Chen W, Xu X, Wang L, Bai G, Xiang W. Low expression of Mfn2 is associated with mitochondrial damage and apoptosis of ovarian tissues in the premature ovarian failure model. *PloS One* (2015) 10(9):e0136421. doi: 10.1371/journal.pone.0136421

18. Luan G, Li G, Ma X, Jin Y, Hu N, Li J, et al. Dexamethasone-induced mitochondrial dysfunction and insulin resistance-study in 3T3-L1 adipocytes and mitochondria isolated from mouse liver. *Molecules.* (2019) 24(10):1982. doi: 10.3390/molecules24101982

19. Li X, Jia P, Huang Z, Liu S, Miao J, Guo Y, et al. Lycopene protects against myocardial ischemia-reperfusion injury by inhibiting mitochondrial permeability transition pore opening. *Drug Des Devel Ther* (2019) 13:2331–42. doi: 10.2147/DDDT.S194753

20. Cioffi F, Senese R, Lasala P, Ziello A, Mazzoli A, Crescenzo R, et al. Fructose-rich diet affects mitochondrial DNA damage and repair in rats. *Nutrients.* (2017) 9 (4):323. doi: 10.3390/nu9040323

21. Gilmer LK, Ansari MA, Roberts KN, Scheff SW. Age-related mitochondrial changes after traumatic brain injury. *J Neurotrauma.* (2010) 27(5):939–50. doi: 10.1089/neu.2009.1181

22. Song N, Jia L, Cao H, Ma Y, Chen N, Chen S, et al. Gypenoside inhibits endothelial cell apoptosis in atherosclerosis by modulating mitochondria through PI3K/Akt/Bad pathway. *BioMed Res Int* (2020) 2020:2819658. doi: 10.1155/2020/2819658

23. Wagner EM. Monitoring gene expression: quantitative real-time rt-PCR. *Methods Mol Biol* (2013) 1027:19–45. doi: 10.1007/978-1-60327-369-5_2

24. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. *Methods.* (2001) 25(4):402–8. doi: 10.1006/meth.2001.1262

25. Moghetti P. Insulin resistance and polycystic ovary syndrome. *Curr Pharm Des* (2016) 22(36):5526–34. doi: 10.2174/1381612822666160720155855

26. O'Reilly M, Gathercole L, Capper F, Arlt W, Tomlinson J. Effect of insulin on AKR1C3 expression in female adipose tissue: *in-vivo* and *in-vitro* study of adipose androgen generation in polycystic ovary syndrome. *Lancet.* (2015) 385 Suppl 1:S16. doi: 10.1016/S0140-6736(15)60331-2

27. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. *Endocr Rev* (2012) 33(6):981–1030. doi: 10.1210/er.2011-1034

28. Guo Y, Qi Y, Yang X, Zhao L, Wen S, Liu Y, et al. Association between polycystic ovary syndrome and gut microbiota. *PloS One* (2016) 11(4):e0153196. doi: 10.1371/journal.pone.0153196

29. Dabravolski SA, Nikiforov NG, Eid AH, Nedosugova LV, Starodubova AV, Popkova TV, et al. Mitochondrial dysfunction and chronic inflammation in polycystic ovary syndrome. *Int J Mol Sci* (2021) 22(8):3923. doi: 10.3390/ijms22083923

30. Duleba AJ, Dokras A. Is PCOS an inflammatory process? *Fertil Steril.* (2012) 97(1):7–12. doi: 10.1016/j.fertnstert.2011.11.023

31. Liu M, Gao J, Zhang Y, Li P, Wang H, Ren X, et al. Serum levels of TSP-1, NF-κB and TGF-β1 in polycystic ovarian syndrome (PCOS) patients in northern China suggest PCOS is associated with chronic inflammation. *Clin Endocrinol (Oxf).* (2015) 83(6):913–22. doi: 10.1111/cen.12951

32. Almeida CP, Ferreira MCF, Silveira CO, Campos JR, Borges IT, Baeta PG, et al. Clinical correlation of apoptosis in human granulosa cells-a review. *Cell Biol Int* (2018) 42(10):1276–81. doi: 10.1002/cbin.11036

33. Shen H, Wang Y. Activation of TGF-β1/Smad3 signaling pathway inhibits the development of ovarian follicle in polycystic ovary syndrome by promoting apoptosis of granulosa cells. *J Cell Physiol* (2019) 234(7):11976–85. doi: 10.1002/jcp.27854

34. Chang RJ, Cook-Andersen H. Disordered follicle development. *Mol Cell Endocrinol* (2013) 373(1-2):51–60. doi: 10.1016/j.mce.2012.07.011

35. Tian Y, Shen W, Lai Z, Shi L, Yang S, Ding T, et al. Isolation and identification of ovarian theca-interstitial cells and granulose cells of immature female mice. *Cell Biol Int* (2015) 39(5):584–90. doi: 10.1002/cbin.10426

36. Huang ST, Chen AP. Traditional Chinese medicine and infertility. *Curr Opin Obstet Gynecol.* (2008) 20(3):211–5. doi: 10.1097/GCO.0b013e3282f88e22

37. Lee CJ, Kapelemera AM, Tsai YZ, Lee CT, Xu MY, Wang CC. Evaluating the therapeutic efficacy of Si-Wu-Tang decoction and concentrated extract in follicular maldevelopment-related menstrual disorders through Pharmacokinetic/Pharmacodynamic studies. *Front Pharmacol* (2020) 11:1245-1245. doi: 10.3389/fphar.2020.01245

38. Lee JC, Pak SC, Lee SH, Lim SC, Bai YH, Jin CS, et al. The effect of herbal medicine on nerve growth factor in estradiol valerate-induced polycystic ovaries in rats. *Am J Chin Med* (2003) 31(6):885–95. doi: 10.1142/S0192415X03001636

39. Xu W, Tang M, Wang J, Wang L. Identification of the active constituents and significant pathways of cangfu daotan decoction for the treatment of PCOS based on network pharmacology. *Evid Based Complement Alternat Med* (2020) 2020:4086864. doi: 10.1155/2020/4086864

40. Xingying L, Qin Y, Qi W. Effects of cangfu daotan decoction combined with clomiphene on serum hormone level and endometri-al receptivity in patients with polycystic ovary syndrome combined with infertility. *China Pharmacy.* (2017) 2826:3698–701.

41. Li Y, Sun J, Wu R, Bai J, Hou Y, Zeng Y, et al. Mitochondrial MPTP: A novel target of ethnomedicine for stroke treatment by apoptosis inhibition. *Front Pharmacol* (2020) 11:352. doi: 10.3389/fphar.2020.00352

42. Landriscina M, Amoroso MR, Piscazzi A, Esposito F. Heat shock proteins, cell survival and drug resistance: the mitochondrial chaperone TRAP1, a potential novel target for ovarian cancer therapy. *Gynecol Oncol* (2010) 117(2):177–82. doi: 10.1016/j.ygyno.2009.10.078

43. Baines CP, Kaiser RA, Purcell NH, Blair NS, Osinska H, Hambleton MA, et al. Loss of cyclophilin d reveals a critical role for mitochondrial permeability transition in cell death. *Nature.* (2005) 434(7033):658–62. doi: 10.1038/nature03434

44. Zamaraeva MV, Sabirov RZ, Maeno E, Ando-Akatsuka Y, Bessonova SV, Okada Y. Cells die with increased cytosolic ATP during apoptosis: a bioluminescence study with intracellular luciferase. *Cell Death Differ* (2005) 12 (11):1390–7. doi: 10.1038/sj.cdd.4401661

45. Sreerangaraja Urs DB, Wu WH, Komrskova K, Postlerova P, Lin YF, Tzeng CR, et al. Mitochondrial function in modulating human granulosa cell steroidogenesis and female fertility. *Int J Mol Sci* (2020) 21(10):3592. doi: 10.3390/ijms21103592

46. Wang T, Zhang J, Hu M, Zhang Y, Cui P, Li X, et al. Differential expression patterns of glycolytic enzymes and mitochondria-dependent apoptosis in PCOS patients with endometrial hyperplasia, an early hallmark of endometrial cancer*In vivo* and the impact of metformin *In vitro*. . *Int J Biol Sci* (2019) 15(3):714–25. doi: 10.7150/ijbs.31425

47. Horbay R, Bilyy R. Mitochondrial dynamics during cell cycling. *Apoptosis.* (2016) 21(12):1327–35. doi: 10.1007/s10495-016-1295-5

48. Benito JM, Godfrey L, Kojima K, Hogdal L, Wunderlich M, Geng H, et al. MLL-rearranged acute lymphoblastic leukemias activate BCL-2 through H3K79 methylation and are sensitive to the BCL-2-Specific antagonist ABT-199. *Cell Rep* (2015) 13(12):2715–27. doi: 10.1016/j.celrep

49. Liu G, Liu S, Xing G, Wang F. lncRNA PVT1/MicroRNA-17-5p/PTEN axis regulates secretion of E2 and P4, proliferation, and apoptosis of ovarian granulosa cells in PCOS. *Mol Ther Nucleic Acids* (2020) 20:205–16. doi: 10.1016/j.omtn.2020.02.007

50. Gong Y, Luo S, Fan P, Zhu H, Li Y, Huang W. Growth hormone activates PI3K/Akt signaling and inhibits ROS accumulation and apoptosis in granulosa cells of patients with polycystic ovary syndrome. *Reprod Biol Endocrinol* (2020) 18 (1):21. doi: 10.1186/s12958-020-00677-x

51. Tai H, Jiang XL, Song N, Xiao HH, Li Y, Cheng MJ, et al. Tanshinone IIA combined with cyclosporine a alleviates lung apoptosis induced by renal ischemia-reperfusion in obese rats. *Front Med (Lausanne).* (2021) 8:617393. doi: 10.3389/fmed.2021.617393

52. Tai H, Jiang XL, Lan ZM, Li Y, Kong L, Yao SC, et al. Tanshinone IIA combined with CsA inhibit myocardial cell apoptosis induced by renal ischemia-reperfusion injury in obese rats. *BMC Complement Med Ther* (2021) 21(1):100. doi: 10.1186/s12906-021-03270-w

53. Chen X, Wang Q, Shao M, Ma L, Guo D, Wu Y, et al. Ginsenoside Rb3 regulates energy metabolism and apoptosis in cardiomyocytes *via* activating PPARα pathway. *BioMed Pharmacother.* (2019) 120:109487. doi: 10.1016/j.biopha.2019.109487

54. Hu M, Zhang Y, Guo X, Jia W, Liu G, Zhang J, et al. Hyperandrogenism and insulin resistance induce gravid uterine defects in association with mitochondrial dysfunction and aberrant reactive oxygen species production. *Am J Physiol Endocrinol Metab* (2019) 316(5):E794–809. doi: 10.1152/ajpendo.00359.2018

55. Lai Y, Lin P, Chen M, Zhang Y, Chen J, Zheng M, et al. Restoration of l-OPA1 alleviates acute ischemic stroke injury in rats *via* inhibiting neuronal apoptosis and preserving mitochondrial function. *Redox Biol* (2020) 34:101503. doi: 10.1016/j.redox.2020.101503

56. de Brito OM, Scorrano L. Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature.* (2008) 456(7222):605–10. doi: 10.1038/nature07534

57. Givvimani S, Pushpakumar SB, Metreveli N, Veeranki S, Kundu S, Tyagi SC. Role of mitochondrial fission and fusion in cardiomyocyte contractility. *Int J Cardiol* (2015) 187:325–33. doi: 10.1016/j.ijcard.2015.03.352

58. Nasrallah CM, Horvath TL. Mitochondrial dynamics in the central regulation of metabolism. *Nat Rev Endocrinol* (2014) 10(11):650–8. doi: 10.1038/nrendo.2014.160

59. Vidoni S, Zanna C, Rugolo M, Sarzi E, Lenaers G. Why mitochondria must fuse to maintain their genome integrity. *Antioxid Redox Signal* (2013) 19(4):379–88. doi: 10.1089/ars.2012.4800

60. Kelly DP, Scarpulla RC. Transcriptional regulatory circuits controlling mitochondrial biogenesis and function. *Genes Dev* (2004) 18(4):357–68. doi: 10.1101/gad.1177604

61. Frank S, Gaume B, Bergmann-Leitner ES, Leitner WW, Robert EG, Catez F, et al. The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis. *Dev Cell* (2001) 1(4):515–25. doi: 10.1016/s1534-5807(01)00055-7

62. Wang B, Nguyen M, Chang NC, Shore GC. Fis1, Bap31 and the kiss of death between mitochondria and endoplasmic reticulum. *EMBO J* (2011) 30(3):451–2. doi: 10.1038/emboj.2010.352

63. Yu R, Jin SB, Lendahl U, Nistér M, Zhao J. Human Fis1 regulates mitochondrial dynamics through inhibition of the fusion machinery. *EMBO J* (2019) 38(8):e99748. doi: 10.15252/embj.201899748

64. Huang B, Ding C, Zou Q, Wang W, Li H. Cyclophosphamide regulates N6-methyladenosine and m6A RNA enzyme levels in human granulosa cells and in ovaries of a premature ovarian aging mouse model. *Front Endocrinol (Lausanne).* (2019) 10:415. doi: 10.3389/fendo.2019.00415

65. Nakagawa H, Hirata Y, Takeda K, Hayakawa Y, Sato T, Kinoshita H, et al. Apoptosis signal-regulating kinase 1 inhibits hepatocarcinogenesis by controlling the tumor-suppressing function of stress-activated mitogen-activated protein kinase. *Hepatology.* (2011) 54(1):185–95. doi: 10.1002/hep.24357

66. Lou G, Li A, Cen Y, Yang Q, Zhang T, Qi J, et al. Selonsertib, a potential drug for liver failure therapy by rescuing the mitochondrial dysfunction of macrophage *via* ASK1-JNK-DRP1 pathway. *Cell Biosci* (2021) 11(1):9. doi: 10.1186/s13578-020-00525-w

67. Ma L, Wei J, Wan J, Wang W, Wang L, Yuan Y, et al. Low glucose and metformin-induced apoptosis of human ovarian cancer cells is connected to ASK1 *via* mitochondrial and endoplasmic reticulum stress-associated pathways. *J Exp Clin Cancer Res* (2019) 38(1):77. doi: 10.1186/s13046-019-1090-6

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 25 November 2022
DOI 10.3389/fendo.2022.1024844

OPEN ACCESS

EDITED BY
Spyridon N Karras,
Aristotle University of Thessaloniki, Greece

REVIEWED BY
Jakub Antoni Kortas,
Gdansk University of Physical Education and Sport, Poland
Maria Kavianpour,
Tehran University of Medical Sciences, Iran

*CORRESPONDENCE
Hugo C. D. Souza
hugocds@fmrp.usp.br

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

RECEIVED 22 August 2022
ACCEPTED 07 November 2022
PUBLISHED 25 November 2022

CITATION
Philbois SV, Ribeiro VB, Tank J, Reis RMd, Gerlach DA and Souza HCD (2022) Cardiovascular autonomic modulation differences between moderate-intensity continuous and high-intensity interval aerobic training in women with PCOS: A randomized trial.
Front. Endocrinol. 13:1024844.
doi: 10.3389/fendo.2022.1024844

Cardiovascular autonomic modulation differences between moderate-intensity continuous and high-intensity interval aerobic training in women with PCOS: A randomized trial

Stella V. Philbois[1], Victor B. Ribeiro[2], Jens Tank[3], Rosana Maria dos Reis[2], Darius A. Gerlach[3] and Hugo C. D. Souza[1,3]*

[1]Laboratory of Physiology and Cardiovascular Physiotherapy, Department of Science of Health, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil, [2]Department of Gynecology and Obstetrics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil, [3]Department of Cardiovascular Aerospace Medicine, Institute of Aerospace Medicine, German Aerospace Center, Cologne, Germany

Background: Moderate-intensity continuous training (MICT) is strongly recommended for polycystic ovarian syndrome (PCOS) treatment. However, recent studies have suggested that high-intensity interval training (HIIT) would promote great benefits for cardiac autonomic control. Therefore, we investigated whether the benefits of HIIT related to cardiovascular autonomic control were greater than those of MICT in women with PCOS.

Methods: Women with PCOS were randomly allocated through a blind draw into three groups: control, MICT, and HIIT. The control group did not undergo exercise, whereas those in the MICT and HIIT groups underwent 16 weeks of aerobic physical training. All groups were evaluated before and after the 16 weeks of intervention in the following aspects: quantification of serum lipids, testosterone, fasting insulin and blood glucose; physical fitness through cardiopulmonary testing; analysis of heart rate variability (HRV) by linear (time domain and frequency domain) and non-linear (symbolic analysis) methods, analysis of blood pressure variability (BPV) and spontaneous baroreflex sensitivity (BRS).

Results: The final analysis, each group comprised 25 individuals. All groups had similar baseline parameters. After 16 weeks, intragroup comparison showed that the MICT and HIIT groups had a reduction in baseline heart rate ($P < 0.001$; $P < 0.001$, respectively) and testosterone levels $P < 0.037$; $P < 0.012$, respectively) associated with an increase in $VO2_{peak}$ (MICT, $P < 0.001$; HIIT, $P < 0.001$). The MICT ($P < 0.36$) and HIIT ($P < 0.17$) groups also showed an increase in cardiac vagal modulation, however only observed in the non-linear analysis. The intergroup comparison showed no differences between the MICT and HIIT groups in any of

the hormonal, metabolic and autonomic parameters evaluated, including testosterone, peak oxygen uptake ($VO2_{peak}$), HRV, BPV and BRS.

Conclusion: HIIT and MICT showed similar results for the different parameters evaluated. This suggests that both training protocols can be recommended for the treatment of PCOS. Brazilian Clinical Trials Registry (RBR-78qtwy).

KEYWORDS

aerobic physical training, cardiovascular autonomic control, heart rate variability, high-intensity interval training, polycystic ovary syndrome

1 Introduction

Polycystic ovary syndrome (PCOS) affects a large proportion of women of reproductive age. Studies have demonstrated a prevalence between 6% and 13%; however, it varies depending on the diagnostic criteria used (1–4). This high prevalence is concerning, since PCOS may lead to insulin resistance, obesity, and cardiovascular autonomic changes, predisposing the development of diabetes mellitus type 2 and cardiovascular diseases (CVDs) (2, 5, 6).

Therapeutic interventions that target the prevention of comorbidities resulting from PCOS include regular physical exercise (3, 4, 7). Some clinical studies demonstrate the long-term effects of moderate-intensity continuous aerobic training (MICT) and its beneficial effects on adjustments in the regulation of hemodynamic, metabolic, and cardiovascular autonomic control, especially insulin sensitivity and cardiac modulation of heart rate variability (HRV) (8–10).

High-intensity interval training (HIIT) is another aerobic physical training protocol that has been used to optimize therapeutic benefits, mainly in patients with different CVDs. Some studies have shown that HIIT promotes greater benefits for cardiorespiratory fitness, as well as hemodynamic and anthropometric parameters, compared to MICT (11–13). However, these therapeutic benefits are still controversial, especially those related to cardiovascular autonomic control (13–18).

Although aerobic physical exercise is well known for improving hemodynamic, metabolic, and cardiovascular autonomic control in people with different diseases, there are limited studies in the literature regarding the effects of physical training on cardiovascular autonomic modulation in women with PCOS. We found few studies, although they did not present an analysis of cardiovascular autonomic control (19–22). Thus, there is a great need to find others training methods that would promote great therapeutic benefits for patients with PCOS. The current study aimed to compare the effects of HIIT and MICT on cardiovascular autonomic control in women with PCOS evaluated using HRV, blood pressure variability (BPV), and baroreflex sensitivity (BRS).

2 Materials and methods

2.1 Sampling

This was a randomized clinical trial. In total, 126 participants aged between 18 and 39 years were enrolled, and 110 were included (Figure 1). All patients were screened at the Gynecology and Obstetrics Clinic of the Ribeirão Preto Medical School's Hospital (HCFMRP/USP). The patients were diagnosed with PCOS according to the Rotterdam Consensus criteria (23). In the end, 75 participants completed the experimental protocol (Figure 1). The participants were randomly allocated to three different experimental groups: control (N = 25), without any exercise training; MICT group (N =25), and HIIT group (N = 25), and the study lasted for 16 weeks. Randomization was performed through a blind draw using numbers one to three, wherein each number corresponded to a specific group. The exclusion criteria were a history of a) smoking, b) cognitive disturbances, c) pregnancy, d) musculoskeletal disorders, e) CVDs, and f) the use of any medication, including contraceptives. The study was approved by the Ethics Committee of the Ribeirão Preto Medical School's Hospital (Protocol number 845.830/2014), the scientific and legal aspects were disclosed to the participants, and all participants signed a free and informed consent form, agreeing to participate. The authors confirm that all ongoing and related trials for this intervention were registered with the Brazilian Clinical Trials Registry (RBR-78qtwy).

2.2 Protocols

Data were collected in the morning during two laboratory visits between 07:00 AM and 10:00 AM, with a 48-hour interval

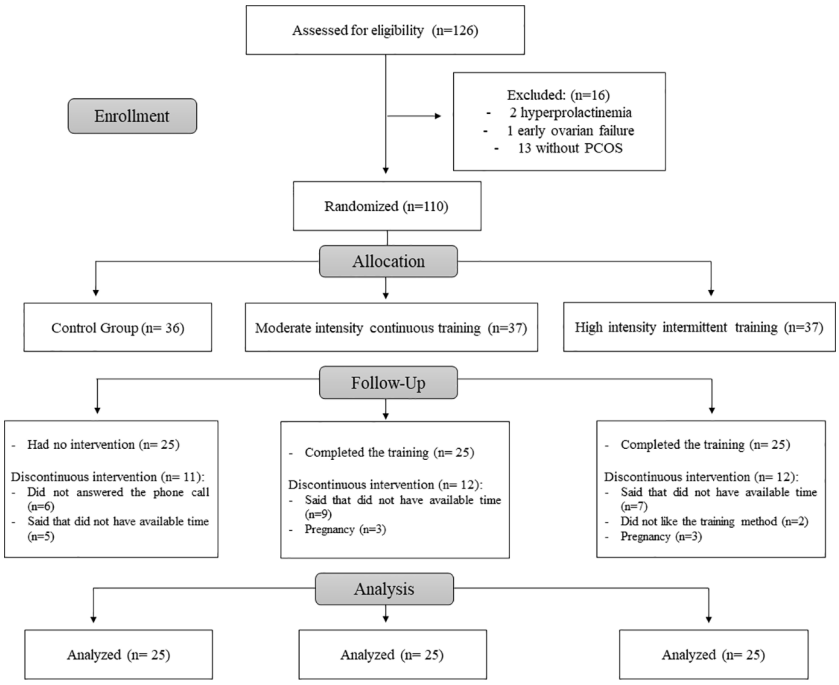


FIGURE 1
A flow diagram of the study.

between visits. Data were collected during the follicular phase for women with regular ovulatory cycles and at any time for those with irregular ovulatory cycles. The first assessment included anthropometric measurements and blood collection performed in the laboratory of the HCFMRP/USP.

The second assessment was performed at the Laboratory of Exercise Physiology and Cardiovascular Physiotherapy of Ribeirão Preto Medical School. During the second visit, the following protocols were completed: anthropometric parameters, cardiovascular autonomic analysis, and cardiorespiratory function test. Each visit lasted approximately two hours.

All participants were asked to avoid exercise and consumption of alcoholic beverages and maintain their usual diet for 48 h prior to the assessments. They were also advised to sleep for at least 7 or 8 h the night before the visits.

2.2.1 Laboratory tests

Blood samples (3.5 ml, BD Vacutainer® EDTA - Becton, Dickin, and Company, Franklin Lakes, NJ, USA) were used to analyze fasting glycemia hexokinase-UV), insulin levels (chemiluminescence immunoassay), triglyceride levels (dehydrogenase), and total cholesterol and fraction levels (esterase-oxidase). All participants were asked to fast for 12 h prior to the assessments. Insulin resistance was assessed using the homeostasis model assessment-insulin resistance index (HOMA-IR) (24).

2.2.2 Anthropometric parameters

Data on body weight and height were obtained using an analog scale with an altimeter (Welmy), while the body mass index (BMI) values were calculated using weight and height expressed as kg/m^2.

2.2.3 Cardiorespiratory function test

Peak oxygen uptake (VO2peak) was assessed by a submaximal exercise test conducted on a treadmill (Super ATL Millenium®, Inbramed/Inbrasport, Brazil) using the modified Bruce protocol. Heart rate (HR) was continuously monitored by electrocardiography with one lead (CM5). The analysis of exhaled gases (VO2 and VCO2) was performed using a metabolic device (PowerLab/8M, ADInstruments, Bella Vista, Australia).

2.2.4 Hemodynamic assessment

Data on systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean blood pressure (MBP) were obtained using digital plethysmography recording equipment (Finometer Pro, Finapres Medical System, Amsterdam, Netherlands). HR data were obtained using an electrocardiographic (ECG) digital recorder through the CM5 lead (ML866 PowerLab).

2.2.5 HRV, BPV, and spontaneous BRS analysis

HRV data were obtained using the RR intervals (RRi) from the ECG records at a sampling frequency of 2000 Hz. The BPV values were obtained from the SBP data recorded beat-to-beat using the Finometer, with a cuff positioned on the middle finger of the right upper limb. The data interface to the microcomputer was performed using a PowerLab4/35 device (ADInstruments),. The data were recorded and stored (Software LabChart 8.0, ADInstruments), for further analysis. The participants were instructed to remain in the supine position for approximately 10 min to stabilize the cardiovascular parameters. After this period, the ECG signals and arterial pulse pressure were recorded simultaneously for another 10 min. The temperature (22°C) and ambient lighting were controlled, and the sessions were performed in a noise-free environment.

The HRV and BPV analyses were performed using custom computer software (CardioSeries v2.4, http://sites.google.com/site/cardioseries). The HRV was assessed by the following three methods: time domain (standard deviation [SD] and the root mean square of successive normal sinus RR-interval [RMSSD]) (25), frequency domain (spectral analysis, fast Fourier transform [FFT]) (25, 26), and nonlinear analysis (symbolic analysis) (27, 28). The BPV was assessed only by frequency domain (spectral analysis) (26). BRS was assessed in the time domain using the sequence technique (29, 30). The computer software CardioSeries v2.4 was also used as previously described (26).

2.2.6 Training protocol

The training sessions were conducted at the Laboratory of Exercise Physiology and Cardiovascular Physiotherapy of the Ribeirão Preto Medical School. After the initial evaluation, both MICT and HIIT participants were submitted to their respective training protocols using a motorized treadmill. These sessions were supervised and monitored three times per week for a total of 16 weeks. MICT used the equivalent intensity of HR corresponding to the sum of HR at rest and 70%–80% of reserve HR, obtaining the following equation: HR recorded at peak cardiopulmonary test – HR at rest. The MICT training sessions had a duration of 1 hour, divided into three phases as follows: 5-min warm-up using intensity lower than the target HR training range (50%–65% of reserve HR), 50-min training using the HR target (70%–80% of reserve HR), and 5-min cool-down, using intensity lower than the training HR (40%–50% of reserve HR).

In contrast, the HIIT session was as follows: 5-min warm-up using intan ensdensitywer than the target HR training range (50%–65% of reserve HR); for the main phase of HIIT, the intensity used for a total of 2-min corresponded to the sum of HR at rest and 85%–90% of the reserve HR, altering with 3-min of intensity corresponding to the sum of HR at rest and 65%–70% of the reserve HR; and the last stage consisted of 5-min cool-down, using intensity lower than the training HR (40%–50% of reserve HR). The two aerobic exercise training protocols, HIIT and MICT, had similar total HR numbers. Every day of physical training, the HR of the participants was beat-by-beat monitored and recorded (Polar RS810). Stored records were quantified for necessary adjustments for the next physical training session. While MICT had a total training duration of 50 min, HIIT had a total duration of 35–45 min. Prior to both training models, during the first 2 weeks of the study, an adaptation period was provided to the participants. All participants underwent sessions of 20–30 min for familiarization and adaptation to the treadmill protocol. The intensity used was equivalent to the sum of HR at rest and 50%–60% of the reserve HR. In addition, if the participant did not have an adherence above 85%, their data were not included in the analysis.

2.3 Statistical analysis

The sample size calculation was performed using the SigmaPlot®, version 11.0 software (Systat Software Inc., San Jose, CA, USA) with a confidence level of 95% and power of 80% using the LF and HF variables in normalized units, with a standard deviation of 15. We initially carried out an exploratory analysis of data through measures of central position and dispersion. A mixed effects linear regression model was adjusted to verify the effect of time and group variables in relation to the outcomes of interest. This model considered intra- and inter-group variability. To verify whether the model was well adjusted to the data, a residual analysis was performed. Comparisons between groups within each time and between times within each group were performed considering orthogonal contrasts. The analyzes were implemented in the SAS program version 9.4. Differences were considered significant at $P < 0.05$.

3 Results

Table 1 presents the means ± standard deviation (SD), while Tables 2, 3 present intragroup (Table 2) and intergroup (Table 3) comparisons between the means of confidence intervals (CI) of the anthropometric characteristics and hemodynamic, hormonal and metabolic parameters. Anthropometric characteristics did not differ in intragroup (Table 2) or intergroup (Table 3). Regarding hemodynamic parameters, the period of 16 weeks (time factor) did not influence the values of heart rate (control group, $P < 0.452$) and mean arterial pressure (control group, $P < 0.184$). In turn, the aerobic physical training (training factor) reduced HR (MICT, $P < .0.001$; HIIT, $P < 0.001$). Both time and training factors had different influences on the metabolic parameters. The time factor reduced VO2peak and increased the testosterone ($P < 0.035$) and cholesterol ($P < 0.009$) levels in the control group, while the training factor increased

VO2peak and decreased the testosterone level in the MICT ($P < 0.001$; $P < 0.037$, respectively) and HIIT ($P < 0.001$; $P < 0.012$, respectively) groups. Finally, both training protocols showed similar effects on all metabolic parameters evaluated, including VO2peak ($P < 0.659$).

Table 4 presents the means ± SD, while Tables 5, 6 present intragroup (Table 5) and intergroup (Table 6) comparisons between the means of CI of all autonomic parameters evaluated. The analysis of HRV using linear methods (time domain and frequency domain) did not show any influence of time and physical training (MICT and HIIT) on cardiac autonomic parameters. In contrast, the symbolic analysis (non-linear method) showed an increase in 2UV% oscillations, which represents vagal modulation, in both trained groups (MICT, $P < 0.036$; HIIT, $P < 0.017$). Tables 5, 6 also present the results of BPV (linear analysis) and BRS, and the results showed that the time and physical training factors did not influence the evaluated parameters.

Figure 2 shows a representative record of the RRi segments of each group before and after 16 weeks of aerobic physical training. It also shows the results of the HRV analysis performed by a non-linear method (symbolic analysis, Figure 2A) and a linear method (spectral analysis, Figure 2B) using Fast Fourier Transform (FFT). In most parameters evaluated, the results were similar between the two methods. Only in the intragroup comparison, the symbolic analysis showed an increase in HRV corresponding to vagal modulation in the MICT and HIIT groups, characterized by an increase in 2UV variations.

4 Discussion

Our results showed that after a 16-week training period, MICT and HIIT groups presented with higher cardiorespiratory fitness than the control, characterized by an increase in VO_{2peak} and a decrease in baseline HR. Both models of physical training reduced testosterone levels, while the control group showed an increase. Metabolic parameters were similar between groups, and these observations may be associated with the maintenance of anthropometric parameters after the 16 weeks. All the

TABLE 1 The descriptive table of characteristics and hemodynamic, hormonal and metabolic parameters obtained before and after the 16-wk physical training (MICT and HIIT groups) or observation period without training (Control group).

	Control (N=25)		MICT (N=25)		HIIT (N=25)	
	Before	**After**	**Before**	**After**	**Before**	**After**
Characteristics						
Age, years	29 ± 5	–	29 ± 5	–	29 ± 4	–
Height, m	1.61 ± 0.07	–	1.62 ± 0.06	–	1.64 ± 0.07	–
Weight, kg	76 ± 15	76 ± 16	73 ± 16	72 ± 16	76 ± 16	75 ± 16
BMI, kg/m^2	29.2 ± 5.4	29.3 ± 5.4	27.7 ± 5.7	27.5± 7.5	27.8 ± 4.2	27.8 ± 4.2
Baseline Cardiovascular Values						
HR (bpm)	69 ± 11	69 ± 10	70 ± 12	66 ± 10	70 ± 10	66 ± 11
SBP (mmHg)	102 ± 11	108 ± 14	106 ± 12	101 ± 12	102 ± 9	103 ± 14
DBP (mmHg)	70 ± 10	71 ± 9	70 ± 10	69 ± 10	68 ± 8	67 ± 11
MAP (mmHg)	81 ± 10	83 ± 10	82 ± 9	80 ± 10	79 ± 7	79 ± 11
Hormonal and Metabolic Values						
VO_{2peak}, mL/min/kg	33.7 ± 5.3	32.3 ± 5.1	32.5 ± 4.7	36.2 ± 5.0 [c]	33.6 ± 3.7	36.9 ± 4.3
Testosterone, ng/dL	86.7 ± 36.9	94.1 ± 42.3	104.7 ± 36.9	87.8 ± 33.3	98.8 ± 41.2	78.6 ± 51.8
Glucose, mg/dL	83.5 ± 7.1	81.8 ± 10.1	82.6 ± 8.4	81.6 ± 6.8	81.9 ± 8.8	81.4 ± 6.6
Insulin, µIU/mL	13.2 ± 9.2	12.8 ± 10.6	12.8 ± 8.1	11.7 ± 8.2	12.1 ± 5.2	11.8 ± 6.3
HOMA-IR	2.69 ± 1.9	2.56 ± 2.3	2.60 ± 1.7	2.41 ± 1.7	2.52 ± 1	2.34 ± 1.3
Triglyceride, mg/dL	117.7 ± 57.5	106 ± 62.9	125 ± 110.3	127.3 ± 91.1	102.4 ± 56.5	110.3 ± 63.6
Cholesterol, mg/dL	189.7 ± 35.4	177.7 ± 24.6	183.4 ± 28	173.2 ± 29.1	180.9 ± 31.1	175 ± 28
HDL, mg/dL	49.6 ± 12.4	47.7 ± 10.6	45.8 ± 8.4	44.7 ± 9.7	48.3 ± 10.6	46.2 ± 9.9
LDL, mg/dL	116.5 ± 33.3	108.6 ± 28.2	109.3 ± 21.8	103.4 ± 24.3	114.3 ± 19.3	107.4 ± 23.7

Values are expressed as means ± SD, standard deviation. MICT, moderate-intensity continuous training; HIIT, high-intensity interval training; BMI, body mass index; HR, heart rate; bpm, beats per minute; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; VO_{2peak}, oxygen uptake at peak exercise; HOMA-IR, homeostatic model assessment for insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

10.3389/fendo.2022.1024844

TABLE 2 Intragroup comparison of baseline hemodynamic, hormonal and metabolic parameters obtained before and after the 16-wk of physical training.

	Control (N=25)		MICT (N=25)		HIIT (N=25)	
	Estimation of difference (CI 95%)	***P value***	**Estimation of difference (CI 95%)**	***P value***	**Estimation of difference (CI 95%)**	***P value***
Baseline hemodynamic parameters						
HR (bpm)	-0.64 (-2.33; 1.06)	.452	4.48 (2.79; 6.17)	< 0.001	4.52 (2.83; 6.22)	< 0.001
SBP (mmHg)	-5.52 (-10.41; -0.62)	.028	4.56 (-0.33; 9.45)	.067	-1.40 (-6.29; 3.49)	.570
DBP (mmHg)	-0.84 (-4.44; 2.76)	.640	0.80 (-2.80; 4.40)	.660	-0.24 (-3.84; 3.36)	.895
MBP (mmHg)	-2.36 (-5.87; 1.15)	.184	2.04 (-1.47; 5.55)	.251	-0.60 (-4.11; 2.91)	.735
Hormonal and Metabolic Values						
VO_{2peak}, mL/min/kg	1.33 (0.06; 2.6)	.040	-3.79 (-5.05; -2.52)	< 0.001	-3.07 (-4.34; -1.81)	< 0.001
BMI, kg/m^2	-0.14 (-0.50; 0.21)	.432	0.16 (-0,19; 0.52)	.368	0.04 (-0,32; 0.39)	.826
Testosterone, ng/dL	-7.36 (-23.01; -8.29)	.035	16.72 (1.07; 32.37)	.037	20.28 (4.63; 35.93)	.012
Glucose, mg/dL	1.66 (-1.0; -4.29)	.023	1.01 (-1.73; 3.75)	.465	0.53 (-2.21; 3.27)	.700
Insulin, µIU/mL	0.22 (-2.34; 2.78)	.862	1.07 (-1.49; 3.63)	.406	0.30 (-2.26; 2.86)	.814
HOMA-IR	0.15 (-0.37; 0.67)	.561	0.20 (-0.32; 0.72)	.444	0.18 (-0.34; 0.69)	.491
Triglyceride, mg/dL	11.72 (-9.54; 32.98)	.276	0.40 (-20.86; 21.66)	.970	2.16 (-19.10; 23.42)	.840
Cholesterol, mg/dL	12.0 (3.0; 21.0)	.009	10.24 (-1.24; 20.24)	.076	6.0 (-3.0; 15.0)	.188
HDL, mg/dL	1.90 (-1.40; 5.21)	.255	1.12 (-2.20; 4.42)	.503	2.16 (-1.15; 5.47)	.197
LDL, mg/dL	7.84 (-1.92; 17.6)	.114	5.92 (-3.84; 15.68)	.231	6.96 (-2.80; 16.72)	.160

Data are presented as means of the confidence intervals (CI) with their respective minimum and maximum values. Statistical analysis was performed by calculating the difference between subtracting the values obtained before and after the 16-week experimental protocol (estimation of difference= before - after). MICT, moderate-intensity continuous training; HIIT, high-intensity interval training; HR, heart rate; bpm, beats per minute; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; VO_{2peak}, oxygen uptake at peak exercise; BMI, body mass index; HOMA-IR, homeostatic model assessment for insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

participants were instructed to maintain a normal diet, and maintenance of anthropometric parameters was expected. However, we expected that both exercise training protocols would increase insulin sensitivity, which did not occur.

Before and after MICT and HIIT, the assessment of BRS and analysis of cardiovascular autonomic modulation using linear methods did show any changes. In this case, MICT and HIIT showed similar cardiovascular autonomic modulation results. In turn, the analysis of HRV through symbolic analysis (non-linear method) showed an increase in vagal modulation only in the HIIT group. In contrast, the comparison between groups showed no differences after 16 weeks. These results contradict the findings of other studies, which have shown that HIIT is more effective than MICT, particularly for some parameters, such as VO_{2peak} and HRV (11–13). However, the prescription of the two training protocols must be considered. In our study, we ensured that both training sessions had similar volumes, controlling not only the daily training time and intensity but also the number of heartbeats during each training session. Monitoring the number of heartbeats ensures that each group has a similar total number of heartbeats per training session (31). This prevents the HIIT group, which has a short training time, from having a greater number of heartbeats per session. During the high metabolic demand (2-min), the training HR reached 85%–90% of the reserve HR, after that, the treadmill's speed and inclination returned to 65%–70% of the reserve HR, that is, the lowest metabolic demand intensity for 3-min. Thus, during the first week of training, especially for sedentary individuals, after reaching 85%–90% of the reserve HR, the exercise intensity decreases (65%–70%) and is not followed by a prompt reduction in HR, which corresponds to this lower intensity. Interestingly, when we consider the rigid pre-determined HIIT interval times, the total number of heartbeats obtained in the HIIT is often higher than the total number obtained in the MICT, even if the latter has a longer training duration. Thus, as mentioned in the methods section, we stipulated a flexible time range for HIIT, which was between 35 and 45 min. This range was used to allow all participants in the HIIT group to obtain similar numbers of total heartbeats compared to the participants in the MICT group. This enabled us to suggest that the most significant results obtained in other studies on the HIIT protocol can be partially explained by a greater volume of training, that is, the number of heartbeats (31). Another important aspect to consider is the variation in BP observed in this study during the physical training sessions, specifically in SBP. SBP reached higher values (≅ 145 mmHg) in the HIIT group than in the MICT group (≅ 130 mmHg). These higher SBP values in HIIT suggest greater endothelial shear stress, promoting prominent adaptations, such as the release of dilating factors derived from the endothelium (32–34). However, we found no statistical differences in resting BP between the training sessions and trained groups. Adherence to the two physical training protocols must also be considered. Despite the greater physical demand in the execution of HIIT, dropouts were slightly lower (N=10) in the HIIT group when compared to those in the MICT group (N=12).

10.3389/fendo.2022.1024844

TABLE 3 Intergroup comparison of baseline hemodynamic, hormonal and metabolic parameters obtained after the 16-wk of physical training.

	Control *vs.* MICT		Control *vs.* HIIT		MICT *vs.* HIIT	
	Estimation of difference (CI 95%)	*P value*	Estimation of difference (CI 95%)	*P value*	Estimation of difference (CI 95%)	*P value*
Baseline hemodynamic parameters						
HR (bpm)	4.12 (-1.60; 9.84)	.155	4.16 (-1.56; 9.88)	.151	0.04 (-5.68; 5.76)	.989
SBP (mmHg)	7.00 (0.21; 13.79)	.044	4.92 (-1.87; 11.71)	.153	-2.08 (-8.87; 4.71)	.544
DBP (mmHg)	1.88 (-3.48; 7.24)	.487	3.32 (-2.04; 8.68)	.221	1.44 (-3.92; 6.80)	.594
MBP (mmHg)	3.56 (-1.86; 8.98)	.195	3.88 (-1.54; 9.30)	.158	0.32 (-5.10; 5.74)	.908
Hormonal and Metabolic Values						
VO_{2peak}, mL/min/kg	-3.95 (-6.63; -1.27)	.005	-4.54 (-7.23; -1.86)	0.001	-0.60 (-3.28; 2.09)	.659
BMI, kg/m^2	1.84 (-1.07; 4.75)	.211	1.57 (-1.34; 4.48)	.285	-0.27 (-3.18; 2.64)	.854
Testosterone, ng/dL	6.20 (-16.74; 29.14)	.591	15.52 (-7.42; 38.46)	.182	9.32 (-13.62; 32.26)	.421
Glucose, mg/dL	0.20 (-4.35; 4.75)	.930	0.44 (-4.11; 4.99)	.848	0.24 (-4.31; 4.79)	.917
Insulin, µIU/mL	1.30 (-3.26; 5.86)	.571	1.19 (-3.37; 5.75)	.605	-0.11 (-4.67; 4.45)	.961
HOMA-IR	1.14 (-0.83; 1.11)	.768	0.22 (-0.75; 1.19)	.652	0.08 (-0.89; 1.05)	.876
Triglyceride, mg/dL	-4.76 (-42.64; 33.12)	.803	-1.36 (-39.24; 36.52)	.943	3.4 (-34.48; 41.28)	.859
Cholesterol, mg/dL	4.56 (-12.08; 21.20)	.587	2.84 (-13.80; 19.48)	.735	-1.72 (-18.36; 14.92)	.837
HDL, mg/dL	3.03 (-2.79; 8.85)	.303	1.56 (-4.26; 7.38)	.596	-1.47 (-7.29; 4.35)	.616
LDL, mg/dL	5.28 (-9.11; 19.67)	.467	1.28 (-13.11; 15.67)	.860	-4.00 (-18.39; 10.38)	.581

Data are presented as means of the confidence intervals (CI) with their respective minimum and maximum values. MICT, moderate-intensity continuous training; HIIT, high-intensity interval training; HR, heart rate; bpm, beats per minute; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; VO_{2peak}, oxygen uptake at peak exercise; BMI, body mass index; HOMA-IR, homeostatic model assessment for insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Regardless of the HRV, BPV, and BRS analysis methods, the clinical relevance of these parameters is widely known as an important predictor of cardiac morbidity and mortality and is often used to investigate autonomic adaptations in cardiac regulation resulting from different diseases (9, 10, 25, 35–37). Moreover, these analysis can be influenced by different metabolic and hormonal variables, such as blood glucose levels, insulin levels, testosterone levels, ovarian hormone levels, obesity, and caffeine intake (15, 26, 38–40). The literature has shown beneficial effects of aerobic physical training on autonomic modulation in individuals with chronic diseases, evidenced by a reduction in sympathetic modulation and an increase in vagal modulation (7, 8, 15–17).

However, in the present study, the participants with PCOS showed modulatory autonomic values similar to those of participants without PCOS, based on other studies that used spectral analysis (linear analysis) as a tool (41, 42). This observation is important because autonomic modulation obtained by spectral analysis seemed intact in these groups. This might be associated with blood glucose and insulin levels, since there were no differences in these parameters between the groups, and the values were within the normal reference range.

In addition, cardiac autonomic modulation has been extensively studied, although there is no consensus among researchers and clinicians regarding the best methodology for the HRV analysis. Since the development of the first computational tools to analyze the physiological mechanisms involved in HRV, several linear and nonlinear methods have been used. The most widely used linear method is the spel analysis, both for clinical and experimental studies (25, 37). Among the various nonlinear methods, symbolic analysis has recently been highlighted (27, 28, 43). This analysis transforms three beats into symbol segments (0V, 1V, 2LV and 2UV) classifying them according to their pattern within the tachogram, created from the delta between the highest and the lowest RRi of the recorded HR (44). Each symbol represents an autonomic modulation response as follows: 0V represents sympathetic modulation, 1V represents both modulations, and 2UV represents vagal modulation (28). Our results suggest a greater sensitivity of symbolic analysis detecting changes in cardiac autonomic modulation, evidenced by the increase in vagal modulation (2UV%) in MICT and HIIT groups, however, in the intergroup comparison, no difference was observed. In this case, further studies are needed to assess whether there are advantages of symbolic (non-linear) analysis over spectral (linear) analysis.

The current study had some limitations: the mean BMI of all groups was classified as overweight, since the body fat percentage may influence cardiovascular autonomic modulation. The dosages of inflammatory markers and oxidative stress could have provided additional important information to differentiate the effects of the two training models. In addition, the application of a physical training period longer than 16 weeks could facilitate the discrimination

10.3389/fendo.2022.1024844

TABLE 4 The descriptive table of cardiovascular autonomic parameters obtained before and after the 16-wk of physical training (MICT and HIIT groups) or observation period without training (Control group).

	Control (N=25)		MICT (N=25)		HIIT (N=25)	
	Before	**After**	**Before**	**After**	**Before**	**After**
HRV - Time domain						
RRi, ms	866 ± 119	865 ± 115	829 ± 114	909 ± 94	823 ± 110	908 ± 126
SD	56 ± 31	57 ± 26	55 ± 23	56 ± 22	54 ± 26	62 ± 37
RMSSD, ms	54 ± 43	52 ± 33	52 ± 32	54 ± 27	48 ± 34	58 ± 45
HRV – Frequency domain						
Variance, ms^2	2495 ± 2783	2906 ± 2507	2827 ± 2445	2990 ± 2352	2245 ± 2156	2515 ± 2521
LF, ms^2	713 ± 741	851 ± 683	771 ± 808	737 ± 561	657 ± 710	620 ± 643
HF, ms^2	1152 ± 1662	1295 ± 1525	1375 ± 1478	1208 ± 1164	791 ± 1052	888 ± 1141
LF, nu	45 ± 14	46 ± 18	42 ± 19	43 ± 14	48 ± 14	44 ± 13
HF, nu	55 ± 14	54 ± 18	58 ± 19	57 ± 14	52 ± 14	56 ± 13
LF/HF Ratio	0,96 ± 0.53	0.99 ± 0.57	0.98 ± 0.84	0.85 ± 0.49	0.96 ± 0.41	0.89 ± 0.53
HRV - Symbolic analysis						
0V %	21.9 ± 12	23.2 ± 15	23.2 ± 12	17.9 ± 11	26.1 ± 13	20.4 ± 9.6
2UV %	22.1 ± 12	20 ± 12	18.7 ± 7.5	22.6 ± 8.9	19.1 ± 9.7	23.5 ± 12
BPV – Frequency domain						
Variance, $mmHg^2$	19 ± 8.7	20.7 ± 8.6	19.5 ± 9.4	20 ± 12.5	23.8 ± 12.1	20.2 ± 10.4
LF, $mmHg^2$	4.9 ± 2.3	5.5 ± 2.2	4.9 ± 2.7	5.6 ± 3.4	6.6 ± 3.1	6.2 ± 3.7
BRS						
Ramp numbers	87 ± 46	82 ± 41	83 ± 48	85 ± 42	86 ± 38	78 ± 35
BEI	0.59 ± 0.14	0.58 ± 0.19	0.55 ± 0.19	0.62 ± 0.12	0.63 ± 0.12	0.6 ± 0.14
UP, ms/mmHg	15.7 ± 10.6	15.4 ± 8.3	14 ± 8.5	16 ± 8.1	13.5 ± 6.6	16 ± 8.3
DOWN, ms/mmHg	16.5 ± 9.4	16.6 ± 7.9	14.4 ± 7.5	16.6 ± 7.5	14.6 ± 6.9	17.4 ± 8.8
GAIN, ms/mmHg	16.2 ± 9.7	16.1 ± 7.9	14.3 ± 7.9	16.5 ± 7.4	14.1 ± 6.7	16.8 ± 8.6

Values are expressed as means ± SD, standard deviation. MICT, moderate-intensity continuous training; HIIT, high-intensity interval training; HRV, heart rate variability; RRi, R-R interval; ms, millisecond; SD, standard deviation; RMSSD, root mean square of successive normal sinus RR-interval; LF, low-frequency; HF, high-frequency; nu, normalized unit; 0V%, patterns with no variation (sympathetic modulation); 2UV, patterns with two unlike variation (vagal modulation); BPV, blood pressure variability; BEI, baroreflex effectiveness index.

TABLE 5 Intragroup comparison of autonomic parameters obtained before and after the 16-wk of physical training.

	Control (N=25)		MICT (N=25)		HIIT (N=25)	
	Estimation of difference (CI 95%)	***P value***	**Estimation of difference (CI 95%)**	***P value***	**Estimation of difference (CI 95%)**	***P value***
HRV – Time domain						
RRi, ms	0.73 (-47.96; 49.43)	.976	-76.64 (-125.34; -27.95)	0.003	-85.02 (-133.72; -36.33)	<0.001
SD, ms	-1.91 (-11.77; 7.94)	.700	-1.56 (-11.41; 8.30)	.754	-7.90 (-17.76; 1.95)	.114
RMSSD, ms	1.25 (-10.23; 12.73)	.829	-1.36 (-12.85; 10.11)	.813	-9.17 (-20.65; 2.31)	.116
HRV – Frequency domain						
Variance, ms^2	-410.42 (-1161.18; 340.35)	.280	-163.50 (-914.27; 587.26)	.666	-270.28 (-1021.05; 480.48)	.475
LF, ms^2	-138.18 (-386.52; 110.15)	.271	33.53 (-214,81; 281.87)	.789	37.23 (-211,11; 285.57)	.766
HF, ms^2	-143.02 (-533.65; 247.61)	.468	166.32 (-224.31; 556.94)	.399	-97.17 (-487.80; 293.46)	.622
LF, nu	-1.34 (-7.87; 5.20)	.685	-1.03 (-7.57; 5.50)	.754	4.32 (-2.21; 10.86)	.191
HF, nu	1.34 (-5.20; 7.87)	.685	1.03 (-5.50; 7.57)	.754	-4.32 (-10.86; 2.21)	.191
LF/HF Ratio	-0.04 (-0.27; 0.19)	.734	0.13 (-0.11; 0.36)	.278	0.08 (-0.16; 0.31)	.519
HRV – Symbolic analysis						
0V %	-1.33 (-7.04; 4.38)	.643	5.27 (-0.44; 10.98)	.070	5.71 (0.004; 11.42)	.050
2UV %	2.07 (-1.53; 5.66)	.255	-3.84 (-7.44; -0.25)	.036	-4.39 (-7.98; -0.79)	.017

(Continued)

10.3389/fendo.2022.1024844

TABLE 5 Continued

	Control (N=25)		MICT (N=25)		HIIT (N=25)	
	Estimation of difference (CI 95%)	***P value***	**Estimation of difference (CI 95%)**	***P value***	**Estimation of difference (CI 95%)**	***P value***
BPV – Frequency domain						
Variance, $mmHg^2$	-1.64 (-6.27; 3.0)	.484	-0.61 (-5.24; 4.03)	.795	3.65 (-0.99; 8.28)	.121
LF, $mmHg^2$	-0.61 (-1.86; 0.65)	.337	-0.70 (-1.96; 0.55)	.267	0.40 (-0.85; 1.66)	.553
BRS						
Ramp numbers	4.95 (-11.19; 21.09)	.543	-1.40 (-17.54; 14.74)	.863	7.95 (-8.19; 24.09)	.330
BEI	0.12 (-0.06; 0.08)	.739	-0.07 (-0,14; 0.0004)	.050	0.03 (-0,04; 0.10)	.429
UP, ms/mmHg	0.35 (-3.79; 4.49)	.867	-2.03 (-6.17; 2.11)	.332	-2.47 (-6.61; 1.67)	.238
DOWN, ms/mmHg	-0.09 (-3.40; 3.22)	.958	-2.20 (-5.51; 1.12)	.190	-2.84 (-6.15; 0.48)	.092
GAIN, ms/mmHg	0.08 (-3.52; 3.68)	.967	-2.21 (-5.81; 1.39)	.226	-2.74 (-6.34; 0.86)	.134

Data are presented as means of the confidence intervals (CI) with their respective minimum and maximum values. Statistical analysis was performed by calculating the difference between subtracting the values obtained before and after the 16-week experimental protocol (estimation of difference= before - after). MICT, moderate-intensity continuous training; HIIT, high-intensity interval training; HRV, heart rate variability; RRi, R-R interval; ms, millisecond; SD, standard deviation; RMSSD, root mean square of successive normal sinus RR-interval; LF, low-frequency; HF, high-frequency; nu, normalized unit; 0V%, patterns with no variation (sympathetic modulation); 2UV, patterns with two unlike variation (vagal modulation); BPV, blood pressure variability; BEI, baroreflex effectiveness index.

TABLE 6 Intergroup comparison of autonomic parameters obtained after the 16-wk of physical training.

	Control *vs.* MICT		Control *vs.* HIIT		MICT *vs.* HIIT	
	Estimation of difference (CI 95%)	***P value***	**Estimation of difference (CI 95%)**	***P value***	**Estimation of difference (CI 95%)**	***P value***
HRV – Time domain						
RRi, ms	-41.03 (-105.09; 23.02)	.206	-43.56 (-107.62; -20.50)	.180	-2.53 (-66.58; 61.53)	.936
SD, ms	1.22 (-14.58; 17.03)	.879	-4.56 (-20.37; 11.24)	.567	-5.79 (-21.59; 10.02)	.468
RMSSD, ms	-1.19 (-21.48; 19.10)	.907	-5.08 (-25.37; 15.21)	.619	-3.89 (-24.18; 16.40)	.703
HRV – Frequency domain						
Variance, ms^2	-84.48 (-1476.25; 1307.28)	.904	390.34 (-1001.43; 1782.10)	.578	474.82 (-916.95; 1866.59)	.499
LF, ms^2	113.52 (-278.60; 505.64)	.566	230.48 (-161.64; 622.60)	.245	116.96 (-275.16; 509.08)	.554
HF, ms^2	87.00 (-677.65; 851.64)	.821	407.01 (-357.63; 1171.65)	.292	320.02 (-444.63; 1084.66)	.407
LF, nu	3.54 (-5.23; 12.31)	.424	2.10 (-6.67; 10.87)	.635	-1.44 (-10.21; 7.33)	.745
HF, nu	-3.54 (-12.31; 5.23)	.424	-2.10 (-10.87; 6.67)	.635	1.44 (-7.33; 10.21)	.745
LF/HF Ratio	0.15 (-0.18; 0.48)	.370	0.10 (-0.23; 0.42)	.560	-0.05 (-0.38; 0.28)	.752
HRV – Symbolic analysis						
0V %	5.29 (-1.59; 12.17)	.130	2.79 (-4.09; 9.67)	.421	-2.50 (-9.38; 4.38)	.471
2UV %	-2.52 (-8.49; 3.46)	.404	-3.46 (-9.43; 2.51)	.251	-0.94 (-6.92; 5.03)	.754
BPV – Spectral Analysis						
Variance, $mmHg^2$	0.52 (-5.35; 6.40)	.859	0.48 (-5.40; 6.35)	.872	-0.05 (-5.92; 5.82)	.987
LF, $mmHg^2$	-0.06 (-1.72; 1.59)	.939	-0.63 (-2.29; 1.03)	.450	-0.57 (-2.23; 1.09)	.497
BRS						
Ramp numbers	2.60 (-26.16; 20.96)	.827	3.79 (-19.78; 27.35)	.750	6.39 (-17.18; 29.95)	.591
BEI	-0.05 (-0.13; 0.04)	.290	-0.03 (-0.11; 0.06)	.528	0.02 (-0.07; 0.11)	.667
UP, ms/mmHg	-0.97 (-5.76; 3.81)	.687	-0.60 (-5.38; 4.18)	.803	0.37 (-4.41; 5.16)	.877

(Continued)

TABLE 6 Continued

	Control *vs.* MICT		Control *vs.* HIIT		MICT *vs.* HIIT	
	Estimation of difference (CI 95%)	*P value*	Estimation of difference (CI 95%)	*P value*	Estimation of difference (CI 95%)	*P value*
DOWN, ms/mmHg	-0.05 (-4.59; 4.48)	.982	-0.86 (-5.40; 3.68)	.707	-0.81 (-5.34; 3.73)	.724
GAIN, ms/mmHg	-0.46 (-5.02; 4.10)	.841	-0.74 (-5.29; 3.82)	.748	-0.28 (-4.83; 4.28)	.904

Data are presented as means of the confidence intervals (CI) with their respective minimum and maximum values.
MICT, moderate-intensity continuous training; HIIT, high-intensity interval training; HRV, heart rate variability; RRi, R-R interval; ms, millisecond; SD, standard deviation; RMSSD, root mean square of successive normal sinus RR-interval; LF, low-frequency; HF, high-frequency; nu, normalized unit; 0V%, patterns with no variation (sympathetic modulation); 2UV, patterns with two unlike variation (vagal modulation); BPV, blood pressure variability; BEI, baroreflex effectiveness index.

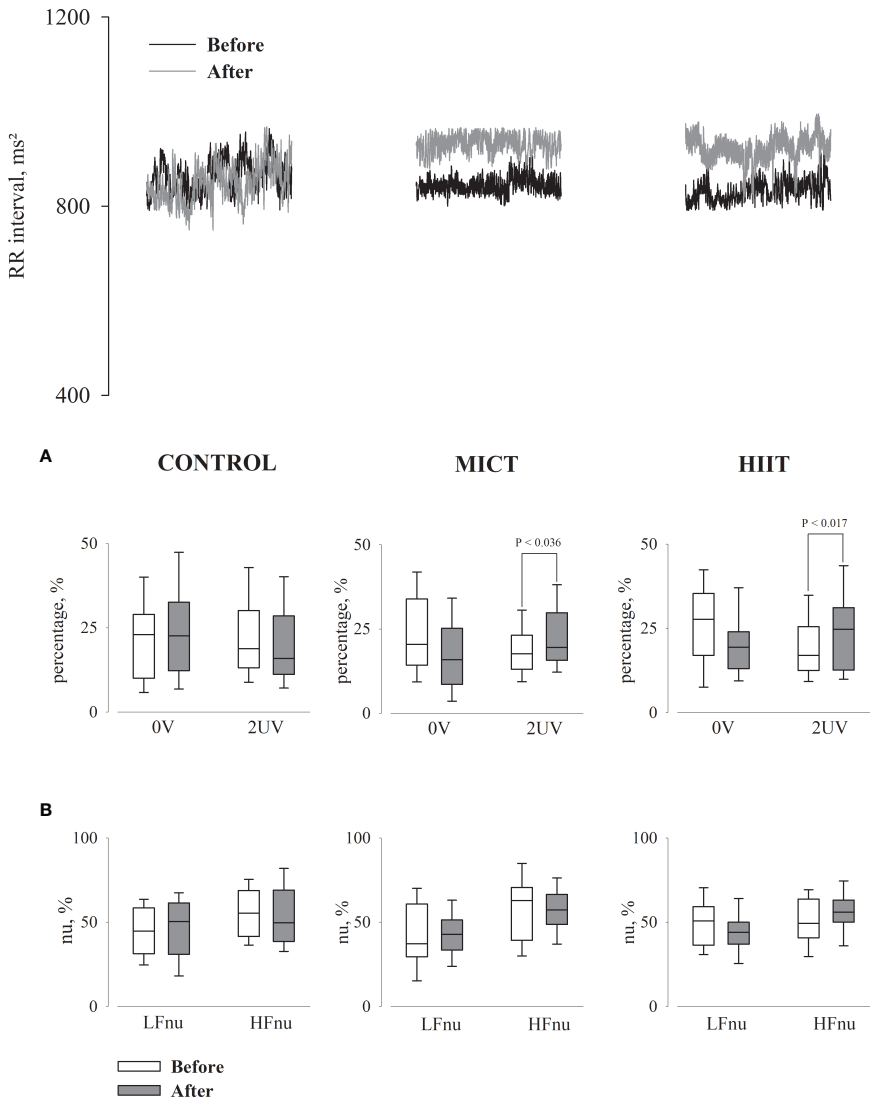


FIGURE 2
Cardiac interval segments obtained during rest in supine position for all groups, before and after 16 weeks of the intervention. **(A)** Graphs showing the symbolic analysis; 0V, patterns with no variation (sympathetic modulation); 2UV, patterns with two unlike variation (vagal modulation). **(B)** Graphs showing the spectral analysis in normalized units (nu); LF, low frequency band (sympathetic modulation); HF, high frequency band (vagal modulation).

of possible differences between the MICT and HIIT. However, it is important to note that these limitations do not invalidate the main findings of this study.

5 Conclusion

In conclusion, MICT and HIIT had similar effects on anthropometric, metabolic and hormonal parameters. Both increased cardiorespiratory fitness and reduced baseline HR and serum testosterone levels. They also did not differ in relation to the effects on cardiovascular autonomic modulation and BRS. In this case, the only finding was the increa in vagal autonomic modulation in both trained groups. However, this finding was only observed in the non-linear analysis. Thus, we did not find greater benefits of HIIT compared to MICT in women with PCOS for all parameters evaluated.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Ribeirão Preto Medical School's Hospital (Protocol number 845.830/2014). The patients/participants provided their written informed consent to participate in this study.

Author contributions

SP participated in the acquisition of data, analysis and interpretation of data, manuscript preparation for the original draft. VR participated in acquisition of data and project administration. JT participated in the interpretation of data and manuscript review for intellectual content. RR participated in the study design, conceptualization, and manuscript review. DG participated in the interpretation of data and statistical analysis. HS participated in the conception, design of the study, analysis and interpretation of data, and manuscript review. All authors have read and approved the final manuscript.

Funding

This work was supported by the São Paulo Research Foundation – FAPESP (Process 2015/14031-0; 2018/08569-6; and 2018/17892-5).

Acknowledgments

We would like to thank all the participants for their commthe itment and adherence to the training protocol.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1024844/full#supplementary-material

References

1. Bozdag G, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO. The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reprod* (2016) 31:2841–55. doi: 10.1093/humrep/dew218

2. Sirmans SM, Pate KA. Epidemiology, diagnosis, and management of polycystic ovary syndrome. *Clin Epidemiol* (2013) 6:1–13. doi: 10.2147/CLEP.S37559

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. doi: 10.1016/j.fertnstert.2011.09.024

4. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the

assessment and management of polycystic ovary syndrome. *Fertil Steril* (2018) 110:364–79. doi: 10.1016/j.fertnstert.2018.05.004

5. Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. *Lancet* (2007) 370:685–97. doi: 10.1016/S0140-6736(07)61345-2

6. Tekin G, Tekin A, Kiliçarslan EB, Haydardedeoğlu B, Katircibaşi T, Koçum T, et al. Altered autonomic neural control of the cardiovascular system in patients with polycystic ovary syndrome. *Int J Cardiol* (2008) 130:49–55. doi: 10.1016/j.ijcard.2007.08.037

7. Pundir J, Charles D, Sabatini L, Hiam D, Jitpiriyaroj S, Teede H, et al. Overview of systematic reviews of non-pharmacological interventions in women with polycystic ovary syndrome. *Hum Reprod Update* (2019) 25:243–56. doi: 10.1093/humupd/dmy045

8. Sá JCF, Costa EC, da Silva E, Tamburús NY, Porta A, Medeiros LF, et al. Aerobic exercise improves cardiac autonomic modulation in women with polycystic ovary syndrome. *Int J Cardiol* (2016) 202:356–61. doi: 10.1016/j.ijcard.2015.09.031

9. Besnier F, Labrunée M, Pathak A, Pavy-Le Traon A, Galès C, Sénard J-M, et al. Exercise training-induced modification in autonomic nervous system: An update for cardiac patients. *Ann Phys Rehabil Med* (2017) 60:27–35. doi: 10.1016/j.rehab.2016.07.002

10. Villafaina S, Collado-Mateo D, Fuentes JP, Merellano-Navarro E, Gusi N. Physical exercise improves heart rate variability in patients with type 2 diabetes: A systematic review. *Curr Diabetes Rep* (2017) 17:110. doi: 10.1007/s11892-017-0941-9

11. Wisløff U, Støylen A, Loennechen JP, Bruvold M, Rognmo Ø, Haram PM, et al. Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation* (2007) 115:3086–94. doi: 10.1161/CIRCULATIONAHA.106.675041

12. Thum JS, Parsons G, Whittle T, Astorino TA. High-intensity interval training elicits higher enjoyment than moderate intensity continuous exercise. *PloS One* (2017) 12:e0166299. doi: 10.1371/journal.pone.0166299

13. Ghardashi-Afousi A, Holisaz MT, Shirvani H, Pishgoo B. The effects of low-volume high-intensity interval versus moderate intensity continuous training on heart rate variability, and hemodynamic and echocardiography indices in men after coronary artery bypass grafting: A randomized clinical trial study. *ARYA Atheroscler* (2018) 14:260–71. doi: 10.22122/arya.v14i6.1781

14. Jaureguizar KV, Vicente-Campos D, Bautista LR, de la Peña CH, Gómez MJA, Rueda MJC, et al. Effect of high-intensity interval versus continuous exercise training on functional capacity and quality of life in patients with coronary artery disease: A RANDOMIZED CLINICAL TRIAL. *J Cardiopulm Rehabil Prev* (2016) 36:96–105. doi: 10.1097/HCR.0000000000000156

15. Masson GS, Borges JP, da Silva PPS, da Nóbrega ACL, Tibiriçá E, Lessa MA. Effect of continuous and interval aerobic exercise training on baroreflex sensitivity in heart failure. *Auton Neurosci* (2016) 197:9–13. doi: 10.1016/j.autneu.2016.03.003

16. Abad CCC, do Nascimento AM, de Souza LE, Figueroa D, Ramona P, Sartori M, et al. Interval and continuous aerobic exercise training similarly increase cardiac function and autonomic modulation in infarcted mice. *J Exerc Rehabil* (2017) 13:257–65. doi: 10.12965/jer.1734914.457

17. Pattyn N, Beckers PJ, Cornelissen VA, Coeckelberghs E, De Maeyer C, Frederix G, et al. The effect of aerobic interval training and continuous training on exercise capacity and its determinants. *Acta Cardiol* (2017) 72:328–40. doi: 10.1080/00015385.2017.1304712

18. Ramos JS, Dalleck LC, Borrani F, Beetham KS, Mielke GI, Dias KA, et al. High-intensity interval training and cardiac autonomic control in individuals with metabolic syndrome: A randomised trial. *Int J Cardiol* (2017) 245:245–52. doi: 10.1016/j.ijcard.2017.07.063

19. Aktaş HŞ, Uzun YE, Kutlu O, Pençe HH, Özçelik F, Çil EÖ, et al. The effects of high intensity-interval training on vaspin, adiponectin and leptin levels in women with polycystic ovary syndrome. *Arch Physiol Biochem* (2022) 128:37–42. doi: 10.1080/13813455.2019.1662450

20. Ribeiro VB, Kogure GS, Lopes IP, Silva RC, Pedroso DCC, Melo AS, et al. Effects of continuous and intermittent aerobic physical training on hormonal and metabolic profile, and body composition in women with polycystic ovary syndrome: A randomized controlled trial. *Clin Endocrinol* (2020) 93:173–86. doi: 10.1111/cen.14194

21. Ribeiro VB, Lopes IP, dos Reis RM, Silva RC, Mendes MC, Melo AS, et al. Continuous versus intermittent aerobic exercise in the improvement of quality of life for women with polycystic ovary syndrome: A randomized controlled trial. *J Health Psychol* (2021) 26:1307–17. doi: 10.1177/1359105319869806

22. Lopes IP, Ribeiro VB, Reis RM, Silva RC, Dutra de Souza HC, Kogure GS, et al. Comparison of the effect of intermittent and continuous aerobic physical training on sexual function of women with polycystic ovary syndrome: Randomized controlled trial. *J Sex Med* (2018) 15:1609–19. doi: 10.1016/j.jsxm.2018.09.002

23. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related ? A3B2 show [#,32] ?>to polycystic ovary syndrome. *Fertil Steril* (2004) 81:19–25. doi: 10.1016/j.fertnstert.2003.10.004

24. Geloneze B, Repetto EM, Geloneze SR, Tambascia MA, Ermetice MN. The threshold value for insulin resistance (HOMA-IR) in an admixtured population IR in the Brazilian metabolic syndrome study. *Diabetes Res Clin Pract* (2006) 72:219–20. doi: 10.1016/j.diabres.2005.10.017

25. . Electrophysiology Task Force of the European Society of Cardiology the North American Society of Pacing. Heart rate variability. *Circulation* (1996) 93:1043–65. doi: 10.1161/01.CIR.93.5.1043

26. Philbois SV, Gastaldi AC, Facioli T de P, Felix ACS, Reis RMD, Fares TH, et al. Women with polycystic ovarian syndrome exhibit reduced baroreflex sensitivity that may be associated with increased body fat. *Arq Bras Cardiol* (2019) 112:424–9. doi: 10.5935/abc.20190031

27. Porta A, Guzzetti S, Montano N, Furlan R, Pagani M, Malliani A, et al. Entropy, entropy rate, and pattern classification as tools to typify complexity in short heart period variability series. *IEEE Trans BioMed Eng* (2001) 48:1282–91. doi: 10.1109/10.959324

28. Porta A, Tobaldini E, Guzzetti S, Furlan R, Montano N, Gnecchi-Ruscone T. Assessment of cardiac autonomic modulation during graded head-up tilt by symbolic analysis of heart rate variability. *Am J Physiol Heart Circ Physiol* (2007) 293:H702–708. doi: 10.1152/ajpheart.00006.2007

29. Bertinieri G, di Rienzo M, Cavallazzi A, Ferrari AU, Pedotti A, Mancia G. A new approach to analysis of the arterial baroreflex. *J Hypertens Suppl* (1985) 3:S79–81.

30. Di Rienzo M, Parati G, Castiglioni P, Tordi R, Mancia G, Pedotti A. Baroreflex effectiveness index: an additional measure of baroreflex control of heart rate in daily life. *Am J Physiology-Regulatory Integr Comp Physiol* (2001) 280:R744–51. doi: 10.1152/ajpregu.2001.280.3.R744

31. Rodrigues JAL, Philbois SV, de Paula Facioli T, Gastaldi AC, de Souza HCD. Should Heartbeats/Training session be considered when comparing the cardiovascular benefits of high-intensity interval aerobic and moderate-intensity continuous training? a critical appraisal. *Sports Med Open* (2020) 6:29. doi: 10.1186/s40798-020-00257-8

32. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* (1980) 288:373–6. doi: 10.1038/288373a0

33. Moncada S, Palmer RM, Gryglewski RJ. Mechanism of action of some inhibitors of endothelium-derived relaxing factor. *Proc Natl Acad Sci USA* (1986) 83:9164–8. doi: 10.1073/pnas.83.23.9164

34. Souza HCD, Terzini GCSV, da Silva VJD, Martins-Pinge MC, Salgado HC, Salgado M-CO. Increased cardiac sympathetic drive and reduced vagal modulation following endothelin receptor antagonism in healthy conscious rats. *Clin Exp Pharmacol Physiol* (2008) 35:751–6. doi: 10.1111/j.1440-1681.2008.04866.x

35. Routledge FS, Campbell TS, McFetridge-Durdle JA, Bacon SL. Improvements in heart rate variability with exercise therapy. *Can J Cardiol* (2010) 26:303–12. doi: 10.1016/s0828-282x(10)70395-0

36. Murad K, Brubaker PH, Fitzgerald DM, Morgan TM, Goff DC, Soliman EZ, et al. Exercise training improves heart rate variability in older patients with heart failure: a randomized, controlled, single-blinded trial. *Congest Heart Fail* (2012) 18:192–7. doi: 10.1111/j.1751-7133.2011.00282.x

37. Billman GE. Heart rate variability - a historical perspective. *Front Physiol* (2011) 2:86. doi: 10.3389/fphys.2011.00086

38. Iellamo F, Manzi V, Caminiti G, Sposato B, Massaro M, Cerrito A, et al. Dose-response relationship of baroreflex sensitivity and heart rate variability to individually-tailored exercise training in patients with heart failure. *Int J Cardiol* (2013) 166:334–9. doi: 10.1016/j.ijcard.2011.10.082

39. Indumathy J, Pal GK, Pal P, Ananthanarayanan PH, Parija SC, Balachander J, et al. Decreased baroreflex sensitivity is linked to sympathovagal imbalance, body fat mass and altered cardiometabolic profile in pre-obesity and obesity. *Metab Clin Exp* (2015) 64:1704–14. doi: 10.1016/j.metabol.2015.09.009

40. De Melo VU, Saldanha RRM, Dos Santos CR, De Campos Cruz J, Lira VA, Santana-Filho VJ, et al. Ovarian hormone deprivation reduces oxytocin expression in paraventricular nucleus preautonomic neurons and correlates with baroreflex impairment in rats. *Front Physiol* (2016) 7:461. doi: 10.3389/fphys.2016.00461

41. Dutra SGV, Pereira APM, Tezini GCSV, Mazon JH, Martins-Pinge MC, Souza HCD. Cardiac autonomic modulation is determined by gender and is independent of aerobic physical capacity in healthy subjects. *PloS One* (2013) 8: e77092. doi: 10.1371/journal.pone.0077092

42. Facioli TP, Gastaldi AC, Dutra SGV, Felix ACS, Philbois SV, Sánchez-Delgado JC, et al. The blood pressure variability and baroreflex sensitivity in healthy participants are not determined by sex or cardiorespiratory fitness. *Blood Press Monit* (2018) 23:260–70. doi: 10.1097/MBP.0000000000000338

43. Guzzetti S, Borroni E, Garbelli PE, Ceriani E, Della Bella P, Montano N, et al. Symbolic dynamics of heart rate variability: a probe to investigate cardiac autonomic modulation. *Circulation* (2005) 112:465–70. doi: 10.1161/CIRCULATIONAHA.104.518449

44. Griffin IJ, Cole TJ, Duncan KA, Hollman AS, Donaldson MD. Pelvic ultrasound measurements in normal girls. *Acta Paediatr* (1995) 84:536–43. doi: 10.1111/j.1651-2227.1995.tb13689.x

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 28 November 2022
DOI 10.3389/fendo.2022.1038184

OPEN ACCESS

EDITED BY
Bianca Bianco,
Faculdade de Medicina do ABC, Brazil

REVIEWED BY
Abbas Alam Choudhury,
VIT University, India
Gengxiang Wu,
Renmin Hospital of Wuhan University, China

*CORRESPONDENCE
Kunming Li
likunming@51mch.com
Liyan Duan
FL1114@126.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

RECEIVED 06 September 2022
ACCEPTED 10 November 2022
PUBLISHED 28 November 2022

CITATION
Zhou H, Xu J, Hong L, Jia Y, Burk LV, Chi F, Zhao M, Guan X, Liu D, Yin X, Zhang Y, Teng X, Duan L and Li K (2022) The alterations of circulating mucosal-associated invariant T cells in polycystic ovary syndrome. *Front. Endocrinol.* 13:1038184. doi: 10.3389/fendo.2022.1038184

The alterations of circulating mucosal-associated invariant T cells in polycystic ovary syndrome

Hong Zhou[1†], Junting Xu[1†], Ling Hong[1†], Yanping Jia[1], Lilo Valerie Burk[2], Fengli Chi[1], Mei Zhao[1], Xiaohong Guan[3], Dan Liu[1], Xiangjie Yin[3], Yiqiao Zhang[4], Xiaoming Teng[1], Liyan Duan[2,5*] and Kunming Li[1*]

[1]Center for Assisted Reproduction, Shanghai First Maternity and Infant Hospital, School of Medicine, Tongji University, Shanghai, China, [2]Department of Gynecology and Obstetrics, University of Duisburg-Essen, Essen, Germany, [3]Department of Obstetrics and Gynecology, Shanghai First Maternity and Infant Hospital, School of Medicine, Tongji University, Shanghai, China, [4]Institute of Pharmacology, University Duisburg-Essen, Essen, Germany, [5]Reproductive Medicine Center, Nanjing Drum Tower Hospital, Nanjing University School of Medicine, Nanjing, China

Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder affecting reproductive age females and an important cause of infertility. Although the etiology is complex and its pathogenesis remains unclear, the pathological process of PCOS is tightly related with the immune dysfunction and gut microbial dysbiosis. Mucosal-associated invariant T (MAIT) cells are a subset of innate-like T cells which can regulate inflammation through the production of cytokines and play a role in regulating the gut microbiota. We aim to evaluate the correlation between characteristics of PCOS and MAIT cells as well as their impact on cytokine secretion.

Methods: Peripheral blood samples were taken from PCOS patients (n=33) and healthy controls (n=30) during 2-5 days of the menstrual period. The frequencies of MAIT cells and T cells were measured by flow cytometry. Cytokines interleukin 17 (IL-17), interleukin 22(IL-22), interferon γ (IFN-γ) and granzyme B were determined by Enzyme-linked immunosorbent assay (ELISA).

Results: The frequency of MAIT cells was significantly reduced in the blood of PCOS patients compared with the controls, and negatively correlated with Body Mass Index (BMI), Homeostatic model assessment- insulin resistance (HOMA-IR) index, and Anti Miillerian Hormone (AMH). Thus, the frequencies of MAIT cells decreased in PCOS patients with abnormal weight (BMI≥24kg/m2), higher HOMA-IR (≥1.5), and excessive AMH (≥8ng/ml). The Cytokine IL-17 was significantly higher in PCOS patients and negatively correlated with the frequency of MAIT cells. Even though the IL-22 was lower in PCOS Patients, no correlation with MAIT cells was detected. In subgroup, CD4+MAIT cells correlated with BMI, AMH, and testosterone (T) levels.

10.3389/fendo.2022.1038184

Conclusion: The frequency change of MAIT cells may play a significant role in the pathogenesis of PCOS. Exploring these interactions with MAIT cells may provide a new target for PCOS treatment and prevention.

KEYWORDS

Mucosal-associated invariant T cells, Polycystic Ovary Syndrome, Interleukin 17, Interleukin 22, CD4+ Mucosal-associated invariant T cells

Introduction

Polycystic ovary syndrome (PCOS) is the most common reproductive endocrine disorder affecting reproductive age females, with an incidence of 5% to 20% worldwide (1). A Chinese community population study found the prevalence of PCOS in China is 5.6% (2).

PCOS has heterogeneous clinical characteristics: polycystic ovarian morphology (PCOM), clinical or biochemical hyperandrogenism, anovulation, and metabolic disorders such as insulin resistance (IR) and obesity (3–5). In the long term, patients with PCOS are at increased risk of diabetes, cardiovascular diseases, endometrial cancer, pregnancy complications and depression due to metabolic disturbance, which result in a heavy global burden (6–10). Although the etiology of PCOS has been studied for decades, the pathogenesis is still unclear and a causative therapy is still missing. Treatments are currently tailored to specific symptoms, include oral contraceptives or hormones to manage the menstrual cycle and control the symptoms of hyperandrogenemia, lifestyle modifications and metformin to improve metabolic syndrome such as insulin sensitivity and obesity, ovulation induction and assisted reproductive techniques to manage the PCOS-induced infertility (11).

Recently, researchers have focused on the hormones and immune cells, including both innate and adaptive immune cells, which have been reported to be a cross-talk in PCOS (12–14). It is well known that the endocrine markers of PCOS are hyperandrogenism and hyperinsulinemia. Previous studies reported a relatively high leukocyte count in PCOS patients with hyperinsulinemia and hyperandrogenemia, which exhibits a low-grade chronic inflammatory state and accounts for a disturbance in T cell polarization (15, 16). T cell polarization markers, such as interferon γ (IFN-γ) and interleukin 17 (IL-17) were increased in PCOS patients (17). However, interleukin 22 (IL-22) secreted by innate lymphocytes leading to an improved IR was decreased in PCOS patients (18). Further results showed that granzyme-B was higher in PCOS patients which was positively correlated with hyperandrogenemia (19).

Mucosal-associated invariant T (MAIT) cells are a kind of unconventional innate-like T cells defined as CD3+CD161 +Vα7.2+cells. They express semi-constant T cell receptor (TCR) composed of TCRα and TCRβ chains (20, 21). This receptor can specifically identify microbial-derived vitamin B metabolites presented by major histocompatibility complex class I-related protein 1(MR1) and plays a crucial role in local and systemic immune-inflammatory responses (22, 23).

MAIT cells are abundant in humans and comprise 1-10% of blood CD3+T cells. They are more enriched in the liver, intestine, and other mucosal tissues (23). Furthermore MAIT cells play a key role in the immune system and other pathologies such as tumors, they can attack healthy cells and thus contribute in certain autoimmune diseases and microbial infection (24–32). Previous studies found that alterations in the frequency and function of MAIT cells in circulation are associated with many metabolic diseases, such as diabetes/IR and obesity (33–37). As we know, PCOS is tightly associated with metabolic disorders, therefor we hypothesize that MAIT cells are related to the disorder of PCOS patients.

Until now, there are five different MAIT subsets which are CD4+CD8-, CD4+CD8+, CD4- CD8-, CD4- CD8αα+ and CD4- CD8αβ+ subset (38, 39). About 95% MAIT cells are CD4- subset (CD4– CD8αα+ or CD4– CD8αβ+ MAIT cells accounts for 80%, CD4- CD8- MAIT constitute 15%), only 5% of MAIT cells are CD4+ MAIT (CD4+CD8- and CD4+CD8+ subsets of MAIT cells) (39). Whereas CD4+ MAIT were focused on less, as this subset may be functionally distinct from others, Zhu et al. found that CD8+ MAIT cells are associated with metabolic dysfunction in PCOS patients (40). To date there has been no research on the role of total MAIT cells in PCOS patients.

We analyzed the frequency and phenotype of circulating MAIT cells (total MAIT cells and CD4+MAIT cells) in the blood of PCOS patients to explore their plausible clinical relevance which may

provide novel therapeutic strategies to interfere with the PCOS disease.

Materials and methods

Patient samples

Ethical approval for this study was granted by the Scientific and Ethical Committee of the Shanghai First Maternity and Infant Hospital affiliated with Tongji University (NO: KS2132). All participating patients signed the informed consent. This study included 33 patients with PCOS and 30 healthy controls. The participants of control group were undergoing *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) due to male factor infertility or tubal factors, and PCOS patients were enrolled from the Endocrinology clinic or undergoing IVF or ICSI diagnosed according to the Rotterdam diagnostic criteria of 2003 (8) at Shanghai First Maternity and Infant Hospital between December 2020 and February 2022. All participants were between 18 to 40 years old, had no history of hereditary or familial diseases, and did not take any drugs. Patients with thyroid dysfunction, diabetes, other endocrine disorders, endometriosis, and autoimmune diseases were excluded. All patients' clinical data are listed in Table 1.

Flow cytometry analysis

100ul of whole blood was incubated for 2o minutes at room temperature in the dark with the following antibodies: anti-CD3 PE (317308, BioLegend, San Diego, USA), anti-CD161 PE-Cyanine 7(339918, BioLegend, San Diego, USA), anti-TCR Vα7.2 APC(351708, BioLegend, San Diego, USA), and anti-CD4 FITC(300505, BioLegend, San Diego, USA), incubated at room temperature, in the dark for 20 minutes. Cell lysis and fixation was performed with 1x RBC Lysis/Fixation Solution (422401, BioLegend, San Diego, USA) for 15 minutes at room temperature in the dark. After centrifuging at 350g for 5 minutes, supernatant was discarded, then washed twice with Cell Staining Buffer. The cell pellet was resuspended in 500μl Cell Staining Buffer (420201, BioLegend, San Diego, USA) and analyzed on BD FACSCalibur flow cytometer using the CellQuestPro Software (BD Biosciences). Data were analyzed using FlowJo Version 10.5.3 software (TreeStar). The serum obtained after centrifugation was stored at -80°C.

Enzyme-linked immunosorbent assay

Serum IL-17(EH6264M, Weiao, Shanghai, China), IL-22 (EH6285M, Weiao, Shanghai, China), IFN-γ (EH6242M, Weiao, Shanghai, China), and granzyme B (EH6217M, Weiao, Shanghai, China) concentrations were measured using commercial ELISA kits according to the manufacturer's protocol and procedure, each serum sample and assay diluent were placed in each well of a 96-well plate coated with IL-17/IL-22/IFN-γ/granzyme B antibody, then incubated for 30 minutes in a 37°C incubator, and each well was washed five times with wash solution, Subsequently, SABC-complexes was added into each well and the reaction was performed for 20 minutes in a 37°C incubator. Again, each well was washed five times with wash solution, after which a TAB reagent was added for 10 minutes in a 37°C incubator. The reaction was quenched by the addition of stop solution. Within 15 minutes, the absorbance was measured at 450 nm using a multifunctional microplate reader. The serum concentration was determined based on a standard concentration curve.

TABLE 1 Clinical and biological characteristics of PCOS patients and healthy controls.

Patients	Control (n=30)	PCOS (n=33)	P-value
Age, (year)	29.83 ± 4.53	29.45 ± 5.25	0.761
BMI, (kg/m^2)	20.90 ± 2.87	23.88 ± 2.81	*<0.001*
T, (ng/ml)	0.27 ± 0.08	0.39 ± 0.12	*<0.001*
AMH, (ng/ml)	4.23 ± 2.90	11.14 ± 5.03	*<0.001*
Basal FSH, (IU/L)	6.35 ± 1.46	6.27 ± 1.27	0.821
Basal LH, (IU/L)	4.54 ± 2.31	10.87 ± 6.42	*<0.001*
LH/FSH	0.74 ± 0.34	1.77 ± 1.14	*<0.001*
Basal E2, (pg/ml)	46.78 ± 24.22	58.42 ± 25.42	0.094
FPG, (mmol/L)	4.54 ± 0.40	4.64 ± 0.38	0.331
FINS, (pmol/L)	6.69 ± 1.85	10.35 ± 5.18	*0.001*
HOMA-IR	1.36 ± 0.42	2.09 ± 1.06	*0.001*
TC, (mmol/L)	4.61 ± 0.78	4.55 ± 0.81	0.827
TG, (mmol/L)	0.84 ± 0.41	0.89 ± 0.33	0.616

Statistical analysis

All statistical analysis was performed using GraphPad Prism version 9.1.0 or OSX (GraphPad Software, La Jolla, CA). Results are expressed as mean ± SD. Groups were compared using the Student t-test or Mann–Whitney U test. Individuals were compared using paired t-test when applicable. Parameter correlation was determined using Pearson correlation coefficients. Levels of significance are indicated as *p < 0.05 or **p < 0.01.

Result

Clinical characteristics of patients

33 PCOS patients and 30 age-matched healthy controls were enrolled in the Shanghai First Maternity and Infant hospital from November 2020 to February 2022. Table 1 shows the main clinical biological characteristics of PCOS and healthy controls included in the study. There were no significant differences in age, basal Follicle-Stimulating Hormone (FSH), estrogen (E2) fasting glucose (FPG), total cholesterol (TC), and triglycerides (TG). While the indicators of total testosterone(T), AMH, Luteinizing Hormone (LH), LH/FSH, Body Mass Index (BMI), fasting insulin (FINS), and HOMA-IR were higher in PCOS patients compared with the healthy controls.

Decreased MAIT cells in peripheral blood CD3+T cells of PCOS patients

Previous studies have suggested that CD3+CD161+TCR Vα7.2+ T cells can be considered MAIT cells (40, 41). Accordingly, the frequency of circulating CD3+CD161+ TCRVα7.2+cells in total CD3+ lymphocytes in individual subjects was determined by flow cytometry (Figure 1A). We first analyzed the frequency of circulating CD3+Vα7.2+CD161+ MAIT cells, the percentages of circulating MAIT cells were significantly lower in PCOS patients than in control (5.29 vs. 7.76%, p<0.01, Figures 1B, C). Concerning the diagnostic accuracy of MAIT cells frequency for PCOS, the ROC curve analysis of MAIT cells in PCOS patients, the AUC was 0.69 (95% CI 0.56–0.82, P <0.05) (Figure 1D). Therefore, PCOS patients had significantly reduced numbers of circulating MAIT cells.

Correlation between MAIT cells clinical characteristics in PCOS patients

Multiple regression analysis was performed to analyze the correlation of BMI, HOMA-IR index, AMH, T, LH/FSH, and MAIT cells frequency. As shown in Figure 2, the frequency of MAIT cells was significantly negatively correlated with BMI, HOMA-IR index, and AMH (R=-0.41, -0.35, -0.4; p=0.0017,

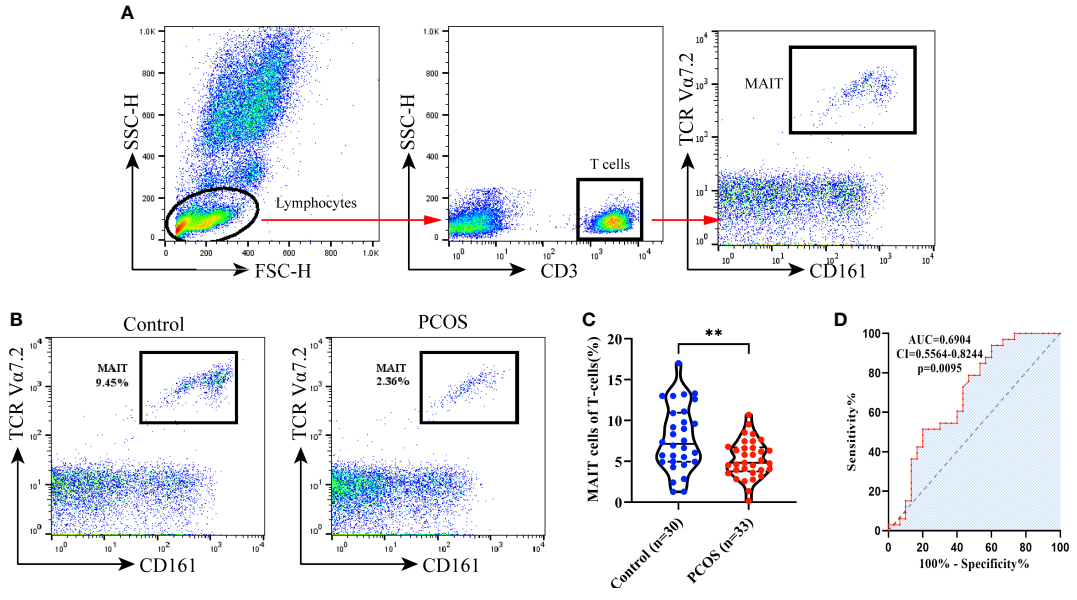


FIGURE 1
Frequency of MAIT cells in controls and PCOS patients. **(A)** Gating strategy for identification of circulating MAIT cells (CD3+ TCRVα7.2+CD161+). **(B)** Representative flow plots show the MAIT cells gated among CD3+ T cells in control and PCOS patients. **(C)** The frequency of MAIT cells in PCOS patients (n = 33) was significantly decreased compared to control (n = 30) (p<0.01). Data represent means ± SEM, **p < 0.01, significantly downregulated compared to control which analyzed by Mann–Whitney U test. **(D)** ROC curves of the MAIT cells in the identification of the PCOS patients (the area under the curve (AUC), confidence interval (CI), and the associated calculated P-value (p) are indicated on each graph).

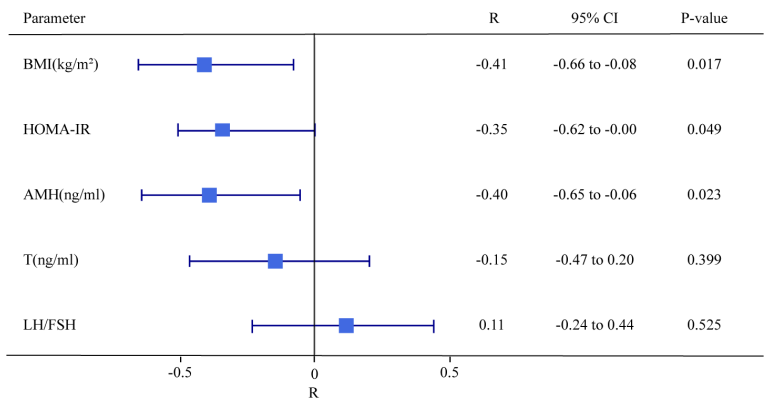


FIGURE 2
Forest plot of the Pearson Correlation between frequencies of MAIT cells of T cells and BMI, HOMI-IR, AMH, T, and LH/FSH in PCOS patients. $P < 0.05$ is considered as significantly different.

0.049, 0.023), and whereas no significant correlation between MAIT cells and LH/FSH or T could be found.

Analysis of MAIT cells in different subgroup of PCOS patients

Due to the frequency of MAIT cells being significantly negatively correlated with BMI, HOMA-IR index, and AMH, 33 PCOS patients were divided into different subgroups by BMI, HOMA-IR, AMH, T, and LH/FSH. We found that the frequencies of MAIT cells of T cells decreased in PCOS patients with abnormal weight [BMI≥24kg/m2 (41)] (Figure 3A), higher HOMA-IR (≥1.5) (42, 43) (Figure 3B), and excessive AMH [≥8ng/ml (44)] (Figure 3C). However, T and LH/FSH do not affect the frequencies of MAIT cells (Figures 3D, E).

CD4+ MAIT cells

Although only 5% of MAIT cells are CD4+ MAIT (CD4+CD8- and CD4+CD8+ subsets of MAIT cells) (39), they may have a distinct role in the immune system. However, a small proportion of CD4+MAIT cells were found in PCOS patients and healthy control group, there was no significant difference in CD4+MAIT cells between the two group (Figures 4A, C). then we analysis the CD4+ cells of T cells, we found that the CD4+ T cells increased in PCOS patients (Figures 4A, B). Interestingly, we found CD4+MAIT cells significantly increased in PCOS patients with abnormal weight (BMI≥24kg/m2), excessive AMH (≥8ng/ml), and excessive T (≥0.4ng/ml). However, CD4+MAIT cells did not show any difference when we divide the subgroups according to HOMA-IR and LH/FSH (Figure 4D).

Correlation between circulating MAIT cells of T cells and cytokines in plasma

MAIT cells can quickly respond to stimulation, which produce a range of cytokines (22). To better demonstrate the function of MAIT cells, we analyzed the relationship between MAIT cells and cytokines in PCOS, we evaluated the level of IL-17, IL-22, IFN-γ, and granzyme B in the plasma of all participants. IL-17 is higher in the plasma of PCOS patients while the IL-22 is less expressed in PCOS patients than in the control group. There were no significant differences in IFN-γ and granzyme B (Figure 5A). In addition, a negative relation between the level of IL-17 in plasma and the frequency of MAIT cells of T cells was found (Figure 5B).

Discussion

This study revealed that the frequency of MAIT cells is decreased in peripheral blood of PCOS patients compared with control group. The decrease of MAIT cells indicates that MAIT cells are associated with the disorder of PCOS. Interestingly, we found that the frequency of MAIT cells were negatively correlated with the clinical features of BMI, HOMA-IR and AMH. It is known that AMH plasma level is a maker of impaired folliculogenesis in patients (45). Therefor we suggest that the altered distribution of MAIT cells can affect the development of PCOS *via* the aspects of metabolism and follicular development.

A previous study showed that blood MAIT cell frequency decreased in obesity and type-2 diabetes, even below the detection limit in severe obesity patients (46). MAIT cells in peripheral blood can be recruited to adipose which may contribute to the decrease MAIT cells number (46). It was reported that 3-6 months after bariatric surgery was performed in obese patients, the frequency of MAIT cells increased (46). As BMI increases, visceral adipose

10.3389/fendo.2022.1038184

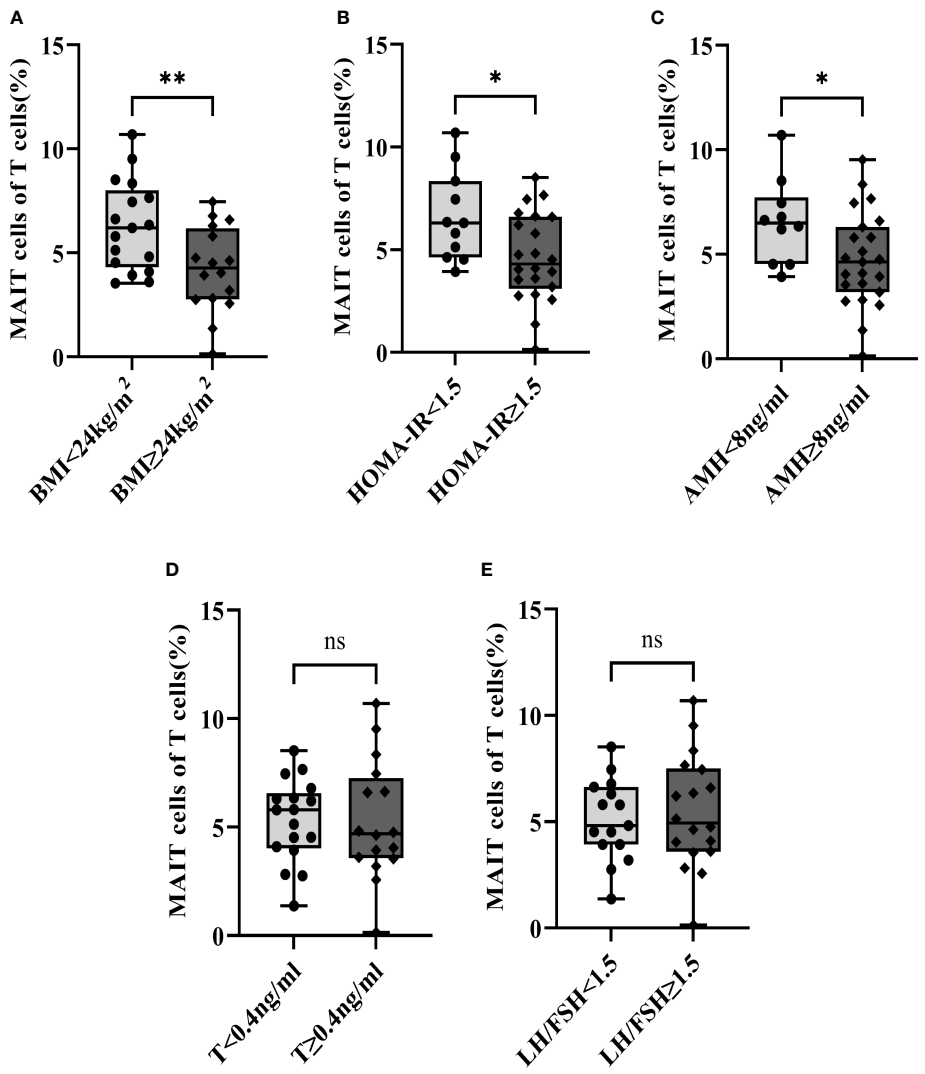


FIGURE 3
Subgroup analysis of the frequencies of MAIT cells of T cells in PCOS patients by BMI, HOMA-IR, AMH, T, and LH/FSH. **(A)** BMI; **(B)** HOMA-IR; **(C)** AMH; **(D)** T; **(E)** LH/FSH. *, p<0.05, **, p<0.01, ns, not significant.

tissue (VAT) exhibits chronic low-grade inflammation, a major factor associated with IR (35). These results are consistent with our results that the frequency of MAIT cells were lower in overweight patients (BMI≥24kg/m^2) and with higher HOMA-IR (≥1.5). Rouxel et al. reported that the frequency of MAIT cells in the peripheral blood of diabetic patients is lower than in healthy people, and this reduction in frequency may be due to the migration of MAIT cells into the pancreas of mice during the development of diabetes, which can also kill human β-cell line (47). Besides, the lower numbers of blood MAIT cells might also lead to cell exhaustion as a previous study has shown they carry a defective BCL2, CD25 and PD-1 expression causing persistent activation (47) (46). However, further studies are needed to verify that MAIT cells are exhausted in PCOS patients.

Our study also observed that the level of IL-17 in plasma increased in PCOS patients and negatively correlated with the frequency of MAIT cells of T cells, which is consistent with the studies on diabetes and obesity (33, 48). Kousei et al. reported that IL-17 can activate Angiotensin II Type 1 Receptor inducing induced IR (49), which may also be the cause of IR in PCOS patients. However, IL-22 was decreased in PCOS which is consistent with Qi et al, who reported that the IL-22 levels in serum and follicular fluid of PCOS patients were decreased (18). IL-22 can be produced by intestinal group 3 innate lymphoid cells (ILC3), and Qi et al. found that transplantation of stool microbiota from mice with PCOS into recipient mice resulted in increased disruption of ovarian function, IR, altered bile acid metabolism which decreased the secretion of IL-22 from

10.3389/fendo.2022.1038184

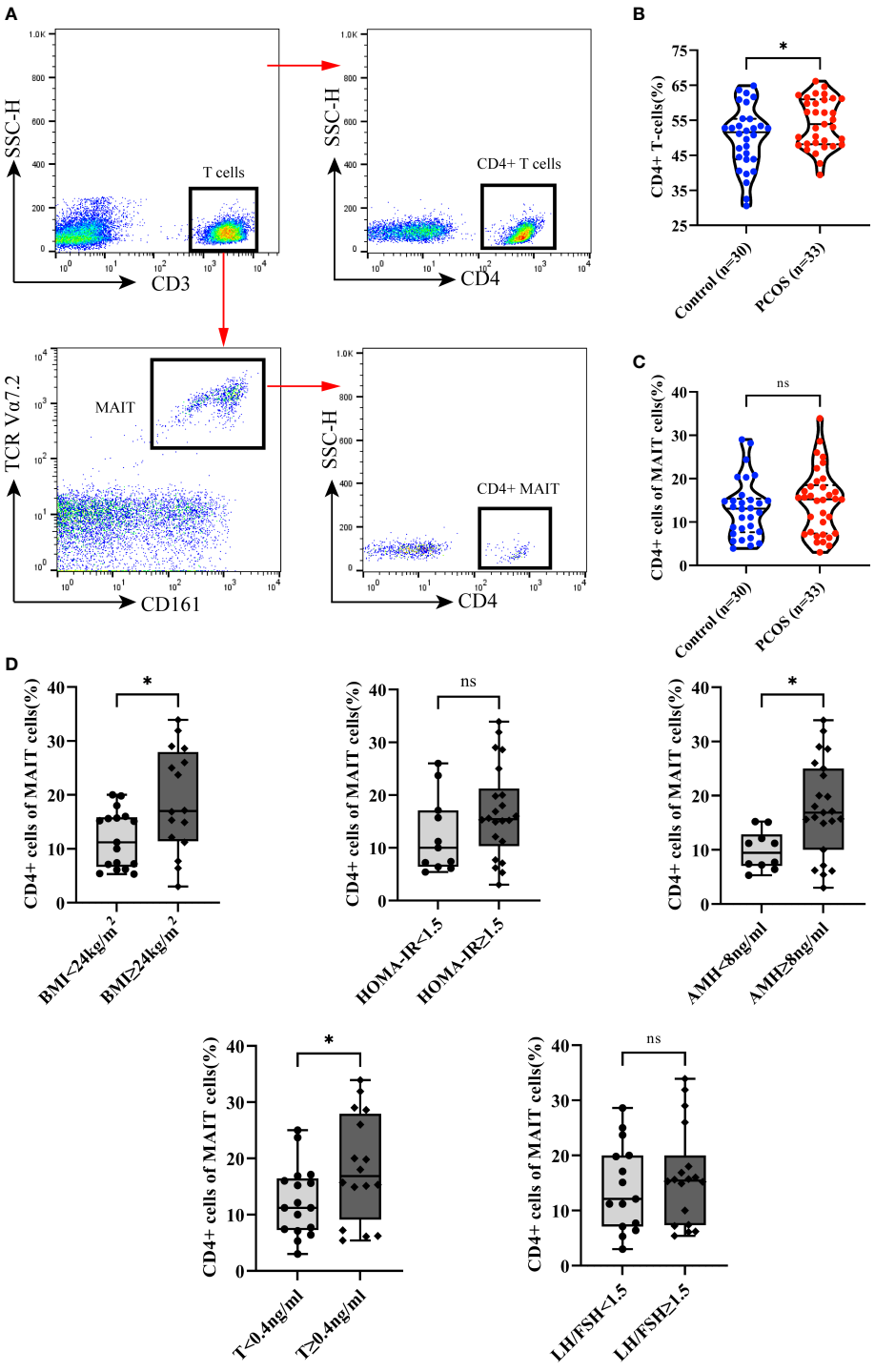


FIGURE 4
CD4+ cells of MAIT cells and T cells between Control and PCOS groups, and subgroup analysis of the CD4+ cells of MAIT cells in PCOS patients by BMI, HOMA-IR, AMH, T, and LH/FSH. **(A)** Gating strategy for CD4+ T cells (CD3+ CD4+) and CD4 MAIT cells (CD3+TCRVα7.2 +CD161+CD4+). **(B)** CD4+ cells of T cells between Control and PCOS groups. **(C)** CD4+ cells of MAIT cells between Control and PCOS groups. **(D)** Subgroup analysis the CD4+ cells of MAIT cells in PCOS patients by BMI, HOMA-IR, AMH, T, and LH/FSH. *p<0.05, ns, not significant.

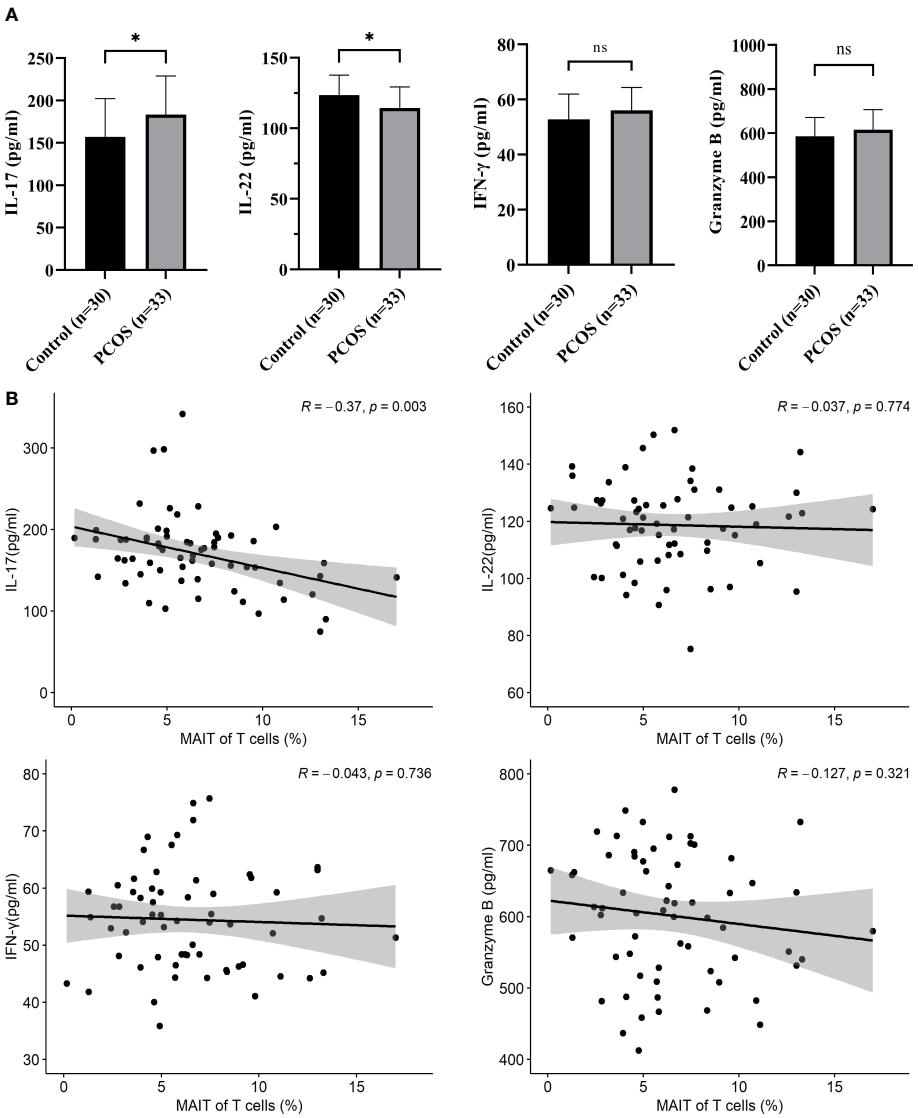


FIGURE 5
Cytokines in the plasma of control and PCOS patients. **(A)** compared L-17, IL-22, IFN-γ and granzyme B between control and PCOS patients. **(B)** The Pearson Correlation between the frequency of MAIT cells and cytokines in plasma. *p<0.05, ns, not significant.

intestinal-type-3 innate lymphoid cells (18). MAIT cells are a kind of innate-like T cell, which are found in the intestine, skin, oral and female genital mucosa. The decrease of IL-22 may have a certain relationship with the changes of MAIT cells. This, however, could not be proven by our study possibly due to the small number of cases, or the MAIT subtype. Therefore, further research is needed which include a large patient cohort longitudinal studies and more subsets of MAIT cells.

Although the CD4+ MAIT cells subset constitutes only a small fraction which usually were ignored for their low frequency, studies have shown that CD4+ MAIT cells can produce more IL-22 than other MAIT subset (38). Therefore CD4+MAIT cells may play a distinct role in disease. Studies that investigated the association between PCOS and IL-22, found that overweight PCOS patients had significantly higher levels of IL-22 in serum compared to healthy controls (14, 50).We speculate that elevated Il-22 may be associated with an increase in CD4+MAIT cells. In our study, we did not find a difference between the control and PCOS group, however the CD4+ T cells increased in PCOS patients, this is consistent with previous literature reports (51), that might be the reason for indifference of CD4+ MAIT between two group. In the subgroup, however, we found that the proportion of CD4+MAIT cells correlates with BMI, AMH, and testosterone levels. CD4 +MAIT were higher in overweight patients (BMI≥24kg/m^2), and patients with higher AMH(≥8ng/ml) and T (≥0.4ng/ml). AMH is regarded as the best serum biomarker of ovarian reserve. Previous

studies revealed that total testosterone is closely related to AMH and plays an important role in follicular growth (52). A study in 2016 found that the number of circulating CD4+ cells strongly affected serum AMH levels (53). Therefore, CD4+MAIT cells may play a key role in ovarian granulosa cell function and follicular physiology.

Conclusion

The outcomes of our study showed that the reduction of MAIT cells and increased frequency of CD4+MAIT cells may contribute to the metabolic disorder and follicular development in PCOS. MAIT cells may act as a predictive marker and could present potential new treatment options. However, the limitation of this study is that we only correlate the frequency to the clinical features, which support the hypothesis that MAIT cells and CD4+MAIT cells play a role in PCOS. Further in-depth research of the correlation between different MAIT cells subsets and PCOS will be performed.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Shanghai First Maternity and Infant Hospital ethics committee (NO: KS2132). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

KL, LD, HZ and JX contributed to study designed and interpretation of data. LD, and KL supervised the experiments. HZ and JX performed the experiments. YJ, XG, LB, DL, XY and YZ assisted with manuscript drafting and critical discussions. LH, FC, MZ and KL collect clinical information. All authors contribute to the article and approved the final manuscript.

Funding

This work was supported by grants from the National Natural Science Foundation of China (Grant No.82201881), the Natural Science Foundation of Shanghai (Grant No.21ZR1450700), and the Medical Consortium of Shanghai Pudong (Grant No. PDYLT2022-14).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Hum Reprod* (2010) 25(2):544–51. doi: 10.1093/humrep/dep399

2. Li R, Zhang Q, Yang D, Li S, Lu S, Wu X, et al. Prevalence of polycystic ovary syndrome in women in China: A Large community-based study. *Hum Reprod* (2013) 28(9):2562–9. doi: 10.1093/humrep/det262

3. Khan KA, Stas S, Kurukulasuriya LR. Polycystic ovarian syndrome. *J Cardiometab Syndr* (2006) 1(2):125–30. doi: 10.1111/j.1559-4564.2006.05675.x

4. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. The androgen excess and pcos society criteria for the polycystic ovary syndrome: The complete task force report. *Fertil Steril* (2009) 91 (2):456–88. doi: 10.1016/j.fertnstert.2008.06.035

5. Ezeh U, Pisarska MD, Azziz R. Association of severity of menstrual dysfunction with hyperinsulinemia and dysglycemia in polycystic ovary syndrome. *Hum Reprod* (2022) 37(3):553–64. doi: 10.1093/humrep/deac001

6. Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. *Lancet* (2007) 370(9588):685–97. doi: 10.1016/S0140-6736(07)61345-2

7. Escobar-Morreale HF. Polycystic ovary syndrome: Definition, aetiology, diagnosis and treatment. *Nat Rev Endocrinol* (2018) 14(5):270–84. doi: 10.1038/nrendo.2018.24

8. Rotterdam EA-SPCWG. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* (2004) 81 (1):19–25. doi: 10.1016/j.fertnstert.2003.10.004

9. Azziz R, Carmina E, Chen Z, Dunaif A, Laven JS, Legro RS, et al. Polycystic ovary syndrome. *Nat Rev Dis Primers* (2016) 2:16057. doi: 10.1038/nrdp.2016.57

10. Avila MA, Bruno RV, Barbosa FC, Andrade FC, Silva AC, Nardi AE. Polycystic ovary syndrome: Implications of metabolic dysfunction. *Rev Col Bras Cir* (2014) 41(2):106–10. doi: 10.1590/s0100-69912014000200006

11. Joham AE, Norman RJ, Stener-Victorin E, Legro RS, Franks S, Moran LJ, et al. Polycystic ovary syndrome. *Lancet Diabetes Endocrinol* (2022) 10(9):668–80. doi: 10.1016/S2213-8587(22)00163-2

12. Krishna MB, Joseph A, Subramaniam AG, Gupta A, Pillai SM, Laloraya M. Reduced tregs in peripheral blood of pcos patients - a consequence of aberrant Il2 signaling. *J Clin Endocrinol Metab* (2015) 100(1):282–92. doi: 10.1210/jc.2014-2401

13. Hu C, Pang B, Ma Z, Yi H. Immunophenotypic profiles in polycystic ovary syndrome. *Mediators Inflammation* (2020) 2020:5894768. doi: 10.1155/2020/5894768

14. Qin L, Xu W, Li X, Meng W, Hu L, Luo Z, et al. Differential expression profile of immunological cytokines in local ovary in patients with polycystic ovarian syndrome: Analysis by flow cytometry. *Eur J Obstet Gynecol Reprod Biol* (2016) 197:136–41. doi: 10.1016/j.ejogrb.2015.12.003

15. Ibanez L, Jaramillo AM, Ferrer A, de Zegher F. High neutrophil count in girls and women with hyperinsulinaemic hyperandrogenism: Normalization with metformin and flutamide overcomes the aggravation by oral contraception. *Hum Reprod* (2005) 20(9):2457–62. doi: 10.1093/humrep/dei072

16. Alanbay I, Ercan CM, Sakinci M, Coksuer H, Ozturk M, Tapan S. A macrophage activation marker chitotriosidase in women with pcos: Does low-grade chronic inflammation in pcos relate to pcos itself or obesity? *Arch Gynecol Obstet* (2012) 286(4):1065–71. doi: 10.1007/s00404-012-2425-0

17. Falahatian S, Haddad R, Pakravan N. Modulatory effects of R10 fraction of garlic (Allium sativum l.) on hormonal levels, T cell polarization, and fertility-related genes in mice model of polycystic ovarian syndrome. . *J Ovarian Res* (2022) 15(1):4. doi: 10.1186/s13048-021-00926-6

18. Qi X, Yun C, Sun L, Xia J, Wu Q, Wang Y, et al. Gut microbiota-bile acid-Interleukin-22 axis orchestrates polycystic ovary syndrome. *Nat Med* (2019) 25 (8):1225–33. doi: 10.1038/s41591-019-0509-0

19. Zarei E, Mohajery H, Aliakbari F, Zadeh FM, Veghari G, Mansourian A. Association of perforin and granzyme-b levels with hyperandrogenism in polycystic ovary syndrome: A case-control study. *Pak J Biol Sci* (2019) 22 (8):393–9. doi: 10.3923/pjbs.2019.393.399

20. Le Bourhis L, Mburu YK, Lantz O. Mait cells, surveyors of a new class of antigen: Development and functions. *Curr Opin Immunol* (2013) 25(2):174–80. doi: 10.1016/j.coi.2013.01.005

21. Porcelli S, Yockey CE, Brenner MB, Balk SP. Analysis of T cell antigen receptor (Tcr) expression by human peripheral blood Cd4-8- Alpha/Beta T cells demonstrates preferential use of several V beta genes and an invariant tcr alpha chain. *J Exp Med* (1993) 178(1):1–16. doi: 10.1084/jem.178.1.1

22. Treiner E, Duban L, Bahram S, Radosavljevic M, Wanner V, Tilloy F, et al. Selection of evolutionarily conserved mucosal-associated invariant T cells by Mr1. *Nature* (2003) 422(6928):164–9. doi: 10.1038/nature01433

23. Toubal A, Nel I, Lotersztajn S, Lehuen A. Mucosal-associated invariant T cells and disease. *Nat Rev Immunol* (2019) 19(10):643–57. doi: 10.1038/s41577-019-0191-y

24. Hinks TS. Mucosal-associated invariant T cells in autoimmunity, immune-mediated diseases and airways disease. *Immunology* (2016) 148(1):1–12. doi: 10.1111/imm.12582

25. Provine NM, Klenerman P. Mait cells in health and disease. *Annu Rev Immunol* (2020) 38:203–28. doi: 10.1146/annurev-immunol-080719-015428

26. Zhang Q, Li P, Zhou W, Fang S, Wang J. Participation of increased circulating mait cells in lung cancer: A pilot study. *J Cancer* (2022) 13(5):1623–9. doi: 10.7150/jca.69415

27. Melo AM, O'Brien AM, Phelan JJ, Kennedy SA, Wood NAW, Veerapen N, et al. Mucosal-associated invariant T cells display diminished effector capacity in oesophageal adenocarcinoma. *Front Immunol* (2019) 10:1580. doi: 10.3389/fimmu.2019.01580

28. Vorwald VM, Davis DM, Van Gulick RJ, Torphy RJ, Borgers JS, Klarquist J, et al. Circulating Cd8(+) mucosal-associated invariant T cells correlate with improved treatment responses and overall survival in anti-Pd-1-Treated melanoma patients. *Clin Transl Immunol* (2022) 11(1):e1367. doi: 10.1002/cti2.1367

29. Ammitzboll C, von Essen MR, Chow HH, McWilliam O, Holm Hansen R, Sellebjerg F. Mait cell subtypes in multiple sclerosis. *J Neuroimmunol* (2020) 339:577117. doi: 10.1016/j.jneuroim.2019.577117

30. Niehaus CE, Strunz B, Cornillet M, Falk CS, Schnieders A, Maasoumy B, et al. Mait cells are enriched and highly functional in ascites of patients with decompensated liver cirrhosis. *Hepatology* (2020) 72(4):1378–93. doi: 10.1002/hep.31153

31. Li Y, Du J, Wei W. Emerging roles of mucosal-associated invariant T cells in rheumatology. *Front Immunol* (2022) 13:819992. doi: 10.3389/fimmu.2022.819992

32. Liu Y, Zhu P, Wang W, Tan X, Liu C, Chen Y, et al. Mucosal-associated invariant T cell dysregulation correlates with conjugated bilirubin level in chronic hbv infection. *Hepatology* (2021) 73(5):1671–87. doi: 10.1002/hep.31602

33. Carolan E, Tobin LM, Mangan BA, Corrigan M, Gaoatswe G, Byrne G, et al. Altered distribution and increased il-17 production by mucosal-associated invariant T cells in adult and childhood obesity. *J Immunol* (2015) 194 (12):5775–80. doi: 10.4049/jimmunol.1402945

34. Magalhaes I, Lehuen A. [Mucosal-associated invariant T cells in obesity and type 2 diabetes]. *Med Sci (Paris)* (2015) 31(8-9):717–9. doi: 10.1051/medsci/20153108005

35. Toubal A, Kiaf B, Beaudoin L, Cagninacci L, Rhimi M, Fruchet B, et al. Mucosal-associated invariant T cells promote inflammation and intestinal dysbiosis leading to metabolic dysfunction during obesity. *Nat Commun* (2020) 11(1):3755. doi: 10.1038/s41467-020-17307-0

36. Fukuda N, Ojima T, Hayata K, Katsuda M, Kitadani J, Takeuchi A, et al. Laparoscopic sleeve gastrectomy for morbid obesity improves gut microbiota balance, increases colonic mucosal-associated invariant T cells and decreases circulating regulatory T cells. *Surg Endosc* (2022) 36(10):7312–24. doi: 10.1007/s00464-022-09122-z

37. Harms RZ, Lorenzo KM, Corley KP, Cabrera MS, Sarvetnick NE. Altered Cd161 bright Cd8+ mucosal associated invariant T (Mait)-like cell dynamics and increased differentiation states among juvenile type 1 diabetics. *PloS One* (2015) 10 (1):e0117335. doi: 10.1371/journal.pone.0117335

38. Gherardin NA, Souter MN, Koay HF, Mangas KM, Seemann T, Stinear TP, et al. Human blood mait cell subsets defined using Mr1 tetramers. *Immunol Cell Biol* (2018) 96(5):507–25. doi: 10.1111/imcb.12021

39. Godfrey DI, Koay HF, McCluskey J, Gherardin NA. The biology and functional importance of mait cells. *Nat Immunol* (2019) 20(9):1110–28. doi: 10.1038/s41590-019-0444-8

40. Zhu M, Xu Y, Li C, Lu Z, Bi K, Wang K, et al. Involvement of impaired Cd8 (+) mucosal-associated invariant T cells and myeloid-derived suppressor cells in polycystic ovary syndrome. *Reprod Biol Endocrinol* (2021) 19(1):175. doi: 10.1186/s12958-021-00861-7

41. Chen C, Lu FC. Department of disease control ministry of health PRC. the guidelines for prevention and control of overweight and obesity in Chinese adults. *BioMed Environ Sci* (2004) 17 Suppl:1–36.

42. Taniguchi A, Fukushima M, Sakai M, Miwa K, Makita T, Nagata I, et al. Remnant-like particle cholesterol, triglycerides, and insulin resistance in nonobese Japanese type 2 diabetic patients. *Diabetes Care* (2000) 23(12):1766–9. doi: 10.2337/diacare.23.12.1766

43. Chang SA, Kim HS, Yoon KH, Ko SH, Kwon HS, Kim SR, et al. Body mass index is the most important determining factor for the degree of insulin resistance in non-obese type 2 diabetic patients in Korea. *Metabolism* (2004) 53(2):142–6. doi: 10.1016/s0026-0495(03)00314-7

44. Yue CY, Lu LK, Li M, Zhang QL, Ying CM. Threshold value of anti-mullerian hormone for the diagnosis of polycystic ovary syndrome in Chinese women. *PloS One* (2018) 13(8):e0203129. doi: 10.1371/journal.pone.0203129

45. Weerakiet S, Lertvikool S, Tingthanatikul Y, Wansumrith S, Leelaphiwat S, Jultanmas R. Ovarian reserve in women with polycystic ovary syndrome who underwent laparoscopic ovarian drilling. *Gynecol Endocrinol* (2007) 23(8):455–60. doi: 10.1080/09513590701485212

46. Magalhaes I, Pingris K, Poitou C, Bessoles S, Venteclef N, Kiaf B, et al. Mucosal-associated invariant T cell alterations in obese and type 2 diabetic patients. *J Clin Invest* (2015) 125(4):1752–62. doi: 10.1172/JCI78941

47. Rouxel O, Da Silva J, Beaudoin L, Nel I, Tard C, Cagninacci L, et al. Cytotoxic and regulatory roles of mucosal-associated invariant T cells in type 1 diabetes. *Nat Immunol* (2017) 18(12):1321–31. doi: 10.1038/ni.3854

48. Bergin R, Kinlen D, Kedia-Mehta N, Hayes E, Cassidy FC, Cody D, et al. Mucosal-associated invariant T cells are associated with insulin resistance in childhood obesity, and disrupt insulin signalling *Via* il-17. *Diabetologia* (2022) 65(6):1012–7. doi: 10.1007/s00125-022-05682-w

49. Ohshima K, Mogi M, Jing F, Iwanami J, Tsukuda K, Min LJ, et al. Roles of interleukin 17 in angiotensin ii type 1 receptor-mediated insulin resistance. *Hypertension* (2012) 59(2):493–9. doi: 10.1161/HYPERTENSIONAHA.111.183178

50. Simkova M, Vitku J, Kolatorova L, Vrbikova J, Vosatkova M, Vcelak J, et al. Endocrine disruptors, obesity, and cytokines - how relevant are they to pcos? *Physiol Res* (2020) 69(Suppl 2):S279–S93. doi: 10.33549/physiolres.934521

51. He S, Mao X, Lei H, Dong B, Guo D, Zheng B, et al. Peripheral blood inflammatory-immune cells as a predictor of infertility in women with polycystic ovary syndrome. *J Inflammation Res* (2020) 13:441–50. doi: 10.2147/JIR.S260770

52. Lv PP, Jin M, Rao JP, Chen J, Wang LQ, Huang CC, et al. Role of anti-mullerian hormone and testosterone in follicular growth: A cross-sectional study. *BMC Endocr Disord* (2020) 20(1):101. doi: 10.1186/s12902-020-00569-6

53. Scherzer R, Bacchetti P, Messerlian G, Goderre J, Maki PM, Seifer DB, et al. Impact of Cd4+ lymphocytes and hiv infection on anti-mullerian hormone levels in a Large cohort of hiv-infected and hiv-uninfected women. *Am J Reprod Immunol* (2015) 73(3):273–84. doi: 10.1111/aji.12332

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 21 December 2022
DOI 10.3389/fendo.2022.935750

OPEN ACCESS

EDITED BY
Bassem Refaat,
Umm Al-Qura University, Saudi Arabia

REVIEWED BY
Ioannis Kyrou,
Coventry University, United Kingdom
Georgios K. Dimitriadis,
King's College Hospital NHS Foundation Trust, United Kingdom

*CORRESPONDENCE
Alexandra E. Butler
aeb91011@gmail.com;
abutler@rcsi.com

†These authors share senior authorship

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

RECEIVED 04 May 2022
ACCEPTED 07 December 2022
PUBLISHED 21 December 2022

CITATION
Moin ASM, Sathyapalan T, Butler AE and Atkin SL (2022) Classical and alternate complement factor overexpression in non-obese weight matched women with polycystic ovary syndrome does not correlate with vitamin D.
Front. Endocrinol. 13:935750.
doi: 10.3389/fendo.2022.935750

Classical and alternate complement factor overexpression in non-obese weight matched women with polycystic ovary syndrome does not correlate with vitamin D

Abu Saleh Md Moin[1], Thozhukat Sathyapalan[2], Alexandra E. Butler[1*†] and Stephen L. Atkin[1†]

[1]Research Department, Royal College of Surgeons in Ireland Bahrain, Adliya, Bahrain, [2]Academic Endocrinology, Diabetes and Metabolism, Hull York Medical School, Hull, United Kingdom

Introduction: Women with polycystic ovary syndrome (PCOS) exhibit complement factor expression changes that may be obesity-driven rather than an intrinsic facet of PCOS; furthermore, complement changes have been associated with vitamin D deficiency, a common feature of PCOS. Therefore, complement pathway proteins and vitamin D levels may be linked in PCOS.

Methods: We measured plasma levels of complement pathway proteins by Slow Off-rate Modified Aptamer (SOMA)-scan plasma protein measurement for the classical (C4, C4a, and C4b) and alternative pathways (C3, C3b, iC3b, properdin, and factors B, D, and H) in weight and age-matched non-obese non-insulin resistant women with PCOS (n = 24) and control women (n = 24). Proteins that differed between groups were correlated with 25-hydroxyvitamin D_3 ($25(OH)D_3$) and 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), measured by isotope-dilution liquid chromatography tandem mass spectrometry.

Results: Women with PCOS had a higher free androgen index and anti-Mullerian hormone, though insulin resistance was comparable to controls; likewise, C-reactive protein, a marker of inflammation, was comparable between cohorts. In the alternative complement pathway, C3, iC3b, and properdin were increased in PCOS ($p < 0.05$), while C4 in the classical pathway was increased ($p < 0.05$). $25(OH)D_3$ levels positively correlated with C3b only in control subjects, with no correlation of $1,25(OH)_2D_3$ with any of the proteins.

Conclusion: In a non-obese PCOS population matched for age, insulin resistance and inflammation, initiating proteins of the classical and alternate

10.3389/fendo.2022.935750

complement cascades were increased. However, a positive correlation with 25 $(OH)D_3$ was only seen for C3b in control subjects, with no correlation to 1,25 $(OH)_2D_3$, suggesting that the increase in complement proteins in PCOS is vitamin D-independent.

KEYWORDS

polycystic ovary syndrome, complement factors, C3, C4, vitamin D

Introduction

In women with polycystic ovary syndrome (PCOS), there is an increased prevalence of type 2 diabetes, hypertension and, potentially, cardiovascular disease (1), the mechanism of which is still unclear, though inflammation has been implicated (2). Increased complement factor proteins in PCOS have been reported for both the classical and alternate cascade pathways, including C3, C4, properdin, factor B, and factor D (3) (Figure 1), though their expression and activation appeared to be dependent upon obesity and insulin resistance (3). Complement protein studies in PCOS have, however, been discrepant in the literature, with a confirmatory report that C3 may be elevated and related to inflammation (4) while, conversely, others report that C3 levels are unchanged (5).

Vitamin D deficiency is very common in women with PCOS, with 67%–85% being severely deficient, and low levels have been reported to correlate with obesity, insulin resistance, and testosterone levels (6, 7); however, it has been suggested that vitamin D does not exacerbate these features in PCOS (8). A systematic review and meta-analysis suggested that vitamin D deficiency was associated with insulin resistance but the significance was lost when BMI was accounted for (9). However, it has been reported that vitamin D insufficiency predicts elevated C3 levels independent of insulin resistance and obesity (10) and therefore, hypothetically, may contribute to the elevation of these proteins in PCOS. Vitamin D_3 (cholecalciferol) is endogenously produced in the skin through the effect of UV-B on 7-dehydrocholesterol that is hydroxylated at position 25 to 25-hydroxyvitamin D_3 ($25(OH)D_3$). $25(OH)D_3$ is transported to the kidney and converted to the active 1,25 $(OH)_2D_3$ by 1-alpha hydroxylase (11). This study was undertaken in a non-obese PCOS population versus controls matched for BMI, insulin resistance, and inflammation to determine whether complement factor proteins were independently associated with PCOS and whether they may be modulated by vitamin D metabolites.

Materials and methods

We determined plasma complement pathway protein levels in women with PCOS (n = 24) and control women (n = 24) attending the Hull IVF clinic (11). Control women were age and body mass index (BMI) matched women with PCOS. Demographic data for both control women and women with PCOS are shown in Table 1 (11). The Rotterdam consensus was used for the diagnosis of PCOS; these criteria are (1) clinical and biochemical hyperandrogenemia, requiring a Ferriman–Gallwey score of >8 and a free androgen index of >4, respectively (2), oligomenorrhea or amenorrhea and (3) polycystic ovaries seen on transvaginal ultrasound (12). Study participants had no other condition or

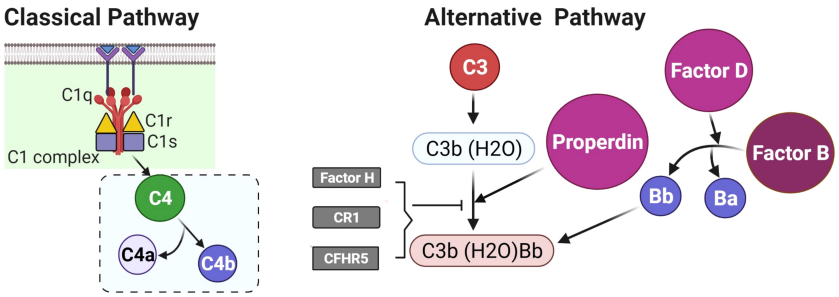


FIGURE 1
A schematic to illustrate the initiating proteins of the classical and alternate complement cascades.

TABLE 1 Demographics, baseline, hormonal, and metabolic parameters of the women with PCOS and control women (mean ± SD). All parameters did not differ other than those marked **=p <0.01.

	Control (n = 24)	PCOS (n = 24)
Age (years)	32.5 ± 4.1	31 ± 6.4
BMI (kg/m^2)	24.8 ± 1.1	25.9 ± 1.8
Fasting glucose (mmol/L)	4.9 ± 0.4	4.7 ± 0.8
HbA1C (mmol/mol)	30.9 ± 6.5	31.8 ± 3.0
HOMA-IR	1.8 ± 1.0	1.9 ± 1.6
SHBG (nmol/L)	104.2 ± 80.3	71.7 ± 62.2
Free androgen index (FAI)	1.3 ± 0.5	4.1 ± 2.9**
CRP (mg L^{-1})	2.34 ± 2.34	2.77 ± 2.57
AMH (ng/ml)	24 ± 13	57 ± 14**
25 hydroxy vitamin D3 (nmol/l)	46.2 ± 23.5	54.0 ± 27.4
1,25 Dihydroxy vitamin D3 (ng/ml)	0.03 ± 0.02	0.04 ± 0.2

BMI, Body Mass Index; HbA1c, glycated hemoglobin; HOMA-IR, Homeostasis model of assessment-insulin resistance; CRP, C reactive protein; SHBG, sex hormone binding globulin; AMH, Anti-Müllerian hormone.

illness and were required to be medication-free for nine months preceding study enrollment. Testing was undertaken to ensure that no patient had any of the following endocrine conditions: non-classical 21-hydroxylase deficiency, hyperprolactinemia, Cushing's disease, or an androgen-secreting tumor. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Yorkshire and The Humber NRES ethical committee, UK. In addition, they were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Fasting blood samples were centrifuged at 3,500*g* for 15 min and placed into aliquots and frozen at −80°C until analysis. The blood samples were analyzed for sex hormone binding globulin (SHBG), insulin (DPC Immulite 200 analyzer, Euro/DPC, Llanberis UK), and plasma glucose (Synchron LX20 analyzer, Beckman-Coulter, High Wycombe, UK). Free androgen index (FAI) was calculated by dividing the total testosterone by SHBG, and then multiplying by 100. Insulin resistance (IR) was calculated using the homeostasis model assessment (HOMA-IR). Serum vitamin D levels and testosterone were quantified using isotope-dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) (11). Circulating levels of complement pathway proteins were determined by Slow Off-rate Modified Aptamer (SOMA)-scan plasma protein measurement (Somalogic, Boulder, CO, USA), the details of which have been previously reported (13). Normalization of raw intensities, hybridization, median signal, and calibration signal were performed based on the standard samples included on each plate, as previously described (14).

We measured plasma complement pathway protein levels for the alternative (C3, C3b, iC3b, properdin, factors B, D, and H) and classical pathways (C4, C4a, and C4b) (3) (Table 2).

Statistics

A power analysis (nQuery version 9, Statsol USA) was undertaken for C3 protein that had been previously reported (3). For 80% power and an alpha of 0.05 with a common standard deviation of 0.37, the number of subjects required was 23. Data trends were visually and statistically evaluated for normality. Independent t-tests were applied to normally distributed data, while non-parametric tests (Mann–Whitney U) were applied to data that violated the assumptions of normality when tested using the Kolmogorov–Smirnov Test. Correlations between vitamin D and the differing complement proteins were undertaken with the Pearson coefficient and shown in Figure 2 and Supplementary Figure 1. All analyses were performed using R version 4.0.0 (R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/).

Results

Baseline data for the 24 non-obese women with PCOS matched for age and BMI with 24 control subjects is shown in Table 1. Insulin resistance and CRP (as a marker of inflammation) did not differ between the two groups. Women with PCOS had an elevated free androgen index and an elevated anti-Mullerian hormone, as expected in PCOS.

The results of the complement factors are shown in Table 2 for both the non-obese women with PCOS and the control

10.3389/fendo.2022.935750

TABLE 2 Complement dysregulation in non-obese non-insulin resistant women with PCOS.

Complement pathway proteins	PCOS	Control	p-value
Properdin	152,592 (42,743)	117,488 (50,041)	0.006
iC3b	7,148 (2,127)	5,991 (1,425)	0.02
C3	65,878 (26,872)	45,742 (18,189)	0.002
C3b	50,982 (28,296)	46,250 (39,450)	0.6
C4	119,057 (28,429)	104,245 (28,069)	0.05
C4a	71,549 (9,802)	73,037 (2,258)	0.43
C4b	349 (186)	335 (202)	0.78
Factor D	733 (99)	693 (150)	0.24
Factor B	30,257 (6,541)	28,172 (5,791)	0.2
Factor H	60,898 (9,191)	59,289 (6,016)	0.43

Complement proteins for the classical (C4, C4a, and C4b) and alternative (C3, iC3, Properdin, Factors B, D, and H) complement pathways in non-obese non-insulin resistant women with polycystic ovary syndrome (PCOS) versus their BMI-matched controls.
Data presented as Mean ± 1 Standard Deviation of Relative Fluorescent Units (RFU).

women. In these non-obese, non-insulin-resistant women with PCOS, there were significant elevations in the levels of alternative pathway complement proteins C3 ($p < 0.002$), iC3b ($p < 0.02$), and properdin ($p < 0.006$), while C4 in the classical pathway was increased ($p < 0.05$); C4a and C4b did not differ between groups.

$25(OH)D_3$ levels correlated with C3 for control subjects, but not for PCOS (Figure 2); however, there was no correlation for $1,25(OH)_2D_3$ with any of the complement proteins in either cohort (Supplementary Figure 1).

Discussion

These data show that, in non-obese women with PCOS that did not differ for insulin resistance and inflammation compared to controls, proteins in the alternative complement activation pathway (C3, iC3, and properdin) were elevated in women with PCOS in comparison with the matched controls; C4, belonging to the classical complement activation pathway, was also increased in PCOS. These data are in accord with those reported by others; of note, in that report, Factors B, D, and H were also elevated but became non-significant when the data was adjusted for BMI (3). C4 was elevated, but its products of activation (C4a and C4b) were no different between women with PCOS and controls, suggesting that activation of C4 was not occurring. Similarly, C3 was elevated, but its product of activation (C3b) was no different between women with PCOS and control women, again suggesting that activation of C3 was not taking place. However, properdin was elevated and, as its action is to stabilize C3 convertase in the alternative pathway, its elevation would prolong complement activation (15).

In this cohort of women with PCOS who were not obese, insulin resistant or demonstrating indices of inflammation greater than control women, this suggests that the alterations of the complement proteins in this study are reflective of the inherent processes in PCOS rather than an epiphenomenon reflective of obesity, insulin resistance or inflammation, as has been previously suggested by mathematical modeling (3). This is important, as many of the cardiovascular risk factors are largely due to obesity and its associated complications rather than the underlying pathophysiology of PCOS. It can, however, be difficult to determine the relative contributions of obesity and insulin resistance to the inherent dysfunction of PCOS (16, 17). Further adding to the complexity, the underlying disease process of PCOS may also be affected by ethnicity (18). The complement protein results reported here are in accord with some studies in PCOS (3, 4) but not others (5); however, those studies had not taken into account the underlying pathophysiology of obesity, insulin resistance and inflammation that are addressed here.

Vitamin D_3 levels correlated positively with C3b in controls only, indicating that vitamin D_3 deficiency was associated with lower C3b protein levels. However, no correlation was seen for the active form, $1,25(OH)_2D_3$. A correlation between vitamin D and C3 was reported to be independent of insulin resistance and BMI in normal controls (10) though, in that study, C3b was not measured specifically. The lack of correlation seen for the active $1,25(OH)_2D_3$ with any of the complement proteins suggests that the $25(OH)D_3$ association seen with C3b alone, together with the fact that C3b plasma protein levels did not differ between groups, may be a chance observation or an epiphenomenon secondary to some unknown factor in the control women. In either case, the data suggest that the changes in complement proteins seen in PCOS are not due to vitamin D levels.

10.3389/fendo.2022.935750

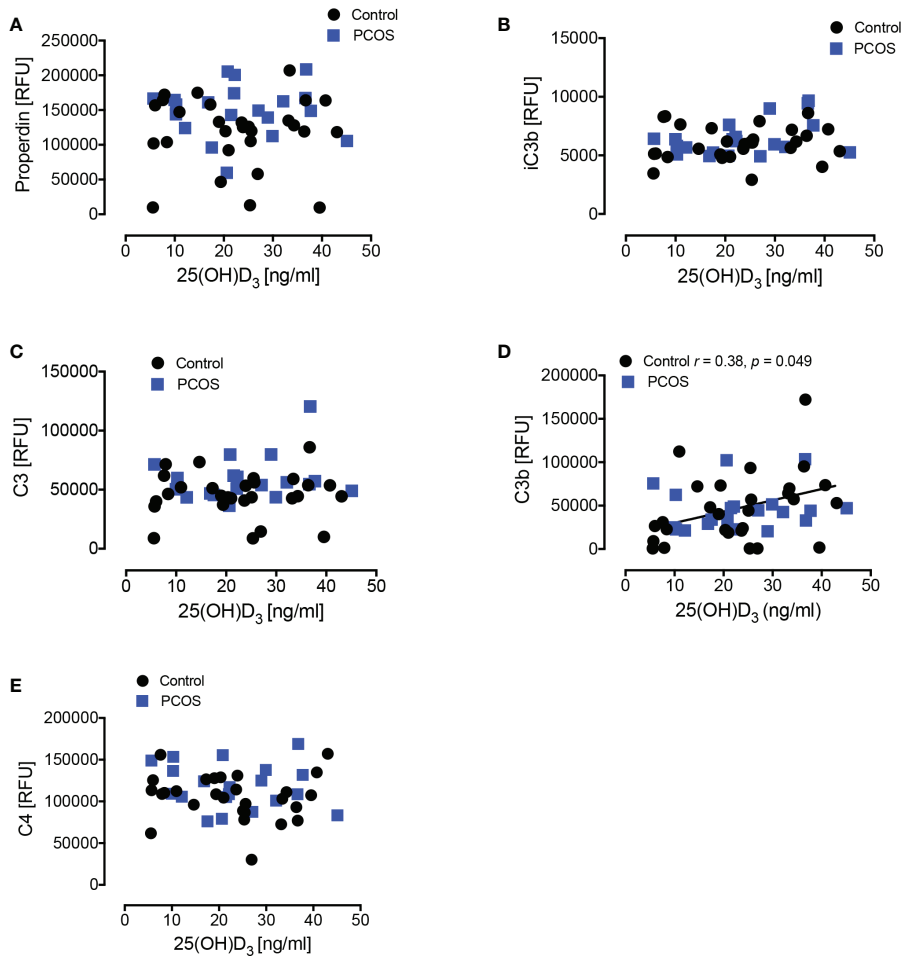


FIGURE 2
Correlations of complement pathway proteins with 25-hydroxy vitamin D_3 [25(OH)D_3]. Correlations of 25(OH)D_3 with properdin **(A)**, iC3b **(B)**, C3 **(C)**, C3b **(D)**, and C4 **(E)** in women with PCOS and control women are shown. Only in controls did C3b show a positive correlation with 25(OH)D_3. RFU, Relative Fluorescent Units.

Limitations

A major concern of any study such as this that describes negative findings is that of reporting a type 2 statistical error due to an inadequate sample size. The initial power analysis was based on that for C3, and significant findings for iC3b, properdin and C4 were found in accord with others (3). The results for Factors B, D, and H are in accord with the modeling that they would become non-significant when BMI is considered and, therefore, in a non-obese population would not differ. As all study subjects were Caucasian, these results may not be generalizable to other ethnic populations. It would be important for future studies to address the role of the PCOS phenotype (19) in the presence and absence of obesity.

In conclusion, in a non-obese PCOS population matched for age, insulin resistance, and inflammation, initiating proteins of the classical and alternate complement cascades were increased in women with PCOS, but their positive correlation with 25(OH)D_3 was only seen for C3b in control women, with no correlation to $1,25(OH)_2D_3$, suggesting that the increase in complement proteins in women with PCOS is independent of vitamin D.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Yorkshire and The Humber NRES ethical

committee, UK. The patients/participants provided their written informed consent to participate in this study.

Author contributions

AM and AB analyzed the data and wrote the manuscript. TS supervised clinical studies and edited the manuscript. SA contributed to study design, data interpretation and the writing of the manuscript. AB is the guarantor of this work. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.935750/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Correlations of complement pathway proteins with 1,25-dihydroxy vitamin D_3 [1,25$(OH)_2D_3$]. No correlation of 1,25$(OH)_2D_3$ was seen with properdin **(A)**, iC3b **(B)**, C3 **(C)**, C3b **(D)** or C4 **(E)** in women with PCOS or control women. Relative Fluorescent Units (RFU).

References

1. Sathyapalan T, Atkin SL. Recent advances in cardiovascular aspects of polycystic ovary syndrome. *Eur J Endocrinol* (2012) 166(4):575–83. doi: 10.1530/EJE-11-0755

2. Carter AM. Complement activation: An emerging player in the pathogenesis of cardiovascular disease. *Scientifica* (Cairo) (2012) 2012:402783. doi: 10.6064/2012/402783

3. Lewis RD, Narayanaswamy AK, Farewell D, Rees DA. Complement activation in polycystic ovary syndrome occurs in the postprandial and fasted state and is influenced by obesity and insulin sensitivity. *Clin Endocrinol (Oxf)* (2021) 94(1):74–84. doi: 10.1111/cen.14322

4. Yang S, Li Q, Song Y, Tian B, Cheng Q, Qing H, et al. Serum complement C3 has a stronger association with insulin resistance than high-sensitivity c-reactive protein in women with polycystic ovary syndrome. *Fertil Steril* (2011) 95(5):1749–53. doi: 10.1016/j.fertnstert.2011.01.136

5. Dehdashtihaghighat S, Mehdizadehkashi A, Arbabi A, Pishgahroudsari M, Chaichian S. Assessment of c-reactive protein and C3 as inflammatory markers of insulin resistance in women with polycystic ovary syndrome: A case-control study. *J Reprod infertility* (2013) 14(4):197-201.

6. Hahn S, Haselhorst U, Tan S, Quadbeck B, Schmidt M, Roesler S, et al. Low serum 25-hydroxyvitamin d concentrations are associated with insulin resistance and obesity in women with polycystic ovary syndrome. *Exp Clin Endocrinol Diabetes* (2006) 114(10):577–83. doi: 10.1055/s-2006-948308

7. Li HW, Brereton RE, Anderson RA, Wallace AM, Ho CK. Vitamin d deficiency is common and associated with metabolic risk factors in patients with polycystic ovary syndrome. *Metabol: Clin Exp* (2011) 60(10):1475–81. doi: 10.1016/j.metabol.2011.03.002

8. Butler AE, Dargham SR, Abouseif A, El Shewehy A, Atkin SL. Vitamin d deficiency effects on cardiovascular parameters in women with polycystic ovary syndrome: A retrospective, cross-sectional study. *J Steroid Biochem Mol Biol* (2021) 211:105892. doi: 10.1016/j.jsbmb.2021.105892

9. Krul-Poel Y, Snackey C, Louwers Y, Lips P, Lambalk C, Laven J, et al. The role of vitamin d in metabolic disturbances in polycystic ovary syndrome (PCOS): A systematic review. *Eur J endocrinology/European Fed Endoc Societies* (2013) 169:853–65. doi: 10.1530/EJE-13-0617

10. Ahmad RMAH, Al-Domi HA. Vitamin d insufficiency predicts elevated levels of complement 3 independent of insulin resistance and BMI. *J Nutr Sci Vitaminol (Tokyo)* (2017) 63(3):155–60. doi: 10.3177/jnsv.63.155

11. Cunningham TK, Allgar V, Dargham SR, Kilpatrick E, Sathyapalan T, Maguiness S, et al. Association of vitamin d metabolites with embryo development and fertilization in women with and without PCOS undergoing subfertility treatment. *Front Endocrinol* (2019) 10:13. doi: 10.3389/fendo.2019.00013

12. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* (2004) 19(1):41–7. doi: 10.1093/humrep/deh098

13. Kahal H, Halama A, Aburima A, Bhagwat AM, Butler AE, Grauman J, et al. Effect of induced hypoglycemia on inflammation and oxidative stress in type 2 diabetes and control subjects. *Sci Rep* (2020) 10(1):4750. doi: 10.1038/s41598-020-61531-z

14. Kraemer S, Vaught JD, Bock C, Gold L, Katilius E, Keeney TR, et al. From SOMAmer-based biomarker discovery to diagnostic and clinical applications: A SOMAmer-based, streamlined multiplex proteomic assay. *PloS One* (2011) 6(10):e26332. doi: 10.1371/journal.pone.0026332

15. Lesher AM, Nilsson B, Song W-C. Properdin in complement activation and tissue injury. *Mol Immunol* (2013) 56(3):191–8. doi: 10.1016/j.molimm.2013.06.002

16. Legro RS. Obesity and PCOS: implications for diagnosis and treatment. *Semin Reprod Med* (2012) 30(6):496-506. doi: 10.1055/s-0032-1328878

17. Moin ASM, Sathyapalan T, Diboun I, Elrayess MA, Butler AE, Atkin SL. Metabolic consequences of obesity on the hypercoagulable state of polycystic ovary syndrome. *Sci Rep* (2021) 11(1):5320. doi: 10.1038/s41598-021-84586-y

18. Butler AE, Abouseif A, Dargham SR, Sathyapalan T, Atkin SL. Metabolic comparison of polycystic ovarian syndrome and control women in middle Eastern and UK Caucasian populations. *Sci Rep* (2020) 10(1):18895. doi: 10.1038/s41598-020-75109-2

19. Neven ACH, Laven J, Teede HJ, Boyle JA. A summary on polycystic ovary syndrome: Diagnostic criteria, prevalence, clinical manifestations, and management according to the latest international guidelines. *Semin Reprod Med* (2018) 36(1):5–12. doi: 10.1055/s-0038-1668085

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 22 December 2022
DOI 10.3389/fendo.2022.1030151

OPEN ACCESS

EDITED BY
Yanting Wu,
Fudan University, China

REVIEWED BY
Li Dan,
Jiangnan University, China
Ju Yang,
Affiliated Hospital of Jiangnan University, China
Yi Gu,
Beijing Children's Hospital, Capital Medical University, China

*CORRESPONDENCE
Xiaoying Ding
xiaoyingding@126.com
Yongde Peng
pengyongde0908@126.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

RECEIVED 28 August 2022
ACCEPTED 05 December 2022
PUBLISHED 22 December 2022

CITATION
Wang X, Gu L, Zhang Y, Xiong C, Peng Y and Ding X (2022) Effects of dehydroepiandrosterone alone or in combination with a high-fat diet and antibiotic cocktail on the heterogeneous phenotypes of PCOS mouse models by regulating gut microbiota. *Front. Endocrinol.* 13:1030151. doi: 10.3389/fendo.2022.1030151

Effects of dehydroepiandrosterone alone or in combination with a high-fat diet and antibiotic cocktail on the heterogeneous phenotypes of PCOS mouse models by regulating gut microbiota

Xuejiao Wang†, Liping Gu†, Yahui Zhang, Chuanhao Xiong, Yongde Peng* and Xiaoying Ding*

Department of Endocrinology and Metabolism, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Objective: Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine and metabolic disease. The gut microbiota is highly correlated with androgen secretion and insulin resistance (IR), which are two potential major pathogenic mechanisms of PCOS. Currently, an antibiotic cocktail (ABX) is often used to construct pseudo germ-free mouse models for studies on the gut microbiota and PCOS. Our work aimed to study the effects of dehydroepiandrosterone (DHEA), a high-fat diet (HFD) and ABX on the heterogeneous phenotypes of PCOS mouse models by regulating the gut microbiota.

Methods: PCOS mouse models were established by subcutaneous injection of DHEA alone or in combination with a HFD in wild-type and pseudo germ-free mice. The changes in ovary morphology and sex hormonal and glycolipid metabolic parameters were evaluated.

Results: Wild-type mice treated with DHEA or DHEA+HFD showed a PCOS-like phenotype of hyperandrogenism, anovulation and polycystic ovaries. The former was combined with hyperinsulinemia and IR, while the latter was combined with glucolipid metabolic disorders, extremely heterogeneous hyperinsulinemia and IR. The phenotype of PCOS mice, especially the metabolic parameters, was correlated with the gut microbiota. The pseudo germ-free mice treated with DHEA or DHEA+HFD also showed a PCOS-like phenotype. However, DHEA could not induce hyperinsulinemia or IR in pseudo germ-free mice. Pseudo germ-free mice treated with DHEA+HFD exhibited decreased serum AMH level, glucolipid metabolic disorders and IR. Compared

with the wild-type mice, the pseudo germ-free mice treated with DHEA showed significantly higher testosterone and lipid levels and lower blood glucose levels, and they did not present with hyperinsulinemia or IR.

Conclusion: A better and stabilized mouse model simulating the pathophysiological defects of PCOS was induced by DHEA alone rather than by DHEA+HFD. The ABX intervention improved glucose metabolic disorders and hyperinsulinemia but aggravated the hyperandrogenism and lipid metabolic disorders of the PCOS mice. This study suggests that the gut microbiota plays an important role in the heterogeneous phenotypes of PCOS mouse models.

KEYWORDS

polycystic ovary syndrome, mouse model, phenotype, dehydroepiandrosterone, high-fat diet, antibiotic cocktail

Introduction

Polycystic ovary syndrome (PCOS) is a common, complex, and heterogeneous endocrine and metabolic disorder that affects 8 to 13% of women of reproductive age and 21% of women in high-risk groups (1). Based on the Rotterdam criteria (2), PCOS can be identified into 4 phenotypes/subtypes, and the presence of insulin resistance (IR) and hyperinsulinemia is common among women with PCOS but are not required for diagnosis (3). Despite significant progress in understanding this disorder over the past 20 years, the heterogeneity of PCOS makes the pathophysiology and treatment still confused. Because of ethical and logistical constraints on clinical studies including women with PCOS, many studies need to be carried out in animal models, especially rodent models.

To date, there are no accepted rodent models of PCOS to comprehensively simulate the pathophysiological process and phenotypes of women with PCOS. Rodent models of PCOS induced by dehydroepiandrosterone (DHEA), dihydrotestosterone or letrozole are widely used in most studies (4–8). These models share the key characteristics of women with PCOS, including hyperandrogenism, disrupted cyclicity, presence of follicular cysts, and some metabolic disorders. However, the above models display high heterogeneity mainly in the presence of metabolic disorders including IR and hyperinsulinemia (9, 10), which leads to difficulties in the repeatability and reliability of further PCOS animal studies. Lai et al. (11) firstly induced a PCOS mice model with distinct metabolic features *via* DHEA combined with a high-fat diet (HFD), confirming the effect of HFD on the heterogeneous phenotypes of PCOS mice.

Studies suggest that the gut microbiota is involved in the genesis of PCOS. In 2012, Tremellen and Pearce proposed a hypothesis that dysbiosis of the gut microbiota is a causative factor of metabolic and reproductive manifestations of PCOS (12). In the past 10 years, studies in both humans and rodent models have demonstrated that alterations of the gut microbiota are associated with PCOS (7, 8, 13, 14). Most evidence has focused on the connection between the gut microbiota and hyperandrogenism and IR, which are the two core pathogenic factors of PCOS. Torres et al. reported a relationship between the gut microbiota and hyperandrogenism, although the genesis is still unclear (15). However, the gut microbiota could cause IR/hyperinsulinemia *via* the immune system and microbiota-gut-brain axis (13, 16, 17). The above evidence proves that the gut microbiota participates in the two core pathogenic factors of PCOS *via* different pathways, suggesting that the gut microbiota may be related to the heterogeneity of PCOS. Our previous study found that the gut microbiota was closely related to the heterogeneous phenotypes of women with PCOS (18). However, the correlation between the gut microbiota and the heterogeneity of PCOS rodent models has not yet been studied.

Based on previous studies of PCOS mouse models, this study aimed to observe whether the gut microbiota plays a role in producing the heterogeneity of PCOS mouse models. We induced PCOS in different mouse models using an injection of DHEA or an injection of DHEA combined with a HFD in wild-type mice and pseudo germ-free mice to observe the effects of the gut microbiota on the phenotypes of PCOS mouse models.

Materials and methods

Animals

Eighty-four female C57BL/6J mice (3 weeks old) were purchased from Shanghai Laboratory Animal Center (SLAC). Five mice per cage were housed in a specific pathogen-free environment at the Shanghai Jiao Tong University animal center

under a standard temperature (22°C ± 3°C), stable humidity, and standard lighting conditions (12 h light/12 h dark cycle), with 24 h free access to irradiated rodent feed and autoclaved water. Body weight was measured weekly.

Establishment of a PCOS model without gut microbiota depletion

Forty mice (6 weeks old) were randomly divided into four groups: the Ctrl, HFD, DHEA and DHEA+HFD groups (n = 10 each group). To induce the PCOS model, the mice in the DHEA and DHEA+HFD groups were given a daily subcutaneous injection of DHEA (6 mg/100 g body weight; D4000-10 g, Sigma Aldrich, USA) dissolved in 0.1 mL of sesame oil for 5 weeks. For the Ctrl group, the mice were fed a normal diet and injected daily with sesame oil. For the HFD group, the mice were fed a HFD (60% of energy provided by fat, D12492, Research Diets, USA) and injected daily with sesame oil. For the DHEA group, the mice were fed a normal diet and injected daily with DHEA. For the DHEA + HFD group, the mice were fed a HFD and injected daily with DHEA.

Establishment of a PCOS model with gut microbiota depletion

To deplete the gut microbiota, forty-four mice (3 weeks old) received a freshly prepared antibiotic cocktail (ABX) in their drinking water that was prepared with ampicillin (1 g/L, A105483), vancomycin (500 mg/L, V105495), metronidazole (1 g/L, M109874), and ciprofloxacin (200 mg/L, C131636) (all from Aladdin, China). After 3 weeks of ABX treatment, thirty-one mice (6 weeks old) were randomly divided into four groups: the ABX group (n = 10), HFD+ABX group (n = 5), DHEA+ABX group (n = 8) and DHEA+HFD+ABX group (n = 8). The mice were injected and fed in the same manner as the mice without gut microbiota depletion and continued to receive ABX water for another 5 weeks.

Oral glucose tolerance test

An oral glucose tolerance test (OGTT) was performed 4 weeks after starting the DHEA treatment. The mice were fasted overnight for 6 hours before the OGTT. The glucose level of blood from the tail vein was measured with a glucometer (Accu-Chek® Performa) before and 15, 30, 60, 90 and 120 min after the administration of glucose (2 g/kg body weight per mL) by oral gavage. Tail vein blood was also collected for serum insulin measurement at 0-, 15-, and 60-min time points. The area under the curve of the glucose levels (AUC-Glucose) was calculated using GraphPad Prism 6.0 software.

Vaginal smears and oestrous cycle determination

Vaginal smears were taken daily at 8:00–9:00 am for 2 weeks before the OGTT to avoid the potential disturbances caused by the OGTT. Vaginal smears were stained with Giemsa for light microscopic determination of oestrous cycle stage. The predominance of nucleated epithelial cells indicated the proestrus stage. The predominance of cornified squamous epithelial cells indicated the oestrus stage. When the number of cornified squamous epithelial cells decreased as numerous white blood cells and nucleated epithelial cells appeared, the mice were in the metoestrus stage. Thin vaginal mucosa with vaginal cells comprising nearly all white blood cells indicated the dioestrus stage. It is worth noting that samples from mice treated with DHEA were all cornified squamous epithelial cells throughout the experiment.

Morphology

After the collection of blood samples, the ovaries were removed and fixed in 4% paraformaldehyde immediately. The tissues were then embedded in paraffin and stained with haematoxylin and eosin (HE). Corpora lutea and antral follicles were counted from the middle section of each ovary. Antral follicles displayed an expanded antral cavity, collapsed walls, and an attenuated and scattered granulosa cell layer.

Serum measurement

At the end of the experiment, blood for the determinations of testosterone (T) and lipid levels was collected from the inner canthus after the mice fasted overnight for 6 hours. Levels of serum T were measured using ^{125}I-labelled radioimmunoassay kits (B10B, Beijing North Institute of Biological Technology, China). The within-assay and between-assay variabilities were 10% and 15%, respectively. Levels of serum lipids were measured using assay kits according to the manufacturer's instructions for the following lipids: triglycerides (TGs; A110-1), total cholesterol (TC, A111-1), high-density lipoprotein cholesterol (HDL-C; A112-1) and low-density lipoprotein cholesterol (LDL-C; A113-1) (all from Nanjing Jiancheng, China).

Levels of serum insulin were measured using an ultrasensitive mouse insulin ELISA kit (90080, Crystal Chem, USA). The area under the curve of the insulin levels (AUC-Insulin) was calculated using GraphPad Prism 6.0 software. HOMA-IR = fasting blood glucose (FBG) (mmol/L) ∗ fasting blood insulin (FINS) (mIU/L)/22.5. Serum anti-Mullerian hormone (AMH) levels were measured using a mouse AMH ELISA kit (YLK-EXS921, Youlike Life Science, China).

Fecal sample collection and 16S rRNA gene sequencing

Fresh fecal samples were collected before sacrifice and were immediately stored at −80°C until analysis. DNA extraction from frozen fecal samples was conducted as previously described (19). All DNA samples were sequenced with a MiSeq reagent kit v3 (600-cycle) (MS-102-3033, Illumina, USA). A sequencing library for the V3–V4 regions of the bacterial 16S rRNA genes was constructed based on the manufacturer's instructions, with some modifications as previously described (20).

Microbiota data analysis

The 16S rRNA gene sequence data were processed and analysed using QIIME2 software (v2018.11) (21). The raw sequence data were demultiplexed and then denoised with the DADA2 pipeline (q2-dada2 plugin) (22) to obtain the amplicon sequence variant (ASV) frequency data table. Alpha diversity metric (observed ASVs), beta diversity metric (Bray–Curtis distance), and principal coordinate analysis (PCoA) were performed using the q2-diversity after rarefying the samples to 11,000 sequences per sample. Taxonomic assignment for ASVs was performed *via* the q2-feature classifier (23) using the SILVA rRNA gene database (24). The observed ASV index was compared using the Kruskal–Wallis test. The treatment-induced structural shifts in the gut microbiota were evaluated using Bray–Curtis distance, visualized by PCoA plot, and assessed by permutation multivariate analysis of variance (PERMANOVA) using the R "vegan" package with 9,999 permutations.

ASVs shared by at least 25% of all samples were considered prevalent ASVs. The correlation coefficients between the ASVs were calculated by the SparCC algorithm (25). The correlations were converted to a correlation distance (1- correlation coefficients) and then clustered into 59 coabundant groups (CAGs) using Ward clustering and PERMANOVA with 9,999 permutations. Spearman correlations between CAGs and parameters were calculated by MATLAB R2014a, and the Benjamini–Hochberg procedure was used to control the false discovery rate.

Statistical analysis

Statistical analysis and data visualization were performed using GraphPad Prism 6.0 software. Data are presented as the means ± SEMs. One-way analysis of variance (ANOVA) followed by the correction of p values with the Tukey post test was used to assess the differences among four groups (i.e., Ctrl, HFD, DHEA and DHEA+HFD or ABX, HFD+ABX, DHEA+ABX and DHEA +HFD+ABX). A two-tailed unpaired Student's t test was used to compare the differences between the two groups (i.e., Ctrl vs. DHEA, ABX vs. DHEA+ABX, Ctrl vs. ABX, and DHEA vs. DHEA+ABX). A difference with a p value < 0.05 was considered statistically significant.

Results

Different treatments induced a PCOS-like phenotype in wild-type mice

All of the mice from the DHEA and DHEA + HFD groups stayed in the dioestrus stage (Figures 1A, B), and the serum T levels were significantly higher in these mice than in mice from the Ctrl and HFD groups (Figure 1C). There was no significant difference in the AMH level among the four groups (Figure 1D). The T level was significantly higher in the DHEA group than in the DHEA+HFD group. Ovaries from the Ctrl and HFD groups contained follicles at different stages of development and several corpora lutea (CL). In contrast, those from the DHEA and DHEA + HFD groups contained several antral follicles and no CL (Figures 1E–G). These results verified the successful establishment of a PCOS-like mouse model with hyperandrogenism, anovulation and polycystic ovaries.

Body weights were similar among all of the groups (Figure 2A). Compared with the Ctrl and DHEA groups, the HFD and DHEA+HFD groups displayed markedly higher FBG levels (Figure 2B) and glucose intolerance (Figures 2E, F). Two mice from the DHEA+HFD group showed extremely heterogeneous hyperinsulinemia and IR. Thus, only the DHEA group displayed significant and stabilized increases in FINS, HOMA-IR and postprandial insulin levels (Figures 2C, D, G, H), indicating hyperinsulinemia and insulin resistance in the DHEA group. In addition, the DHEA and DHEA + HFD groups displayed significantly increased TC and LDL-C levels compared with the other groups, while the mice on a HFD displayed a reduced trend for TG levels (Figures 2I–L).

We performed V3-V4 16S rRNA gene sequencing to compare the diversity and composition of the gut microbiota of the four groups. The observed ASVs showed a significant reduction in alpha diversity in the HFD group compared to the Ctrl and DHEA groups, but no significant difference was observed among the Ctrl, DHEA and DHEA+HFD groups (Figure 3A). In the context of beta diversity based on Bray–Curtis distance (Figures 3B, C), the overall microbial structure showed significant differences among the four groups, but the HFD might contribute to a more severe effect. We constructed a coabundance network between the 520 ASVs that were shared by at least 25% of all samples and clustered the ASVs into 59 coabundant groups (CAGs) (Table S1). Of these, CAG2, mainly containing ASVs from *Bifidobacterium* and *Lactobacillus*, was significantly increased in the DHEA and DHEA+HFD groups. CAG2 was also positively correlated with the T level, the number

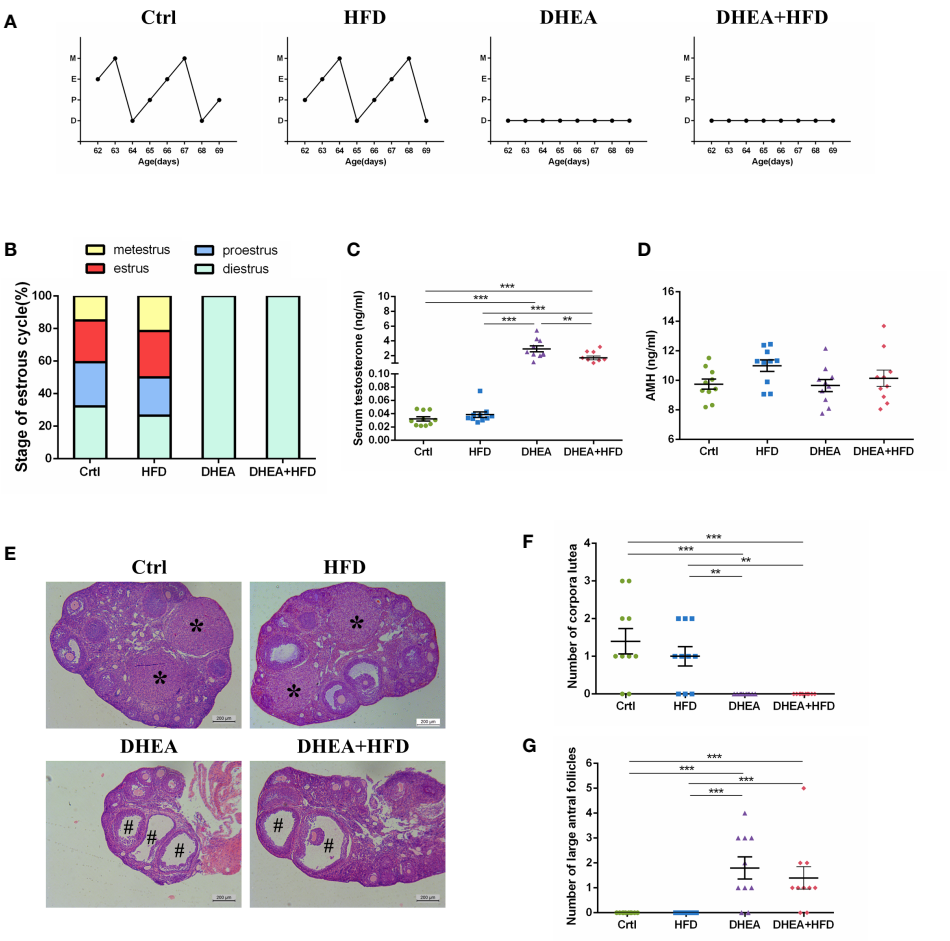


FIGURE 1
The stage of the oestrous cycle, ovarian morphology and serum T level in wild-type mice. **(A)** Representative oestrous cycle of one mouse from each group. **(B)** The proportion of each oestrous cycle stage in each group. **(C)** Serum testosterone level. **(D)** Serum AMH level. **(E)** Representative HE staining of ovarian tissue from one mouse from each group. * indicates corpora lutea, and # indicates large antral follicles. The number of **(F)** corpora lutea and **(G)** large antral follicles of one ovary from each group. Data are reported as the means ± SEMs. $^{**}P$ <0.01, and $^{***}P$ < 0.001. AMH, anti-Mullerian hormone.

of antral follicles and the TG level and negatively correlated with the AUC-Glucose and FBG levels. CAG6 composed of ASVs from *Alistipes* and *Turicibacter*, CAG9, CAG15 and CAG17 composed of ASVs from *Muribaculaceae*, CAG16 composed of ASVs from *Lactobacillus* and *Muribaculaceae*, and CAG18 composed of ASVs from *Lachnospiraceae* were enriched in the Ctrl and DHEA groups. Additionally, they were negatively correlated with the AUC-Glucose and FBG levels, and positively correlated with the TG levels. In contrast, CAG36 and CAG46 composed of ASVs from *Lachnospiraceae*, CAG38 composed of ASVs from *Blautia*, CAG47 composed of ASVs from *Desulfovibrionaceae* and *Blautia*, CAG54 composed of ASVs from *Alistipes* and *Muribaculaceae*, and CAG56 composed of ASVs from *Bacteroides* and *Alistipes* were enriched in the HFD and DHEA+HFD groups and showed an opposite trend in their correlations with the disease phenotypes (Figures 3D, E and Table S1).

Different treatments induced a PCOS-like phenotype in pseudo germ-free mice

Quantitative real-time PCR and viable bacterial plate counts confirmed that more than 99.99% of the gut bacteria were depleted after 3 weeks of ABX treatment. These results verified the successful establishment of a pseudo germ-free mouse model.

Similar to wild-type mice, all of the mice from the DHEA+ABX and DHEA + HFD+ABX groups displayed disrupted oestrous cycles, increased serum T levels, several antral follicles and no CL

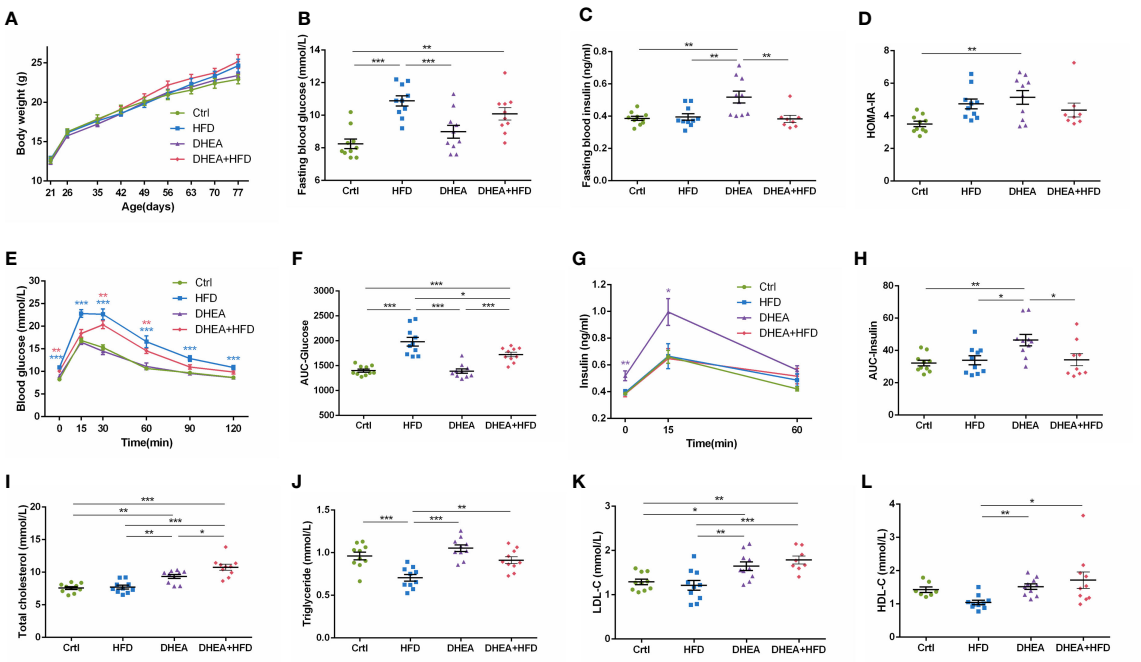


FIGURE 2
Glucolipid metabolism in wild-type mice. **(A)** Body weight. **(B)** Fasting blood glucose levels. **(C)** Fasting blood insulin levels. **(D)** HOMA-IR. **(E)** Serum glucose levels during the OGTT. **(F)** AUC-Glucose. **(G)** Serum insulin levels during the OGTT. **(H)** AUC-Insulin. **(I)** Total cholesterol. **(J)** Triglycerides. **(K)** LDL-C. **(L)** HDL-C. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. HOMA-IR, homeostasis model assessment for insulin resistance index; AUC, area under the curve; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

(Figures 4A–G). The AMH level in DHEA + HFD+ABX group was significantly lower than that of the ABX and DHEA+ABX groups (Figure 4D). These results suggest that DHEA can also induce the PCOS-like mouse model in pseudo germ-free mice. However, pseudo germ-free mice in the ABX group displayed a reduced oestrous stage and no CL (Figures 4A, B, E, F), indicating oligo- or anovulation in pseudo germ-free mice.

For all mice, body weights decreased progressively during the first week of ABX treatment and gradually recovered thereafter. At the end of the experiment, the body weight of the HFD+ABX group was significantly higher than that of the ABX group (Figure 5A). Similar to wild-type mice, the HFD+ABX and DHEA+HFD+ABX groups displayed markedly higher FBG levels (Figure 5B) and glucose intolerance (Figures 5E, F) than the ABX and DHEA +ABX groups. There was no significant difference in the FINS level among the four groups (Figure 5C). The AUC-Insulin level of the DHEA+ABX and DHEA+HFD+ABX groups was not significantly higher than that of the ABX group (Figures 5G, H). The HFD+ABX and DHEA+HFD+ABX groups displayed a significantly increased HOMA-IR index (Figure 5D). In addition, the TC, TG and LDL-C levels of the DHEA+ABX group were significantly higher than those of the ABX group, and the TC level of the DHEA+HFD+ABX group was significantly higher than that of the ABX group (Figures 5I–L).

Effect of gut microbiota depletion on the phenotype of PCOS mouse models

To explore the effect of gut microbiota depletion on the phenotype of PCOS mouse models, we compared the reproductive endocrine and metabolism indicators of wild-type and pseudo germ-free mice. The disrupted oestrous cycles, AMH level, numbers of antral follicles and CL were not significantly different between the DHEA and DHEA+ABX groups (Figures 6A, B, D–G). In contrast, the DHEA+ABX group displayed significantly higher serum T levels than the DHEA group (Figure 6C).

The decrease in body weight induced by ABX treatment was restored to the level of the wild-type mice after 6 weeks, and there was no significant difference among the four groups at the end of the experiment (Figure 7A). The mice in the ABX and DHEA+ABX groups displayed markedly lower FBG levels (Figure 7B) and were less glucose-intolerant (Figures 7E, F) than the Ctrl and DHEA groups. Unlike the wild-type mice, the FINS, HOMA-IR, 15 min insulin and AUC-Insulin levels (Figures 7C, D, G, H) of the ABX and DHEA+ABX groups were not significantly different. In addition, the DHEA+ABX group displayed significantly higher TC levels and markedly higher TG levels than the other three groups (Figures 7I–L).

10.3389/fendo.2022.1030151

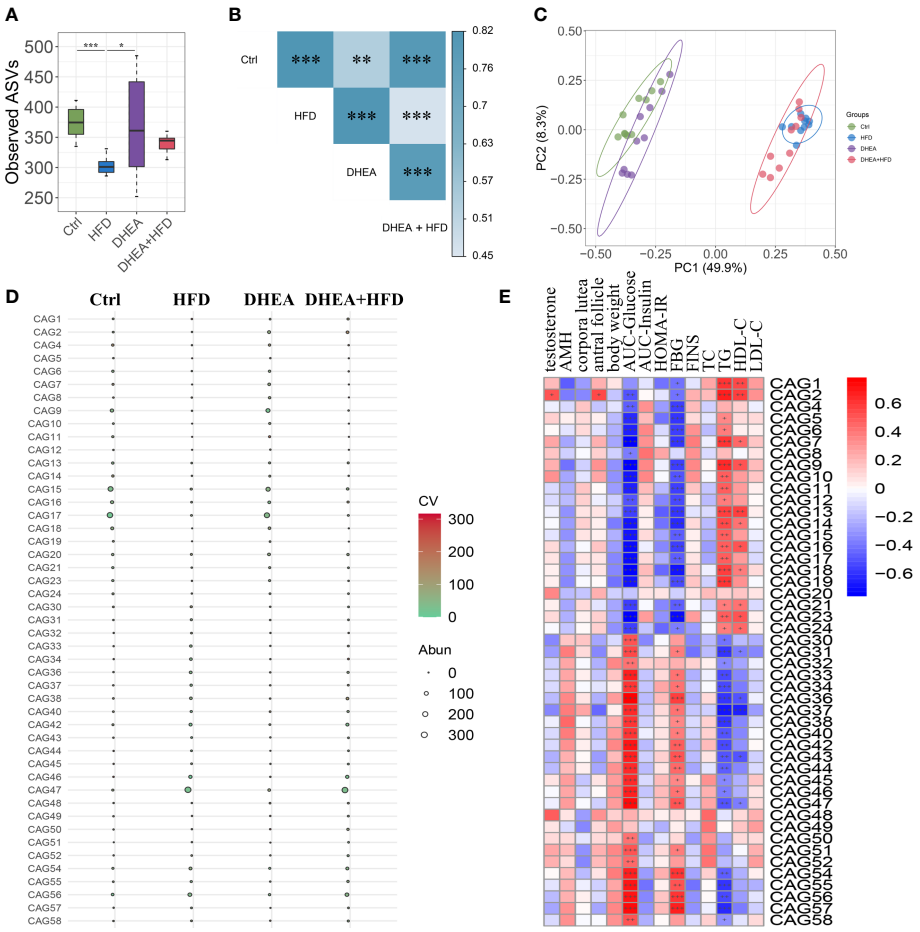


FIGURE 3
The gut microbial structure was altered in PCOS mice. Alpha diversity measured by **(A)** observed ASVs. Data are expressed as the mean ± SEM. *P < 0.05 and ***P < 0.001. **(B)** A PERMANOVA test was performed on the basis of the Bray–Curtis distances. The colors of the blocks indicate the distance, and the asterisks denote significant differences between different groups. ** P <0.01 and *** P < 0.001. **(C)** Principal coordinates analysis (PCoA) performed on the basis of the Bray–Curtis distances. PC1, principal coordinate 1; PC2, principal coordinate 2. **(D)** Bubble plot shows the variation in the average abundance of CAGs in each group. The size and color of the circles represent the average abundance and coefficient of variance (CV) of each CAG, respectively. **(E)** Correlations between phenotypic data and CAGs.

Discussion

PCOS mouse models are often heterogeneous, especially in terms of metabolic features, but studies are still limited. Our current study first indicated that the gut microbiota was associated with heterogeneous phenotypes in PCOS mice. In this study, we induced PCOS in mice by DHEA administration or DHEA administration together with a HFD and ABX to observe the differences in reproductive and metabolic phenotypes. PCOS mice induced by DHEA alone showed reproductive disorders and hyperinsulinemia, and the HFD mainly aggravated glucolipid metabolic disorders. Glucolipid metabolic disorders were associated with alterations in the gut microbiota. The PCOS mice with gut microbiota depletion showed improvements in hyperinsulinemia and glucose metabolic disorders and more serious hyperandrogenism and lipid disorders.

In the past decade, the number of studies on PCOS has increased exponentially. A number of studies suggest that women with PCOS exhibit hyperandrogenism, multi-cystic ovaries, hyperinsulinemia, glucolipid metabolism disorders and obesity, which are related to the gut microbiota (14, 26, 27). However, women with PCOS often show high clinical heterogeneity, and our previous study found that the clinical heterogeneity of PCOS was related to the gut microbiota (18). Combined with reviews of PCOS rodent models over the past decades, we found that PCOS rodent models are often variable (9, 10). Lai et al. (11) used a HFD combined with DHEA to induce PCOS mouse models and found that HFD treatment did not affect the reproductive phenotype of DHEA-treated mice but

10.3389/fendo.2022.1030151

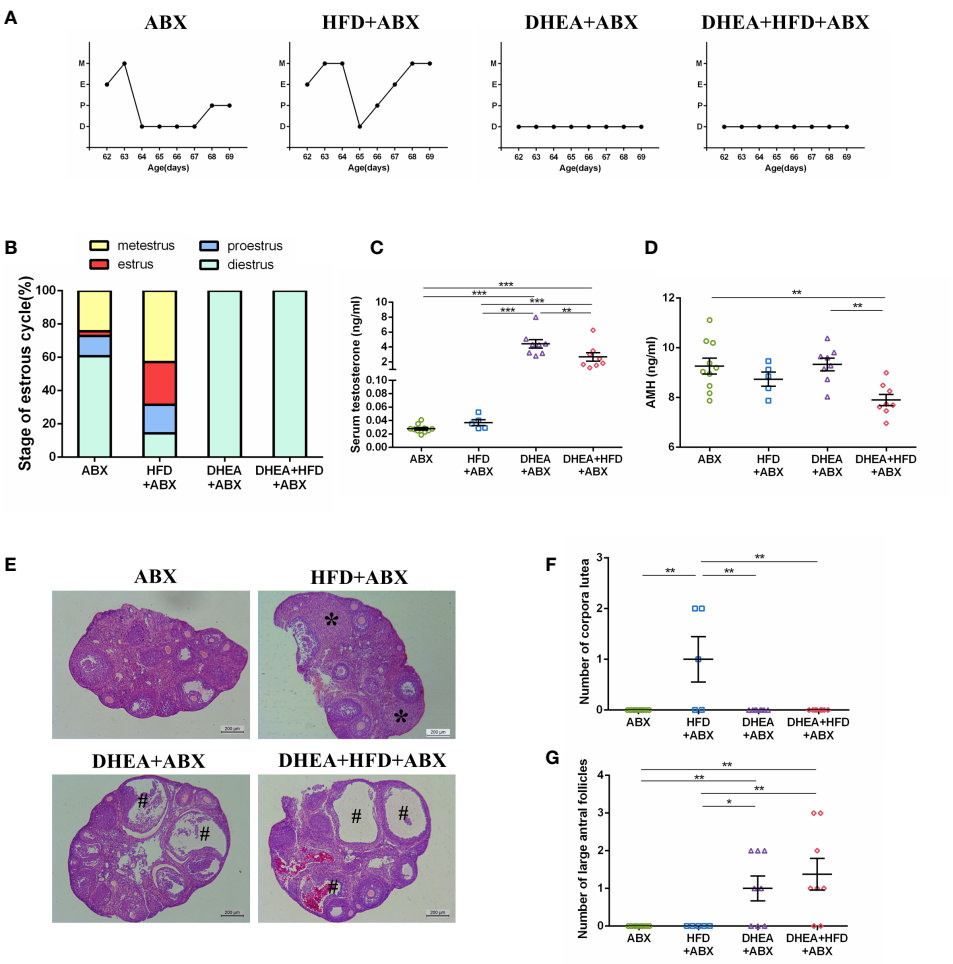


FIGURE 4
The stage of the oestrous cycle, ovarian morphology and serum T level in pseudo germ-free mice. **(A)** Representative oestrous cycle of one mouse from each group. **(B)** The proportion of each oestrous cycle stage in each group. **(C)** Serum testosterone level. **(D)** Serum AMH level. **(E)** Representative HE staining of ovarian tissue from one mouse from each group. * indicates corpora lutea, and # indicates large antral follicles. The number of **(F)** corpora lutea and **(G)** large antral follicles of one ovary from each group. Data are reported as the means ± SEMs. $^{**}P$ <0.01, and $^{***}P$ < 0.001. AMH, anti-Mullerian hormone.

caused significant metabolic alterations. Considering the effects of a HFD on metabolism in DHEA-treated mice, we treated C57BL/6 mice with DHEA alone or in combination with a HFD and studied the heterogeneity and its relationship with the gut microbiota.

Consistent with previous studies (5, 11), DHEA-treated and DHEA+HFD-treated PCOS mice showed disturbed cyclicity, multi-cystic ovaries and hyperandrogenism. Recently, Hohos et al. (28) found that mice fed a HFD for 10 weeks exhibited ovulatory dysfunction, which appears to be mediated through the dysregulation of ovarian Edn2 expression. In our study, HFD-fed mice did not show ovulatory dysfunction, and these conflicting results might be due to the time of HFD feeding. Thus, ovulatory dysfunction was caused by the effect of DHEA. It is well known that the HFD is frequently used to induce obesity, IR and hyperinsulinemia (29). However, we found that DHEA-treated mice showed hyperinsulinemia and IR, while DHEA+HFD-treated mice did not show this, except the two mice with extreme values. This may be due to the short duration of HFD treatment in our study. In addition, DHEA could lead to hyperinsulinemia by affecting granulosa cells (30). Our study suggested that DHEA could better induce PCOS in mice with hyperandrogenism and hyperinsulinemia than DHEA+HFD. Mice fed a 60% HFD showed impaired glucose tolerance, but the DHEA-treated mice showed normal glucose tolerance. The above result indicates that the impaired glucose tolerance was mainly induced by HFD. Therefore, we could conclude that the physiological and pathological pathways for reproductive and metabolic disorders in DHEA-induced PCOS mice may be different.

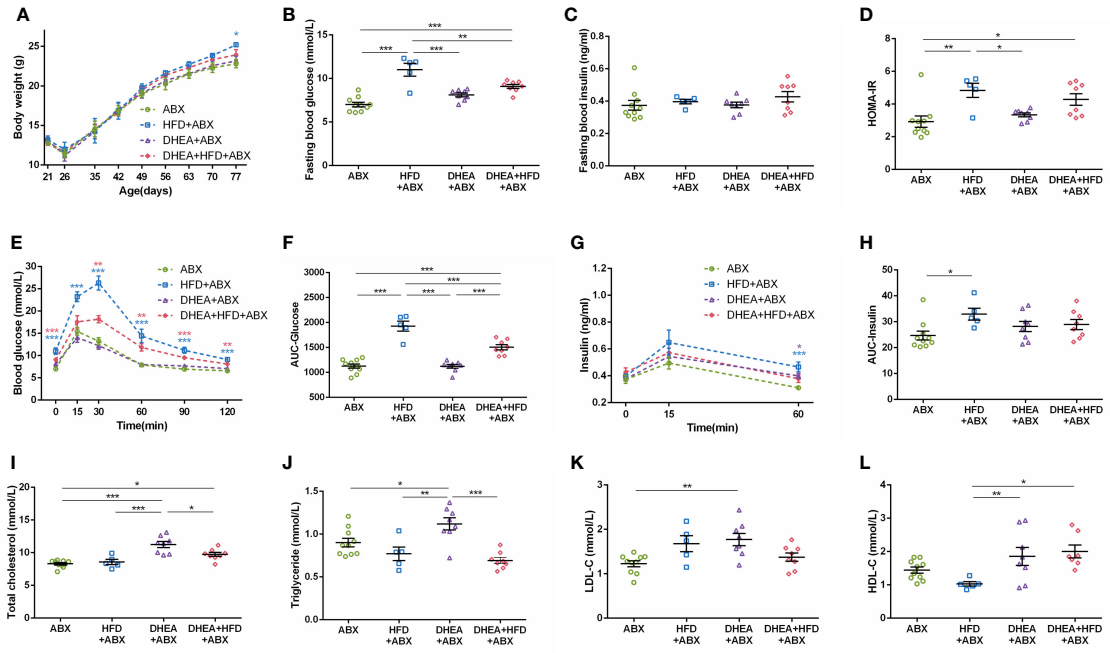


FIGURE 5
Glucolipid metabolism in pseudo germ-free mice. **(A)** Body weights. **(B)** Fasting blood glucose levels. **(C)** Fasting blood insulin levels. **(D)** HOMA-IR. **(E)** Serum glucose levels during the OGTT. **(F)** AUC-Glucose. **(G)** Serum insulin levels during the OGTT. **(H)** AUC-Insulin. **(I)** Total cholesterol. **(J)** Triglycerides. **(K)** LDL-C. **(L)** HDL-C. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. HOMA-IR, homeostasis model assessment for insulin resistance index; AUC, area under the curve; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

We found that the gut microbiota was altered in PCOS mice induced by DHEA or combined with HFD. Up to now, only one study reported that the relative abundance of *Lactobacillus* was lower in mice with prenatal androgen exposure, and no study reported the alteration of *Bifidobacterium* in PCOS mice (31). Our study showed the relative abundance of *Bifidobacterium* and *Lactobacillus* was significantly increased in the mice treated with DHEA, indicating the enrichment of *Bifidobacterium* and *Lactobacillus* by androgen exposure. Previous studies showed that *Bifidobacterium* and *Lactobacillus* could improve hyperandrogenism, obesity and IR of PCOS (32–34). However, our study showed the relative abundance of *Bifidobacterium* was positively correlated with the level of testosterone. These results suggest that the causal relationship between *Bifidobacterium* and androgen remains to be further explored. Moreover, a HFD might play a more critical role in shaping the gut microbiota structure than DHEA, emphasizing the important influence of diet on the host gut microbiota (35). Our study showed that the *Bacteroides*, *Blautia* and *Desulfovibrionaceae* specifically enriched in mice treated with HFD were positively correlated with the AUC-Glucose and FBG levels, while *Turicibacter* and *Lactobacillus* specifically inhibited in mice treated with HFD showed an opposite trend correlation with the disease phenotypes. Sun et al. (36) and Jiang et al. (16) pointed out that two strains of *Bacteroides* affected the host metabolic disorders and inflammation of host *via* the gut microbiota–bile acid axis. Lin et al. (37) reported an increase in the abundance of *Blautia* genera was correlated with the alterations of bile acids in rats treated with HFD. *Blautia* and *Desulfovibrionaceae*, the HFD-dependent taxa, were related to the glucose homeostasis (38). In this study, Zhao et al. also found that the enrichment of *Turicibacter* and *Bifidobacterium* was related to the improvement of glucose homeostasis. One analysis of the alteration of gut microbiota richness and structure in women with PCOS found that the dysbiosis of the gut microbiota was more severe in obese PCOS women than in nonobese women (17). These results suggest that changes in the gut microbiota mainly affected the metabolic disorders more than reproductive disorders by regulating bile acid metabolism and inflammation of the host.

In pseudo germ-free mice, DHEA treatment could still induce reproductive disorders. Han et al. (4) also induced PCOS in rats with disturbed cyclicity, multi-cystic ovaries and hyperandrogenism by treatment with DHEA+ABX. The above results indicated that the gut microbiota could not prevent the occurrence of reproductive disorders in PCOS rodent models induced by DHEA. The ABX-treated mice showed disturbed cyclicity and anovulation, while the HFD+ABX-treated mice showed normal ovulation. It is interesting that this phenomenon was associated with the change in adipose tissue weight (Figure S2). Serum AMH is

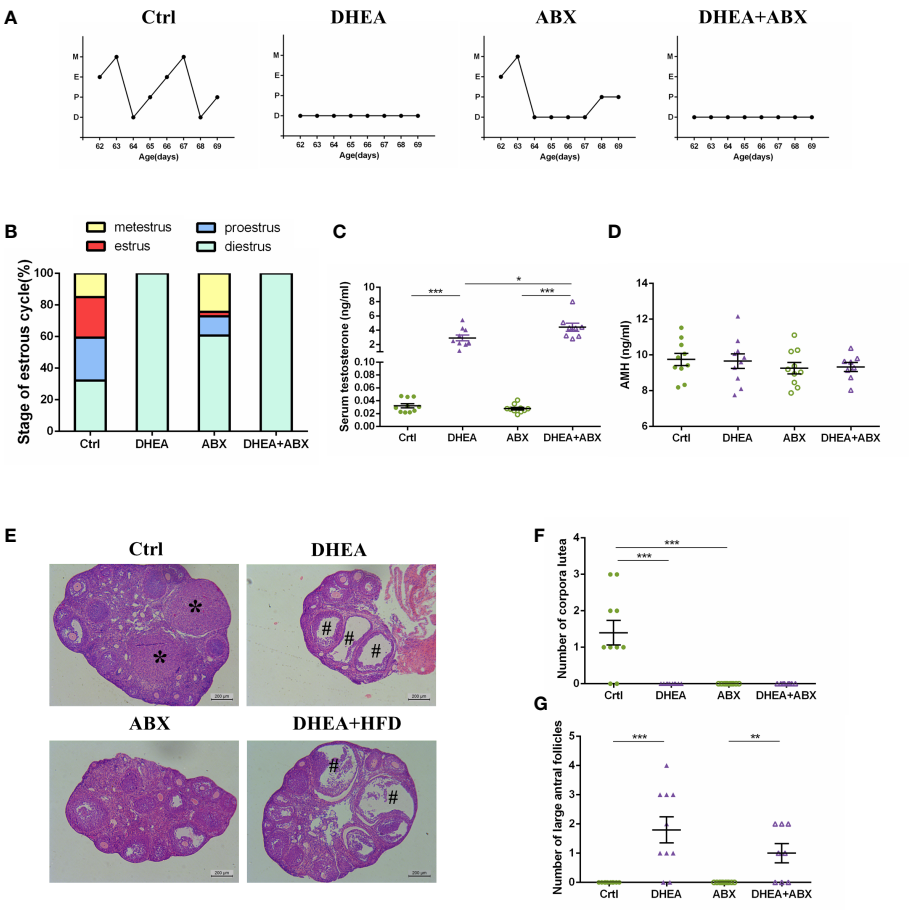


FIGURE 6
The stage of the oestrous cycle, ovarian morphology and serum T level of wild-type mice compared to pseudo germ-free mice. **(A)** Representative oestrous cycle of one mouse from each group. **(B)** The proportion of each oestrous cycle stage in each group. **(C)** Serum testosterone level. **(D)** Serum AMH level. **(E)** Representative HE staining of ovarian tissue from one mouse from each group. * indicates corpora lutea, and # indicates large antral follicles. The number of **(F)** corpora lutea and **(G)** large antral follicles of one ovary from each group. Data are reported as the means ± SEMs. $^{**}P$ <0.01, and $^{***}P$ < 0.001. AMH, anti-Mullerian hormone.

the preferred ovarian reserve marker. PCOS rats showed significant decrease of AMH level in serum and increase of AMH protein expression in ovaries (39). In our study, serum AMH level was only significantly decreased in the DHEA+HFD+ABX group. However, the serum AMH level of the ABX-treated mice was not decreased, indicating the reproductive function is normal in pseudo germ-free mice. The underlying mechanisms require further investigation. AMH is an indicator that was tested four years later than the original experiment. Because of the long storage time and freeze-thawing of the samples, the result of AMH needs to be further verified. A Vietnamese study reported that lean PCOS women presented with anovulation, higher serum testosterone levels and a low metabolic disease risk (40). In a 2007 study, Martin et al. (41) found that caloric restriction led females become ceased cycling and underwent endocrine masculinization. However, until now, studies on lean PCOS are limited. Gut microbiota depletion did not prevent the glucose metabolic disorders induced by a HFD. Fleissner et al. (42) found that the absence of the gut microbiota in germ-free mice could not protect mice from diet-induced obesity. Metabolic disorders were not affected solely by the gut microbiota.

By comparing wild-type and pseudo germ-free mice, we found that gut microbiota depletion did not affect the occurrence of oestrous cycle disorders, serum AMH levels or ovarian morphological changes induced by DHEA but aggravated hyperandrogenism. Consistent with our results, Markle et al. found that the serum T level of germ-free female mice is significantly higher than that of SPF female mice and that the transplantation of the gut microbiota from male mice to germ-free female mice significantly increased the serum T level of the recipients (43). The serum T levels of PCOS mice in our study seemed to be negatively correlated with body fat weight. This phenomenon that might be due to the conversion of androgens to oestrogens by the aromatase enzyme that is expressed in fat tissue (44). These results suggest that the gut microbiota is not a

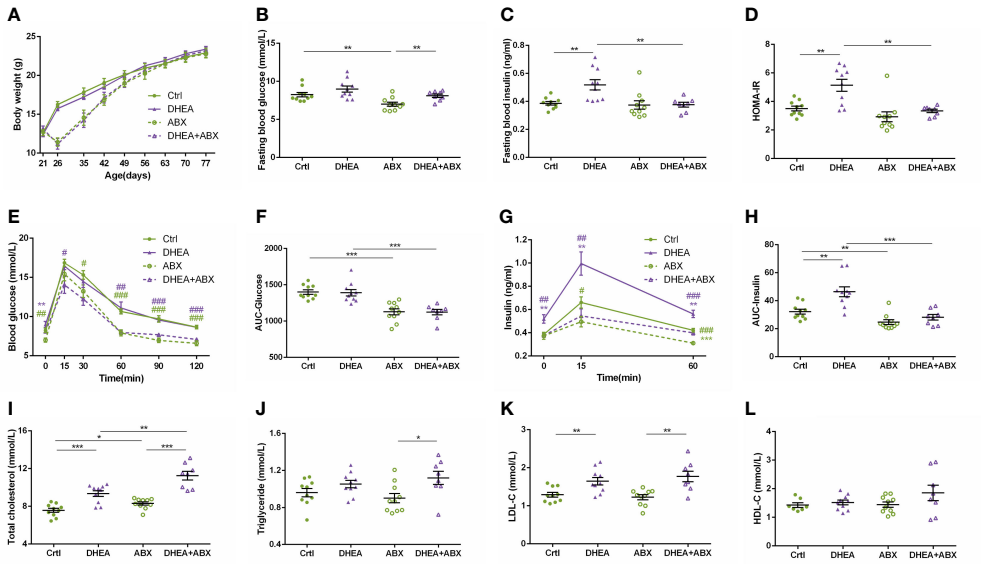


FIGURE 7
Glucolipid metabolism of wild-type mice compared to pseudo germ-free mice. **(A)** Body weights. **(B)** Fasting blood glucose levels. **(C)** Fasting blood insulin levels. **(D)** HOMA-IR. **(E)** Serum glucose levels during the OGTT. **(F)** AUC-Glucose. **(G)** Serum insulin levels during the OGTT. **(H)** AUC-Insulin. **(I)** Total cholesterol. **(J)** Triglycerides. **(K)** LDL-C. **(L)** HDL-C. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. HOMA-IR, homeostasis model assessment for insulin resistance index; AUC, area under the curve; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

direct factor in the development of hyperandrogenemia. The blood glucose level of PCOS mice was decreased after ABX intervention. ABX-induced gut microbiota depletion alters metabolic homeostasis by affecting colonic metabolism. Zarrinpar et al. (45) found that antibiotic-induced microbiome depletion decreased luminal *Firmicutes* and *Bacteroidetes* and thereby decreased luminal short-chain fatty acids (SCFAs). This was especially true for butyric acid, which can provide intestinal epithelial cells with energy. In addition, the body weight and parametrial fat weight of pseudo germ-free mice were lower than wild-type mice (Figure S3). Therefore, the decrease of blood glucose levels might correlate with emaciation. It suggested that we should pay attention to the effect of ABX on blood glucose of the host in future studies. ABX-induced gut microbiota depletion promoted glucose uptake in brown adipose tissue and the cecum (46). ABX-induced gut microbiota depletion could reduce the content of lipopolysaccharide in the ileum and inhibited the TLR4-related inflammatory pathways (47). Therefore, ABX treatment improved the chronic metabolic inflammation of the host. However, the PCOS mice treated with ABX showed more severe hyperlipemia. Although recent studies suggested that ABX could reduce lipid levels of HFD-fed mice, two studies reported the liver lipid accumulation by ABX treatment in tacrolimus-treated mice and the ABX discontinuity in db/db mice, which might be due to the reduced SCFAs and bile acid metabolism (48, 49). The specific mechanism for this still requires further study.

Our study focused on the correlational relationship between the gut microbiota and the heterogeneity of PCOS mouse models. Next, fecal microbiota transplantation of PCOS women to germ-free mice is needed to explore the causal relationship and mechanisms of the gut microbiota in the heterogeneity of PCOS.

Our work described the phenotypic differences in PCOS induced by different methods in mice and revealed that the phenotype of PCOS mice was correlated with the gut microbiota. Compared to DHEA+HFD, the PCOS mouse model induced by DHEA alone better simulates the stabilized pathophysiological defects of this disease. ABX intervention improved glucose metabolic disorders and hyperinsulinemia but aggravated hyperandrogenism and lipid metabolic disorders in PCOS mice. These findings provide new insight into the establishment of PCOS rodent models and studies about the pathophysiological mechanisms of PCOS in the future.

Data availability statement

The raw sequence data presented in the study are deposited in the NCBI Sequence Read Archive database, accession number PRJNA672803.

Ethics statement

The animal study was reviewed and approved by the Institutional Animal Care and Use Committee of Shanghai Jiaotong University (A2017062).

Author contributions

XW performed the animal experiments, DNA extraction and sequencing, bioinformatics and statistical analysis. LG performed the animal experiment and statistical analysis. YZ and CX performed the animal experiments. YP designed the study and performed quality control. XD designed the study and wrote the manuscript. All authors contributed to the article and approved the submitted version.

Funding

The study was supported by the National Natural Science Foundation of China (81870594), the Shanghai Jiao Tong University Research Funding on Medical, Engineering Interdisciplinary Project (YG2019GD05), the Clinical Research Plan of SHDC (SHDC2020CR1016B), the Multicenter Clinical Research Project of Shanghai Jiao Tong University School of Medicine (DLY201824), and the National Key Research and Development Program of China (2021YFC2501600, 2021YFC2501601).

Acknowledgments

We are grateful to all the participants in the study. Chenhong Zhang and her team are gratefully acknowledged for providing their excellent technical support and designing the experiments.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1030151/full#supplementary-material

References

1. Bozdag G, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO. The prevalence and phenotypic features of polycystic ovary syndrome: A systematic review and meta-analysis. *Hum Reprod* (2016) 31(12):2841–55. doi: 10.1093/humrep/dew218

2. The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* (2004) 19(1):41–7. doi: 10.1093/humrep/deh098

3. Meier RK. Polycystic ovary syndrome. *Nurs Clin North Am* (2018) 53(3):407–20. doi: 10.1016/j.cnur.2018.04.008

4. Han Q, Wang J, Li W, Chen ZJ, Du Y. Androgen-induced gut dysbiosis disrupts glucolipid metabolism and endocrinal functions in polycystic ovary syndrome. *Microbiome* (2021) 9(1):101. doi: 10.1186/s40168-021-01046-5

5. Xue J, Li X, Liu P, Li K, Sha L, Yang X, et al. Inulin and metformin ameliorate polycystic ovary syndrome *via* anti-inflammation and modulating gut microbiota in mice. *Endocr J* (2019) 66(10):859–70. doi: 10.1507/endocrj.EJ18-0567

6. Zhang F, Ma T, Tong X, Liu Y, Cui P, Xu X, et al. Electroacupuncture improves metabolic and ovarian function in a rat model of polycystic ovary syndrome by decreasing white adipose tissue, increasing brown adipose tissue, and modulating the gut microbiota. *Acupunct Med* (2022) 40(4):347–59. doi: 10.1177/09645284211056663

7. Zhang F, Ma T, Cui P, Tamadon A, He S, Huo C, et al. Diversity of the gut microbiota in dihydrotestosterone-induced PCOS rats and the pharmacologic effects of Diane-35, probiotics, and berberine. *Front Microbiol* (2019) 10:175. doi: 10.3389/fmicb.2019.00175

8. Yang YL, Zhou WW, Wu S, Tang WL, Wang ZW, Zhou ZY, et al. Intestinal flora is a key factor in insulin resistance and contributes to the development of polycystic ovary syndrome. *Endocrinology* (2021) 162(10):bqab118. doi: 10.1210/endocr/bqab118

9. Noroozzadeh M, Behboudi-Gandevani S, Zadeh-Vakili A, Ramezani Tehrani F. Hormone-induced rat model of polycystic ovary syndrome: A systematic review. *Life Sci* (2017) 191:259–72. doi: 10.1016/j.lfs.2017.10.020

10. Corrie L, Gulati M, Singh SK, Kapoor B, Khursheed R, Awasthi A, et al. Recent updates on animal models for understanding the etiopathogenesis of polycystic ovarian syndrome. *Life Sci* (2021) 280:119753. doi: 10.1016/j.lfs.2021.119753

11. Lai H, Jia X, Yu Q, Zhang C, Qiao J, Guan Y, et al. High-fat diet induces significant metabolic disorders in a mouse model of polycystic ovary syndrome. *Biol Reprod* (2014) 91(5):127. doi: 10.1095/biolreprod.114.120063

12. Tremellen K, Pearce K. Dysbiosis of gut microbiota (DOGMA)–a novel theory for the development of polycystic ovarian syndrome. *Med Hypotheses* (2012) 79(1):104–12. doi: 10.1016/j.mehy.2012.04.016

13. Liu R, Zhang C, Shi Y, Zhang F, Li L, Wang X, et al. Dysbiosis of gut microbiota associated with clinical parameters in polycystic ovary syndrome. *Front Microbiol* (2017) 8:324. doi: 10.3389/fmicb.2017.00324

14. Insenser M, Murri M, Del Campo R, Martinez-Garcia MA, Fernandez-Duran E, Escobar-Morreale HF. Gut microbiota and the polycystic ovary syndrome: Influence of sex, sex hormones, and obesity. *J Clin Endocrinol Metab* (2018) 103(7):2552–62. doi: 10.1210/jc.2017-02799

15. Torres PJ, Siakowska M, Banaszewska B, Pawelczyk L, Duleba AJ, Kelley ST, et al. Gut microbial diversity in women with polycystic ovary syndrome correlates with hyperandrogenism. *J Clin Endocrinol Metab* (2018) 103(4):1502–11. doi: 10.1210/jc.2017-02153

16. Qi X, Yun C, Sun L, Xia J, Wu Q, Wang Y, et al. Gut microbiota-bile acid-interleukin-22 axis orchestrates polycystic ovary syndrome. *Nat Med* (2019) 25 (8):1225–33. doi: 10.1038/s41591-019-0509-0

17. Zeng B, Lai Z, Sun L, Zhang Z, Yang J, Li Z, et al. Structural and functional profiles of the gut microbial community in polycystic ovary syndrome with insulin resistance (IR-PCOS): A pilot study. *Res Microbiol* (2019) 170(1):43–52. doi: 10.1016/j.resmic.2018.09.002

18. Wang X, Xu T, Liu R, Wu G, Gu L, Zhang Y, et al. High-fiber diet or combined with acarbose alleviates heterogeneous phenotypes of polycystic ovary syndrome by regulating gut microbiota. *Front Endocrinol (Lausanne)* (2021) 12:806331. doi: 10.3389/fendo.2021.806331

19. Godon JJ, Zumstein E, Dabert P, Habouzit F, Moletta R. Molecular microbial diversity of an anaerobic digestor as determined by small-subunit rDNA sequence analysis. *Appl Environ Microbiol* (1997) 63(7):2802–13. doi: 10.1128/aem.63.7.2802-2813.1997

20. Ghosh S, Yang X, Wang L, Zhang C, Zhao L. Active phase prebiotic feeding alters gut microbiota, induces weight-independent alleviation of hepatic steatosis and serum cholesterol in high-fat diet-fed mice. *Comput Struct Biotechnol J* (2021) 19:448–58. doi: 10.1016/j.csbj.2020.12.011

21. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* (2019) 37(8):852–7. doi: 10.1038/s41587-019-0209-9

22. Callahan BJ, Mcmurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from illumina amplicon data. *Nat Methods* (2016) 13(7):581–3. doi: 10.1038/nmeth.3869

23. Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, et al. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* (2018) 6(1):90. doi: 10.1186/s40168-018-0470-z

24. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* (2013) 41(Database issue):D590–596. doi: 10.1093/nar/gks1219

25. Friedman J, Alm EJ. Inferring correlation networks from genomic survey data. *PloS Comput Biol* (2012) 8(9):e1002687. doi: 10.1371/journal.pcbi.1002687

26. Zhao X, Jiang Y, Xi H, Chen L, Feng X. Exploration of the relationship between gut microbiota and polycystic ovary syndrome (PCOS): A review. *Geburtshilfe und Frauenheilkunde* (2020) 80(2):161–71. doi: 10.1055/a-1081-2036

27. Zhou L, Ni Z, Yu J, Cheng W, Yu C. Correlation between fecal metabolomics and gut microbiota in obesity and polycystic ovary syndrome. *Front Endocrinol (Lausanne)* (2020) 11:628. doi: 10.3389/fendo.2020.00628

28. Hohos NM, Elliott EM, Giornazi A, Silva E, Rice JD, Skaznik-Wikiel ME. High-fat diet induces an ovulatory defect associated with dysregulated endothelin-2 in mice. *Reproduction* (2021) 161(3):307–17. doi: 10.1530/REP-20-0290

29. Nagy C, Einwallner E. Study of *In vivo* glucose metabolism in high-fat diet-fed mice using oral glucose tolerance test (OGTT) and insulin tolerance test (ITT). *J Vis Exp* (2018) (131):56672. doi: 10.3791/56672

30. Tao X, Chen L, Cai L, Ge S, Deng X. Regulatory effects of the AMPKalpha-SIRT1 molecular pathway on insulin resistance in PCOS mice: An in vitro and *in vivo* study. *Biochem Biophys Res Commun* (2017) 494(3-4):615–20. doi: 10.1016/j.bbrc.2017.09.154

31. Sherman SB, Sarsour N, Salehi M, Schroering A, Mell B, Joe B, et al. Prenatal androgen exposure causes hypertension and gut microbiota dysbiosis. *Gut Microbes* (2018) 9(5):400–21. doi: 10.1080/19490976.2018.1441664

32. Zhang J, Sun Z, Jiang S, Bai X, Ma C, Peng Q, et al. Probiotic bifidobacterium lactis V9 regulates the secretion of sex hormones in polycystic ovary syndrome patients through the gut-brain axis. *mSystems* (2019) 4(2):e00017–19. doi: 10.1128/mSystems.00017-19

33. Kaur I, Suri V, Sachdeva N, Rana SV, Medhi B, Sahni N, et al. Efficacy of multi-strain probiotic along with dietary and lifestyle modifications on polycystic ovary syndrome: a randomised, double-blind placebo-controlled study. *Eur J Nutr* (2022) 61(8):4145–54. doi: 10.1007/s00394-022-02959-z

34. Samimi M, Dadkhah A, Haddad Kashani H, Tajabadi-Ebrahimi M, Seyed Hosseini E, Asemi Z. The effects of synbiotic supplementation on metabolic status in women with polycystic ovary syndrome: A randomized double-blind clinical trial. *Probiotics Antimicrob Proteins* (2019) 11(4):1355–61. doi: 10.1007/s12602-018-9405-z

35. Zhang CH, Zhang MH, Wang SY, Han RJ, Cao YF, Hua WY, et al. Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. *Isme J* (2010) 4(2):312–3. doi: 10.1038/ismej.2009.144

36. Sun L, Xie C, Wang G, Wu Y, Wu Q, Wang X, et al. Gut microbiota and intestinal FXR mediate the clinical benefits of metformin. *Nat Med* (2018) 24 (12):1919–29. doi: 10.1038/s41591-018-0222-4

37. Lin H, An Y, Tang H, Wang Y. Alterations of bile acids and gut microbiota in obesity induced by high fat diet in rat model. *J Agric Food Chem* (2019) 67 (13):3624–32. doi: 10.1021/acs.jafc.9b00249

38. Zhao Q, Hou D, Fu Y, Xue Y, Guan X, Shen Q. Adzuki bean alleviates obesity and insulin resistance induced by a high-fat diet and modulates gut microbiota in mice. *Nutrients* (2021) 13(9):3240. doi: 10.3390/nu13093240

39. Zhang Y, Xu L. Comparative study of DHEA and letrozole induced polycystic ovary syndrome in post-pubertal rats. *Gynecol Endocrinol* (2022) 38 (5):425–31. doi: 10.1080/09513590.2022.2052843

40. Cao NT, Le MT, Nguyen VQH, Pilgrim J, Le VNS, Le DD, et al. Defining polycystic ovary syndrome phenotype in Vietnamese women. *J Obstet Gynaecol Res* (2019) 45(11):2209–19. doi: 10.1111/jog.14097

41. Martin B, Pearson M, Kebejian L, Golden E, Keselman A, Bender M, et al. Sex-dependent metabolic, neuroendocrine, and cognitive responses to dietary energy restriction and excess. *Endocrinology* (2007) 148(9):4318–33. doi: 10.1210/en.2007-0161

42. Fleissner CK, Huebel N, Abd El-Bary MM, Loh G, Klaus S, Blaut M. Absence of intestinal microbiota does not protect mice from diet-induced obesity. *Br J Nutr* (2010) 104(6):919–29. doi: 10.1017/S0007114510001303

43. Markle JG, Frank DN, Mortin-Toth S, Robertson CE, Feazel LM, Rolle-Kampczyk U, et al. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science* (2013) 339(6123):1084–8. doi: 10.1126/science.1233521

44. Frisch RE. Fatness, menarche, and female fertility. *Perspect Biol Med* (1985) 28(4):611–33. doi: 10.1353/pbm.1985.0010

45. Zarrinpar A, Chaix A, Xu ZZ, Chang MW, Marotz CA, Saghatelian A, et al. Antibiotic-induced microbiome depletion alters metabolic homeostasis by affecting gut signaling and colonic metabolism. *Nat Commun* (2018) 9(1):2872. doi: 10.1038/s41467-018-05336-9

46. Li M, Li L, Li B, Hambly C, Wang G, Wu Y, et al. Brown adipose tissue is the key depot for glucose clearance in microbiota depleted mice. *Nat Commun* (2021) 12(1):4725. doi: 10.1038/s41467-021-24659-8

47. Zhao Z, Cheng W, Qu W, Shao G, Liu S. Antibiotic alleviates radiation-induced intestinal injury by remodeling microbiota, reducing inflammation, and inhibiting fibrosis. *ACS Omega* (2020) 5(6):2967–77. doi: 10.1021/acsomega.9b03906

48. Fu L, Qiu Y, Shen L, Cui C, Wang S, Xie Y, et al. The delayed effects of antibiotics in type 2 diabetes, friend or foe? *J Endocrinol* (2018) 238(2):137–49. doi: 10.1530/JOE-17-0709

49. Han Y, Jiang X, Ling Q, Wu L, Wu P, Tang R, et al. Antibiotics-mediated intestinal microbiome perturbation aggravates tacrolimus-induced glucose disorders in mice. *Front Med* (2019) 13(4):471–81. doi: 10.1007/s11684-019-0686-8

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 17 January 2023
DOI 10.3389/fendo.2022.993619

OPEN ACCESS

EDITED BY
Lisa Owens,
St. James's Hospital, Ireland

REVIEWED BY
Vasantha Padmanabhan,
University of Michigan, United States
Fatme Al Anouti,
Zayed University, United Arab Emirates

*CORRESPONDENCE
Jacqueline A. Boyle
jacqueline.boyle@monash.edu

†These authors share senior authorship

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

RECEIVED 13 July 2022
ACCEPTED 06 December 2022
PUBLISHED 17 January 2023

CITATION
Neven ACH, Mousa A, Boyle JA and Teede HJ (2023) Endocrine and metabolic interactions in healthy pregnancies and hyperinsulinemic pregnancies affected by polycystic ovary syndrome, diabetes and obesity. *Front. Endocrinol.* 13:993619. doi: 10.3389/fendo.2022.993619

Endocrine and metabolic interactions in healthy pregnancies and hyperinsulinemic pregnancies affected by polycystic ovary syndrome, diabetes and obesity

Adriana C. H. Neven[1], Aya Mousa[1], Jacqueline A. Boyle[2,3*†] and Helena J. Teede[1†]

[1]Monash Centre for Health Research and Implementation (MCHRI), School of Public Health and Preventive Medicine, Monash University, Clayton, VIC, Australia, [2]Monash Department of Obstetrics and Gynaecology, Monash Health, Clayton, VIC, Australia, [3]Eastern Health Clinical School, Monash University, Box Hill, VIC, Australia

During pregnancy, the fetoplacental unit is key in the pronounced physiological endocrine changes which support pregnancy, fetal development and survival, birth and lactation. In healthy women, pregnancy is characterized by changes in insulin sensitivity and increased maternal androgen levels. These are accompanied by a suite of mechanisms that support fetal growth, maintain glucose homeostasis and protect both mother and fetus from adverse effects of pregnancy induced insulin and androgen excess. In pregnancies affected by endocrine, metabolic disorders such as polycystic ovary syndrome (PCOS), diabetes and obesity, there is an imbalance of beneficial and adverse impacts of pregnancy induced endocrine changes. These inter-related conditions are characterized by an interplay of hyperinsulinemia and hyperandrogenism which influence fetoplacental function and are associated with adverse pregnancy outcomes including hypertensive disorders of pregnancy, macrosomia, preterm delivery and caesarean section. However, the exact underlying mechanisms and relationships of the endocrine and metabolic milieu in these disorders and the impact they have on the prenatal endocrine environment and developing fetus remain poorly understood. Here we aim to review the complex endocrine and metabolic interactions in healthy women during normal pregnancies and those in pregnancies complicated by hyperinsulinemic disorders (PCOS, diabetes and obesity). We also explore the relationships between these endocrine and metabolic differences and the fetoplacental unit, pregnancy outcomes and the developing fetus.

KEYWORDS

hyperandrogenism, hyperinsulinemia, polycystic ovary syndrome, obesity, gestational diabetes (GDM), type 1 diabetes (T1D), type 2 diabetes, pregnancy

1 Introduction

During pregnancy, pronounced physiological endocrine changes support maintenance of pregnancy, fetal development and survival, birth and lactation. Hormonal factors including progesterone, estrogens, androgens, and glucocorticoids are important during critical developmental windows of pregnancy (1). Increasingly, links between maternal endocrine metabolic conditions and pregnancy outcomes are recognized, with insulin resistance and hyperandrogenism central in these interactions. During normal progression of pregnancy, insulin resistance (IR) increases in the mother to provide energy for the growing fetus and (2) androgens increase, regulating key processes during pregnancy and parturition (3). These changes are balanced by pregnancy-specific mechanisms that are activated to maintain glucose homeostasis and to protect both the mother and fetus from pregnancy-induced insulin and androgen excess (4, 5).

Endocrine metabolic disorders such as polycystic ovary syndrome (PCOS), type 1 and type 2 diabetes (T1D, T2D), gestational diabetes (GDM) and maternal obesity are common and the prevalence of these conditions is rising globally, in line with increasing obesity. These conditions influence the endocrine environment and impact of the balance of beneficial and protective mechanisms in pregnancy. PCOS, the most common endocrinopathy in reproductive age women, affects 8 to 21% of this population. (6) PCOS is diagnosed (Rotterdam criteria) based on the presence of two of three features: oligo- or anovulation, hyperandrogenism (clinical or biochemical), and polycystic ovary morphology on ultrasound (7). Pre-existing diabetes is estimated to affect around 2% of pregnancies, doubling from 1990 to 2020 (8), while diabetes in pregnancy (DIP), either from pre-existing diabetes or GDM, affects approximately 16% of pregnancies globally (9). Overweight and obesity, characterized by an elevated body mass index (BMI) of 25 kg/m (2) and 30 kg/m (2) respectively, currently affects 26% and 25% of US women of reproductive age (20-39 years) (10). Globally, 18% and 20% of pregnant women start pregnancy overweight or obese and 47% gain gestational weight above that recommended by guidelines (11). These conditions are interrelated, with GDM prevalence closely associated with maternal obesity (12) and with PCOS (13).

According to the Barker hypothesis, the health and development of children is directly affected by fetal programming *in utero* during critical stages of development in early embryonic and fetal life (14). Critically, the presence of PCOS, diabetes or obesity is associated with higher rates of hypertensive disorders of pregnancy, macrosomia, pre-term delivery and caesarean sections, among other adverse impacts (15–19). These conditions may also lead to unfavorable long-term developmental programming in offspring and manifest as higher rates of metabolic disorders in adulthood, adverse cardiometabolic profiles and even mortality (16, 20). However, the exact underlying mechanisms and relationships between the endocrine and metabolic milieu in these disorders and the mechanisms and impacts they may have on the developing fetus are not fully understood.

A complex interplay of hyperinsulinemia and hyperandrogenism alters the endocrine milieu in pregnant women with PCOS, diabetes and obesity. In non-pregnant women with PCOS, insulin resistance (IR) and compensatory hyperinsulinemia contribute to hyperandrogenic traits as insulin facilitates androgen secretion (21). In turn, androgen excess itself facilitates metabolic dysfunction (21). This persists in pregnancy when women with PCOS are reported to have higher IR, insulin and androgens compared to women without PCOS (22–26). Systemic hyperinsulinemia in pregnancy also occurs with endogenous factors such as obesity (27), GDM (28) and T2D or exogenous factors (such as insulin use in type 1 diabetes or GDM) (29). Associations between maternal hyperinsulinemia and adverse neonatal outcomes are poorly understood, but some studies point to a role of hyperinsulinemia in macrosomia, neurological disorders and endothelial dysfunction in the neonate and impaired glucose tolerance in childhood (30, 31). These endocrine metabolic conditions are also characterized by hyperandrogenism. Fetal exposure to high androgen concentrations is associated with virilization (32–34), intra-uterine growth restriction (IUGR) (34, 35), placental differentiation (36), reproductive and metabolic dysfunction (37–42), adverse cardiac programming (43–45), and behavioral outcomes later in life (46–48). Hyperandrogenism is also associated with high levels of Anti-Müllerian hormone (AMH) in women with PCOS (49). AMH is a member of the transforming growth factor beta (TGFβ) family, produced by granulosa cells of the ovarian (pre-) antral follicles. Most non-pregnant women with PCOS exhibit higher levels of AMH than women without PCOS, with a positive correlation with androstenedione (A4) and testosterone (T) (49). Further, a positive correlation between AMH levels and HOMA-IR has been reported in non-obese women with PCOS (50). Elevated AMH concentrations have also been seen in prepubescent girls with T1D, suggesting a stimulatory effect of insulin therapy on granulosa cells (51). Recently, a number of cohort studies have reported elevated levels of AMH in pregnant women with PCOS versus controls (22, 23, 25, 52, 53), and positive correlations between AMH and maternal total T levels (53). Altered levels of AMH are associated with increased risk of miscarriage (53), lower live birth rates in women with PCOS undergoing assisted reproductive technology (54), milder forms of Mullerian anomalies (22), preterm delivery (55–57) and PCOS features in adulthood (53).

With the rising incidence of these endocrine metabolic disorders in pregnancy, an improved understanding of their impact on pregnancy and on the developing fetus and child is pertinent. Whilst this is a broad topic with other factors involved such as lipid metabolism, growth factors and cytokines, here we

will focus on core hormonal factors linking endocrine and metabolic dysfunction - hyperinsulinemia, hyperandrogenism and AMH. This review aims to explore the complex endocrine and metabolic interactions in healthy women during normal pregnancies and those in pregnancies complicated by hyperinsulinemic disorders (PCOS, diabetes and obesity). We also explore the relationships between these endocrine and metabolic differences and the fetoplacental unit, the developing fetus and child health.

2 Endocrine and metabolic interactions in healthy pregnant women

During pregnancy, pronounced physiological endocrine changes occur to support fetal development and survival, birth and lactation. Effective exchange of nutritive and metabolic products is essential for intrauterine life. Although the endocrine systems are compartmentalized, processes within the fetal, placental and maternal compartments, complement each other, functioning as a unit that utilizes building materials from the maternal compartment for steroidogenic activities (58, 59). The fetus influences maternal adaptations, and thereby its own growth and development. Moreover, mechanisms involved in the regulation and metabolism of sex steroids during pregnancy could be dependent on adaptations in the maternal/placental compartment, determined by the fetal gender (60). Relative contributions of sex steroids shift from maternal ovaries and adrenals to the fetoplacental unit. The fetoplacental unit also acts as a barrier to some substances crossing to the fetus, with maternal hormones larger than 0.7 kDA barely passing the placenta. This ensures that the fetal endocrine environment is largely independent of maternal hormones. Whilst steroids are highly lipophilic and cross the placenta in both directions, most of them are metabolized en route (59). Although the placenta functions as a hypothalamic-pituitary-end organ-like entity, placental function is more complex.

In the sections below, we will describe the endocrine actions of the fetoplacental unit in relation to reproductive and steroid hormones and the impact of insulin, androgens and AMH on these hormones in healthy pregnant women.

2.1 Actions of the fetoplacental unit

Taking over from LH eight days after ovulation, hCG supports survival of the corpus luteum. A maximum level is reached at 8-10 weeks of gestation, then decreases to 10,000-20,000 IU/L by 18-20 weeks and remains at that level to term. Similar to the pituitary secretion of gonadotropins, inhibin and progesterone are inhibiting factors and estrogen and activin are enhancing factors of GnRH-hCG regulation. Maternal serum FSH concentrations are almost undetectable, and LH concentrations slowly decline until birth (3). Elevated levels of hCG in the second trimester are associated with miscarriage, small-for-gestational age infants, pre-eclampsia and preterm delivery (58).

2.1.1 Progesterone

Progesterone prepares and maintains the endometrium to allow implantation, is important in maternal immunologic responses to fetal antigens and has a role in parturition. Initially, the corpus luteum is responsible for its production until about 10 weeks of gestation. After a transition period, the placental unit functions as the major source of progesterone synthesis and maternal circulating levels increase across pregnancy to reach a peak concentration (in order of 130 ng/ml) in the third trimester (59) whilst the fetal contribution is negligible (58). The precursor for progesterone derives from maternal cholesterol and production is therefore independent of uteroplacental perfusion or fetal wellbeing. Firstly, cholesterol is converted to pregnenolone by cytochrome P450scc (CYP11A1). Secondly, Pregnenolone is converted to progesterone by type 1 3β-hydroxysteroid dehydrogenase (HSD3B1) (59). The fetus uses progesterone to synthesize biologically important corticosteroids such as cortisol and aldosterone (58).

2.1.2 Estrogens

Three forms of estrogen are produced in women: estrone (E1), estradiol (E2) and estriol (E3) (61). During pregnancy, E1 and E2 production is increased about a 100-fold, and E3 secretion by about a 1000-fold. This increase is due mainly to estrogen production in the placenta (59). Estrogens contribute to progesterone production, maternal cardiovascular adaptations, blood volume and uteroplacental blood flow. They also regulate genes involved in cholesterol supply to the placenta which is important for fetal and placental steroid hormone production (62). Additionally, estrogens exert effects on the developing fetus by maintaining intrauterine homeostasis, promoting maturation of fetal organs, regulating the fetal neuroendocrine system and regulating timing of parturition (63).

Human placental estrogen synthesis depends on DHEA and its sulfated form (DHEAS), produced from maternal androgens in the early months of gestation and derived from fetal androgens by the 20th week of pregnancy (64). Further conversion of DHEAS to E1 and E2 requires four key enzymes. Placental sulfatases convert DHEAS into DHEA. Placental type I 3β-hydroxysteroid dehydrogenase (HSD)/Δ5Δ4isomerase (HSD3B1), converts DHEA into Δ4-androstenedione. Aromatase irreversibly converts Δ4-androstenedione into estrone (E1). Finally, E1 is converted into E2 by 17-β-HSD type 1 (HSD17B1) and then delivered to the maternal circulation (64). Placental E3 formation is carried

out by 16a hydroxylation in the fetal liver (58), and then metabolized by placental sulfate, 3β-hydroxysteroid dehydrogenase, 17-hydroxysteroid oxidoreductase and maternal P450 aromatase to yield estriol (59). Moreover, the human fetal liver responds by increasing production of SHBG in order to bind estrogen in the circulation and limit levels of bioactive estrogens (65). These processes all ensure that biological effects of potent steroids are blocked. After birthing this ability disappears and estrogen concentrations decline rapidly in the postpartum period (3). Because estrogen production is dependent on fetal and placental steroidogenic cooperation, the amount of estrogen (especially estriol) present in the maternal blood or urine reflects fetal and placental enzymatic capability and, hence, well-being (58). In fact, Wan et al. reported lower levels of estrogen in preeclampsia and speculate that this reduction may be due to the impairment of placental function in preeclampsia (66).

2.1.3 Activin and inhibin

Inhibin, produced by the placenta, shows increased levels during pregnancy (3). It peaks at 8 weeks gestation, then decreases (67), and increases again in the third trimester to 100-fold more than that during the normal menstrual cycle (3). Inhibin and estrogen account for the suppression of maternal gonadotropins during pregnancy (58). Activin A is also increased during pregnancy, with stable levels from 8 to 24 weeks and then increasing to a 100-fold of that during the normal menstrual cycle (58). Inhibin and Activin act as regulators within the placenta for the production of GnRH, hCG and steroids; inhibin is inhibitory and activin is stimulatory. Abnormal concentrations of Inhibin A have been associated with miscarriage, fetal growth restriction, gestational diabetes, and pre-eclampsia (68, 69). Elevated levels of Activin-A are associated with preeclampsia (70–72).

A summary of steroid synthesis during pregnancy is provided in Figure 1 with details in Table 1.

2.2 Metabolic factors influencing actions of the feto-placental unit

To understand the potential impacts of high levels of insulin, androgens and AMH, an understanding of their actions in normal pregnancies is critical. The sections below describe the normal actions of insulin, androgens and AMH and their relation to the hormones described in the previous sections.

2.2.1 Insulin

Depending on the requirements of pregnancy, insulin sensitivity shifts. At 12–14 weeks' gestation, insulin sensitivity is somewhat increased to promote the uptake of glucose into adipose stores in preparation for the energy demands later in pregnancy. However, insulin sensitivity then declines throughout pregnancy as a result of local and placental

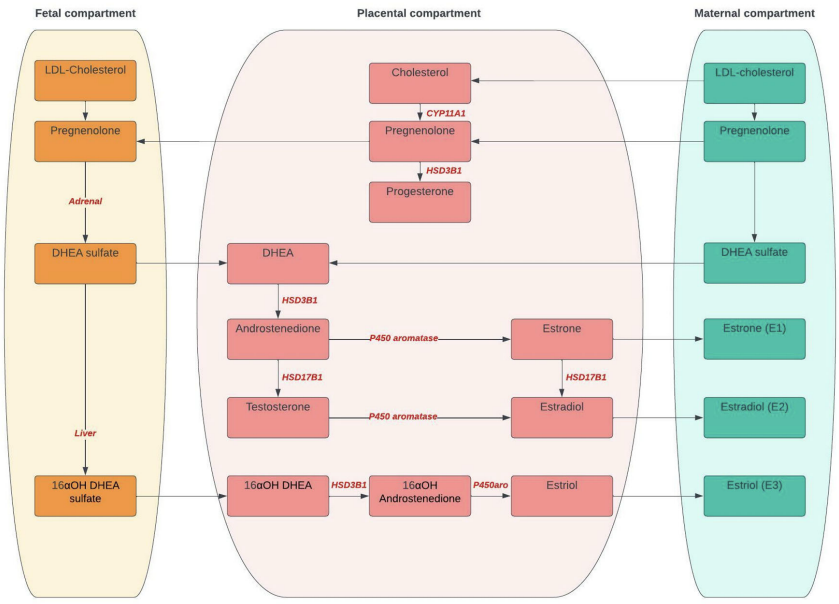


FIGURE 1
Steroid synthesis during pregnancy. CYP11A1, Cytochrome P450 side-change cleavage enzyme; HSD3B1, 3β-hydroxysteroid dehydrogenase 1; HSD17B1, 17-β-HSD type 1; DHEA, Dehydroepiandrosterone; 16αOH; 16α-hydroxydehydro.

TABLE 1 Physiology of the feto-placental unit and the influence of metabolic factors in healthy pregnant women vs pregnant women with hyperinsulinemic disorders.

	Actions in healthy pregnant women	Actions in pregnant women with hyperinsulinemic disorders
GnRH	- Regulates placental steroidogenesis and release of prostaglandins and human chorionic gonadotrophin (hcG). GnRH secretion from the fetoplacental unit is controlled similarly to the hypothalamus and is increased by estrogen, activin-A, insulin and prostaglandins, and inhibited by progesterone and inhibin (58). - The GnRH receptor is highly expressed in the fetal adrenal gland as well, which shows another possible pathway by which the placenta can influence fetal adrenal function (58).	- Studies lacking
LH	- Slowly declines until birth (3)	- Studies lacking
FSH	- Almost undetectable (3)	- Studies lacking
Estrogens (E1, E2, E3)	- Contribute to progesterone production, maternal cardiovascular adaptations, blood volume, uteroplacental blood flow, cholesterol supply to the placenta, maintaining intrauterine homeostasis, maturation of fetal organs, timing of parturition, regulation of fetal neuroendocrine system - Estrone and estradiol production increased about a 100-fold, estriol secretion by about a 1000-fold. - Placental estrogen synthesis depends on DHEA (S) from maternal and fetal androgens	- E2 levels similar in PCOS vs non-PCOS (22, 73, 74) - E1 and E2 lower in T2D and GDM compared to healthy controls (75)
Progesterone	- Prepares and maintains endometrium, important in maternal immunologic responses to fetal antigens, role in parturition (58). - Produced as a result of placental-maternal cooperation (58) - Maternal circulating levels increase to high concentration in third trimester (59). - Used by the fetus to synthesize corticosteroids such as cortisol and aldosterone (58)	- Studies lacking
Inhibin B and Activin	- Produced by the placenta, increased levels during pregnancy (3). - Regulators for the production of GnRH, hCG and steroids; inhibin is inhibitory and activin stimulatory (58).	- Studies lacking
AMH	- Levels decrease as a result of hormonal suppression and inhibition of folliculogenesis (76, 77). - Relationship with other reproductive hormones in pregnancy have not been established	- Elevated in women with PCOS vs controls (22, 23, 25, 52, 53). - No significant differences between pregnant women with T2D/GDM vs without T2D/GDM (78, 79). - Elevated levels correlated with maternal total testosterone levels (22, 25, 49) - Elevated levels correlated with diminished placental metabolism of T to estradiol (53) - Elevated levels found in umbilical artery and vein in women with PCOS vs controls (22) - Impact of elevated levels include risk of miscarriage and preterm delivery (53, 55–57)
SHBG	- Increased production in order to bind estrogen and T (65).	- Lower levels among women with GDM in women with and without PCOS (27, 80–82). - Inverse association with fasting insulin and insulin resistance (82).
Insulin	- Insulin sensitivity declines throughout pregnancy as a result of placental hormones resulting in slightly elevated blood glucose levels to support the growing fetus (73–75). - Inhibits hepatic SHBG production (3).	- GDM is characterized by lack of insulin action, b-cell impairment and insulin resistance (83). - Higher insulin levels and HOMA-IR scores in women with PCOS and GDM (26) - Hyperinsulinemia precedes development of later gestation hyperglycemia and GDM (81, 82, 84–86)

(Continued)

TABLE 1 Continued

	Actions in healthy pregnant women	Actions in pregnant women with hyperinsulinemic disorders
		- Maternal and fetal hyperinsulinemia also associated with maternal obesity (87). - May directly stimulate androgen production or inhibit placental aromatase activity (24, 88). - Limited studies to confirm diagnostic thresholds for hyperinsulinemia in pregnancy (89) - Fetal hyperinsulinemia associated with macrosomia, neurological disorders, endothelial dysfunction and metabolic complications later in life (30, 31)
Androgens (DHEA, DHEA-S, A4, T and DHT)	- Rise of testosterone levels during pregnancy (3) as a result of decrease in metabolic clearance and adipose tissue aromatization (90, 91). - Effects on genital development and maintenance of pregnancy and initiation of parturition (92). - A4 levels peak in third trimester (3). Associated with fetal size, duration of gestation and onset of labor (93). - DHEAS major source of estriol production in fetal-placental unit (92). - Women and their fetus largely protected from androgen excess by SHBG and placental aromatization (4, 65).	- Elevated levels in pregnant women with PCOS (22–25, 94) and obesity (60). - Conflicting results in pregnant women with T2D/GDM (75, 95) - Decreased E2/T ratios in women with T2D and GDM (75). - Alterations of steroidogenesis in placenta or fetus may account for altered androgen levels (96). - Effects of fetal exposure to high androgen concentrations include virilization, IUGR, reproductive function including PCOS phenotypes, metabolic dysfunction later in life (97).

hormones, including estrogen, progesterone, leptin, cortisol, placental cytokines (especially TNF-α), placental lactogen (hPL) and placental growth hormone that promote IR (98, 99). This results in slightly elevated blood glucose levels as a means to provide energy to support the growing fetus. The state of IR also promotes endogenous glucose production and breakdown of fat stores with further increase in blood glucose concentrations (100). Hypertrophy and hyperplasia of pancreatic β-cells and increased glucose-stimulated insulin secretion compensate for these changes to maintain glucose homeostasis, as shown in animal studies (101). Insulin has been found to inhibit hepatic SHBG production (3) and, low SHBG concentrations might even reflect IR better than fasting glucose or insulin levels (102, 103).

2.2.2 Androgens

The fetoplacental unit, the adrenals and potentially adipose tissue have been assumed to be responsible for maternal androgen levels in pregnancy (90, 97). During normal progression of pregnancy, levels of total testosterone (T) increase by 70%, with peak levels in the third trimester of pregnancy (3). Among factors contributing to the rise of T are the decrease in metabolic clearance of T (91) and adipose tissue aromatization (90). Among others, T exerts effects on genital development during early gestation (3) and on the maintenance of pregnancy and initiation of parturition (92). Maternal serum A4 concentrations start to increase in the later part of pregnancy, with peak levels in the third trimester (3). A4 levels have been associated with nausea, fetal size, duration of gestation and onset of labour (93). DHEAS plays a role in cervical ripening through activation of collagenase activity induced by the enhanced conversion to E2 (104). It is also a major source of E3 production in the fetal-placental unit (92). It has been shown to decline during pregnancy (105). However, recent studies suggest a more fluctuating pattern of DHEAS throughout gestation (3).

Pregnant women and their infants remain largely unaffected by the changes in androgen concentrations due to pregnancy-specific mechanisms that are activated to protect both the mother and fetus from this androgen excess. The increased levels of SHBG bind T, while the cytochrome P450 aromatase enzyme converts androgens into E2. The fetal liver subsequently converts E2 to E3, which is then excreted in maternal urine. Placental aromatization is so efficient, that little androgen presented to the placenta escapes (4). It has been proposed that the increase in SHBG during pregnancy protects the fetus from exposure to maternal androgens, however, reciprocally, maternal SHBG may protect mothers from androgens originating from the fetus (65). This has been demonstrated in a clinical case report that showed profound and transient maternal virilization resulting from very low plasma SHBG, whereas the twin daughters did not show any evidence of

excessive androgen exposure (106). Something similar has been demonstrated in patients with a fetus with a defective P450 aromatase gene, which resulted in a spillover of fetal androgens into the maternal circulation (107). This highlights the importance of SHBG and placental aromatase in protecting the mother from fetal androgens.

2.2.3 Anti-Müllerian hormone

During pregnancies of women without hyperinsulinemic disorders, AMH levels significantly drop during the course of pregnancy and immediately after birth, (first trimester: 1.69 ng/ml (IQR 0.71-3.10), second trimester: 0.8 ng/ml (IQR 0.48-1.41), third trimester: 0.5 ng/ml (IQR 0.18-1.00)) but subsequently increases over the first four days postpartum (76). This decrease throughout the three trimesters of pregnancy most likely results in the inhibition of folliculogenesis in pregnancy (78). Hormones such as estrogen may regulate AMH production (77), however some studies have not found associations between AMH levels and E1 in pregnancies in women with or without T2D and GDM (78, 108). The relationship between other reproductive hormones (e.g. LH, activin, inhibin) and AMH production has not been established. Although possible involvement of these factors in regulating gravid AMH levels cannot be excluded, other factors, such as Follistatin (109) may also regulate AMH levels during pregnancy.

In summary, in normal pregnancies, insulin sensitivity declines as a result of local and placental hormones resulting in slightly elevated blood glucose levels to support the growing fetus, with a concomitant inhibition of hepatic SHBG production. Androgen levels increase due to decreased metabolic clearance and adipose tissue aromatization to regulate key processes during pregnancy and parturition. Mechanisms such as altered SHBG that binds T and conversion of androgens into E2 by cytochrome P450 are in place to protect both the mother and fetus from androgen excess. Levels of AMH decrease as a result of hormonal suppression and inhibition of folliculogenesis. Relationships between AMH and other reproductive hormones in pregnancy are not extensively studied.

3 Hormonal and metabolic interactions in pregnant women with hyperinsulinemic disorders

The interrelationships between hyperinsulinemia, androgen excess, and AMH are potentially reflected in pregnancies complicated by hyperinsulinemic disorders. The preconception state of hyperinsulinemia and/or androgen and AMH excess might drive the mechanisms underlying fetoplacental endocrine dysfunction. However, because hyperinsulinemic conditions including PCOS, diabetes (GDM, T1D and T2D), and obesity often appear concurrently, no one condition explains the altered endocrine milieu and eventual adverse maternal and neonatal outcomes in these women. The following section aims to assess the endocrine and metabolic factors, focusing on insulin, androgens and AMH (and their interactions) in women with PCOS, diabetes and obesity and how they impact the fetoplacental unit.

3.1 Actions of the fetoplacental unit and the influence of metabolic factors in women with hyperinsulinemic pregnancies

Clinical studies on placental GnRH, hCG, estrogens, progesterone, activin and inhibin in women with hyperinsulinemic conditions in pregnancy are scarce. Studies have reported differences in some hormones in pregnant women with and without PCOS, but do not specifically compare the fetoplacental unit between these groups or address interactions between androgens, estrogens, AMH and pituitary hormones in the mothers and the fetus (22). Maternal E2 levels appear similar in women with and without PCOS (22, 73, 74). A recent study found that E1 and E2 levels were lower in women with T2D and GDM than in healthy controls during the second half of pregnancy (75). Data on cord blood estrogens show conflicting outcomes. Lower E1 concentrations were found in cord blood from girls and women with PCOS compared with controls, but there were no differences in E2 and E3 in these studies (74, 88). Anderson et al. (73) reported decreased E2 levels in female PCOS cord blood samples compared with controls, and lower E2 levels have been noted in cord blood compared to maternal levels (22, 110). Few studies report on estrogens in relation with other reproductive and metabolic hormones.

3.1.1 Hyperinsulinemia

Whereas increased IR during pregnancy is an expected physiological state, complications arise when these changes are augmented by pre-existing hyperinsulinemia and related metabolic dysfunction. When there is a relative lack of insulin action in body tissues to overcome IR, GDM ensues (83). Critical to the pathophysiology of GDM is β-cell impairment aggravated by tissue IR during pregnancy. These impairments usually exist prior to pregnancy and can be progressive. Women with a history of GDM were almost 10 times more likely to develop T2DM than those with a normoglycemic pregnancy with pooled cumulative incidence estimated to be around 16% for studies with more than 10 years of follow up (111). Reduced insulin-stimulated glucose uptake further contributes to hyperglycemia which overburdens the β-cell, producing an additional insulin response and worsening β-cell dysfunction (5). IR, which

includes a failure of insulin signaling, further exacerbates β-cell dysfunction (5).

The mechanisms underlying GDM are thought to be similar to other disorders of insulin sensitivity such as T2DM, prediabetes, obesity and PCOS. De Wilde et al. (26) demonstrated that in 22 women with PCOS and GDM, insulin levels and homeostasis model assessment of insulin resistance (HOMA-IR) scores were higher before conception and at each sampling point in pregnancy compared to women with PCOS who did not develop GDM, independently of BMI. Insulin sensitivity in women with PCOS who develop GDM is potentially not as high as in normal glucose-tolerant women in the first trimester resulting in hyperinsulinemia to keep glucose concentrations within normal ranges. Additionally, insulin levels and HOMA-IR levels were already significantly increased prior to conception in the GDM group compared with the non-GDM group. These findings support the hypothesis that the risk of developing GDM is already present early in pregnancy. A growing body of evidence suggests that hyperinsulinemia in early gestation precedes later gestation hyperglycemia and GDM development (89, 112–114). Associations have also been found between maternal obesity and maternal and fetal hyperinsulinemia (87).

Insulin interacts with other important metabolic markers. High levels of insulin may contribute to fetal hyperandrogenism exposure by directly stimulating androgen production (24). Furthermore, insulin, Insulin-like growth factor 1 (IGF-I) and IGF-II have been shown to inhibit placental aromatase activity (88). This is important, given the exacerbated IR and increased levels of insulin in pregnant women with PCOS. In turn, a decrease in P450 aromatase activity has been described in GDM and pre-eclampsia (115, 116). Importantly, a recent meta-analysis of prospective studies found an inverse association of SHBG with fasting insulin and IR (80). SHBG levels were lower among women who subsequently developed GDM compared to those who did not, independent of adiposity (80). Similarly, lower preconception SHBG concentrations are also associated with GDM in women with PCOS (81, 82). The study by de Wilde et al. (26) also demonstrated that in 22 women with PCOS and GDM, SHBG levels were significantly lower before conception and in the second trimester. Low plasma SHBG in pregnancy was also associated with hyperandrogenism (106). We therefore hypothesize that like outside pregnancy, hyperinsulinemia in these disorders and subsequent direct stimulation of androgen production, and inhibition of SHBG (that binds androgens), contributes to hyperandrogenism. In pregnancy, inhibition of placental aromatase activity is likely to exacerbate this phenomenon.

Hyperinsulinemia in pregnancy may impact maternal and fetal health. Routine assessments and standardized diagnostics of hyperinsulinemia in pregnancy are lacking. Therefore, associations between pathological hyperinsulinemia and maternal and neonatal outcomes are not well understood. However, it is proposed that hyperinsulinemia has effects on maternal hemodynamic adaptations, potentially increasing the risk of pre-eclampsia, stillbirth and intrauterine growth restriction (84). Studies have reported increased insulin in cord blood of babies born to mothers with T2D and GDM compared to controls (85, 86). Fetal hyperinsulinism is associated with increased risk of later metabolic complications (30). Further, some studies point to a role of hyperinsulinemia in fetal development such as macrosomia, neurological disorders, endothelial dysfunction in the neonate (31), offspring hyperinsulinemia and cardiac hypertrophy (84). North et al. (89) hypothesize that hyperinsulinemia in women with "borderline" glucose intolerance may explain pivotal observations from The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) cohort that showed an increased risk of fetal overgrowth, primary caesarean delivery, elevated cord-blood Connecting peptide (C-peptide) and neonatal hypoglycemia in mothers with mild-to-moderately elevated glucose levels (19). Importantly, metformin use in pregnancy might enhance insulin sensitivity, reduce insulin resistance and fetal hyperinsulinemia and in turn, reduce neonatal adiposity (117). However, it's use in pregnancy continues to be investigated to determine efficacy and safety for preventing or treating GDM (118).

Human data on the effects of exogenous insulin use in pregnancy in relation to other reproductive factors or pregnancy outcomes is scarce. Insulin does not cross the placenta and in animal studies, exogenous insulin has no effects on embryo or fetal development (119). However, insulin receptors are expressed on the placenta and may be a means through which insulin mediates effects on the fetus through action on the placenta (84). A recent meta-analysis compared outcomes between women with GDM with insulin use, GDM without insulin use and no GDM (120). In studies with no insulin use, when adjusted for confounders such as BMI, women with GDM had increased odds of caesarean section, preterm delivery, low one-minute Apgar scores, macrosomia and infant born large for gestational age compared with women without GDM. In studies with insulin use, when adjusted for confounders, women with GDM had increased odds of having an infant born large for gestational age, with respiratory distress syndrome, neonatal jaundice, and/or requiring admission to the neonatal intensive care unit compared to women without GDM (120). Furthermore, an increase in placental weight in women with GDM that used insulin versus women with GDM that were controlled with diet and exercise only was observed (121). In pregnant women with T1D, the use of insulin lispro was associated with an increased risk of macrosomia (122). Importantly, a high incidence of macrosomia was found despite overall good glycemic control, as assessed by HbA1c. It was proposed that the acute pulsatile rise and fall of maternal blood glucose levels which occur after food intake (hyperglycemia) and insulin treatment (hypoglycemia), respectively, might result in increased fetal release of

endogenous insulin leading to increased placental weight and macrosomia (121, 122). However, clinical implications of hyperinsulinemia and links to perinatal and neonatal outcomes remain poorly understood. There is limited research to confirm diagnostic thresholds for hyperinsulinemia in to insulin metabolism become potentially harmful are needed (89).

Overall, studies show relative hyperinsulinemia during pregnancy in women with PCOS GDM, and maternal obesity, compared to healthy pregnancies without these conditions. Hyperinsulinemia contributes to fetal hyperandrogenism exposure by stimulating androgen production, inhibiting placental aromatase activity and inhibiting SHBG. Hyperinsulinemia may also increase risks of PE, stillbirth, IUGR, macrosomia, and neurological disorders, endothelial dysfunction and later metabolic complications in the offspring. Finally, similar to the exogenous action of insulin on androgen excess in women with T1D, we hypothesize that the use of insulin in women with GDM (with or without PCOS) might also aggravate hyperinsulinemia in pregnancy; however, this phenomenon and its potential complications await further study.

3.1.2 Hyperandrogenism

The adaptations which occur in pregnancy to protect both the mother and fetus from pregnancy-induced androgen excess might not be sufficient in pregnant women with underlying hyperinsulinemic pregnancies, as evidenced by elevated levels of androgens in pregnant women with PCOS. Several studies found elevated serum androgen levels, including T (22–25, 94), A4 (23, 24, 94), DHEAS and free androgen index (FAI) (24, 25, 94) in pregnant women with PCOS. This may be a potential source of fetal androgen excess and induce effects on the development of the fetus, even if virilization of a female fetus is not observed (24). Results regarding T and GDM are conflicting. Some report that women who develop GDM have significantly higher T concentrations compared with controls (95), while others demonstrated hyperandrogenemia in pregnant women with T2D, but not in GDM (75). Additionally, decreased estrogen and E/T ratios were found in women with T2D and women with GDM (75). In the latter study, BMI and T levels were positively associated in the T2D and GDM groups (75). Maternal obesity has also been found to be associated with elevated maternal serum T concentrations (60).

In relation to the impact of androgen excess on fetal health, some studies have used cord blood to investigate the intrauterine fetal environment in women with PCOS (22, 73, 74, 88, 123, 124). Levels of DHEA and DHEAS in fetal cord blood were generally not associated with PCOS (73, 74, 88, 95), but studies regarding T and A4 have produced conflicting results. A recent meta-analysis of seven studies (n= 570) found no significant differences in cord blood T levels between women with and without PCOS, irrespective of neonatal sex (125). The authors suggest that T may be quickly degraded and converted by placental aromatase into E2 when passing through the placenta. However, cord blood A4 levels in female newborns were significantly lower in PCOS than in the control group (125). As explained in section 2, cord blood androgens are derived from both fetal adrenal as well as placental steroidogenesis. The fetal adrenals produce DHEAS *in utero*, which is transformed into A4, T, and E2 by the placenta (126). Alterations of steroidogenesis in the fetus or an abnormality in placental steroidogenesis could potentially account for the decreased A4 levels (125). Similarly, Kelly et al. (96) proposed that maternal androgens may exert a programming effect on placental and/or fetal steroidogenesis to alter androgen levels within the fetal-placental unit. This concept is supported by Maliqueo et al. (88), wherein placental tissue from women with PCOS had increased 3β-hydroxysteroid dehydrogenase 1 enzymatic activity and decreased aromatase activity compared with non-PCOS controls inducing accumulation of androgenic substrate. This is in line with findings of lower E1 and E2 serum levels in women with GDM and lower E2/T ratios suggesting a lower conversion of T to estrogens (75).

Despite the fact that maternal androgen excess was not associated with elevated T concentrations in cord blood, hyperandrogenism in pregnancy is clinically relevant given the numerous reports, both from animal models and clinical studies, on the adverse effects of fetal exposure to high androgen concentrations. Importantly, umbilical cord blood is only a representation of the end of pregnancy rather than the entire gestation period. The effects of fetal exposure to high androgen concentrations have been reviewed in detail elsewhere and include virilization (32–34), IUGR (34, 35), placental differentiation culminating in low birth weight (36), reproductive function including PCOS like phenotypes (37–39), metabolic dysfunction (37, 39–42), adverse cardiac programming (43–45), and behavioral outcomes later in life (46–48).

To what extent high levels of androgens directly impact maternal and fetal health is not clear, given that androgens are converted to estrogens. In animal studies, examining direct effects of DHT, a nonaromatizable androgen, was associated with decreased placental weight and cotreatment of T with an androgen antagonist prevented placental changes, suggesting placental changes were mediated at least in part by androgenic action (96). Additionally, evidence from studies in rhesus monkeys indicates that gestational T and DHT treatment induces maternal hyperinsulinemia and insulin resistance in addition to elevating circulating androgen levels in both maternal and fetal compartments (42, 127). This may result in reprogramming of insulin target tissues in offspring such as liver and adipose tissue, leading to hyperglycemia in adulthood (128). As such, effects of hyperandrogenism on fetal development and pregnancy outcomes could be mediated *via* insulin and further altered by the impact of hyperinsulinemic states. Importantly, the inhibitory action of insulin and AMH on placental aromatase action might contribute to changes in sexual

steroids and insulin levels during pregnancy. Placental steroidogenesis possibly follows different pathways in hyperinsulinemic pregnancies, which results in a different ratio of E/T fractions during gestation and in offspring.

In summary, studies have found relatively higher levels of androgens in pregnant women with PCOS and obesity, whereas this is less clear in T2D and GDM. Reports of cord blood levels of androgens are scarce and conflicting. Different androgen profiles within the fetal-placental unit may be reflective of abnormalities of steroidogenesis in the fetus or placenta. Hyperandrogenism in pregnancy is associated with adverse short- and long-term neonatal outcomes. Rather than a function of direct androgenic action only, effects from hyperandrogenism on fetal development and pregnancy outcomes are potentially mediated by insulin and further altered by hyperinsulinemic states.

3.1.3 Anti-Müllerian hormone

As described in section 2, AMH levels decrease successively during pregnancy, mostly between the first and second trimester. Several studies show elevated levels of AMH during pregnancy in women with PCOS versus controls (22, 23, 25, 52, 53). In fact, high AMH levels in women with PCOS are maintained throughout the entire pregnancy (23). However, studies have not found significantly different AMH concentrations between women with diabetes (T2D; GDM) and healthy pregnant women (78, 79). In relation to obesity, studies found that AMH was negatively associated with maternal BMI (22, 108, 129).

Interactions between AMH and other hormones in pregnancy have recently been investigated by Tata et al. (53) who measured AMH in a cohort of 66 pregnant women with PCOS and 63 control women, at gestational week 16-19. AMH was significantly higher in pregnant lean women with PCOS compared with the control group but not in obese women with PCOS versus obese control women. Furthermore, AMH was significantly higher in lean women with PCOS who had hyperandrogenism than in those without. No differences between obese women with PCOS with and without hyperandrogenism were detected, potentially as the impact of obesity may negate PCOS effects on AMH. An animal model that treated pregnant mice with AMH resulted in maternal neuroendocrine-driven T excess and diminished placental metabolism of T to E2. Authors suggest that this could be a viable route by which maternal and placental T can be transferred to the human fetus (53). Although not extensively studied, lower levels of E1 and E2 in pregnant women with T2D and GDM and in female cord blood of women with PCOS support this suggestion, and could be reflective of increased AMH levels leading to decreased placental metabolism of T, however a direct relationship has not been established (73, 74, 78, 88).

Several other studies report a positive correlation between elevated AMH levels and maternal total T levels (both in women with PCOS as well as controls) (22, 23, 25). It is thought that the ovaries may contribute to maternal serum concentrations of T. In addition, high AMH may influence placental T production. This is in line with findings in prenatal AMH-exposed mice, in which AMH exposure was associated with elevated T levels in the dams and offspring. Treatment of pregnant mice with AMH resulted in a masculinization of the exposed female fetus and PCOS-like phenotypic traits in adulthood (reproductive and neuroendocrine) (53). Lastly, although studies in non-pregnant adolescents with PCOS show a positive association between serum AMH and HOMA-IR, no significant associations of AMH with insulin, HOMA-IR or IGF-1 have been observed in pregnant women (78, 108).

Elevated levels of AMH potentially impact the developing fetus. Studies have found elevated serum AMH levels in the umbilical vein at time of delivery in newborns of women with PCOS compared with healthy controls (110). Importantly, higher levels of AMH were observed in neonates born from women with PCOS and maternal hyperandrogenism or maternal BMI higher than 30 kg/m^2 (110). On the other hand, no significant associations were observed between maternal AMH concentrations and AMH in female offspring (110), whereas AMH was higher in male fetuses, than in the mother (22). This suggests that AMH concentrations in umbilical cord blood might differ from that in maternal serum because there is no passage of AMH from fetus to mother (22, 110). Instead, elevated AMH concentrations could be a result of placental passage from the mother and increased AMH production by the fetal compartment. Detti et al. (22) hypothesize that genetic inheritance of PCOS by the fetus causes increased AMH production by the embryonal/fetal granulosa and Sertoli cells.

Studies investigating associations between levels of AMH and pregnancy outcomes are scarce. One study showed increased risk of miscarriage in association with prenatal exposure to AMH (53). Further, high serum AMH levels were associated with lower live birth rates in women with PCOS undergoing assisted reproductive technology (54). Studies have reported no association between maternal AMH and infant birthweight (25, 79, 129, 130). Increased incidence of PCOS is observed in women with sub-separate uteri. Detti et al. (22) hypothesize that higher AMH concentrations could contribute to development of milder forms of Mullerian anomalies by delay/arrest in Mullerian duct fusion and inner wall reabsorption (22, 110). AMH may also be associated with preterm delivery (55–57). Kaing et al. (57) examined women with PCOS and high AMH who conceived after ovulation induction, and found that 62% of women who delivered preterm had AMH levels above the 75th percentile. Women with PCOS who delivered preterm had notably higher AMH than their term counterparts (11.1 vs 5.4 ng/mL). Similarly, a recent retrospective cohort study with patients with PCOS who had undergone IVF/ICSI showed that for patients with a BMI ≥ 24 kg/m (2) plus serum AMH > 6.45 ng/ml (75th percentile), the

risk of preterm birth was 2.1 times that in the AMH <2.71 ng/ml group (55).Associations with other maternal and perinatal outcomes remain uncertain. Most existing studies do not show statistically increased risk of other adverse maternal and perinatal outcomes in women with elevated AMH levels (25, 55, 130). Prenatal AMH treatment in mice resulted in PCOS features in adulthood: hyperandrogenism, LH elevation, sporadic ovulation, and fertility defects. However, these mice did not show weight alterations, leading to the assumption that prenatal AMH could predispose to the lean PCOS phenotype in adulthood (53).

In summary, elevated levels of AMH during pregnancy have been associated with T excess, and diminished metabolism of T to E2. However, associations between AMH and insulin or HOMA-IR in pregnancy remains to be determined. Elevated AMH may be associated with preterm birth in women with PCOS, however associations with other maternal and neonatal outcomes remain uncertain.

4 Limitations and future directions

While an abnormal prenatal endocrine milieu could possibly reflect a deranged feto-placental unit in women with hyperinsulinemic disorders, studies specifically addressing relationships and associations between insulin, androgens, pituitary hormones, estrogens, AMH and placental steroidogenesis are scarce. Studies are often cross-sectional in design, do not adjust for confounding factors such as nutrition and physical activity and longitudinal studies on maternal hormones do not always include early pregnancy samples. Furthermore, they are often limited by small sample sizes and to increase the power, the conclusions drawn from these studies need to be further studied with larger sample sizes. Data regarding subsequent alterations in the intrauterine environment, often studied in cord blood have produced conflicting results and overall evidence is scarce. Hormonal dysregulation in women with PCOS, diabetes or obesity may impact placental development and function. However, placental studies (looking at macroscopic and microscopic changes) in pregnancies impacted by these conditions are limited.

In order to study the interrelationships between insulin, androgens and AMH, investigating placental tissue could be an important additional mode of research.

Hyperinsulinemia in pregnancies affected by PCOS, obesity or diabetes seems largely a result of pre-existing hyperinsulinemia and related metabolic dysfunction paired with IR in pregnancy exacerbating B-cell dysfunction. However, further research is warranted on the factors that promote IR more than the physiological state of IR hPL for example, contributes to IR and secretion of insulin. Pregnancies affected by obesity and diabetes show altered hPL levels: lower concentrations have been shown in obesity and increased levels in GDM. Disruptions in hPL are also thought to be associated with an increased prevalence of placental dysfunction and increased fetal growth (131). Likewise, TNF-α, a proinflammatory cytokine produced by the placenta and other placental adipokines (e.g. Chemerin, Apelin, Omentin) also likely play a role in development of IR in pregnancy (131). In order to understand the pathways by which IR occurs, factors associated with IR in women with hyperinsulinemic pregnancies should be further explored.

Studies of diagnostic thresholds of insulin levels in pregnancy are lacking. Therefore, in order to study associations between pathological hyperinsulinemia and maternal and neonatal outcomes, criteria need to be established to determine when normal hyperinsulinemia in pregnancy results in levels that result in clinically relevant adverse outcomes. In addition, data on insulin levels and IR in pregnancies affected by metabolic and endocrine disorders is notably lacking. Longitudinal measurements of insulin and IR in pregnancy would be beneficial. The possible associations between maternal hyperinsulinemia and offspring health highlight the need for further study into the interactions and mechanisms between maternal insulin and fetal development and health through pregnancy, post birth and into childhood.

Whilst studies show that hyperinsulinemia is likely to result in decreased levels of SHBG (leading to increased free T), direct stimulation of androgens and inhibition of placental aromatase activity, future research is needed to substantiate associations between insulin levels in pregnancy and the maternal and prenatal endocrine milieu. Human association studies of the various hyperinsulinemic states, coupled with animal models that ablate variables of interest could help clarify these links. Furthermore, the use of insulin in women across a range of hyperinsulinemic disorders in pregnancy, the potential concurrent worsening of hyperinsulinemia in pregnancy, related hyperandrogenism and its potential complications have not been studied previously.

The effects of hyperandrogenism in pregnancy on developmental programming have been widely studied. However, questions regarding the pathways by which androgens impact maternal and fetal health remain unanswered. Whilst evidence is clear regarding the contribution of hyperinsulinemia on hyperandrogenism, few studies examine the effects of androgens on insulin and insulin sensitivity. Observational studies of various hyperandrogenic states coupled with further explorations using animal models will enhance understanding of how hyperandrogenism interacts with other variables such as estrogens, AMH, insulin and potential other markers in pregnancy.

The hyperandrogenic phenotype is likely to be underreported in maternal samples as observational studies do not always include a full assessment of the different androgens. This will influence interpretation of gestation AMH levels across PCOS phenotypes. Future research, with bigger sample sizes and

full assessment of hyperandrogenism is warranted to confirm elevated levels of AMH during pregnancy between groups, to support the hypothesis that high AMH levels lead to decreased placental metabolism of T and to assess associations between AMH and insulin and HOMA-IR. Animal studies could explore what contributes to higher levels of AMH in pregnancy. In addition, the physiologically low AMH levels observed during the second half of pregnancy may require more sensitive tests to find a significant difference between groups under study. Previous studies assayed serum AMH using the widely clinically used assay AMH/MIS ELISA kit (Immunotech-Beckman, Marseilles, France) (75, 79). Due to a lower threshold of detection, a newer commercially available AMH enzyme-linked assay (pico AMH ELISA, AnshLabs) might be more appropriate for the measurement of low AMH concentrations (132). Further, studies are needed to investigate associations between high AMH levels and pregnancy and fetal and offspring health and to elucidate the potential actions of high AMH on the maternal and placental adaptations in pregnancy. Reports so far show no conclusive results and the polycystic ovarian morphology, reflected by high AMH levels, might not drive mechanisms behind adverse outcomes in pregnant women with PCOS.

Other factors important for pregnancy, birth, lactation, child development and survival, such as hCG, activin, inhibin, hPL and placental cytokines have not been widely studied in relation to hyperinsulinemic pregnancies but might be potential markers relevant to explain altered hormonal states in pregnancies affected by hyperinsulinemic disorders. For example, while Inhibin and Activin act as regulators within the placenta for GnRH, hCG and steroids, to our knowledge no reports are currently present on their actions in hyperinsulinemic pregnancies. Additionally, hyperinsulinemia could stimulate production of leptin which could amplify inflammation (133).

Interestingly, upon analyzing T and P450 aromatase in normal weight and obese women, Maliqueo et al. (2017) only found an increment of T and lower P450 aromatase in obese women with male fetuses. The authors therefore suggest that regulation and metabolism of sex steroids in pregnancy could be dependent on maternal/placental adaptations determined by fetal gender (60). In relation to pregnancies affected by hyperinsulinemic disorders, future research is warranted to understand the mechanisms by which fetal gender may impact placental function.

5 Conclusions

This comprehensive review compares physiological changes in normal pregnancies to those in women with pre-existing hyperinsulinemic conditions including PCOS, diabetes and obesity. We highlight how the maternal, placental and fetal compartments are separate, but functionally complement each other in healthy pregnancies. Yet in pregnant women with pre-existing hyperinsulinemic disorders, it appears that abnormal levels of insulin, androgens, estrogens and AMH are reflective of a deranged fetoplacental unit, ultimately leading to adverse pregnancy and neonatal outcomes. Hyperinsulinemia in these disorders and subsequent direct stimulation of androgen production, inhibition of SHBG and inhibition of placental aromatase activity, contributes to hyperandrogenism. Effects of hyperandrogenism could be mediated *via* insulin and altered placental steroidogenesis. Elevated levels of AMH contribute to T excess, and diminished metabolism of T to E2. With the high prevalence of hyperinsulinemic endocrine metabolic conditions including PCOS, diabetes and obesity in pregnancy, more research, including longitudinal sampling, and consideration of severity of hyperinsulinemia and interaction across these linked conditions is needed. A focus on placental and fetal steroidogenesis and hormonal interactions in these conditions is important. Finally, further study on the impacts of exogenous insulin administered in women with T1D, T2D and GDM, is needed, in relation to important endocrine and metabolic markers, and impact on fetal health.

Author contributions

AN collated and reviewed the literature and wrote the first draft of the manuscript. AM, JB, and HT reviewed and edited the manuscript. All authors provided intellectual input in line with the ICMJE criteria for authorship and have approved the final version for publication.

Funding

AN is a PhD student supported by the Monash International Tuition Scholarship (MITS) and Research Training Program Stipend (RTP stipend). AM is supported by a Peter Doherty Biomedical Research Fellowship provided by the National Health and Medical Research Council (NHMRC) of Australia. HT is supported by an NHMRC Investigator Fellowship. This body of work is funded by an NHMRC Centre for Research Excellence on Women's Health in Reproductive Life.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Banker M, Puttabyatappa M, O'Day P, et al. Association of maternal-neonatal steroids with early pregnancy endocrine disrupting chemicals and pregnancy outcomes. *J Clin Endocrinol Metab* (2021) 106(3):665–87. doi: 10.1210/clinem/dgaa909

2. Ryan EA. Hormones and insulin resistance during pregnancy. *Lancet* (2003) 362(9398):1777–8. doi: 10.1016/S0140-6736(03)14942-2

3. Kuijper EA, Ket JC, Caanen MR, Lambalk CB. Reproductive hormone concentrations in pregnancy and neonates: a systematic review. *Reprod BioMed Online* (2013) 27(1):33–63. doi: 10.1016/j.rbmo.2013.03.009

4. Thompson EAJr., Siiteri PK. The involvement of human placental microsomal cytochrome p-450 in aromatization. *J Biol Chem* (1974) 249 (17):5373–8. doi: 10.1016/S0021-9258(20)79736-X

5. Plows JF, Stanley JL, Baker PN, Reynolds CM, Vickers MH. The pathophysiology of gestational diabetes mellitus. *Int J Mol Sci* (2018) 19(11). doi: 10.3390/ijms19113342

6. Bozdag G, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO. The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reprod* (2016) 31(12):2841–55. doi: 10.1093/humrep/dew218

7. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* (2004) 81(1):19–25.

8. Chivese T, Hoegfeldt CA, Werfalli M, et al. IDF diabetes atlas: The prevalence of pre-existing diabetes in pregnancy - a systematic reviewand meta-analysis of studies published during 2010-2020. *Diabetes Res Clin Pract* (2022) 183:109049. doi: 10.1016/j.diabres.2021.109049

9. Wang H, Li N, Chivese T, et al. IDF diabetes atlas: Estimation of global and regional gestational diabetes mellitus prevalence for 2021 by international association of diabetes in pregnancy study group's criteria. *Diabetes Res Clin Pract* (2022) 183:109050. doi: 10.1016/j.diabres.2021.109050

10. Shrestha A, Prowak M, Berlandi-Short V-M, Garay J, Ramalingam L. Maternal obesity: A focus on maternal interventions to improve health of offspring. *Front Cardiovasc Med* (2021) 8. doi: 10.3389/fcvm.2021.696812

11. Goldstein RF, Abell SK, Ranasinha S, et al. Association of gestational weight gain with maternal and infant outcomes: A systematic review and meta-analysis. *JAMA* (2017) 317(21):2207–25. doi: 10.1001/jama.2017.3635

12. Deputy NP, Kim SY, Conrey EJ, Bullard KM. Prevalence and changes in preexisting diabetes and gestational diabetes among women who had a live birth - united states, 2012-2016. *MMWR Morb Mortal Wkly Rep* (2018) 67(43):1201–7. doi: 10.15585/mmwr.mm6743a2

13. Joham AE, Ranasinha S, Zoungas S, Moran L, Teede HJ. Gestational diabetes and type 2 diabetes in reproductive-aged women with polycystic ovary syndrome. *J Clin Endocrinol Metab* (2014) 99(3):E447–452. doi: 10.1210/jc.2013-2007

14. Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME. Growth *in utero*, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *BMJ (Clinical Res ed)* (1989) 298(6673):564–7. doi: 10.1136/bmj.298.6673.564

15. Bahri Khomami M, Joham AE, Boyle JA, et al. Increased maternal pregnancy complications in polycystic ovary syndrome appear to be independent of obesity-a systematic review, meta-analysis, and meta-regression. *Obes Rev* (2019) 20(5):659–74. doi: 10.1111/obr.12829

16. Alexopoulos AS, Blair R, Peters AL. Management of preexisting diabetes in pregnancy: A review. *Jama* (2019) 321(18):1811–9. doi: 10.1001/jama.2019.4981

17. Kjerulff LE, Sanchez-Ramos L, Duffy D. Pregnancy outcomes in women with polycystic ovary syndrome: a metaanalysis. *Am J Obstet Gynecol* (2011) 204 (6):558.e551–556. doi: 10.1016/j.ajog.2011.03.021

18. Marchi J, Berg M, Dencker A, Olander EK, Begley C. Risks associated with obesity in pregnancy, for the mother and baby: a systematic review of reviews. *Obes Rev* (2015) 16(8):621–38. doi: 10.1111/obr.12288

19. Metzger BE, Lowe LP, Dyer AR, et al. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med* (2008) 358(19):1991–2002.

20. Gunning MN, Sir Petermann T, Crisosto N, et al. Cardiometabolic health in offspring of women with PCOS compared to healthy controls: a systematic review and individual participant data meta-analysis. *Hum Reprod update* (2020) 26 (1):103–17. doi: 10.1093/humupd/dmz036

21. Escobar-Morreale HF. Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. *Nat Rev Endocrinol* (2018) 14(5):270–84. doi: 10.1038/nrendo.2018.24

22. Detti L, Christiansen ME, Francillon L, et al. Serum anti-müllerian hormone (AMH) in mothers with polycystic ovary syndrome (PCOS) and their term fetuses. *Syst Biol Reprod Med* (2019) 65(2):147–54. doi: 10.1080/19396368.2018.1537385

23. Piltonen TT, Giacobini P, Edvinsson Å, et al. Circulating antimüllerian hormone and steroid hormone levels remain high in pregnant women with polycystic ovary syndrome at term. *Fertil Steril* (2019) 111(3):588–596.e581.

24. Sir-Petermann T, Maliqueo M, Angel B, Lara HE, Pérez-Bravo F, Recabarren SE. Maternal serum androgens in pregnant women with polycystic ovarian syndrome: possible implications in prenatal androgenization. *Hum Reprod* (2002) 17(10):2573–9. doi: 10.1093/humrep/17.10.2573

25. Valdimarsdottir R, Valgeirsdottir H, Wikström AK, et al. Pregnancy and neonatal complications in women with polycystic ovary syndrome in relation to second-trimester anti-müllerian hormone levels. *Reprod BioMed Online* (2019) 39 (1):141–8. doi: 10.1016/j.rbmo.2019.02.004

26. de Wilde MA, Goverde AJ, Veltman-Verhulst SM, et al. Insulin action in women with polycystic ovary syndrome and its relation to gestational diabetes. *Hum Reproduction* (2015) 30(6):1447–53. doi: 10.1093/humrep/dev072

27. Alvarez-Blasco F, Botella-Carretero JI, San Millán JL, Escobar-Morreale HF. Prevalence and characteristics of the polycystic ovary syndrome in overweight and obese women. *Arch Intern Med* (2006) 166(19):2081–6. doi: 10.1001/archinte.166.19.2081

28. Lo JC, Feigenbaum SL, Escobar GJ, Yang J, Crites YM, Ferrara A. Increased prevalence of gestational diabetes mellitus among women with diagnosed polycystic ovary syndrome: a population-based study. *Diabetes Care* (2006) 29 (8):1915–7. doi: 10.2337/dc06-0877

29. Escobar-Morreale HF, Roldán-Martín MB. Type 1 diabetes and polycystic ovary syndrome: Systematic review and meta-analysis. *Diabetes Care* (2016) 39 (4):639–48. doi: 10.2337/dc15-2577

30. Silverman BL, Metzger BE, Cho NH, Loeb CA. Impaired glucose tolerance in adolescent offspring of diabetic mothers. *Relationship to fetal hyperinsulinism. Diabetes Care* (1995) 18(5):611–7.

31. Sobrevia L, Salsoso R, Fuenzalida B, et al. Insulin is a key modulator of fetoplacental endothelium metabolic disturbances in gestational diabetes mellitus. *Front Physiol* (2016) 7. doi: 10.3389/fphys.2016.00119

32. Merke DP, Bornstein SR. Congenital adrenal hyperplasia. *Lancet* (2005) 365 (9477):2125–36. doi: 10.1016/S0140-6736(05)66736-0

33. Spitzer RF, Wherrett D, Chitayat D, et al. Maternal luteoma of pregnancy presenting with virilization of the female infant. *J Obstet Gynaecol Can* (2007) 29 (10):835–40. doi: 10.1016/S1701-2163(16)32642-1

34. Manikkam M, Crespi EJ, Doop DD, et al. Fetal programming: prenatal testosterone excess leads to fetal growth retardation and postnatal catch-up growth in sheep. *Endocrinology* (2004) 145(2):790–8. doi: 10.1210/en.2003-0478

35. Wolf CJ, Hotchkiss A, Ostby JS, LeBlanc GA, Gray LEJr.. Effects of prenatal testosterone propionate on the sexual development of male and female rats: a dose-response study. *Toxicol Sci* (2002) 65(1):71–86. doi: 10.1093/toxsci/65.1.71

36. Beckett EM, Astapova O, Steckler TL, Veiga-Lopez A, Padmanabhan V. Developmental programing: impact of testosterone on placental differentiation. *Reproduction* (2014) 148(2):199–209. doi: 10.1530/REP-14-0055

37. Padmanabhan V, Veiga-Lopez A. Animal models of the polycystic ovary syndrome phenotype. *Steroids* (2013) 78(8):734–40. doi: 10.1016/j.steroids.2013.05.004

38. Abbott DH, Barnett DK, Bruns CM, Dumesic DA. Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome? *Hum Reprod Update* (2005) 11(4):357–74.

39. Padmanabhan V, Veiga-Lopez A. Sheep models of polycystic ovary syndrome phenotype. *Mol Cell Endocrinol* (2013) 373(1-2):8–20. doi: 10.1016/j.mce.2012.10.005

40. Speiser PW, Serrat J, New MI, Gertner JM. Insulin insensitivity in adrenal hyperplasia due to nonclassical steroid 21-hydroxylase deficiency. *J Clin Endocrinol Metab* (1992) 75(6):1421–4.

41. Yan X, Dai X, Wang J, Zhao N, Cui Y, Liu J. Prenatal androgen excess programs metabolic derangements in pubertal female rats. *J Endocrinol* (2013) 217 (1):119–29. doi: 10.1530/JOE-12-0577

42. Abbott DH, Bruns CR, Barnett DK, et al. Experimentally induced gestational androgen excess disrupts glucoregulation in rhesus monkey dams and their female offspring. *Am J Physiol Endocrinol Metab* (2010) 299(5):E741–751. doi: 10.1152/ajpendo.00058.2010

43. King AJ, Olivier NB, Mohankumar PS, Lee JS, Padmanabhan V, Fink GD. Hypertension caused by prenatal testosterone excess in female sheep. *Am J Physiol Endocrinol Metab* (2007) 292(6):E1837–1841. doi: 10.1152/ajpendo.00668.2006

44. Blesson CS, Chinnathambi V, Hankins GD, Yallampalli C, Sathishkumar K. Prenatal testosterone exposure induces hypertension in adult females *via* androgen receptor-dependent protein kinase cδ-mediated mechanism. *Hypertension* (2015) 65(3):683–90. doi: 10.1161/HYPERTENSIONAHA.114.04521

45. Vyas AK, Hoang V, Padmanabhan V, Gilbreath E, Mietelka KA. Prenatal programming: adverse cardiac programming by gestational testosterone excess. *Sci Rep* (2016) 6:28335. doi: 10.1038/srep28335

46. Auyeung B, Ahluwalia J, Thomson L, et al. Prenatal versus postnatal sex steroid hormone effects on autistic traits in children at 18 to 24 months of age. *Mol Autism* (2012) 3(1):17. doi: 10.1186/2040-2392-3-17

47. Roberts EK, Flak JN, Ye W, Padmanabhan V, Lee TM. Juvenile rank can predict male-typical adult mating behavior in female sheep treated prenatally with testosterone. *Biol Reprod* (2009) 80(4):737–42. doi: 10.1095/biolreprod.108.073429

48. Roberts EK, Padmanabhan V, Lee TM. Differential effects of prenatal testosterone timing and duration on phenotypic and behavioral masculinization and defeminization of female sheep. *Biol Reprod* (2008) 79(1):43–50. doi: 10.1095/biolreprod.107.067074

49. Piltonen T, Morin-Papunen L, Koivunen R, Perheentupa A, Ruokonen A, Tapanainen JS. Serum anti-müllerian hormone levels remain high until late reproductive age and decrease during metformin therapy in women with polycystic ovary syndrome. *Hum Reprod* (2005) 20(7):1820–6. doi: 10.1093/humrep/deh850

50. Tokmak A, Kokanali D, Timur H, Kuntay Kokanali M, Yilmaz N. Association between anti-mullerian hormone and insulin resistance in non-obese adolescent females with polycystic ovary syndrome. *Gynecol Endocrinol* (2016) 32 (11):926–30. doi: 10.1080/09513590.2016.1193140

51. Codner E, Iñiguez G, Hernández IM, et al. Elevated anti-müllerian hormone (AMH) and inhibin b levels in prepubertal girls with type 1 diabetes mellitus. *Clin Endocrinol (Oxf)* (2011) 74(1):73–8. doi: 10.1111/j.1365-2265.2010.03887.x

52. Köninger A, Kampmeier A, Schmidt B, et al. Trends in anti-müllerian hormone concentrations across different stages of pregnancy in women with polycystic ovary syndrome. *Reprod BioMed Online* (2018) 37(3):367–74. doi: 10.1016/j.rbmo.2018.05.011

53. Tata B, Mimouni NEH, Barbotin AL, et al. Elevated prenatal anti-müllerian hormone reprograms the fetus and induces polycystic ovary syndrome in adulthood. *Nat Med* (2018) 24(6):834–46. doi: 10.1038/s41591-018-0035-5

54. Tal R, Seifer CM, Khanimov M, Seifer DB, Tal O. High serum antimullerian hormone levels are associated with lower live birth rates in women with polycystic ovarian syndrome undergoing assisted reproductive technology. *Reprod Biol Endocrinol* (2020) 18(1):20. doi: 10.1186/s12958-020-00581-4

55. Du M, Zhang J, Yu X, Guan Y. Elevated anti-müllerian hormone is an independent risk factor for preterm birth among patients with overweight polycystic ovary syndrome. *Front Endocrinology* (2021) 12.

56. Hu KL, Liu FT, Xu H, Li R, Qiao J. High antimüllerian hormone levels are associated with preterm delivery in patients with polycystic ovary syndrome. *Fertil Steril* (2020) 113(2):444–452.e441.

57. Kaing A, Jaswa EA, Diamond MP, Legro RS, Cedars MI, Huddleston HG. Highly elevated level of antimüllerian hormone associated with preterm delivery in polycystic ovary syndrome patients who underwent ovulation induction. *Fertil Steril* (2020). doi: 10.1016/j.fertnstert.2020.06.015

58. Fritz MA, Speroff L. *Clinical gynecologic endocrinology and infertility (8th ed.)*. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins (2011).

59. Morel Y, Roucher F, Plotton I, Goursaud C, Tardy V, Mallet D. Evolution of steroids during pregnancy: Maternal, placental and fetal synthesis. *Ann Endocrinol (Paris)* (2016) 77(2):82–9. doi: 10.1016/j.ando.2016.04.023

60. Maliqueo M, Cruz G, Espina C, et al. Obesity during pregnancy affects sex steroid concentrations depending on fetal gender. *Int J Obes (Lond)* (2017) 41 (11):1636–45. doi: 10.1038/ijo.2017.159

61. Simoni RD, Hill RL, Vaughan M. The discovery of estrone, estriol, and estradiol and the biochemical study of reproduction. *work Edward Adelbert Doisy. J Biol Chem* (2002) 277(28):e1–2.

62. Chatuphonprasert W, Jarukamjorn K, Ellinger I. Physiology and pathophysiology of steroid biosynthesis, transport and metabolism in the human placenta. *Front Pharmacol* (2018) 9(1027). doi: 10.3389/fphar.2018.01027

63. Kaludjerovic J, Ward WE. The interplay between estrogen and fetal adrenal cortex. *J Nutr Metab* (2012) 2012:837901. doi: 10.1155/2012/837901

64. Berkane N, Liere P, Oudinet J-P, et al. From pregnancy to preeclampsia: A key role for estrogens. *Endocrine Rev* (2017) 38(2):123–44. doi: 10.1210/er.2016-1065

65. Hammond GL. Diverse roles for sex hormone-binding globulin in reproduction. *Biol Reprod* (2011) 85(3):431–41. doi: 10.1095/biolreprod.111.092593

66. Wan J, Hu Z, Zeng K, et al. The reduction in circulating levels of estrogen and progesterone in women with preeclampsia. *Pregnancy Hypertens* (2018) 11:18–25. doi: 10.1016/j.preghy.2017.12.003

67. Phupong V, Hanprasertpong T, Honsawek S. First trimester serum inhibin a in normal pregnant women. *Arch Gynecology Obstetrics* (2008) 277(4):307–10. doi: 10.1007/s00404-007-0491-5

68. Singnoi W, Wanapirak C, Sekararithi R, Tongsong T. A cohort study of the association between maternal serum inhibin-a and adverse pregnancy outcomes: a population-based study. *BMC Pregnancy Childbirth* (2019) 19(1):124. doi: 10.1186/s12884-019-2266-y

69. Yue CY, Zhang CY, Ni YH, Ying CM. Are serum levels of inhibin a in second trimester predictors of adverse pregnancy outcome? *PloS One* (2020) 15(5): e0232634. doi: 10.1371/journal.pone.0232634

70. Tarca AL, Romero R, Benshalom-Tirosh N, et al. The prediction of early preeclampsia: Results from a longitudinal proteomics study. *PloS One* (2019) 14(6): e0217273. doi: 10.1371/journal.pone.0217273

71. Lai J, Pinas A, Syngelaki A, Poon LC, Nicolaides KH. Maternal serum activin-a at 30-33 weeks in the prediction of preeclampsia. *J Matern Fetal Neonatal Med* (2013) 26(8):733–7. doi: 10.3109/14767058.2012.755167

72. Muttukrishna S, North RA, Morris J, et al. Serum inhibin a and activin a are elevated prior to the onset of pre-eclampsia. *Hum Reprod* (2000) 15(7):1640–5. doi: 10.1093/humrep/15.7.1640

73. Anderson H, Fogel N, Grebe SK, Singh RJ, Taylor RL, Dunaif A. Infants of women with polycystic ovary syndrome have lower cord blood androstenedione and estradiol levels. *J Clin Endocrinol Metab* (2010) 95(5):2180–6. doi: 10.1210/jc.2009-2651

74. Caanen MR, Kuijper EA, Hompes PG, et al. Mass spectrometry methods measured androgen and estrogen concentrations during pregnancy and in newborns of mothers with polycystic ovary syndrome. *Eur J Endocrinol* (2016) 174(1):25–32. doi: 10.1530/EJE-15-0699

75. Villarroel C, Salinas A, López P, et al. Pregestational type 2 diabetes and gestational diabetes exhibit different sexual steroid profiles during pregnancy. *Gynecol Endocrinol* (2017) 33(3):212–7. doi: 10.1080/09513590.2016.1248933

76. Köninger A, Kauth A, Schmidt B, et al. Anti-mullerian-hormone levels during pregnancy and postpartum. *Reprod Biol Endocrinol* (2013) 11:60. doi: 10.1186/1477-7827-11-60

77. Pankhurst MW, Clark CA, Zarek J, Laskin CA, McLennan IS. Changes in circulating ProAMH and total AMH during healthy pregnancy and post-partum: A longitudinal study. *PloS One* (2016) 11(9):e0162509–e0162509. doi: 10.1371/journal.pone.0162509

78. Villarroel C, Salinas A, López P, et al. Anti-müllerian hormone in type 2 and gestational diabetes during the second half of pregnancy: relationship with sexual steroid levels and metabolic parameters. *Gynecol Endocrinol* (2018) 34(2):120–4. doi: 10.1080/09513590.2017.1359824

79. Gerli S, Favilli A, Brozzetti A, et al. Anti-mullerian hormone concentration during the third trimester of pregnancy and puerperium: a longitudinal case-control study in normal and diabetic pregnancy. *Endocrine* (2015) 50(1):250–5. doi: 10.1007/s12020-014-0515-4

80. Li M-Y, Rawal S, Hinkle SN, et al. Sex hormone-binding globulin, cardiometabolic biomarkers, and gestational diabetes: A longitudinal study and meta-analysis. *Matern Fetal Med* (2020) 2(1):2–9. doi: 10.1097/FM9.0000000000000037

81. Veltman-Verhulst SM, van Haeften TW, Eijkemans MJ, de Valk HW, Fauser BC, Goverde AJ. Sex hormone-binding globulin concentrations before conception as a predictor for gestational diabetes in women with polycystic ovary syndrome. *Hum Reprod* (2010) 25(12):3123–8. doi: 10.1093/humrep/deq272

82. Zhang YJ, Jin H, Qin ZL, et al. Predictors of gestational diabetes mellitus in chinese women with polycystic ovary syndrome: A cross-sectional study. *Gynecol Obstet Invest* (2016) 81(3):220–4. doi: 10.1159/000440618

83. Yamashita H, Shao J, Friedman JE. Physiologic and molecular alterations in carbohydrate metabolism during pregnancy and gestational diabetes mellitus. *Clin Obstet Gynecol* (2000) 43(1):87–98. doi: 10.1097/00003081-200003000-00009

84. Hufnagel A, Dearden L, Fernandez-Twinn DS, Ozanne SE. Programming of cardiometabolic health: the role of maternal and fetal hyperinsulinaemia. *J Endocrinol* (2022) 253(2):R47–r63. doi: 10.1530/JOE-21-0332

85. Westgate JA, Lindsay RS, Beattie J, et al. Hyperinsulinemia in cord blood in mothers with type 2 diabetes and gestational diabetes mellitus in new zealand. *Diabetes Care* (2006) 29(6):1345–50. doi: 10.2337/dc05-1677

86. Uebel K, Pusch K, Gedrich K, Schneider KT, Hauner H, Bader BL. Effect of maternal obesity with and without gestational diabetes on offspring subcutaneous and preperitoneal adipose tissue development from birth up to year-1. *BMC Pregnancy Childbirth* (2014) 14:138. doi: 10.1186/1471-2393-14-138

87. Catalano PM, Presley L, Minium J, Hauguel-de Mouzon S. Fetuses of obese mothers develop insulin resistance. *utero. Diabetes Care* (2009) 32(6):1076–80. doi: 10.2337/dc08-2077

88. Maliqueo M, Lara HE, Sánchez F, Echiburú B, Crisosto N, Sir-Petermann T. Placental steroidogenesis in pregnant women with polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol* (2013) 166(2):151–5. doi: 10.1016/j.ejogrb.2012.10.015

89. North S, Zinn C, Crofts C. Hyperinsulinemia during pregnancy across varying degrees of glucose tolerance: An examination of the kraft database. *J Obstetrics Gynaecology Res* (2021) 47(5):1719–26. doi: 10.1111/jog.14731

90. Kallak TK, Hellgren C, Skalkidou A, et al. Maternal and female fetal testosterone levels are associated with maternal age and gestational weight gain. *Eur J Endocrinol* (2017) 177(4):379–88. doi: 10.1530/EJE-17-0207

91. Saez JM, Forest MG, Morera AM, Bertrand J. Metabolic clearance rate and blood production rate of testosterone and dihydrotestosterone in normal subjects, during pregnancy, and in hyperthyroidism. *J Clin Invest* (1972) 51(5):1226–34. doi: 10.1172/JCI106917

92. Makieva S, Saunders PT, Norman JE. Androgens in pregnancy: roles in parturition. *Hum Reprod Update* (2014) 20(4):542–59. doi: 10.1093/humupd/dmu008

93. Jaffe RB. Fetoplacental endocrine and metabolic physiology. *Clin Perinatol* (1983) 10(3):669–93. doi: 10.1016/S0095-5108(18)30958-8

94. Andræ F, Abbott D, Stridsklev S, et al. Sustained maternal hyperandrogenism during PCOS pregnancy reduced by metformin in non-obese women carrying a male fetus. *J Clin Endocrinol Metab* (2020) 105(12):3762–70. doi: 10.1210/clinem/dgaa605

95. Thadhani R, Wolf M, Hsu-Blatman K, Sandler L, Nathan D, Ecker JL. First-trimester sex hormone binding globulin and subsequent gestational diabetes mellitus. *Am J Obstet Gynecol* (2003) 189(1):171–6. doi: 10.1067/mob.2003.343

96. Kelley AS, Smith YR, Padmanabhan V. A narrative review of placental contribution to adverse pregnancy outcomes in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* (2019) 104(11):5299–315. doi: 10.1210/jc.2019-00383

97. Hakim C, Padmanabhan V, Vyas AK. Gestational hyperandrogenism in developmental programming. *Endocrinology* (2016) 158(2):199–212.

98. Barbour LA, McCurdy CE, Hernandez TL, Kirwan JP, Catalano PM, Friedman JE. Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes. *Diabetes Care* (2007) 30(Supplement 2):S112. doi: 10.2337/dc07-s202

99. Di Cianni G, Miccoli R, Volpe L, Lencioni C, Del Prato S. Intermediate metabolism in normal pregnancy and in gestational diabetes. *Diabetes Metab Res Rev* (2003) 19(4):259–70. doi: 10.1002/dmrr.390

100. Phelps RL, Metzger BE, Freinkel N. Carbohydrate metabolism in pregnancy. XVII. diurnal profiles of plasma glucose, insulin, free fatty acids, triglycerides, cholesterol, and individual amino acids in late normal pregnancy. *Am J Obstet Gynecol* (1981) 140(7):730–6. doi: 10.1016/0002-9378(81)90731-6

101. Parsons JA, Brelje TC, Sorenson RL. Adaptation of islets of langerhans to pregnancy: increased islet cell proliferation and insulin secretion correlates with the onset of placental lactogen secretion. *Endocrinology* (1992) 130(3):1459–66.

102. Smirnakis KV, Plati A, Wolf M, Thadhani R, Ecker JL. Predicting gestational diabetes: choosing the optimal early serum marker. *Am J Obstet Gynecol* (2007) 196(4):410.e411–416. doi: 10.1016/j.ajog.2006.12.011

103. Spencer K, Yu CK, Rembouskos G, Bindra R, Nicolaides KH. First trimester sex hormone-binding globulin and subsequent development of preeclampsia or other adverse pregnancy outcomes. *Hypertens Pregnancy* (2005) 24(3):303–11. doi: 10.1080/10641950500281068

104. Mochizuki M, Maruo T. Effect of dehydroepiandrosterone sulfate on uterine cervical ripening in late pregnancy. *Acta Physiol Hung* (1985) 65 (3):267–74.

105. Tagawa N, Hidaka Y, Takano T, Shimaoka Y, Kobayashi Y, Amino N. Serum concentrations of dehydroepiandrosterone and dehydroepiandrosterone sulfate and their relation to cytokine production during and after normal pregnancy. *Clin Chim Acta* (2004) 340(1-2):187–93. doi: 10.1016/j.cccn.2003.10.018

106. Hogeveen KN, Cousin P, Pugeat M, Dewailly D, Soudan B, Hammond GL. Human sex hormone-binding globulin variants associated with hyperandrogenism and ovarian dysfunction. *J Clin Invest* (2002) 109(7):973–81. doi: 10.1172/JCI0214060

107. Jones ME, Boon WC, McInnes K, Maffei L, Carani C, Simpson ER. Recognizing rare disorders: aromatase deficiency. *Nat Clin Pract Endocrinol Metab* (2007) 3(5):414–21. doi: 10.1038/ncpendmet0477

108. Nelson SM, Stewart F, Fleming R, Freeman DJ. Longitudinal assessment of antimüllerian hormone during pregnancy–relationship with maternal adiposity, insulin, and adiponectin. *Fertility Sterility* (2010) 93(4):1356–8. doi: 10.1016/j.fertnstert.2009.07.1676

109. Köninger A, Kampmeier A, Mach P, et al. Tight interplay in early pregnancy between follistatin and anti-mullerian hormone in women with polycystic ovarian syndrome (PCOS). *Arch Gynecology Obstetrics* (2018) 297 (5):1307–16. doi: 10.1007/s00404-018-4718-4

110. Zhou S, Lu D, Wen S, Sheng Y, Kang D, Xu L. Elevated anti-müllerian hormone levels in newborns of women with polycystic ovary syndrome: a systematic review and meta-analysis based on observational studies. *Reprod Sci* (2022) 29(1):301–11. doi: 10.1007/s43032-021-00652-w

111. Vounzoulaki E, Khunti K, Abner SC, Tan BK, Davies MJ, Gillies CL. Progression to type 2 diabetes in women with a known history of gestational diabetes: systematic review and meta-analysis. *Bmj* (2020) 369:m1361.

112. Bitó T, Földesi I, Nyári T, Pál A. Prediction of gestational diabetes mellitus in a high-risk group by insulin measurement in early pregnancy. *Diabetes Med* (2005) 22(10):1434–9. doi: 10.1111/j.1464-5491.2005.01634.x

113. Lapolla A, Dalfrà MG, Mello G, et al. Early detection of insulin sensitivity and beta-cell function with simple tests indicates future derangements in late pregnancy. *J Clin Endocrinol Metab* (2008) 93(3):876–80. doi: 10.1210/jc.2007-1363

114. Zhang N-J, M-f T, Li H-P, Zhao F, Wang F-H. The relationship between patterns of insulin secretion and risks of gestational diabetes mellitus. *Int J Gynecology Obstetrics* (2020) 150(3):318–23. doi: 10.1002/ijgo.13200

115. Uzelac PS, Li X, Lin J, et al. Dysregulation of leptin and testosterone production and their receptor expression in the human placenta with gestational diabetes mellitus. *Placenta* (2010) 31(7):581–8. doi: 10.1016/j.placenta.2010.04.002

116. Hähnel ME, Martin JD, Michael CA, Hähnel R. Metabolism of androstenedione by placental microsomes in pregnancy hypertension. *Clin Chim Acta* (1989) 181(1):103–8. doi: 10.1016/0009-8981(89)90322-7

117. Brown FM, Wyckoff J, Rowan JA, Jovanovic L, Sacks DA, Briggs GG. Metformin in pregnancy: its time has not yet come. *Diabetes Care* (2006) 29 (2):485–6. doi: 10.2337/diacare.29.02.06.dc05-2098

118. Mousa A, Løvvik T, Hilkka I, et al. Metformin in pregnancy study (MiPS): protocol for a systematic review with individual patient data meta-analysis. *BMJ Open* (2020) 10(5):e036981. doi: 10.1136/bmjopen-2020-036981

119. Blum AK. Insulin use in pregnancy: An update. *Diabetes Spectr* (2016) 29 (2):92–7. doi: 10.2337/diaspect.29.2.92

120. Ye W, Luo C, Huang J, Li C, Liu Z, Liu F. Gestational diabetes mellitus and adverse pregnancy outcomes: systematic review and meta-analysis. *BMJ* (2022) 377:e067946. doi: 10.1136/bmj-2021-067946

121. Arshad R, Karim N, Ara Hasan J. Effects of insulin on placental, fetal and maternal outcomes in gestational diabetes mellitus. *Pak J Med Sci* (2014) 30 (2):240–4.

122. Evers IM, de Valk HW, Mol BW, ter Braak EW, Visser GH. Macrosomia despite good glycaemic control in type i diabetic pregnancy; results of a nationwide study in the netherlands. *Diabetologia* (2002) 45(11):1484–9.

123. Daan NM, Koster MP, Steegers-Theunissen RP, Eijkemans MJ, Fauser BC. Endocrine and cardiometabolic cord blood characteristics of offspring born to mothers with and without polycystic ovary syndrome. *Fertil Steril* (2017) 107 (1):261–268.e263. doi: 10.1016/j.fertnstert.2016.09.042

124. Mehrabian F, Kelishadi R. Comparison of the metabolic parameters and androgen level of umbilical cord blood in newborns of mothers with polycystic ovary syndrome and controls. *J Res Med Sci* (2012) 17(3):207–11.

125. Duan C, Pei T, Li Y, Cao Q, Chen H, Fu J. Androgen levels in the fetal cord blood of children born to women with polycystic ovary syndrome: a meta-analysis. *Reprod Biol Endocrinol* (2020) 18(1):81. doi: 10.1186/s12958-020-00634-8

126. Kallen CB. Steroid hormone synthesis in pregnancy. *Obstetrics Gynecology Clinics North America* (2004) 31(4):795–816. doi: 10.1016/j.ogc.2004.08.009

127. Padmanabhan V, Veiga-Lopez A. Developmental origin of reproductive and metabolic dysfunctions: androgenic versus estrogenic reprogramming. *Semin Reprod Med* (2011) 29(3):173–86. doi: 10.1055/s-0031-1275519

128. Padmanabhan V, Veiga-Lopez A, Abbott DH, Recabarren SE, Herkimer C. Developmental programming: impact of prenatal testosterone excess and postnatal weight gain on insulin sensitivity index and transfer of traits to offspring of overweight females. *Endocrinology* (2010) 151(2):595–605. doi: 10.1210/en.2009-1015

129. Vanky E, Carlsen SM. Androgens and antimüllerian hormone in mothers with polycystic ovary syndrome and their newborns. *Fertil Steril* (2012) 97(2):509–15. doi: 10.1016/j.fertnstert.2011.11.021

130. Tokmak A, Güney G, Aksoy RT, et al. May maternal anti-mullerian hormone levels predict adverse maternal and perinatal outcomes in preeclampsia? *J Matern Fetal Neonatal Med* (2015) 28(12):1451–6. doi: 10.3109/14767058.2014.955007

131. Parrettini S, Caroli A, Torlone E. Nutrition and metabolic adaptations in physiological and complicated pregnancy: Focus on obesity and gestational diabetes. *Front Endocrinol (Lausanne)* (2020) 11:611929. doi: 10.3389/fendo.2020.611929

132. Welsh P, Smith K, Nelson SM. A single-centre evaluation of two new anti-mullerian hormone assays and comparison with the current clinical standard assay. *Hum Reprod* (2014) 29(5):1035–41. doi: 10.1093/humrep/deu036

133. Nuamah MA, Yura S, Sagawa N, et al. Significant increase in maternal plasma leptin concentration in induced delivery: a possible contribution of pro-inflammatory cytokines to placental leptin secretion. *Endocr J* (2004) 51(2):177–87. doi: 10.1507/endocrj.51.177

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 23 January 2023
DOI 10.3389/fendo.2023.1008675

OPEN ACCESS

EDITED BY
Eleonora Porcu,
University of Bologna, Italy

REVIEWED BY
Manvendra Pratap Singh,
Massachusetts General Hospital and Harvard Medical School, United States
Shang Li,
Shanghai Jiao Tong University, China
Pratibha G,
Manipal College of Dental Sciences, Manipal

*CORRESPONDENCE
Donglei Wu
wu1582224766@163.com

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

RECEIVED 01 August 2022
ACCEPTED 03 January 2023
PUBLISHED 23 January 2023

CITATION
Dou Y, Xin J, Zhou P, Tang J, Xie H, Fan W, Zhang Z and Wu D (2023) Bidirectional association between polycystic ovary syndrome and periodontal diseases. *Front. Endocrinol.* 14:1008675. doi: 10.3389/fendo.2023.1008675

Bidirectional association between polycystic ovary syndrome and periodontal diseases

Yang Dou[1], Jinglei Xin[2], Peng Zhou[2], Jianming Tang[3], Hongliang Xie[3], Wanting Fan[3], Zheng Zhang[3] and Donglei Wu[3]*

[1]Department of Stomatology, Shenzhen Baoan Women's and Children's Hospital, Jinan University, Shenzhen, Guangdong, China, [2]Department of Stomatology, Guangdong Women and Children hospital, Guangzhou, Guangdong, China, [3]Department of Stomatology, Shenzhen People's Hospital, Shenzhen, Guangdong, China

Polycystic ovary syndrome (PCOS) and periodontal disease (PDD) share common risk factors. The bidirectional interaction between PCOS and PDD has been reported, but until now, the underlying molecular mechanisms remain unclear. Endocrine disorders including hyperandrogenism (HA) and insulin resistance (IR) in PCOS disturb the oral microbial composition and increase the abundance of periodontal pathogens. Additionally, PCOS has a detrimental effect on the periodontal supportive tissues, including gingiva, periodontal ligament, and alveolar bone. Systemic low-grade inflammation status, especially obesity, persistent immune imbalance, and oxidative stress induced by PCOS exacerbate the progression of PDD. Simultaneously, PDD might increase the risk of PCOS through disturbing the gut microbiota composition and inducing low-grade inflammation and oxidative stress. In addition, genetic or epigenetic predisposition and lower socioeconomic status are the common risk factors for both diseases. In this review, we will present the latest evidence of the bidirectional association between PCOS and PDD from epidemiological, mechanistic, and interventional studies. A deep understanding on their bidirectional association will be beneficial to provide novel strategies for the treatment of PCOS and PDD.

KEYWORDS

polycystic ovary syndrome, periodontal diseases, host immune, inflammation, oral micro biota

1 Introduction

Polycystic ovary syndrome (PCOS), the most common heterogeneous disorder in women with high prevalence rate of 5%–10% (1), is characterized by hyperandrogenism (HA), oligomenorrhea or amenorrhea, polycystic ovary, and low-grade inflammation (2). PCOS contributes to menstrual cycle abnormalities, pregnancy complications, long-term metabolic disorders, cardiovascular diseases, and even increases cancer risk (2). Periodontal diseases (PDDs) are multifactorial, highly prevalent chronic inflammatory disorders in the oral cavity. It is widely recognized that the symbiotic relationship between oral microbiota and the host is essential for the homeostasis in oral microecology (3). Oral flora dysbiosis renders dominance of periodontal

pathogens, impacts tooth-supporting tissues (gingiva, periodontal ligament, and alveolar bone), and even leads to tooth loss (4). Systemic diseases including diabetes mellitus (DM), obesity, and metabolic syndrome (MS) are independent risk factors for PDD (5). Interestingly, PCOS and PDD have common risk factors, including metabolic syndrome (6), obesity, DM, and cardiovascular disease (7). Recently, several studies have explored and confirmed the association between PCOS and PDD (8–11). However, the cause-and-effect relationship between PCOS and PDD and their molecular mechanisms remain undefined. In this review, we will focus on recent studies to discuss the potential mechanisms between PCOS and PDD.

2 Epidemiological evidence of the bidirectional relationship between PCOS and PDD

There are 15 published papers on the bidirectional relationship between PCOS and PDD, including 14 cross-sectional studies and 1 randomized controlled trial. A recent cross-sectional study indicated that women who are infertile, in comparison with those who are fertile, are more vulnerable to PDD with signs of increased probing depth (PD) and clinical attachment loss (CAL). However, no difference were observed in oral-health-related quality of life (OHRQoL) scores between the two groups, which might reveal a poor periodontal status and weak awareness of oral health care in infertile women (12). PCOS as an important cause of infertility might be associated with PDD. Recently published studies have provided evidence to support the interaction network between PCOS and PDD (9–11). PCOS may have an impact on gingival inflammation and *vice versa*. Several cross-sectional studies unanimously concluded that PCOS increases the risk of PDD (13–18). Women with PCOS tend to manifest poor periodontal conditions, such as positive bleeding on probing (BOP), deep PD, and high plaque index (PI). Interestingly, controlling PCOS with oral contraceptives and metformin mitigates periodontal inflammation (19). A retrospective cohort study involving 48,820 subjects showed that PDD multiplies the risk of PCOS (20). The bidirectional relationship between PCOS and PDD was discussed in recent meta-analysis research. The risk of PDD is increased by 28% in women with PCOS, and the risk of PCOS is increased by 46% in women with PDD (9). In addition, a higher PD rate is observed in PCOS patients than in healthy women in a recent case–control study. However, there was no differentiation between PCOS and healthy women in other periodontal parameters, such as PI, BOP, and CAL (21). Although no causal relationship between genetic liability for PCOS and periodontitis was identified based on a recent bidirectional Mendelian randomization analysis (22), strong evidence supports the mutual effect between PCOS and PDD. The potential mechanisms between PCOS and PDD are discussed in the following sections (Table 1).

3 Potential mechanisms by which PCOS might increase the risk of PDD

PCOS is featured with endocrine disorders, including HA, insulin resistance (IR), and estrogen reduction. Endocrine disorder aggravates the development of PDD. We summarize the potential mechanisms of endocrine disorders between PCOS and PDD in the following four aspects (Figure 1).

3.1 PCOS alters the composition of oral microbiota

The initiation and progression of periodontal lesions depend on the interactions between host and oral microecology. Within the oral ecosystem, local microbial metabolism, systemic stress-induced dysbiosis, especially hormonal imbalance, toxins, and inflammatory cytokines might result in 10-fold higher migration of immune cells from the gingival sulcus (36). Once the host immune system is continuously activated, chronic inflammation will develop. Periodontitis is mainly caused by the disturbance of subgingival biofilm ecosystems. *Fusobacterium nucleatum* (*F.n*) is one of the anaerobic bacteria in supra- and subgingival biofilms. *F.n* serves as a bridge to assist the interspecies coaggregation, including *Porphyromonas gingivalis* (*P.g*), *Treponema denticola* (*T.d*), and *Streptococcus gordonii* (*S.g*), and accelerates biofilm formation *via* outer membrane proteins. In addition, *F.n* breaks the epithelial barrier and engages in attacking the lymphocytes, resulting in the host immune imbalance (37). Accumulating evidence suggests that the alteration of the oral microbial community in PCOS women might increase the risk of PDD. Increased number of *Fusobacterium* and decreased number of Actinobacteria were observed in the salivary microbiome from PCOS women (38). Actinobacteria are more abundant in the periodontium in a healthy state than that in the inflammatory state (39) and plays an important role in maintaining oral microbial homeostasis. A decreased proportion of phylum Actinobacteria was observed in the salivary microbiome in PCOS when compared with healthy women, but no relationship was found between saliva alpha diversity or beta diversity and serum testosterone and inflammatory markers in PCOS (40, 41). Higher abundances of *F.n* and *Tannerella forsythia* (*T.f*) were observed in gingival crevicular fluid (GCF) in patients with comorbidity of PCOS and gingivitis rather than patients with gingivitis alone (23), which suggests a detrimental effect of PCOS on periodontal microecology (Table 2). Interestingly, a similar change was observed in the abundance of *F.n* and Actinobacteria in DM patients accompanied with PDD (43).

Sex hormones may also contribute to the differences in the oral microbiota. A higher abundance of *F.n* subspecies, *fusiforme/vincentii*, was observed in women compared with men (44). Additionally, increased abundance of Actinobacteria has been reported in postmenarcheal girls compared with premenarcheal girls (45). Moreover, endocrine disorder alters the microbiota composition in female individuals at reproductive age. The level of estradiol (E2) was positively correlated with the number of green complex bacteria (*Capnocytophaga gingivalis*) in subgingival microflora in adolescent girls with PCOS (42). Similarly, alteration of the composition of oral microbial community was observed in various endocrine system diseases, such as DM and obesity (41). A recent study demonstrated that diabetes increased the pathogenicity of the oral microbiota by enhancing the expression of interleukin (IL)-17 in mice. Moreover, mice infected with DM-related oral

10.3389/fendo.2023.1008675

TABLE 1 Summary of studies investigating the association between PCOS and PDD.

Authors Year	Study style	Periodontal Index	Other Index	Sample	Group	Inclusion criteria of PD	Inclusion criteria of PCOS	Confounders variables assessed
Akcali (2014) (23)	Case-control	PI, BOP, PPD	/	/	PCOS +HP:45 PCOS +GG:35 NP+GG: 20 NP+HP: 25	BOP >50% sites, PPD< 3mm at 90% sites, no sign of PD	Rotterdam criteria (2003)	hyperandrogenism, DM, hyperprolactemia, congenital adrenal hyperplasia, thyroid disorders, Cushing's syndrome, HBP, CVD, hepatic or renal dysfunction, oral contraceptives, steroid hormones, insulin-sensitizing. BMI>30 kg/m^2;
Rahiminejad (2015) (17)	Case-control	PI, BOP, CAL,	/	/	PCOS:98 HC:98	/	Rotterdam criteria (2003)	Pregnancy, smoking, malignancies, osteoporosis, antibiotics, periodontal treatment, BMI >25 kg/m^2, IGT
Işık (2020) (21)	Case-control	BOP, PI, GI, CAL,PD	/	/	PCOS:116 HC:90	/	Rotterdam criteria (2003)	Androgen-secreting tumors, congenital adrenal hyperplasia, Cushing's syndrome, hyperprolactinemia, thyroid disorders, hypertension, hepatic or renal dysfunction, CAM, DM, chronic inflammatory disease, malignancy, pregnancy, AIDS, smoking, alcohol drinking, periodontal treatment, oral contraceptive agents, antipsychotic, antiepileptic, steroid hormones, antihypertensive, insulin sensitizing drugs, antibiotics, or anti-inflammatory drugs
Ozcaka (2012) (16)	Case–control	PPD, BOP, PI	IL-6	GCF, saliva, serum	PCOS +HP:30 PCOS +GG:31 HC:12	BOP >50% sites, PPD <3 mm at 90% sites, no sign of PD, >20 teeth	Rotterdam criteria (2003)	BMI > 30 kg/m^2, androgen-secreting tumors, congenital adrenal hyperplasia, thyroid disorders, DM, hyperprolactinemia, Cushing´s syndrome, hypertension, hepatic and renal dysfunction, oral contraceptives, steroid hormones, insulin-sensitizing drugs, alcohol, smokers
Ozcaka (2013) (24)	Case–control	PPD, BOP, PI	IL-17	GCF, saliva, serum	PCOS +HP:30 PCOS +GG:31 HC:12	BOP >50% sites, PPD< 3mm at 90% sites, no sign of PD, >20 teeth	Rotterdam criteria (2003)	BMI > 30 kg/m^2, hyperandrogenism, thyroid disorders, hyperprolactinemia, CVD,DM, high BP, oral contraceptives, steroid hormone, insulin-sensitizing drugs
Akcali (2015) (13)	Case–control	PI, BOP, PPD	MMP-8, TIMP1	Saliva, serum	PCOS +HP:45 PCOS +GG:35 NP+GG: 20 NP+HP: 25	BOP >50% sites, PPD <3 mm at 90% sites, no sign of PD	Rotterdam criteria (2003)	Hyperandrogenism, DM, hyperprolactemia, congenital adrenal hyperplasia, thyroid disorders, Cushing's syndrome, HBP, CVD, hepatic or renal dysfunction, oral contraceptives, steroid hormones, insulin-sensitizing. BMI >30 kg/m^2
Akcali (2017) (25)	Case–control	PI, BOP, PPD	MMP-8, TIMP1, MPO, NE	Saliva, serum	PCOS +HP:45 PCOS +GG:35 NP+GG: 20 NP+HP: 25	BOP >50% sites, PPD <3 mm at 90% sites, no sign of PD	Rotterdam criteria (2003)	Hyperandrogenism, DM, hyperprolactemia, congenital adrenal hyperplasia, thyroid disorders, Cushing's syndrome, HBP, CVD, hepatic or renal dysfunction, oral contraceptives, steroid hormones, insulin-sensitizing. BMI>30 kg/m^2
Dursun (2011) (15)	Cross-section	PPD, CAL, BOP, GI	MPO, NO	GCF	PCOS:25 HC:27	/	Rotterdam criteria (2003) (26)	Cushing syndrome, congenital adrenal hyperplasia, hyperprolactinemia, thyroid dysfunction, and androgen-secreting tumors, smokers, oral contraceptives, BMI>30 kg/m^2; IGT
Saglam (2018) (27)	Case–control	BOP, PI, GI PPD, CAL	MDA, 8-OHdG, TAS	Serum, saliva	PCOS:22 PCOS +CP:22 NP +CP:22 NP +HP:22	PPD ≥5 mm, CAL ≥ 6 mm	Rotterdam criteria (2003)	BMI > 25 kg/m^2, HbA1c >6,5%, OGTT-2h > 200 mg/dl, antibiotics, oral contraceptives, steroid hormones, hypertensive medications, insulin-sensitizing drugs, periodontal treatment, androgen-secreting tumors, congenital adrenal hyperplasia, thyroid disorders, DM, hyperprolactinemia, Cushing's syndrome

(Continued)

TABLE 1 Continued

Authors Year	Study style	Periodontal Index	Other Index	Sample	Group	Inclusion criteria of PD	Inclusion criteria of PCOS	Confounders variables assessed
Varadan (2019) (18)	Case–control	BOP, PPD, PI, mGI	MDA, MPO	Serum, saliva	PCOS:30 HC:30	/	Rotterdam criteria (2003)	Cushing's syndrome, adrenal hyperplasia, hyperprolactinemia, thyroid dysfunction, androgen secreting tumors, history of systemic disease, pregnancy, interfering drugs (antibiotics, oral contraceptives, chemotherapeutic), periodontal treatment, smoking, tobacco, and alcohol consumption
Dharuman (2022) (28)	Cross-section	BOP, PD, PISA	AOPP	Serum; saliva	PCOS:12 CP:12 PCOS +CP:12 HC:12	2012 definition (29)	Rotterdam criteria (2003)	Smoking, pregnant, history of systemic disease other than PCOS, consumed medications within the past 3 months, and had periodontal therapy.
Saljoughi (2020) (30)	/	BOP, CAL, PD, raidograph	Visfatin	GCF	PCOS +HP:25 NP+CP: 23 PCOS +CP:30 NP+HP: 32	> 35 years and > 30% sites with CAL ≥3 mm, and PPD ≥5 mm with BOP (+).	Rotterdam criteria (2003)	Pregnancy, interfering drugs (antibiotics, oral contraceptives, antihypertensive, DM drugs), infection, thyroid disorders, hyperprolactinemia, diabetes, hypertension, malignancies, osteoporosis, obesity, overweight, smoking, alcohol consumption.
Zia (2022) (31)	Cross-section	PD, CAL, PI, GI	ALP, BMD, CTX, VD	Serum	PCOS:40 CP:40 PCOS +CP:40 HC:20	2017 classification (32)	Rotterdam criteria (2003)	BMI, hypo- or hyperthyroidism, hypogonadism, Cushing's syndrome, androgen-secreting tumors, smoking, drugs-like oral contraceptives, anti-inflammatory, antibiotics, and others that could affect periodontal status in the last 6 months
Deepti (2017) (33)	RCT	PI, GI, BOP, CAL, PD	hsCRP HOMA	serum	SPR +MI:30 OHI +MI:30	≥20 teeth; moderate periodontitis (34)	Androgen Excess Society/2006 criteria (35)	androgen-secreting tumors, congenital adrenal hyperplasia, and thyroid dysfunction; nephrotic syndrome; chronic renal failure; significant cardiovascular disease; DM; active cancer within the last 5 years; smokers and women who are alcohol dependent; history of systemic antibiotics or oral contraceptives usage within last 3 months; periapical pathology or other oral inflammatory conditions; and any periodontal treatment within 6 months prior to study
Porwal (2014) (19)	Case-control	BOP, PPD, CAL, GI	hsCRP	Serum	PCOS:41 PCOS+T: 45 HC:40	/	Rotterdam criteria (2003)	BMI > 30 kg/m^2, thyroid disorders, hyperprolactinemia, androgen-secreting tumors, chronic inflammatory diseases, DM, CVD, cancer, smoking, alcohol, antibiotics, periodontal treatment, aggressive periodontitis

AIDS, acquired immunodeficiency syndrome; ALP, alkaline phosphatase; advanced oxidative protein products, AOPP; BMD, bone mineral density; BMI, body mass index; BOP, bleeding on probing; CAL, clinical attachment loss; CTX, C-terminal telopeptides of type I collagen; CP, chronic periodontitis GCF, gingival crevicular fluid; GI, gingival index; GG, gingivitis; HC, healthy control women; hsCRP, high-sensitivity C-reactive protein; IL, interleukin; IGT, impaired glucose tolerance; MDA, malondialdehyde; MMP, matrix metalloproteinase; MPO, myeloperoxidase; NA, not applicable; NE, neutrophil elastase; NP, non-polycystic ovary syndrome; NO, nitric oxide; PCOS, polycystic ovary syndrome; PCOS+T, PCOS treatment; PI, plaque index; PISA, periodontal inflamed surface area; PPD, periodontal probing depth; RCT, randomized controlled trial; TAS, total antioxidant status; TIMP, tissue inhibitors of MMP-I; TNF-α, tumor necrosis factor-α, VD, 25-hydroxyvitamin D; /, no available.

microbiota manifest inflammation and bone loss in periodontal tissues (46), which confirms that oral microbiota is implicated in DM patients. In addition, altered composition of salivary bacteria is also observed in overweight women (47).

Both HA and IR affect the composition of the microbiota and metabolic activity in PCOS. HA was associated with decreased alpha diversity and alteration of specific Bacteroidetes and Firmicutes in gut microbiota in women with PCOS (48). PCOS accompanied by IR alters the composition of the gut microbial community (48) and oral microbiota. Numerous observational and interventional studies have linked IR to PDD (49). IR was positively correlated with the abundances of *Granulicatella*, *Veillonella*, *Streptococcus*, and *Scardovia* in supragingival plaque by 16s rDNA sequencing in patients with metabolism-associated fatty liver disease (50). A previous study has demonstrated that IR was associated with the abundance of 22 individual taxa in human subgingival plaque (51). Hence, endocrine disorder in PCOS might increase the risk of PDD by altering the composition of oral microbiota.

Microbial metabolomics is the key to probe into the relationship between the alteration of oral microbial composition in PCOS and the occurrence of PDD. Altered composition of salivary bacteria in PCOS women causes changes in host metabolism (38), including oxidative phosphorylation, methane metabolism, nitrogen metabolism, butanoate metabolism, molecular chaperones, folding catalysts, and membrane and intracellular structural molecules. More importantly, consistently upregulated methane metabolism and downregulated

10.3389/fendo.2023.1008675

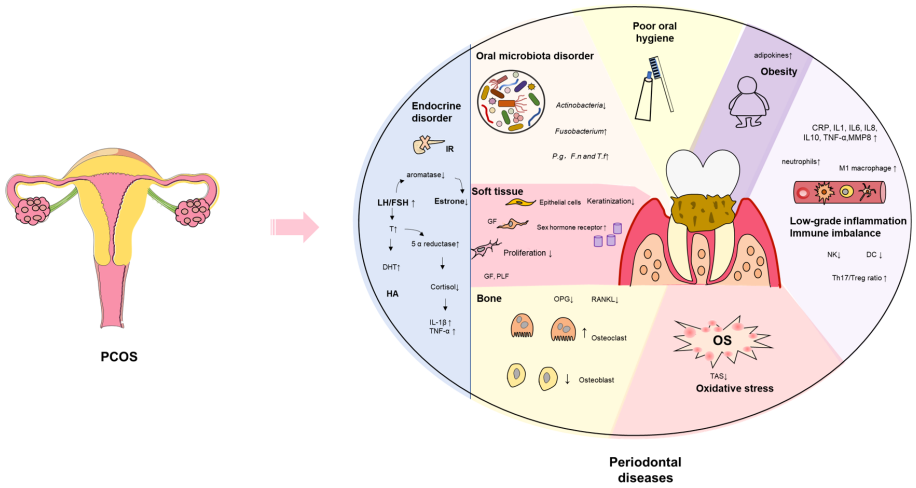


FIGURE 1
Schematic representation of the association between PCOS and PDD. PCOS is characterized by low-grade inflammation, immune imbalance, endocrine disorder, and oxidative stress. The above microenvironment has an adverse implication for oral microbiome, periodontium, and alveolar bone. In addition, PCOS women show a propensity for obesity and have a poor oral hygiene, which increases the risk of PDD. PCOS, polycystic ovary syndrome; T, testosterone; DHT, Dihydro testosterone; HA, hyperandrogenism; IR, insulin resistance; LH/FSH, luteinizing hormone/follicle stimulating hormone; *F.n*, *Fusobacterium nucleatum*; *P.g*, *Porphyromonas gingivalis*; *T.f*, *Tannerella forsythia;* GF, gingival fibroblasts; PLF, periodontal ligament fibroblasts; OPG, osteoprotegerin; RANKL, receptor activator of NF-κB ligand; MDA, malondialdehyde; MPO, myeloperoxidase; No, nitric oxide; TAS, total antioxidant status; NK, natural killer cells; DC, dendritic cell;IL, interleukin; TNF, tumor necrosis factor.

chaperones and folding catalysts are observed in PCOS (38). Interestingly, both butanoate and methane metabolism in the subgingival microbiota are significantly over-activated in patients with periodontitis (52). Butanoate production has been known to play an important role in periodontal disease (53). Butyrate, the metabolite of periodontal pathogens (including *F.n* and *P.g*) (54), inhibits cell cycle of gingival fibroblasts and promotes apoptosis (55) by inducing reactive oxygen species (56). Therefore, more studies should focus on the effects of the oral microbiome in PCOS on host metabolism.

3.2 PCOS promotes bone resorption

PCOS has a negative effect on bone metabolism. PCOS women are at high risk of osteoporosis (57). Another study revealed a decreased bone mineral density (BMD) in the spine and femur and less bone formation with decreased osteocalcin in PCOS patients with a body mass index (BMI) <27 kg/m^2 (58). Zia et al. evaluated the serum levels of bone metabolism and bone turnover markers (BTMs) in PCOS accompanied with PDD. Increased level of C-terminal telopeptides of type I collagen (CTX, bone resorption marker) and decreased level of alkaline phosphatase (ALP, bone formation marker) were found in patients with comorbidity of PCOS and PDD than in patients with PDD alone (31), suggesting that PCOS worsens the bone metabolism of the alveolar bone around the periodontal tissue. In addition, PCOS enhances the levels of PD and CAL in PDD patients with continuing alveolar bone resorption (31, 33).

HA, estrogen reduction, and IR are responsible for the alteration of bone metabolism in PCOS (57). Elevated luteinizing hormone (LH)/follicle stimulating hormone (FSH) ratio in PCOS decreases the activity of aromatase, which converts androgens into estrogens, leading to hyperandrogenism (57). Although androgen plays an important role in maintaining BMD in men, it does not exert the same function in women with PCOS (57). Excessive androgen has an adverse effect on bone anabolism in women. The increase in 5α reductase in PCOS enhances the conversion of testosterone to dihydrotestosterone (DHT), which inhibits the expression of cortisol. Subsequently, cortisol inactivation increases the expression of IL-1β and tumor necrosis factor alpha (TNF-α) and contributes to bone resorption. In addition, estrogen reduction in PCOS decreases bone density, deteriorates microarchitecture, and increases fracture risk (59). IR in PCOS women inhibits the expression of

TABLE 2 Alteration of composition of oral microbial community in PCOS women.

Case group	Control group	Sample types	Methods	Result*
Adolescent PCOS (42)	Health	GCF	qPCR	*P.m* and *T.d*↓
PCOS (40)	Health	Saliva	16S rRNA	Actinobacteria↓
PCOS (38)	Health	Saliva	16S rRNA	Fusobacterium↑Actinobacteria↓
PCOS and gingivitis (23)	Gingivitis	Saliva	qPCR	*P.g*, *F.n* and *T.f*↑

GCF, gingival crevicular fluid; F.n, Fusobacterium nucleatum; PCOS, polycystic ovary syndrome; P.g, Porphyromonas gingivalis; P.m, Peptostreptococcus micros; qPCR, quantitative real-time polymerase chain reaction; T.d, Treponema denticola; T.f, Tannerella forsythia; *the trend for compared with control group. ↑, increased level; ↓, decreased level.

osteoprotegerin (OPG) and induces RANKL expression, which are responsible for bone resorption (57). Decreased levels of vitamin D and increased levels of parathyroid hormone (PTH) and calcitonin in PCOS promote bone resorption (57). These findings suggest that hormonal imbalance in PCOS might increase bone resorption and lead to the development of PDD.

3.3 PCOS increases infection susceptibility in soft tissues

Poor oral hygiene and the inflammation status of gingival tissues are closely related to periodontitis. Higher PI and gingival index (GI) are observed in PCOS patients than in healthy controls (31), which indicates poor oral hygiene and soft tissue inflammation in PCOS patients. Accumulated evidence demonstrated that periodontal tissues are hormone sensitive and that gonadal hormones modulate the periodontium including fibroblasts and the epithelium (60, 61). Hence, endocrine dysfunction in PCOS might impact the periodontium. The tissue specificity of sex steroid hormone mainly depends on the expression of specific hormone receptors (62). Extensive studies have supported the high expression of estrogen receptors in periodontal tissues. Estrogen receptors (ERs), but not progesterone, are highly expressed in gingival tissues of PCOS (63). During inflammation, estrogen receptors are expressed in gingival tissues by 10-fold than normal state (64). Meanwhile, androgen receptors have been detected in the nuclei of basal gingival epithelial cells and gingival fibroblasts (65). Collectively, androgens and estrogens are preferentially localized and retained in periodontal tissues (66). Moreover, inflammation increases the metabolism activity of androgens in gingival tissues (67–69). Testosterone can be metabolized to 5α-dihydrotestosterone, 4α-androstenedione, and 5α-androstanediols in human gingival fibroblasts *in vitro* (70). In addition, sex hormones also mediate the action of periodontal ligament fibroblasts, gingival fibroblasts, and epithelial cells in the gingiva. Estrogen plays an important role in maintaining the epithelial barrier in the periodontium (60). It has been reported that estrogen stimulates the proliferation and keratinization of gingival epithelium, increases the downgrowth of epithelial attachment, and accelerates the proliferation of fibroblasts (62). Decreased estrogen contributed to the thinning of oral mucosa through reducing the epithelial keratinization and collagen formation in connective tissues (71, 72). Progesterone, however, is not conducive to the repair and maintenance of the periodontium (60). Progesterone inhibited the proliferation of human gingival fibroblasts *ex vivo* (73). In addition, progesterone suppressed the collagen synthesis in periodontal ligament fibroblast (74). In summary, androgens might modulate the gingival tissues in PCOS by affinity for androgen receptors, elevation of androgen metabolism, and inhibition of fibroblast proliferation.

3.4 PCOS causes immune imbalance

Immune–endocrine interactions play an important role between PCOS and PDD. However, no study has directly explored the effects of PCOS-induced immune dysfunction on the development of PDD. PCOS-induced innate and adaptive immune imbalance might promote the pathogenic effects by periodontal pathogens. High levels of neutrophils and high ratio of neutrophil-to-lymphocyte in PCOS indicate low-grade inflammation status (75, 76). In addition, a shift from M2- to M1-polarized macrophage was observed in a dehydroepiandrosterone-induced mouse model of PCOS, which caused chronic inflammation (77). The increased M1/M2 ratio accelerated alveolar bone resorption in periodontal tissues (78). In addition, decreased natural killer (NK) cells and dendritic cells (DC) have been reported in PCOS women (79), which might enhance susceptibility to periodontitis (80). T lymphocytes play an important role in adaptive immune response, while the dysfunction of T lymphocytes might accelerate microorganism invasion. PCOS patients are characterized by Th1/Th2 imbalance and increase in $CD4^+CD28^-$ T cell and Th17/Treg ratio (79).

Sex hormones have been shown to influence the immune system in the periodontium. Increased immune cells were observed in oral and sulcular gingival epithelium during pregnancy (81). Androgens play an important role in adaptive immunity and innate inflammatory response and maintain the homeostasis of periodontal tissues (82). Furthermore, sex hormone disorders in PCOS have been shown to mediate the production of cytokines (62). E2 level was negatively correlated with interferon gamma (IFN-γ) level and positively correlated with PD-1 level in serum CD4+ and CD8+ T cells of infertile women with PCOS (83). This immune network is complicated and can be an interesting topic for future research.

3.5 PCOS elevates the systemic inflammation

PCOS is characterized by low-grade chronic inflammation, which is considered as a key contributor to the development of PDD. In fact, systemic inflammation in metabolic diseases creates a chronic inflammatory status for periodontal tissues (84). A large number of studies revealed that multiple inflammatory cytokines contributed to the interaction between PCOS and PDD, including IL-6, IL-17, and TNF-α. Reduced estrogen in PCOS increases the expression of proinflammatory cytokines, including IL-1, IL-6, IL-8, IL-10, TNF-α, and granulocyte colony-stimulating factor (GCSF), which creates an inflammatory microenvironment for the development of PDD (71, 72). In addition, a higher expression of matrix metalloproteinase (MMP)-8 was observed in the serum and saliva of women with comorbidity of PCOS and gingivitis compared with that in systemically healthy individuals with gingivitis (13). A positive correlation between MMP-8 levels and poor periodontal conditions including PD, BOP, and PI was detected in PCOS, which suggests a deteriorative role of PCOS for PDD (13). Similarly, elevated serum CRP level in PCOS reveals systemic inflammation and increases the risk of PDD (19, 33).

3.6 PCOS triggers oxidative stress

The imbalance of oxidation/antioxidant capacity contributes to oxidative damage, called oxidative stress (OS). The PCOS-induced OS

has an impact on gingival inflammation. A higher level of malondialdehyde (MDA) (a lipid peroxidation product indicating OS) in serum and GCF was detected in women with PCOS, which was positively correlated with gingival inflammation (18, 27). In addition, the contents of nitric oxide (NO) and myeloperoxidase (MPO) were higher in women with PCOS than in healthy women (14). Furthermore, women with PCOS and PDD exhibited higher serum levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), MPO, MDA, and lower total antioxidant status (TAS) than women with PCOS alone (25, 27). Hence, PCOS might enhance systemic lipid peroxidation and oxidative DNA damage and contribute to the development of PDD.

3.7 PCOS with obesity increases the expression of inflammation mediators

Epidemiological data showed that obesity affects 30%–70% PCOS women (85). Although not all morbidly obese women develop PCOS, adipose tissue is important for the development and maintenance of PCOS (85, 86). Adipokines including adiponectin leptin, resistin, visfatin, and retinol-binding protein 4 (RBP4) are mainly secreted by adipose tissues and involved in the glucolipid metabolism and IR (87). Disturbed expression of adipokines has been found in PCOS, which has an impact on the secretion of sex steroid, such as IR (87). A recent study identified a significantly elevated level of visfatin in GCF of women with comorbidity of PCOS and PDD, compared with that of women with PDD alone (30). In addition, more than 50 cytokines and inflammatory mediators are released from adipose tissues and responsible for the regulation of inflammation, glucose metabolism, and energy balance. Increased levels of CRP, IL-6, and TNF-α were detected in PCOS, which contributes to low-grade chronic inflammation and increases the risk of PDD (87). In summary, obesity in PCOS results in IR and low-grade chronic inflammation and accelerates PDD development through altering the expression of adipokines and inflammation mediators.

3.8 PCOS therapy mitigates periodontal inflammation

Porwal et al. (19) compared the morbidity in two groups of patients with PCOS receiving and not receiving drug treatment. A lower frequency of moderate periodontitis was observed in PCOS with the drug treatment group. Furthermore, the authors also evaluated the effect of drug treatment on periodontal clinical parameters and serum hsCRP levels in PCOS. Periodontal parameters including BOP, PD, and CAL were significantly improved by drug treatment. In addition, drug treatment caused lower serum level of hsCRP in PCOS. Interestingly, there were no statistical difference in the serum level of hsCRP between PCOS patients receiving drug treatment and healthy controls. The authors concluded that PCOS-induced systemic inflammatory responses might function as a pivotal role in the development of PDD. However, further studies are needed to focus on the impacts of drug therapy for PCOS on periodontal status based on before-and-after study in the same patient.

4 Potential mechanisms by which PDD might increase the risk of PCOS

4.1 PDD leads to low-grade inflammation and oxidative damage

PDD is characterized by chronic inflammation induced by the subgingival biofilm. Various studies have demonstrated that multiple proinflammatory cytokines and reactive oxygen species (ROS) are involved in the systemic effects of PDD on systemic diseases, such as DM, MS, and obesity (88). PDD increases the risk of PCOS by mediating the anti-inflammatory and proinflammatory pathways. Higher concentrations of IL-6 in GCF, saliva, and serum were found in PCOS women with gingivitis, compared with PCOS women with healthy periodontium (16). Another study found that the expression of anti-inflammatory cytokine IL-17E was decreased in PCOS women with gingivitis, compared with PCOS women with healthy periodontium (24). In addition, PDD increases the oxidative damage and induces OS. Dharuman et al. found higher levels of advanced oxidation protein products (AOPP, a marker of oxidative damage) in the serum and saliva of PCOS women accompanied with PDD, when compared with that of PCOS women with good periodontal heath (28) (Figure 2). Hence, PDD might function as a risk factor for PCOS through promoting inflammation and oxidative damage.

4.2 PDD promotes IR

It has been confirmed that IR is accompanied by chronic and low-grade inflammation (89). Several proinflammatory cytokines are induced by PDD, including IL-1, IL-6, and TNF-α, and play critical roles in the progression of IR. Periodontitis exacerbates IR (90) and impairs the host immune response (49), which finally contributes to the development of PCOS. Subgingival flora dysbiosis might function as an aggravating factor for IR by increasing the proteobacteria levels (51). In addition, high abundance of *P.g*, a major pathogen in periodontitis, can exacerbate IR (84). Enhanced IR was observed in high-fat-fed rats after injection with *P.g*, which reveals a positive relationship between *P.g* abundance and IR and bone loss (49). The outer membrane vesicles from *P.g* decreased the insulin sensitivity through delivering gingipains to the liver (91). Another study indicated that *P.g* promoted IR in high-fat-diet-fed mice by increasing plasma levels of branched-chain amino acids (BCAAs) (92). IR alters the oral microflora composition and *vice versa* (84). In addition, PDD impaired the β-cell function by downregulating the IL-12 levels and contributed to the development of DM (93, 94). The above findings suggest the possible harmful effects of periodontal pathogens on IR.

4.3 PDD induces dysbiosis of intestinal flora

Intestinal flora dysbiosis has an impact on IR and PCOS occurrence by regulating IL-22 (95). Mice transplanted with fecal microbiota from PCOS women were characterized by ovarian dysfunction, infertility, and

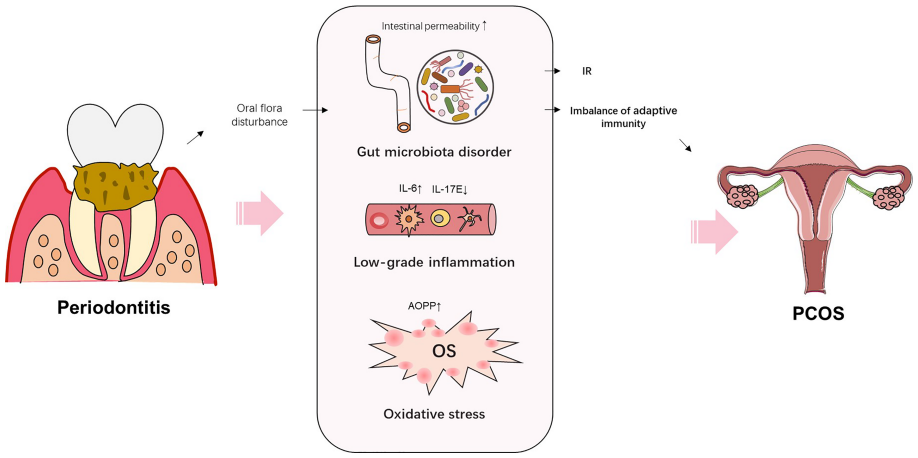


FIGURE 2
Schematic representation of the potential mechanisms by which periodontitis increases the risk of PCOS.

IR, which was similar to PCOS symptoms (95). Elevated abundance of *Bacteroides vulgatus* was identified in gut microbiota of PCOS women (95). In addition, the gut microbiota disturbance in PCOS participates in the alteration of host metabolism. The levels of glycodeoxycholic acid and tauroursodeoxycholic acid were decreased in the stool and serum of PCOS women (95). Another study demonstrated a low concentration of 5-hydroxyindoleacetic acid (5-HIAA) in the serum of PCOS women (96). Disturbance of the salivary microbiota in PDD leads to dysbiosis of the gut microbiota (97). The salivary microbes in periodontitis persists in the intestine and induces intestinal microbiota dysbiosis (97). The altered composition of the gut microbiota was characterized by the enrichment of *Porphyromonadaceae* and *F.n* in mice with severe periodontitis. In addition, transplantation of PDD-related salivary microbes into the colon could initiate the inflammation in the colon by upregulating the levels of proinflammatory cytokines and chemokines in mice (97). Administration of *P.g* for mice altered the composition and function of the intestinal microbiota and even increased the intestinal permeability (90). Both tryptophan and choline metabolisms play important roles in the *P.g*-induced MS (90). In addition, the alterations of serum metabolome markers (including 5-HIAA, indole-3-acetaldehyde, P-salicylic acid, and phosphatidylcholine) were observed in mice with *P.g* administration, which is closely associated with gut microbiota. Hence, periodontitis might render individuals more susceptible to PCOS through altering the intestinal microbiota and host metabolism and increasing intestinal permeability. However, further studies are needed to clarify the effects of PDD-induced oral microbiota disorder on PCOS.

4.4 Periodontal therapy improves PCOS

It is essential to investigate the impact of periodontal therapy on PCOS. Deepti et al. (33) evaluated the alteration of anthropometric parameters and metabolic and periodontal parameters in periodontitis patients with PCOS after periodontal therapy for 6 months. A combination of oral hygiene instructions (OHI) or scaling and root planing (SRP) with myo-inositol (MI) significantly decreased the serum level of hsCRP in PCOS women with periodontitis. Interestingly, PDD patients with PCOS receiving SRP and MI intervention exhibited the improved body mass index (BMI) compared with those receiving OHI and MI intervention. No statistical difference was observed in serum LH/FSH, testosterone, prolactin, HOMR, and lipid profiles in both groups at 6-month follow-up. In addition, the BMI and the modified Ferriman–Gallwey score (MFG), which assess hair growth, remained high in the two groups at 6-month follow-up when compared with healthy controls. The above evidence indicates that periodontal therapy alleviates PCOS by reducing the low-grade inflammation. Hence, good oral hygiene practices and regular oral health examination are recommended for women with PCOS.

5 Common risk factors for PCOS and PDD

5.1 Genetic/epigenetic predisposition

There is little evidence reporting the impact of genetic or epigenetic modification in both PDD and PCOS. Peroxisome-proliferator-activated receptor gamma (PPAR-γ), primarily expressed in adipose tissues, exerts an influence of insulin sensitivity through regulating glucolipid metabolism (98). Both PCOS and PDD have gene polymorphisms of PPAR-γ (88, 99), which might function as a cross-link between PDD and PCOS. In addition, epigenetic alteration occurs in both diseases to regulate transcriptional events without changing the DNA sequence. Decreased methylation levels of TNF-α, COX2, IFN-γ, and immune-related genes were found in PDD (100). There were increased methylation levels of PPARG, PPARGC1A, and CYP19A1 in adipose tissues, peripheral blood, and ovarian tissues, respectively, which regulate the ovarian functions in PCOS (101, 102). Future researchers should focus on the mechanism of epigenetic alterations for the association between PCOS and PDD.

5.2 Low socioeconomic status

Environmental factors have significant impact on the development of PCOS and PDD. Several studies have confirmed that women with low socioeconomic status have higher incidence of PCOS (103). Meanwhile, individuals with low socioeconomic status have a higher risk of moderate-to-severe periodontitis (104, 105). Individuals with lower socioeconomic status are prone to have adverse health behaviors, including smoking, sedentary lifestyles, poor oral hygiene, and poor nutritional diet (106). Food habits with high consumption of fatty and salty foods increase risk of PCOS and PDD (107, 108). Hence, women with PCOS or PDD should pay more attention to lifestyle modification, including quitting smoking and keeping healthy with exercise and a nutritious diet. More research is needed on the effect of lifestyle intervention on patients from a lower socioeconomic stratum, having either PCOS or PDD.

6 Conclusion

Multiple lines of evidence confirmed the bidirectional relationships between PCOS and PDD. Endocrine disorders, low-grade inflammation, immune imbalance, and OS in PCOS deteriorate the periodontal microenvironment, while PDD accelerates the development of low-grade inflammation, OS, and IR and increases the risk of PCOS. However, only a few intervention studies revealed the mechanisms underlying the causal relation between PCOS and PDD. In addition, both PCOS and PDD share common risk factors including genetic or epigenetic predisposition and low socioeconomic status. Further longitudinal studies are needed to elucidate the shared pathophysiology between PCOS and PDD.

Author contributions

YD, JX, DW, and JT conceived the study question, and all authors participated in the study design. JX created the first draft of the manuscript. HX, PZ, ZZ, and WF made substantial contributions to drafting the article. YD revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was funded by the Medical Science and Technology Research Foundation of Guangdong Province (No. B2021053) and Shenzhen People's Hospital for young and middle-aged Co-PI foster project (SYJCYJ202105).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

Publisher's note

References

1. Hart R. Polycystic ovarian syndrome–prognosis and treatment outcomes. *Curr Opin Obstet Gynecol* (2007) 19:529–35. doi: 10.1097/GCO.0b013e3282f10e22

2. Fauser BC, 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. doi: 10.1016/j.fertnstert.2011.09.024

3. Kim EH, Kim S, Kim HJ, Jeong HO, Lee J, Jang J, et al. Prediction of chronic periodontitis severity using machine learning models based on salivary bacterial copy number. *Front Cell Infect Microbiol* (2020) 10:571515. doi: 10.3389/fcimb.2020.571515

4. Memedovski Z, Czerwonka E, Han J, Mayer J, Luce M, Klemm LC, et al. Classical and alternative activation of rat microglia treated with ultrapure porphyromonas gingivalis lipopolysaccharide in vitro. *Toxins (Basel)* (2020) 12:333. doi: 10.3390/toxins12050333

5. Li Y, Lu Z, Zhang X, Yu H, Kirkwood KL, Lopes-Virella MF, et al. Metabolic syndrome exacerbates inflammation and bone loss in periodontitis. *J Dent Res* (2015) 94:362–70. doi: 10.1177/0022034514561658

6. Lim SS, Kakoly NS, Tan JWJ, Fitzgerald G, Bahri Khomami M, Joham AE, et al. Metabolic syndrome in polycystic ovary syndrome: a systematic review, meta-analysis and meta-regression. *Obes Rev* (2019) 20:339–52. doi: 10.1111/obr.12762

7. Jacewicz-Swiecka M, Kowalska I. Polycystic ovary syndrome and the risk of cardiometabolic complications in longitudinal studies. *Diabetes Metab Res Rev* (2018) 34:e3054. doi: 10.1002/dmrr.3054

8. Marquez-Arrico CF, Silvestre-Rangil J, Gutierrez-Castillo L, Martinez-Herrera M, Silvestre FJ, Rocha M. Association between periodontal diseases and polycystic ovary syndrome: A systematic review. *J Clin Med* (2020) 9:1586. doi: 10.3390/jcm9051586

9. Machado V, Escalda C, Proença L, Mendes JJ, Botelho J. Is there a bidirectional association between polycystic ovarian syndrome and periodontitis? a systematic review and meta-analysis. *J Clin Med* (2020) 9:1961. doi: 10.3390/jcm9061961

10. Kellesarian SV, Malignaggi VR, Kellesarian TV, Al-Kheraif AA, Alwageet MM, Malmstrom H, et al. Association between periodontal disease and polycystic ovary syndrome: a systematic review. *Int J Impot Res* (2017) 29:89–95. doi: 10.1038/ijir.2017.7

11. Tanguturi SC, Nagarakanti S. Polycystic ovary syndrome and periodontal disease: Underlying links- a review. *Indian J Endocrinol Metab* (2018) 22:267–73. doi: 10.4103/ijem.IJEM_577_17

12. Machado V, Botelho J, Proenca L, Mendes JJ. Comparisons of periodontal status between females referenced for fertility treatment and fertile counterparts: A pilot case-control study. *Int J Environ Res Public Health* (2020) 17:5281. doi: 10.3390/ijerph17155281

13. Akcali A, Bostanci N, Ozcaka O, Ozturk-Ceyhan B, Gumus P, Tervahartiala T, et al. Elevated matrix metalloproteinase-8 in saliva and serum in polycystic ovary syndrome and association with gingival inflammation. *Innate Immun* (2015) 21:619–25. doi: 10.1177/1753425915572172

14. Dursun E, Akalin FA, Guncu GN, Cinar N, Aksoy DY, Tozum TF, et al. Periodontal disease in polycystic ovary syndrome. *Fertil Steril* (2011) 95:320–3. doi: 10.1016/j.fertnstert.2010.07.1052

15. Greenhill C. Reproductive endocrinology: PCOS raises risk of periodontitis. *Nat Rev Endocrinol* (2010) 6:656–6. doi: 10.1038/nrendo.2010.184

16. Ozcaka O, Ceyhan BO, Akcali A, Bicakci N, Lappin DF, Buduneli N. Is there an interaction between polycystic ovary syndrome and gingival inflammation? *J Periodontol* (2012) 83:1529–37. doi: 10.1902/jop.2012.110588

17. Rahiminejad ME, Moaddab A, Zaryoun H, Rabiee S, Khodadoustan A. Comparison of prevalence of periodontal disease in women with polycystic ovary syndrome and healthy controls. *Dent Res J (Isfahan)* (2015) 12:507–12. doi: 10.4103/1735-3327.170547

18. Varadan M, Gopalkrishna P, Bhat PV, Kamath SU, Krithishree S, Thriveni GK, et al. Influence of polycystic ovary syndrome on the periodontal health of Indian women

visiting a secondary health care centre. *Clin Oral Investig* (2019) 23:3249–55. doi: 10.1007/s00784-018-2741-2

19. Porwal S, Tewari S, Sharma RK, Singhal SR, Narula SC. Periodontal status and high-sensitivity c-reactive protein levels in polycystic ovary syndrome with and without medical treatment. *J Periodontol* (2014) 85:1380–9. doi: 10.1902/jop.2014.130756

20. Tong C, Wang YH, Yu HC, Chang YC. Increased risk of polycystic ovary syndrome in Taiwanese women with chronic periodontitis: A nationwide population-based retrospective cohort study. *J Womens Health (Larchmt)* (2019) 28:1436–41. doi: 10.1089/jwh.2018.7648

21. Isik Y, Telatar GY, Neselioglu S, Bicer C, Gurlek B. Evaluation of periodontal status in different phenotypes of polycystic ovary syndrome in untreated patients of early reproductive age: A case-control study. *J Obstet Gynaecol Res* (2020) 46:459–65. doi: 10.1111/jog.14179

22. Wu P, Zhang X, Zhou P, Zhang W, Li D, Lv M, et al. Assessment of bidirectional relationships between polycystic ovary syndrome and periodontitis: Insights from a mendelian randomization analysis. *Front Genet* (2021) 12:644101. doi: 10.3389/fgene.2021.644101

23. Akcali A, Bostanci N, Ozcaka O, Ozturk-Ceyhan B, Gumus P, Buduneli N, et al. Association between polycystic ovary syndrome, oral microbiota and systemic antibody responses. *PloS One* (2014) 9:e108074. doi: 10.1371/journal.pone.0108074

24. Ozcaka O, Buduneli N, Ceyhan BO, Akcali A, Hannah V, Nile C, et al. Is interleukin-17 involved in the interaction between polycystic ovary syndrome and gingival inflammation? *J Periodontol* (2013) 84:1827–37. doi: 10.1902/jop.2013.120483

25. Akcali A, Bostanci N, Ozcaka O, Gumus P, Ozturk-Ceyhan B, Tervahartiala T, et al. Gingival inflammation and salivary or serum granulocyte-secreted enzymes in patients with polycystic ovary syndrome. *J Periodontol* (2017) 88:1145–52. doi: 10.1902/jop.2017.170043

26. T.R.E.A.-S.P. Consensus and W. Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod (Oxford, England)* (2004) 19:41–7. doi: 10.1093/humrep/deh098

27. Saglam E, Canakci CF, Sebin SO, Saruhan N, Ingec M, Canakci H, et al. Evaluation of oxidative status in patients with chronic periodontitis and polycystic ovary syndrome: A cross-sectional study. *J Periodontol* (2018) 89:76–84.

28. Dharuman S, Ajith Kumar S, Kanakasabapathy Balaji S, Vishwanath U, Parameshwari RP, Santhanakrishnan M. Evaluation of levels of advanced oxidative protein products in patients with polycystic ovary syndrome with and without chronic periodontitis: A cross-sectional study. *Int J Fertil Steril* (2022) 16:55–9.

29. Eke PI, Page RC, Wei L, Thornton-Evans G, Genco RJ. Update of the case definitions for population-based surveillance of periodontitis. *J Periodontol* (2012) 83:1449–54. doi: 10.1902/jop.2012.110664

30. Saljoughi F, Nasri K, Bayani M. Gingival crevicular fluid levels of visfatin in patients with chronic periodontitis and polycystic ovary syndrome. *Obstetr Gynecol Sci* (2020) 63:87. doi: 10.5468/ogs.2020.63.1.87

31. Zia A, Hakim S, Khan AU, Bey A, Ateeq H, Parveen S, et al. Bone markers and bone mineral density associates with periodontitis in females with poly-cystic ovarian syndrome. *J Bone Miner Metab* (2022) 40:487–97. doi: 10.1007/s00774-021-01302-6

32. Caton JG, Armitage G, Berglundh T, Chapple ILC, Jepsen S, Kornman KS, et al. A new classification scheme for periodontal and peri-implant diseases and conditions - introduction and key changes from the 1999 classification. *J Periodontol* (2018) 89 Suppl 1:S1–8.

33. Deepti, Tewari S, Narula SC, Singhal SR, Sharma RK. Effect of non-surgical periodontal therapy along with myo-inositol on high-sensitivity c-reactive protein and insulin resistance in women with polycystic ovary syndrome and chronic periodontitis: A randomized controlled trial. *J Periodontol* (2017) 88:999–1011. doi: 10.1902/jop.2017.170121

34. Page RC, Eke PI. Case definitions for use in population-based surveillance of periodontitis. *J Periodontol* (2007) 78:1387–99. doi: 10.1902/jop.2007.060264

35. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an androgen excess society guideline. *J Clin Endocrinol Metab* (2006) 91:4237–45. doi: 10.1210/jc.2006-0178

36. Freire M, Nelson KE, Edlund A. The oral host-microbial interactome: An ecological chronometer of health? *Trends Microbiol* (2020) 29:551–61. doi: 10.1016/j.tim.2020.11.004

37. Chen Y, Shi T, Li Y, Huang L, Yin D. Fusobacterium nucleatum: The opportunistic pathogen of periodontal and peri-implant diseases. *Front Microbiol* (2022) 13:860149. doi: 10.3389/fmicb.2022.860149

38. Li N, Li Y, Qian C, Liu Q, Cao W, Ma M, et al. Dysbiosis of the saliva microbiome in patients with polycystic ovary syndrome. *Front Cell Infect Microbiol* (2020) 10:624504. doi: 10.3389/fcimb.2020.624504

39. Ko Y, Lee EM, Park JC, Gu MB, Bak S, Ji S. Salivary microbiota in periodontal health and disease and their changes following nonsurgical periodontal treatment. *J Periodontal Implant Sci* (2020) 50:171–82. doi: 10.5051/jpis.2020.50.3.171

40. Lindheim L, Bashir M, Munzker J, Trummer C, Zachhuber V, Pieber TR, et al. The salivary microbiome in polycystic ovary syndrome (PCOS) and its association with disease-related parameters: A pilot study. *Front Microbiol* (2016) 7:1270. doi: 10.3389/fmicb.2016.01270

41. Gao L, Xu T, Huang G, Jiang S, Gu Y, Chen F. Oral microbiomes: more and more importance in oral cavity and whole body. *Protein Cell* (2018) 9:488–500. doi: 10.1007/s13238-018-0548-1

42. Wendland N, Opydo-Szymaczek J, Mizgier M, Jarzabek-Bielecka G. Subgingival microflora in adolescent females with polycystic ovary syndrome and its association with oral hygiene, gingivitis, and selected metabolic and hormonal parameters. *Clin Oral Investig* (2020) 25:1485–96. doi: 10.1007/s00784-020-03456-5

43. Matsha TE, Prince Y, Davids S, Chikte U, Erasmus RT, Kengne AP, et al. Oral microbiome signatures in diabetes mellitus and periodontal disease. *J Dent Res* (2020) 99:658–65. doi: 10.1177/0022034520913818

44. Henne K, Schilling H, Stoneking M, Conrads G, Horz HP. Sex-specific differences in the occurrence of fusobacterium nucleatum subspecies and fusobacterium periodonticum in the oral cavity. *Oncotarget* (2018) 9:20631–9. doi: 10.18632/oncotarget.25042

45. Mervish NA, Hu J, Hagan LA, Arora M, Frau C, Choi J, et al. Associations of the oral microbiota with obesity and menarche in inner city girls. *J Child Obes* (2019) 4:2.

46. Xiao E, Mattos M, Vieira GHA, Chen S, Correa JD, Wu Y, et al. Diabetes enhances IL-17 expression and alters the oral microbiome to increase its pathogenicity. *Cell Host Microbe* (2017) 22:120–128 e4. doi: 10.1016/j.chom.2017.06.014

47. Goodson JM, Groppo D, Halem S, Carpino E. Is obesity an oral bacterial disease? *J Dent Res* (2009) 88:519–23. doi: 10.1177/0022034509338353

48. Thackray VG. Sex, microbes, and polycystic ovary syndrome. *Trends Endocrinol Metab* (2019) 30:54–65. doi: 10.1016/j.tem.2018.11.001

49. Blasco-Baque V, Garidou L, Pomie C, Escoula Q, Loubieres P, Le Gall-David S, et al. Periodontitis induced by porphyromonas gingivalis drives periodontal microbiota dysbiosis and insulin resistance *via* an impaired adaptive immune response. *Gut* (2017) 66:872–85. doi: 10.1136/gutjnl-2015-309897

50. Zhao F, Dong T, Yuan KY, Wang NJ, Xia FZ, Liu D, et al. Shifts in the bacterial community of supragingival plaque associated with metabolic-associated fatty liver disease. *Front Cell Infect Microbiol* (2020) 10:581888. doi: 10.3389/fcimb.2020.581888

51. Demmer RT, Breskin A, Rosenbaum M, Zuk A, LeDuc C, Leibel R, et al. The subgingival microbiome, systemic inflammation and insulin resistance: The oral infections, glucose intolerance and insulin resistance study. *J Clin Periodontol* (2017) 44:255–65. doi: 10.1111/jcpe.12664

52. Ikeda E, Shiba T, Ikeda Y, Suda W, Nakasato A, Takeuchi Y, et al. Japanese Subgingival microbiota in health vs disease and their roles in predicted functions associated with periodontitis. *Odontology* (2020) 108:280–91. doi: 10.1007/s10266-019-00452-4

53. May A, Brandt BW, El-Kebir M, Klau GW, Zaura E, Crielaard W, et al. metaModules identifies key functional subnetworks in microbiome-related disease. *Bioinformatics* (2016) 32:1678–85. doi: 10.1093/bioinformatics/btv526

54. Cueno ME, Ochiai K. Re-discovering periodontal butyric acid: New insights on an old metabolite. *Microb Pathog* (2016) 94:48–53. doi: 10.1016/j.micpath.2015.10.006

55. Kurita-Ochiai T, Seto S, Suzuki N, Yamamoto M, Otsuka K, Abe K, et al. Butyric acid induces apoptosis in inflamed fibroblasts. *J Dent Res* (2008) 87:51–5. doi: 10.1177/154405910808700108

56. Chang MC, Tsai YL, Chen YW, Chan CP, Huang CF, Lan WC, et al. Butyrate induces reactive oxygen species production and affects cell cycle progression in human gingival fibroblasts. *J Periodontal Res* (2013) 48:66–73. doi: 10.1111/j.1600-0765.2012.01504.x

57. Krishnan A, Muthusami S. Hormonal alterations in PCOS and its influence on bone metabolism. *J Endocrinol* (2017) 232:R99–R113. doi: 10.1530/JOE-16-0405

58. Piovezan JM, Premaor MO, Comim FV. Negative impact of polycystic ovary syndrome on bone health: a systematic review and meta-analysis. *Hum Reprod Update* (2019) 25:633–45. doi: 10.1093/humupd/dmz020

59. Emmanuelle NE, Marie-Cecile V, Florence T, Jean-Francois A, Francoise L, Coralie F, et al. Critical role of estrogens on bone homeostasis in both Male and female: From physiology to medical implications. *Int J Mol Sci* (2021) 22:1568.

60. Guncu GN, Tozum TF, Caglayan F. Effects of endogenous sex hormones on the periodontium–review of literature. *Aust Dent J* (2005) 50:138–45. doi: 10.1111/j.1834-7819.2005.tb00352.x

61. Mealey BL, Moritz AJ. Hormonal influences: effects of diabetes mellitus and endogenous female sex steroid hormones on the periodontium. *Periodontol 2000* (2003) 32:59–81. doi: 10.1046/j.0906-6713.2002.03206.x

62. Mariotti A, Mawhinney M. Endocrinology of sex steroid hormones and cell dynamics in the periodontium. *Periodontol* (2013) 2000 61:69–88. doi: 10.1111/j.1600-0757.2011.00424.x

63. Asnani KP, Hingorani D, Kheur S, Deshmukh V, Romanos GE. Expression of nuclear receptors of gingiva in polycystic ovarian syndrome: a preliminary case study. *Aust Dent J* (2014) 59:252–7. doi: 10.1111/adj.12176

64. Staffolani N, Guerra M, Pugliese M, Cardinale G, Gulino A. Hormonal receptors in gingival inflammation. *Minerva Stomatol* (1989) 38:823–6.

65. Ojanotko-Harri A, Forssell H, Laine M, Hurttia H, Blauer M, Tuohimaa P. Immunohistochemical detection of androgen receptors in human oral mucosa. *Arch Oral Biol* (1992) 37:511–4. doi: 10.1016/0003-9969(92)90108-K

66. ElAttar TM, Hugoson A. The *in vitro* conversion of female sex steroid, oestrone, in normal and inflamed human gingiva. *Arch Oral Biol* (1974) 19:425–9. doi: 10.1016/0003-9969(74)90147-2

67. Vittek J, Rappaport SC, Gordon GG, Munnangi PR, Southren AL. Concentration of circulating hormones and metabolism of androgens by human gingiva. *J Periodontol* (1979) 50:254–64. doi: 10.1902/jop.1979.50.5.254

68. ElAttar TM. The *in vitro* conversion of male sex steroid, (1,2-3-H)-androstenedione in normal and inflamed human gingiva. *Arch Oral Biol* (1974) 19:1185–90. doi: 10.1016/0003-9969(74)90250-7

69. Ojanotko A, Nienstedt W, Harri MP. Metabolism of testosterone by human healthy and inflamed gingiva in vitro. *Arch Oral Biol* (1980) 25:481–4. doi: 10.1016/0003-9969(80)90055-2

70. Sooriyamoorthy M, Harvey W, Gower DB. The use of human gingival fibroblasts in culture for studying the effects of phenytoin on testosterone metabolism. *Arch Oral Biol* (1988) 33:353–9. doi: 10.1016/0003-9969(88)90069-6

71. Jafri Z, Bhardwaj A, Sawai M, Sultan N. Influence of female sex hormones on periodontium: A case series. *J Nat Sci Biol Med* (2015) 6:S146–9. doi: 10.4103/0976-9668.166124

72. Massler M. Oral manifestations during the female climacteric (the postmenopausal syndrome). *Oral Surg Oral Med Oral Pathol* (1951) 4:1234–43. doi: 10.1016/0030-4220(51)90081-3

73. Fukuda H. Experimental studies on the effect of sex hormones on the proliferation of cells derived from the gingival tissue in culture. *Shikwa Gakuho* (1971) 71:1214–32.

74. Thomson ME, Pack AR. Effects of extended systemic and topical folate supplementation on gingivitis of pregnancy. *J Clin Periodontol* (1982) 9:275–80. doi: 10.1111/j.1600-051X.1982.tb02067.x

75. Delano MJ, Kelly-Scumpia KM, Thayer TC, Winfield RD, Scumpia PO, Cuenca AG, et al. Neutrophil mobilization from the bone marrow during polymicrobial sepsis is dependent on CXCL12 signaling. *J Immunol* (2011) 187:911–8. doi: 10.4049/jimmunol.1100588

76. Herlihy AC, Kelly RE, Hogan JL, O'Connor N, Farah N, Turner MJ. Polycystic ovary syndrome and the peripheral blood white cell count. *J Obstet Gynaecol* (2011) 31:242–4. doi: 10.3109/01443615.2011.553693

77. Xie Q, Xiong X, Xiao N, He K, Chen M, Peng J, et al. Mesenchymal stem cells alleviate DHEA-induced polycystic ovary syndrome (PCOS) by inhibiting inflammation in mice. *Stem Cells Int* (2019) 2019:9782373. doi: 10.1155/2019/9782373

78. Sun X, Gao J, Meng X, Lu X, Zhang L, Chen R. Polarized macrophages in periodontitis: Characteristics, function, and molecular signaling. *Front Immunol* (2021) 12:763334. doi: 10.3389/fimmu.2021.763334

79. Luan YY, Zhang L, Peng YQ, Li YY, Liu RX, Yin CH. Immune regulation in polycystic ovary syndrome. *Clin Chim Acta* (2022) 531:265–72. doi: 10.1016/j.cca.2022.04.234

80. Xiao W, Dong G, Pacios S, Alnammary M, Barger LA, Wang Y, et al. FOXO1 deletion reduces dendritic cell function and enhances susceptibility to periodontitis. *Am J Pathol* (2015) 185:1085–93. doi: 10.1016/j.ajpath.2014.12.006

81. Raber-Durlacher JE, Leene W, Palmer-Bouva CC, Raber J, Abraham-Inpijn L. Experimental gingivitis during pregnancy and post-partum: immunohistochemical aspects. *J Periodontol* (1993) 64:211–8. doi: 10.1902/jop.1993.64.3.211

82. Shiau HJ, Reynolds MA. Sex differences in destructive periodontal disease: exploring the biologic basis. *J Periodontol* (2010) 81:1505–17. doi: 10.1902/jop.2010.100045

83. Li Z, Peng A, Feng Y, Zhang X, Liu F, Chen C, et al. Detection of T lymphocyte subsets and related functional molecules in follicular fluid of patients with polycystic ovary syndrome. *Sci Rep* (2019) 9:6040. doi: 10.1038/s41598-019-42631-x

84. Minty M, Canceil T, Serino M, Burcelin R, Terce F, Blasco-Baque V. Oral microbiota-induced periodontitis: a new risk factor of metabolic diseases. *Rev Endocr Metab Disord* (2019) 20:449–59. doi: 10.1007/s11154-019-09526-8

85. Vrbikova J, Hainer V. Obesity and polycystic ovary syndrome. *Obes Facts* (2009) 2:26–35. doi: 10.1159/000194971

86. Barber TM, McCarthy MI, Wass JA, Franks S. Obesity and polycystic ovary syndrome. *Clin Endocrinol (Oxf)* (2006) 65:137–45. doi: 10.1111/j.1365-2265.2006.02587.x

87. Spritzer PM, Lecke SB, Satler F, Morsch DM. Adipose tissue dysfunction, adipokines, and low-grade chronic inflammation in polycystic ovary syndrome. *Reproduction* (2015) 149:R219–27. doi: 10.1530/REP-14-0435

88. Jepsen S, Suvan J, Deschner J. The association of periodontal diseases with metabolic syndrome and obesity. *Periodontol* (2020) 2000 83:125–53. doi: 10.1111/prd.12326

89. Gurav AN. Periodontitis and insulin resistance: casual or causal relationship? *Diabetes Metab J* (2012) 36:404–11. doi: 10.4093/dmj.2012.36.6.404

90. Dong Z, Lv W, Zhang C, Chen S. Correlation analysis of gut microbiota and serum metabolome with porphyromonas gingivalis-induced metabolic disorders. *Front Cell Infect Microbiol* (2022) 12:858902. doi: 10.3389/fcimb.2022.858902

91. Seyama M, Yoshida K, Fujiwara N, Ono K, Eguchi T, Kawai H, et al. Outer membrane vesicles of porphyromonas gingivalis attenuate insulin sensitivity by delivering gingipains to the liver. *Biochim Biophys Acta Mol Basis Dis* (2020) 1866:165731. doi: 10.1016/j.bbadis.2020.165731

92. Tian J, Liu C, Zheng X, Jia X, Peng X, Yang R, et al. Porphyromonas gingivalis induces insulin resistance by increasing BCAA levels in mice. *J Dent Res* (2020) 99:839–46. doi: 10.1177/0022034520911037

93. Thouvenot K, Turpin T, Taile J, Clement K, Meilhac O, Gonthier MP. Links between insulin resistance and periodontal bacteria: Insights on molecular players and therapeutic potential of polyphenols. *Biomol* (2022) 12:378. doi: 10.3390/biom12030378

94. Liu Y, Zhang Q. Periodontitis aggravated pancreatic beta-cell dysfunction in diabetic mice through interleukin-12 regulation on klotho. *J Diabetes Investig* (2016) 7:303–11. doi: 10.1111/jdi.12410

95. Qi X, Yun C, Sun L, Xia J, Wu Q, Wang Y, et al. Gut microbiota-bile acid-interleukin-22 axis orchestrates polycystic ovary syndrome. *Nat Med* (2019) 25:1225–33. doi: 10.1038/s41591-019-0509-0

96. Shi X, Zhang L, Fu S, Li N. Co-Involvement of psychological and neurological abnormalities in infertility with polycystic ovarian syndrome. *Arch Gynecol Obstet* (2011) 284:773–8. doi: 10.1007/s00404-011-1947-1

97. Bao J, Li L, Zhang Y, Wang M, Chen F, Ge S, et al. Periodontitis may induce gut microbiota dysbiosis *via* salivary microbiota. *Int J Oral Sci* (2022) 14:32. doi: 10.1038/s41368-022-00183-3

98. Shaikh N, Mukherjee A, Shah N, Meherji P, Mukherjee S. Peroxisome proliferator activated receptor gamma gene variants influence susceptibility and insulin related traits in Indian women with polycystic ovary syndrome. *J Assist Reprod Genet* (2013) 30:913–21. doi: 10.1007/s10815-013-0025-y

99. Orio FJr., Matarese G, Di Biase S, Palomba S, Labella D, Sanna V, et al. Exon 6 and 2 peroxisome proliferator-activated receptor-gamma polymorphisms in polycystic ovary syndrome. *J Clin Endocrinol Metab* (2003) 88:5887–92. doi: 10.1210/jc.2002-021816

100. Suzuki S, Yamada S. Epigenetics in susceptibility, progression, and diagnosis of periodontitis. *Jpn Dent Sci Rev* (2022) 58:183–92. doi: 10.1016/j.jdsr.2022.06.001

101. Bruni V, Capozzi A, Lello S. The role of genetics, epigenetics and lifestyle in polycystic ovary syndrome development: the state of the art. *Reprod Sci* (2022) 29:668–79. doi: 10.1007/s43032-021-00515-4

102. Sagvekar P, Kumar P, Mangoli V, Desai S, Mukherjee S. DNA Methylome profiling of granulosa cells reveals altered methylation in genes regulating vital ovarian functions in polycystic ovary syndrome. *Clin Epigenet* (2019) 11:61. doi: 10.1186/s13148-019-0657-6

103. Merkin SS, Azziz R, Seeman T, Calderon-Margalit R, Daviglus M, Kiefe C, et al. Socioeconomic status and polycystic ovary syndrome. *J Womens Health (Larchmt)* (2011) 20:413–9. doi: 10.1089/jwh.2010.2303

104. Schuch HS, Nascimento GG, Peres KG, Mittinty MN, Demarco FF, Correa MB, et al. The controlled direct effect of early-life socioeconomic position on periodontitis in a birth cohort. *Am J Epidemiol* (2019) 188:1101–8. doi: 10.1093/aje/kwz054

105. Schuch HS, Peres KG, Singh A, Peres MA, Do LG. Socioeconomic position during life and periodontitis in adulthood: a systematic review. *Community Dent Oral Epidemiol* (2017) 45:201–8. doi: 10.1111/cdoe.12278

106. Barkley GS. Factors influencing health behaviors in the national health and nutritional examination survey, III (NHANES III). *Soc Work Health Care* (2008) 46:57–79. doi: 10.1300/J010v46n04_04

107. Iwasaki M, Manz MC, Moynihan P, Yoshihara A, Muramatsu K, Watanabe R, et al. Relationship between saturated fatty acids and periodontal disease. *J Dent Res* (2011) 90:861–7. doi: 10.1177/0022034511405384

108. Hajivandi L, Noroozi M, Mostafavi F, Ekramzadeh M. Food habits in overweight and obese adolescent girls with polycystic ovary syndrome (PCOS): a qualitative study in Iran. *BMC Pediatr* (2020) 20:277. doi: 10.1186/s12887-020-02173-y

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 15 February 2023
DOI 10.3389/fendo.2023.951099

OPEN ACCESS

EDITED BY
Yanting Wu,
Fudan University, China

REVIEWED BY
Ricardo Azziz,
University of Alabama at Birmingham, United States
Donna F. Vine,
University of Alberta, Canada

*CORRESPONDENCE
Licy L. Yanes Cardozo
lyanes@umc.edu

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

RECEIVED 13 June 2022
ACCEPTED 26 January 2023
PUBLISHED 15 February 2023

CITATION
Pruett JE, Romero DG and Yanes Cardozo LL (2023) Obesity-associated cardiometabolic complications in polycystic ovary syndrome: The potential role of sodium-glucose cotransporter-2 inhibitors. *Front. Endocrinol.* 14:951099. doi: 10.3389/fendo.2023.951099

Obesity-associated cardiometabolic complications in polycystic ovary syndrome: The potential role of sodium-glucose cotransporter-2 inhibitors

Jacob E. Pruett[1], Damian G. Romero[1,2,3,4] and Licy L. Yanes Cardozo[1,2,3,4,5]*

[1]Department of Cell and Molecular Biology, University of Mississippi Medical Center, Jackson, MS, United States, [2]Mississippi Center of Excellence in Perinatal Research, University of Mississippi Medical Center, Jackson, MS, United States, [3]Women's Health Research Center, University of Mississippi Medical Center, Jackson, MS, United States, [4]Cardiovascular-Renal Research Center, University of Mississippi Medical Center, Jackson, MS, United States, [5]Department of Medicine, University of Mississippi Medical Center, Jackson, MS, United States

Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder in reproductive-age women. PCOS is characterized by androgen excess, oligo/anovulation, and polycystic appearance of the ovaries. Women with PCOS have an increased prevalence of multiple cardiovascular risk factors such as insulin resistance, hypertension, renal injury, and obesity. Unfortunately, there is a lack of effective, evidence-based pharmacotherapeutics to target these cardiometabolic complications. Sodium-glucose cotransporter-2 (SGLT2) inhibitors provide cardiovascular protection in patients with and without type 2 diabetes mellitus. Although the exact mechanisms of how SGLT2 inhibitors confer cardiovascular protection remains unclear, numerous mechanistic hypotheses for this protection include modulation of the renin-angiotensin system and/or the sympathetic nervous system and improvement in mitochondrial function. Data from recent clinical trials and basic research show a potential role for SGLT2 inhibitors in treating obesity-associated cardiometabolic complications in PCOS. This narrative review discusses the mechanisms of the beneficial effect of SGLT2 inhibitors in cardiometabolic diseases in PCOS.

KEYWORDS

polycystic ovary syndrome (PCOS), obesity, SGLT 2 inhibitors, cardiometabolic complications, hypertension, insulin resistance, mitochondrial dysfunction, women's health

Introduction

Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder in reproductive-age women, affecting 5-20% of this population (1–3). While there has been evidence of women with PCOS since the time of Hippocrates (4), the syndrome was first described by Stein and Leventhal in 1935 in their article "Amenorrhea associated with bilateral polycystic ovaries" (5). Stein and Leventhal described a series of cases focusing on amenorrhea in women with polycystic ovaries as determined by bimanual pelvic exam and/or pneumoroentgenography (5). However, they also noted physical signs of androgen excess in most cases (5). Since the 1930s, our understanding of the disease has evolved. However, the etiology of PCOS remains unknown, and there are still many gaps in our knowledge and disagreements in the field, in part due to three coexisting sets of criteria for PCOS diagnosis (6–8).

PCOS, a diagnosis of exclusion, can be characterized by using three different sets of diagnostic criteria: the National Institutes of Health (NIH) 1990 criteria, the Rotterdam 2003 criteria, and the Androgen Excess-PCOS Society (AE-PCOS) 2006 criteria (6–8) (Figure 1). The NIH and AE-PCOS criteria consider androgen excess a requirement for diagnosis. Androgen excess can be defined as either biochemical or clinical evidence of high levels of androgens (6–8). Therefore, if a woman presents to the clinic with hirsutism (as defined by the Ferriman-Gallwey Scale) (9), excessive acne after puberty, or male-pattern balding, then measurement of serum androgens is not necessary for the PCOS diagnosis *per se*. However, biochemical testing, including testosterone levels, is required to eliminate other possible causes of androgen excess, as PCOS is a diagnosis of exclusion. Workup to exclude elevated prolactin, Cushing syndrome, non-classic congenital adrenal hyperplasia, thyroid dysfunction, androgen-producing tumors, and exogenous administration of androgens should be done before conferring the PCOS diagnosis (6–8). In particular, if total serum testosterone is above 200 ng/dL, if dehydroepiandrosterone sulfate is above 800 µg/dL, if signs of excess androgen progress rapidly, or if the voice changes, then the possibility of an androgen-producing tumor in the adrenal gland or ovary should be thoroughly investigated before diagnosing the patient with PCOS (10). Women with PCOS will have elevated levels of androgens, but they should not approach male levels (10, 11). The Rotterdam criteria, the most common criteria used in the clinic, requires the presence of two of the three PCOS' characteristics (Figure 1) generating 4 different phenotypes. As a result, there are women diagnosed with PCOS with and without hyperandrogenism. In recent years, several lines of evidence suggest that women with hyperandrogenic PCOS have worsened cardiovascular profiles (12). Unfortunately, there are no safe and effective therapeutic agents to decrease the levels or block the action of androgens in women. Furthermore, the heterogeneity of the clinical manifestation of PCOS suggests that the involvement of multiple pathophysiological pathways as suggested by GWAS studies (13, 14). Thereby, novel and effective therapeutic agents are needed for safe and effective PCOS clinical management.

Sodium-glucose cotransporter-2 (SGLT2) inhibitors provide cardiovascular protection in patients with and without type 2 diabetes mellitus (T2DM). Although the exact mechanisms of how SGLT2 inhibitors confer cardiovascular protection remains unclear, numerous mechanistic hypotheses have been postulated, including modulation of the renin-angiotensin system and/or the sympathetic nervous system and improvement in mitochondrial function. Data from recent clinical trials and basic research show a potential role for SGLT2 inhibitors in treating obesity-associated cardiometabolic complications targeting those abnormal pathophysiological systems in PCOS. This narrative review discusses the mechanisms of the beneficial effect of SGLT2 inhibitors in cardiometabolic diseases in PCOS.

Cardiovascular disease in PCOS

Cardiovascular disease is the number one cause of death among females in the United States (15). Unfortunately, PCOS is associated with multiple cardiovascular risk factors, such as obesity, hypertension, insulin resistance, dyslipidemia, and renal injury (16–19). While it is clear that women with PCOS have increased prevalence of cardiovascular risk factors, there is debate about whether or not these cardiovascular risk factors translate into increased cardiovascular events (20). In 2006, a study including ~11,000 women with PCOS from California (USA) showed no

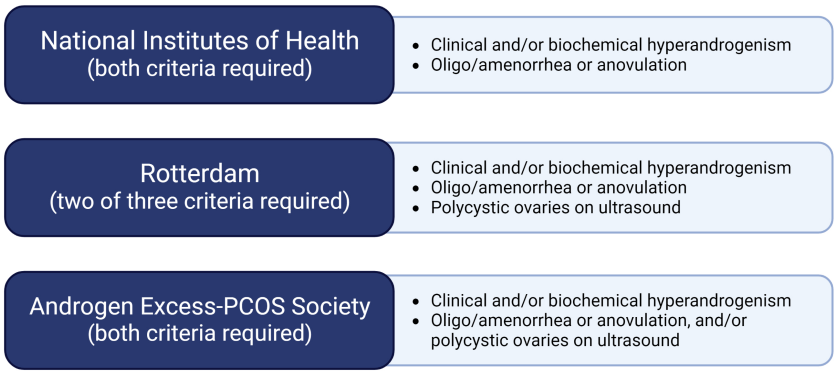


FIGURE 1
PCOS diagnosis criteria.

increased risk of coronary artery disease, cerebrovascular disease, or peripheral arterial disease in PCOS (17). Later, in 2015, a study including ~20,000 Danish women with PCOS also showed no increased risk of cardiovascular disease (21). However, when the same Danish population was reanalyzed broadening the definition of CVD to include hypertension and dyslipidemia, women with PCOS showed a ~2-fold increase in CVD events (22). The negative findings of the American and some of the Danish studies were in spite of both studies showing increased prevalence of cardiovascular risk factors, such as hypertension, dyslipidemia, and T2DM, in women with PCOS (17, 21). Conversely, in 2020, a meta-analysis showed that women with PCOS have an increased risk of cardiovascular disease and stroke (23). Furthermore, in 2021, a study including ~175,000 British women with PCOS demonstrated an increased risk for cardiovascular events, including myocardial infarction, angina, and revascularization in young women with PCOS (24). Participants were matched with controls for body mass index (BMI) on a 1:1 ratio in a total of 350,000 women, giving unprecedented strength to the work of Berni et al. (20, 24). Although there are multiple pharmacological agents used to manage the cardiometabolic complications in PCOS, their safety and effectiveness to prevent or ameliorate cardiovascular disease and mortality in PCOS are limited (25).

Although the etiology of the syndrome remains unknown, hyperandrogenemia may constitute a key mechanism underlying the cardiovascular risk factors in PCOS. We have demonstrated that hyperandrogenemia in female rats elicits several cardiovascular risk factors also present in women with PCOS (Figure 2). More recently, we demonstrated the potential benefit of SGLT2 inhibitors in body composition and blood pressure in such PCOS experimental model. Therefore, novel pharmacotherapies, such as sodium-glucose cotransporter-2 inhibitors, could simultaneously target multiple mechanisms of the pathophysiology of the cardiometabolic complications associated with PCOS.

SGLT2 inhibitors: Discovery and use beyond type 2 diabetes mellitus

Discovery of SGLT2 and its function

As the kidney filters blood, it must exercise precise, selective control over the reabsorption of electrolytes, carbohydrates, and amino acids while excreting waste products like urea. In the 1930s, with experiments from Shannon and Fisher, glucose in the mammalian nephron was shown to go "into reversible combination with some element in the tubule cells, present in constant but limited amount," and this mystery element was proposed to be the rate-limiting step of glucose reabsorption from the nephron (26). In the 1970s, Scriver et al. hypothesized that there might be a G1/G2 system to reabsorb hexoses in the kidney, with G1 having a low-capacity, and low affinity for glucose. In contrast, G2 would have a high-capacity, high affinity. Inspired by familial renal glucosuria, Scriver et al. also hypothesized that only the G1 system was present in the intestines while G2 was not (27). Experiments from Turner and Moran in the 1980s gave further evidence for two different sodium-dependent glucose transporters in the proximal tubule of the nephron (28). The G1 and G2 systems were later characterized as low-capacity, high-affinity, or high-capacity, low-affinity and were recognized as SGLT1 and SGLT2, respectively (29). SGLT2 expression was localized to the renal cortex, while SGLT1 was localized to the renal medulla and intestine (29, 30). As SGLT2 has a high capacity for glucose, it was later confirmed that SGLT2 is explicitly responsible for the vast majority of renal glucose reabsorption (31).

Discovery of SGLT2 inhibitors

By inhibiting SGLT2, blood glucose could be lowered independently of insulin *via* its glucosuric effect, thus minimizing the risk of hypoglycemia observed with other antidiabetic agents (32). However, there were multiple drawbacks to using phlorizin, an early SGLT2 inhibitor naturally found in apple trees (33, 34), such as poor absorption in the gut and its concurrent inhibition of SGLT1 (35). Lack of functioning SGLT1 can lead to diarrhea, as seen with hereditary glucose/galactose malabsorption, so the search began for selective and orally available drugs to inhibit SGLT2 (36). Out of this search came the burgeoning drug class of gliflozins, which were based on the structure of the o-glucoside phlorizin (37, 38). While some gliflozins, such as the o-glucoside sergliflozin, were not successful candidates for managing glucose homeostasis (37), many c-glucosides made it through phase III clinical trials, such as empagliflozin, dapagliflozin, and canagliflozin, which are the SGLT2 inhibitors that are widely used in clinical practice nowadays (38, 39).

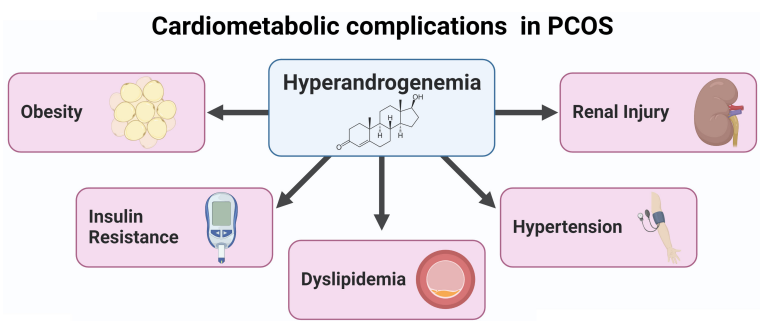


FIGURE 2
Cardiometabolic complications in PCOS.

SGLT2 inhibitors confer cardiovascular protection in patients with and without diabetes

Around the time that currently available SGLT2 inhibitors were progressing through clinical trials, the United States Food and Drug Administration (FDA) issued an additional requirement for phase III clinical trials of antidiabetic medications for T2DM (40). Because of evidence that thiazolidinediones, PPARγ agonists that act as insulin sensitizers (41), may increase cardiovascular risk in patients with T2DM even while improving glycemic control, the FDA began recommending that all new antidiabetic medications also needed to demonstrate cardiovascular safety (40, 42).

Intriguingly, with the landmark EMPA-REG study (43), empagliflozin was shown to reduce the risk of cardiovascular death in T2DM, becoming the first antidiabetic medicine proven to do so (40, 43). Additionally, EMPA-REG showed preservation of renal function with empagliflozin (44). Later with the CANVAS (45), DECLARE-TIMI 58 (46), and CREDENCE (47) trials, the SGLT2 inhibitors canagliflozin and dapagliflozin were also shown to decrease the risk of cardiovascular death and renal failure. These benefits have been proposed to be independent of changes in glycemic status (47) as the reduction in blood glucose with SGLT2 inhibition is modest (43, 45, 47), suggesting that the positive benefit of SGLT2 inhibitors could be possible in patients without overt T2DM. Additionally, in terms of side effects, there was no increased risk of hypoglycemia or acute kidney injury with SGLT2 inhibition (43, 45–47). However, there are some notable side effects of this drug class. SGLT2 inhibition does increase the risk of mycotic genital infection (43, 45–47). More rarely, SGLT2 inhibition also increases the risk of diabetic ketoacidosis (46, 47), in particular euglycemic diabetic ketoacidosis (48), often occurring with surgery or illness. Euglycemic diabetic ketoacidosis is a severe and life-threatening complication that can be overlooked by providers because of the normal range blood glucose in this condition, so this is an important consideration for anyone taking or prescribing gliflozins.

More recently, with multiple trials such as EMPEROR-Reduced (49), EMPEROR-Preserved (50), DAPA-HF (51), and DAPA-CKD (52), even in the absence of T2DM, SGLT2 inhibition benefited patients with chronic kidney disease and patients with heart failure (with either reduced or preserved ejection fraction). These findings implicated a potential role of SGLT2 inhibition in cardiovascular or renal disease for various conditions. However, exactly how SGLT2 inhibition produces this cardiovascular/renal protection independently of its effect on glycemia is uncertain and is an open question. There are multiple hypotheses concerning these mechanisms (53, 54).

SGLT2 inhibition in women with PCOS

PCOS is associated with insulin resistance, obesity, renal injury, mitochondrial dysfunction, and activation of both the Sympathetic Nervous System (SNS) and Renin-Angiotensin System (RAS). SGLT2 inhibitors have demonstrated improvements in all these disease states, suggesting they may be a promising novel therapy to improve women's healthcare in PCOS (Figure 3). Recently three small clinical trials used SGLT2 inhibitors to improve the cardiometabolic complications of patients with PCOS with exciting results (see summary in Table 1). In the trial by Javed et al., empagliflozin decreased body weight, body mass index, and fat mass in overweight women with PCOS compared to metformin (55). However, there was no decrease in insulin resistance or blood pressure, though it should be noted that the patients in this study had normal blood pressure at baseline (55). In the trial by Elkind-Hirsch et al., they explored if there were a synergistic effect between SGLT2 inhibition with dapagliflozin and glucagon-like peptide-1 receptor agonism (GLP-1RA) with exenatide in obese women with PCOS (56). Their data show that dapagliflozin and exenatide have an additive effect to further reduce body weight and fat mass than either drug individually can, which is likely due to their differing mechanisms of action. Combination therapy of SGLT2 and GLP-1RA could constitute a promising therapeutic tool to ameliorate cardiometabolic complications in PCOS women.

More recently, Tan et al. reported that licogliflozin decreased insulin resistance and circulating dehydroepiandrosterone sulfate (DHEAS) in women with PCOS, with a similar tendency in other circulating androgens (57). However, licogliflozin is not yet approved by the FDA and is more promiscuous than other SGLT2 inhibitors, having only a 30-fold selectivity for SGLT2 over SGLT1 (58), instead of the over 2,500-fold selectivity that empagliflozin has for SGLT2 (38). Higher-quality clinical trials are needed to better define the

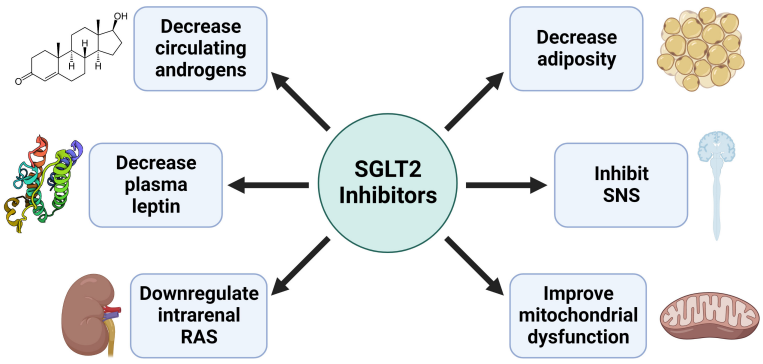


FIGURE 3
Potential mechanisms by which SGLT2 inhibitors improve the cardiometabolic complications in PCOS.

TABLE 1 Clinical trials of SGLT2 inhibitors in PCOS.

Trial	SGLT2 Inhibitor	Number of Participants	Duration of Trial	Findings
Javed et al. 2019 (55)	Empagliflozin	39	12 weeks	↓ Body weight, BMI, and fat mass
Elkind-Hirsch et al. 2021 (56)	Dapagliflozin	92	24 weeks	Additive effect in ↓ body weight and fat mass with GLP-1RA
Tan et al. 2021 (57)	Licogliflozin	20	2 weeks	↓ Insulin Resistance and DHEAS

↓, decrease.

potential role of selective SGLT2 inhibitors in treating the cardiometabolic complications in women with PCOS.

Insulin resistance and SGLT2 inhibition in PCOS

While not part of the diagnostic criteria for PCOS, insulin resistance is frequently present in lean and obese women with PCOS (16, 59). In PCOS patients using the sequential euglycemic insulin clamp technique, insulin infusion leads to elevations in testosterone (60). Mechanistically, insulin and insulin-like growth factors synergize with luteinizing hormone to increase androgen synthesis in ovarian theca cells (61). Furthermore, insulin decreases circulating sex hormone-binding globulin (SHBG) by decreasing its hepatic synthesis, leading to increased circulating free testosterone (61), which can bind and activate the androgen receptor. Moreover, testosterone can be reduced to 5α-dihydrotestosterone (DHT) in peripheral tissues to become the most potent endogenous agonist of the androgen receptor (62). However, short-term androgen administration in women also decreases insulin sensitivity, as demonstrated by both hyperglycemic and euglycemic hyperinsulinemic clamps (63). Furthermore, androgen administration in female rats leads to insulin resistance (64, 65). Thus, it is still unclear if insulin resistance causes androgen excess in women with PCOS or whether androgen excess leads to the constellation of cardiometabolic dysfunctions, including insulin resistance, in women with PCOS.

To ameliorate insulin resistance in PCOS, the first-line therapy for T2DM, metformin, is often used off-label (66, 67). However, there is an ongoing debate whether or not metformin is beneficial for insulin resistance in PCOS patients, as a recent meta-analysis showed that metformin had no impact on fasting blood glucose or insulin in overweight women with PCOS (68, 69). Small short-term clinical trials have shown that the FDA-approved anti-obesity glucagon-like peptide-1 receptor agonists (GLP-1 RAs) improve insulin resistance in PCOS patients (70) and may be superior to metformin (71). Therefore, there is a great need for new therapeutics targeting insulin resistance and T2DM in PCOS.

We previously demonstrated that hyperandrogenic female (HAF) experimental PCOS model generated with female Sprague Dawley rats chronically implanted with subcutaneous DHT pellets (7.5 mg/90 days) exhibit similar cardiometabolic complications to those in women with PCOS. The HAF rats exhibit increased food intake, obesity with an expansion of both subcutaneous and visceral fat depots, insulin resistance, and elevated blood pressure, that closely mimic the cardiometabolic complications in women with PCOS (65, 72). This review will focus on the HAF cardiometabolic complications; however, there are multiple animal experimental models of PCOS, each one with pros and cons to model the human disease as summarized in recent reviews (73, 74).

We recently demonstrated that SGLT2 inhibition did not improve insulin resistance in the HAF rats, but it did improve adiposity and elevated blood pressure, suggesting that this agent could exhibit cardioprotective effects in women with PCOS. Whether SGLT2 inhibitors could be used to prevent the progression of insulin resistance to diabetes in women with PCOS remains unknown.

Hypertension and SGLT2 inhibition in PCOS

The systemic renin-angiotensin system

Multiple studies have demonstrated an increased prevalence of hypertension in women with PCOS (17, 18). Moreover, blood pressure could be only mildly elevated as demonstrated in 24-hour ambulatory blood pressure measurements in women with PCOS (75). Obesity is associated with hypertension, but women with PCOS, even with normal weight, can have elevated blood pressure (76). The specific mechanism(s) that lead to elevated blood pressure or hypertension in PCOS remain a matter of debate.

One promising mechanism is dysregulation of the renin-angiotensin system (77, 78). The RAS plays a significant role in long-term blood pressure control, and it may lead to hypertension when inappropriately activated (79). The systemic RAS begins with the macula densa in the distal portion of the nephron (80). When it senses low sodium, the macula densa releases the enzyme renin from juxtaglomerular cells. Renin will then cleave angiotensinogen, released mainly from the liver, into angiotensin I (Ang I). Angiotensin-converting enzyme (ACE), which is expressed primarily in the lung, will then convert Ang I into angiotensin II (Ang II). Then, Ang II can bind to the Ang II Type 1 Receptor (AT1R) or the Ang II Type 2 Receptor (AT2R) (80, 81). Through AT1R, Ang II constricts the efferent arteriole, causing an increase in glomerular filtration rate to increase sodium delivery to the macula densa. Furthermore, Ang II stimulates aldosterone release by the adrenal gland *via* AT1R, which will increase sodium reabsorption by principal cells of the collecting duct of the nephron. Alternatively, *via* AT2R, Ang II causes vasodilation to decrease blood pressure. Furthermore,

Ang II can be converted by angiotensin-converting enzyme 2 (ACE2) into the heptapeptide angiotensin (1–7) that reduces blood pressure and insulin resistance *via* the Mas receptor (81, 82).

The intrarenal renin-angiotensin system

In addition to the systemic RAS, several tissue-specific renin-angiotensin systems exist, such as in the kidneys, heart, and adipose tissue (83). We will focus on the intrarenal RAS for this review as SGLT2, an essential player in this work, is predominantly expressed in the kidney but not in the heart or the adipose tissue (84). In the nephron, AT1R lines the lumen of the proximal tubule, distal tubule, and collecting duct (85) and can stimulate angiotensinogen synthesis in the proximal tubule with Ang II-activation (86). Renin is produced in the proximal tubule (87, 88), distal tubule (89), and principal cells of the collecting duct (89), allowing for angiotensin I to be produced within the nephron. Meanwhile, ACE is expressed along the brush border of the proximal tubule (90) and within cells of the collecting duct (91), allowing for Ang I to be produced within the nephron. When Ang II binds to the AT1R of the principal cells of the collecting duct, it increases the activity of epithelial sodium channels, which can further increase blood pressure (86, 91, 92). The detailed mechanism of the regulation of blood pressure by epithelial sodium channels can be found elsewhere (93). Thus, the intrarenal RAS appears to have a positive feedback loop where intratubular Ang II can lead to the formation of more intratubular Ang II, which can lead to increases in blood pressure (86).

Hypertension in PCOS: Targeting the renin-angiotensin system

The RAS, a central regulator of blood pressure, is modulated by androgens. Women with PCOS have dysregulation of the RAS with high circulating levels of renin (77, 94), the rate-limiting enzyme of the RAS (95). Plasma ACE2, which converts Ang II into the vasodilator angiotensin (1–7), is also decreased in women with PCOS (94). In a case series of four women with PCOS, treatment with telmisartan, an AT1R blocker, normalized blood pressure, reduced androgen levels, and improved the menstrual cycle (78). In a preclinical hyperandrogenemic female (HAF) rat model of PCOS, mRNA expression of renal angiotensinogen and ACE is increased (65). Furthermore, the ACE inhibitor enalapril reduces blood pressure in aged HAF rats more than in controls (96). However, compensatory alterations in the RAS have been shown in PCOS, as circulating angiotensinogen is decreased in women with PCOS (94). Moreover, intrarenal ACE2 is upregulated while intrarenal renin is downregulated in HAF rat model of PCOS (97). The upregulation of the ACE2 could be a protective mechanism to counteract the activation of the classical arm of the RAS; however, this hypothesis needs to be tested. Altogether, these data implicate the RAS is at least partially responsible for the increased blood pressure observed in PCOS. ACE inhibitors or AT1R blockers are widely used as antihypertensive drugs in the general population. However, due to their potential teratogenic, fetotoxic, and miscarriage-associated risks during pregnancy, ACE inhibitors and AT1R blockers are rarely used in the clinic in PCOS women of reproductive age (98–100). Thereby, agents that impact the RAS safely and effectively are needed to treat hypertension in women with PCOS.

SGLT2 and the renin-angiotensin system

RAS blockers are part of the standard of care for chronic kidney disease (both with and without diabetes mellitus) and heart failure. In landmark clinical trials showing cardiovascular and renal protection by SGLT2 inhibition, most patients were on some form of RAS blocker at baseline (43, 45–47, 49–52). In other words, the benefit of SGLT2 inhibition in clinical trials typically occurred on a background of RAS blockade. How might RAS blockade be working with SGLT2 inhibition, though?

As SGLT2 inhibition decreases sodium reabsorption in the proximal tubule, one would expect that there would be increased sodium delivery to the macula densa, thus reducing renin release and RAS activation. However, what has been found experimentally is more complex. Concerning the first part of the systemic RAS cascade, in a retrospective analysis of patients with hypertension and T2DM, no significant change was observed in plasma renin activity (PRA) with SGLT2 inhibition (101). Meanwhile, diabetic male mice treated with empagliflozin had decreased PRA; however, empagliflozin caused no change in PRA in control mice (102). Furthermore, a small observational study showed in diabetic patients that PRA was initially increased after one month of SGLT2 inhibition but returned to normal after three months of treatment (103). The variability observed in PRA with SGLT2 inhibition could be partly due to volume contraction from SGLT2 inhibition. The glucosuria from SGLT2 inhibition causes osmotic diuresis, which may decrease extracellular volume to trigger the release of renin (53). However, with time, the elevated antidiuretic hormone can compensate for the decrease in volume from SGLT2 inhibition (104), which may restore renin levels to normal.

Renin is far from the only component of the RAS reported to respond to SGLT2 inhibition, and the results are equally as mixed as those of renin. In a mouse model of T2DM, Woods et al. found that renal cortex angiotensinogen mRNA and protein expression was decreased by SGLT2 inhibition; however, renal ACE and AT1R mRNA expression was unchanged (105). Meanwhile, in a rat model of T2DM, Shin et al. found that AT1R protein expression was decreased in the renal cortex with SGLT2 inhibition (106). Furthermore, in diabetic Dahl salt-sensitive rats, SGLT2 inhibition was shown to work synergistically, instead of additively, with ACE inhibition to reduce blood pressure (107). Meanwhile, Bautista et al. found in male rats that Ang II increases renal SGLT2 independent of blood pressure changes and that inhibiting Ang II formation or AT1R decreases renal SGLT2 (108). Therefore, at least in male rodents, SGLT2 inhibitors and RAS blockade may work together synergistically, which may translate to the importance of patients having both types of pharmacotherapies to treat their cardiometabolic disease.

Recently, we reported that SGLT2 inhibition in HAF rats downregulates intrarenal ACE and AT1R mRNA, which was accompanied by a slight decrease in mean arterial pressure (97). However, intrarenal ACE2 mRNA, which is part of the vasodilatory

arm of the RAS, was also downregulated by SGLT2 inhibition in HAF rats (97). If the upregulation of the ACE2 in the kidney is a compensatory mechanism to combat the androgen deleterious effect, one can speculate that due to the beneficial effect of SGLT2 inhibitors, this is not further needed. These data suggest that SGLT2 inhibition could work synergistically with RAS blockers to reduce blood pressure in HAF rats, similar as in male rodents (107).

Sympathetic nervous system and SGLT2 in PCOS

Another possible mechanism for hypertension in women with PCOS is an activation of the SNS. Using heart rate variability to measure autonomic dysfunction, women with PCOS matched with controls for body mass index and blood pressure have increased SNS activity and decreased parasympathetic activity (109). Furthermore, adrenergic blockade with terazocin and propranolol in young HAF rats effectively decreases blood pressure (110). Renal denervation, which reduces sympathetic activity in the kidney, also reduces blood pressure in HAF rats and in women with PCOS (110, 111). The SNS may be activated in PCOS women and HAF rats because of elevations in the adipokine leptin in the circulation (65, 112). Leptin secretion by the adipose tissue is upregulated in obesity. When leptin is chronically elevated, it increases blood pressure by stimulating the sympathetic nervous system *via* melanocortin 4 receptor (MC4R) in pro-opiomelanocortin (POMC) neurons (113). Activation of the renal SNS can also activate the RAS (113) and promote sodium retention, actions that, if sustained over time, can increase blood pressure. Consequently, it is possible to speculate that adrenergic blockade could reduce RAS activation in PCOS. The detailed mechanism by which MC4R regulates blood pressure has been recently reviewed (114).

Adrenergic blockade is part of the standard of care in particular clinical conditions such as heart failure with reduced ejection fraction (115). To the best of our knowledge, randomized clinical trials with adrenergic blockade have not been yet performed in women with PCOS. However, while used to treat some individuals with essential hypertension, adrenergic blockade is not the gold standard, with some studies showing a lack of cardiovascular protection with this drug class (116, 117). Therefore, while the SNS appears to be upregulated in overweight or obese women with PCOS, whether or not direct adrenergic blockade would be beneficial in attenuating their hypertension is still unclear.

There are some evidence that SGLT2 inhibitors could target the SNS to exhibit their cardioprotective effects. Activation of the SNS leads to vasoconstriction and an increase in heart rate, leading to an increase in blood pressure (80). With the potential volume contraction from the osmotic diuresis caused by SGLT2 inhibition, one would not be surprised to observe a compensatory increase in heart rate. However, data from phase II/III clinical trials in patients with T2DM show that SGLT2 inhibition is associated with a decrease in heart rate (118, 119). Why might that be? A study by Herat and colleagues recently demonstrated in Schlager mice, a model of neurogenic hypertension with sympathetic activation, that SGLT2 inhibition decreases SNS innervation of the kidney, accompanied by a reduction of renal norepinephrine (120). However, precisely how SGLT2 regulates renal SNS is uncertain. A recent meta-analysis in patients with T2DM demonstrated that SGLT2 inhibition was associated with decreased circulating leptin (121), and we recently found that SGLT2 inhibition decreases plasma leptin in HAF rats (97). As leptin is known to stimulate the renal SNS, reduced circulating leptin may contribute to how SGLT2 regulates the renal SNS.

Mitochondrial dysfunction and SGLT2 inhibitors in PCOS

The mitochondrion is an essential organelle for eukaryotic organisms. It is known as "the powerhouse of the cell" because it conducts oxidative phosphorylation, a process necessary to generate enough energy for complex organisms to function (80). Mitochondrial dysfunction can be defined as when mitochondria cannot provide ATP for the cell while minimizing an overflow of naturally formed reactive oxygen species (ROS) from damaging the rest of the cell (122, 123). Methods to measure ROS in humans or animals include assessing total antioxidant capacity in serum or measuring markers of oxidative damage, such as lipid peroxidation, through 2-thiobarbituric acid reactive substances assay (124). Mitochondrial dysfunction has been linked to diabetes (122, 125), metabolic syndrome (125), heart failure (126), chronic kidney disease (127), and PCOS (128).

Women with PCOS have decreased mitochondrial DNA, a marker of mitochondrial content or volume, in circulating leukocytes (129, 130). Decreased inner mitochondrial membrane potential, altered mitochondrial structure, and increased ROS have also been demonstrated in the oocytes of a PCOS mouse model (131). Furthermore, lean women with PCOS have decreased circulating total antioxidant capacity and increased malondialdehyde, indicating increased oxidative stress (59). The mitochondrion is an organizing center for cellular metabolism (80), so there are significant implications for insulin resistance and obesity in PCOS. Additionally, excess oxidative stress can lead to inflammation, further worsening insulin resistance, obesity, and blood pressure in PCOS (132, 133). Therefore, targeting mitochondrial dysfunction may be a promising therapeutic avenue in patients with PCOS. Yilmaz et al. demonstrated in lean women with PCOS that rosiglitazone, a peroxisome proliferator-activated receptor-γ (PPARγ) agonist that stimulates mitochondrial biogenesis (41), increases circulating total antioxidant capacity, decreases circulating malondialdehyde, and decreases insulin resistance (59). However, PPARγ has multiple functions outside of stimulating mitochondrial biogenesis (134), so it is uncertain if specifically increasing mitochondrial content improves these parameters in women with PCOS. Furthermore, body mass index increases with the thiazolidinedione rosiglitazone in women with PCOS (59). However, there are concerns about thiazolidinediones and cardiovascular risk (42), limiting excitement for using PPARγ agonists in women with PCOS. Exploring the potential role of other pharmacotherapeutics that improve mitochondrial function, such as SGLT2 inhibitors (105, 135–138), is a promising new direction in the field.

SGLT2 inhibition has been found to improve mitochondrial function in various ways. In the heart of male rodents with T2DM, SGLT2 inhibition decreases ROS production (135) and also increases the expression of nuclear respiratory factor 1 (NRF1) and PPARγ coactivator 1-α (PGC1α) (136), which positively regulate mitochondrial biogenesis (134). SGLT2 inhibition also decreases markers of oxidative stress in the blood while increasing the activity of the antioxidant enzyme superoxide dismutase (136). Meanwhile, in the kidney of male rodents with T2DM, SGLT2 inhibition normalizes mitochondrial morphology (preventing mitochondria from becoming excessively round or fragmented) while decreasing urinary 8-isoprostane and 8-hydroxydeoxyguanosine, which are markers of oxidative stress (105, 137). In white adipose tissue of male rodents with T2DM, SGLT2 inhibition similarly increased the expression of NRF1 and PGC1α as it did in the heart, which was associated with an increase in mitochondrial DNA, a marker of mitochondrial content (136, 138). The diseases currently indicated for SGLT2 inhibitor use, such as T2DM, heart failure, and chronic kidney disease, have been linked to mitochondrial dysfunction (125–127), so improving mitochondrial function may be an essential pathway for the beneficial effects seen in patients on SGLT2 inhibitors. However, more studies are needed to explore the therapeutic potential of SGLT2 inhibition on mitochondrial dysfunction in women, especially those with PCOS. We recently demonstrated that hyperandrogenemia in the HAF rat model of PCOS causes the expansion of white adipose tissue, which is associated with decreases in mitochondrial content and function in both subcutaneous and visceral adipose tissue (139). Treatment with SGLT2 inhibitors increased the frequency of small adipocytes in visceral adipose tissue without affecting mitochondrial dysfunction in white adipose tissue, oxidative stress, or insulin resistance in the HAF rat model (139). Our study suggests that targeting mitochondrial dysfunction in PCOS may be necessary to improve insulin resistance and that hyperandrogenemia blunts the beneficial effect of SGLT2 inhibitors in the HAF rat model of PCOS.

Perspective and clinical implications

PCOS is the most common endocrine disorder in reproductive-age women (1–3). Patients with PCOS have an increased incidence of major adverse cardiovascular events (24), likely driven by the increased incidence of cardiovascular risk factors in this population, such as hypertension, insulin resistance, renal injury, and obesity (16–19) (Figure 2). Unfortunately, there is a lack of effective, evidence-based pharmacotherapeutics targeted at cardiometabolic disease (25). Meanwhile, SGLT2 inhibitors have been rapidly expanding their clinical indications because of their cardiovascular protection in patients with and without T2DM (43, 47, 51, 52). However, whether and exactly how SGLT2 inhibitors confer cardiovascular protection in PCOS women, with and without diabetes remains to be elucidated pending high quality large clinical trials. Limited clinical data have suggested that women with PCOS have renal and cardiac target organ injury (140). Moreover, a recent study has shown that women with PCOS have a higher risk of preeclampsia/eclampsia, peripartum cardiomyopathy, and heart failure during hospitalizations for delivery (141). Women with PCOS who have increased cardiovascular risk factors as the conditions mentioned above could benefit from SGLT2 inhibitors, pending confirmation with clinical trials. The numerous mechanistic hypotheses for cardiovascular protection include deactivation of the RAS and/or the SNS as well as improvement in mitochondrial function, all of which are abnormal in women with PCOS. Data from recent small clinical trials and basic research show promise for SGLT2 inhibitors in treating some of the cardiometabolic complications in PCOS.

Author contributions

JP and LY drafted the manuscript. JP, DR, and LY reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by National Institutes of Health National Institute of General Medical Sciences grant P20GM121334 (LY and DR) and National Institute of Diabetes and Digestive and Kidney Diseases grant R21DK113500 (DR). JP was supported by National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases fellowship F30DK127527. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Acknowledgments

Figures generated with BioRender.com.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Bozdag G, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO. The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reprod* (2016) 31(12):2841–55. doi: 10.1093/humrep/dew218

2. March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Hum Reprod* (2010) 25(2):544–51. doi: 10.1093/humrep/dep399

3. Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R. Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern united states: a prospective study. *J Clin Endocrinol Metab* (1998) 83 (9):3078–82. doi: 10.1210/jc.83.9.3078

4. Hippocrates. *The aphorisms of Hippocrates: With a translation into Latin and English*. (1822) Coar T, editor. London: Longman p. 140.

5. Stein IF. Amenorrhea associated with bilateral polycystic ovaries. *Am J Obstet Gynecol* (1935) 29:181–91. doi: 10.1016/S0002-9378(15)30642-6

6. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum reproduction* (2004) 19(1):41–7. doi: 10.1093/humrep/deh098

7. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. Criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an androgen excess society guideline. *J Clin Endocrinol Metab* (2006) 91(11):4237–45. doi: 10.1210/jc.2006-0178

8. Zawadski JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif A, Givens JR, Haseltine FP, Merrian GR editors. *Polycystic ovary syndrome*. Boston: Blackwell Scientific Publications (1992). p. 377–84.

9. Bode DV, Seehusen DA, Baird D. Hirsutism in women. *Am Fam Physician* (2012) 85(4):373–80.

10. Sheehan MT. Polycystic ovarian syndrome: diagnosis and management. *Clin Med Res* (2004) 2(1):13–27. doi: 10.3121/cmr.2.1.13

11. Travison TG, Vesper HW, Orwoll E, Wu F, Kaufman JM, Wang Y, et al. Harmonized reference ranges for circulating testosterone levels in men of four cohort studies in the united states and Europe. *J Clin Endocrinol Metab* (2017) 102(4):1161–73. doi: 10.1210/jc.2016-2935

12. Yanes Cardozo LL, Romero DG, Reckelhoff JF. Cardiometabolic features of polycystic ovary syndrome: role of androgens. *Physiology* (2017) 32(5):357–66. doi: 10.1152/physiol.00030.2016

13. Dapas M, Lin FT, Nadkarni GN, Sisk R, Legro RS, Urbanek M, et al. Distinct subtypes of polycystic ovary syndrome with novel genetic associations: An unsupervised, phenotypic clustering analysis. *PLoS Med* (2020) 17(6):e1003132. doi: 10.1371/journal.pmed.1003132

14. Day F, Karaderi T, Jones MR, Meun C, He C, Drong A, et al. Large-Scale genome-wide meta-analysis of polycystic ovary syndrome suggests shared genetic architecture for different diagnosis criteria. *PLoS Genet* (2018) 14(12):e1007813. doi: 10.1371/journal.pgen.1007813

15. Heron M. Deaths: Leading causes for 2019. *Natl Vital Stat Reports: Natl Center Health Stat* (2021) 70(9):1–114. doi: 10.15620/cdc:104186

16. Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS. Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. *Endocr Rev* (2015) 36(5):487–525. doi: 10.1210/er.2015-1018

17. Lo JC, Feigenbaum SL, Yang J, Pressman AR, Selby JV, Go AS. Epidemiology and adverse cardiovascular risk profile of diagnosed polycystic ovary syndrome. *J Clin Endocrinol Metab* (2006) 91(4):1357–63. doi: 10.1210/jc.2005-2430

18. Elting MW, Korsen TJ, Bezemer PD, Schoemaker J. Prevalence of diabetes mellitus, hypertension and cardiac complaints in a follow-up study of a Dutch PCOS population. *Hum Reprod* (2001) 16(3):556–60. doi: 10.1093/humrep/16.3.556

19. Song Y, Ye W, Ye H, Xie T, Shen W, Zhou L. Serum testosterone acts as a prognostic indicator in polycystic ovary syndrome-associated kidney injury. *Physiol Rep* (2019) 7(16):e14219. doi: 10.14814/phy2.14219

20. Romero DG, Yanes Cardozo LL. Cardiovascular events in polycystic ovary syndrome: Is the debate settled for good? *J Clin Endocrinol Metab* (2021) 106(12): e5258–e9. doi: 10.1210/clinem/dgab509

21. Glintborg D, Hass Rubin K, Nybo M, Abrahamsen B, Andersen M. Morbidity and medicine prescriptions in a nationwide Danish population of patients diagnosed with polycystic ovary syndrome. *Eur J Endocrinol* (2015) 172(5):627–38. doi: 10.1530/EJE-14-1108

22. Glintborg D, Rubin KH, Nybo M, Abrahamsen B, Andersen M. Cardiovascular disease in a nationwide population of Danish women with polycystic ovary syndrome. *Cardiovasc diabetology* (2018) 17(1):1–12. doi: 10.1186/s12933-018-0680-5

23. Zhang J, Xu J-H, Qu Q-Q, Zhong G-Q. Risk of cardiovascular and cerebrovascular events in polycystic ovarian syndrome women: a meta-analysis of cohort studies. *Front Cardiovasc Med* (2020) 7:552421. doi: 10.3389/fcvm.2020.552421

24. Berni TR, Morgan CL, Rees DA. Women with polycystic ovary syndrome have an increased risk of major cardiovascular events: a population study. *J Clin Endocrinol Metab* (2021) 106(9):e3369–e80. doi: 10.1210/clinem/dgab392

25. Yanes Cardozo LL, Romero DG. Management of cardiometabolic complications in polycystic ovary syndrome: Unmet needs. *FASEB J* (2021) 35(11):e21945. doi: 10.1096/fj.202002526RR

26. Shannon JA, Fisher S. The renal tubular reabsorption of glucose in the normal dog. *Am J Physiology-Legacy Content* (1938) 122(3):765–74. doi: 10.1152/ajplegacy.1938.122.3.765

27. Scriver CR, Chesney RW, McInnes RR. Genetic aspects of renal tubular transport: diversity and topology of carriers. *Kidney Int* (1976) 9(2):149–71. doi: 10.1038/ki.1976.18

28. Turner RJ, Moran A. Heterogeneity of sodium-dependent d-glucose transport sites along the proximal tubule: evidence from vesicle studies. *Am J Physiology-Renal Physiol* (1982) 242(4):F406–F14. doi: 10.1152/ajprenal.1982.242.4.F406

29. You G, Lee W-S, Barros EJ, Kanai Y, Huo T-L, Khawaja S, et al. Molecular characteristics of na+-coupled glucose transporters in adult and embryonic rat kidney. *J Biol Chem* (1995) 270(49):29365–71. doi: 10.1074/jbc.270.49.29365

30. Hediger M, Kanai Y, You G, Nussberger S. Mammalian ion-coupled solute transporters. *J Physiol* (1995) 482(suppl):7–17. doi: 10.1113/jphysiol.1995.sp020559

31. Vallon V, Platt KA, Cunard R, Schroth J, Whaley J, Thomson SC, et al. SGLT2 mediates glucose reabsorption in the early proximal tubule. *J Am Soc Nephrology* (2011) 22(1):104–12. doi: 10.1681/ASN.2010030246

32. Filippas-Ntekouan S, Filippatos TD, Elisaf MS. SGLT2 inhibitors: are they safe? *Postgrad Med* (2018) 130(1):72–82. doi: 10.1080/00325481.2018.1394152

33. Ehrenkranz JR, Lewis NG, Ronald Kahn C, Roth J. Phlorizin: a review. *Diabetes Metab Res Rev* (2005) 21(1):31–8. doi: 10.1002/dmrr.532

34. Rieg T, Vallon V. Development of SGLT1 and SGLT2 inhibitors. *Diabetologia* (2018) 61(10):2079–86. doi: 10.1007/s00125-018-4654-7

35. Pajor AM, Randolph KM, Kerner SA, Smith CD. Inhibitor binding in the human renal low- and high-affinity na+/glucose cotransporters. *J Pharmacol Exp Ther* (2008) 324:985–91. doi: 10.1124/jpet.107.129825

36. Turk E, Martín MG, Wright EM. Structure of the human na+/glucose cotransporter gene SGLT1. *J Biol Chem* (1994) 269:15204–9. doi: 10.1016/S0021-9258(17)36592-4

37. White JR. Apple trees to sodium glucose co-transporter inhibitors: a review of SGLT2 inhibition. *Clin Diabetes* (2010) 28(1):5–10. doi: 10.2337/diaclin.28.1.5

38. Grempler R, Thomas L, Eckhardt M, Himmelsbach F, Sauer A, Sharp D, et al. Empagliflozin, a novel selective sodium glucose cotransporter-2 (SGLT-2) inhibitor: characterisation and comparison with other SGLT-2 inhibitors. *Diabetes Obes Metab* (2012) 14(1):83–90. doi: 10.1111/j.1463-1326.2011.01517.x

39. Braunwald E. SGLT2 inhibitors: the statins of the 21st century. *Eur Heart J* (2022) 43(11):1029–30. doi: 10.1093/eurheartj/ehab765

40. Smith RJ, Goldfine AB, Hiatt WR. Evaluating the cardiovascular safety of new medications for type 2 diabetes: time to reassess? *Diabetes Care* (2016) 39(5):738–42. doi: 10.2337/dc15-2237

41. Bogacka I, Xie H, Bray GA, Smith SR. Pioglitazone induces mitochondrial biogenesis in human subcutaneous adipose tissue in vivo. *Diabetes* (2005) 54(5):1392–9. doi: 10.2337/diabetes.54.5.1392

42. Rosen CJ. The rosiglitazone story–lessons from an FDA advisory committee meeting. *New Engl J Med* (2007) 357(9):844–6. doi: 10.1056/NEJMp078167

43. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *New Engl J Med* (2015) 373(22):2117–28. doi: 10.1056/NEJMoa1504720

44. Wanner C, Inzucchi SE, Lachin JM, Fitchett D, von Eynatten M, Mattheus M, et al. Empagliflozin and progression of kidney disease in type 2 diabetes. *New Engl J Med* (2016) 375(4):323–34. doi: 10.1056/NEJMoa1515920

45. Neal B, Perkovic V, Mahaffey KW, De Zeeuw D, Fulcher G, Erondu N, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *New Engl J Med* (2017) 377(7):644–57. doi: 10.1056/NEJMoa1611925

46. Wiviott SD, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A, et al. Dapagliflozin and cardiovascular outcomes in type 2 diabetes. *New Engl J Med* (2019) 380(4):347–57. doi: 10.1056/NEJMoa1812389

47. Perkovic V, Jardine MJ, Neal B, Bompoint S, Heerspink HJ, Charytan DM, et al. Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. *New Engl J Med* (2019) 380(24):2295–306. doi: 10.1056/NEJMoa1811744

48. Blau JE, Tella SH, Taylor SI, Rother KI. Ketoacidosis associated with SGLT2 inhibitor treatment: analysis of FAERS data. *Diabetes Metab Res Rev* (2017) 33(8):e2924. doi: 10.1002/dmrr.2924

49. Packer M, Anker SD, Butler J, Filippatos G, Pocock SJ, Carson P, et al. Cardiovascular and renal outcomes with empagliflozin in heart failure. *New Engl J Med* (2020) 383(15):1413–24. doi: 10.1056/NEJMoa2022190

50. Anker SD, Butler J, Filippatos G, Ferreira JP, Bocchi E, Böhm M, et al. Empagliflozin in heart failure with a preserved ejection fraction. *New Engl J Med* (2021) 385(16):1451–61. doi: 10.1056/NEJMoa2107038

51. McMurray JJ, Solomon SD, Inzucchi SE, Køber L, Kosiborod MN, Martinez FA, et al. Dapagliflozin in patients with heart failure and reduced ejection fraction. *New Engl J Med* (2019) 381(21):1995–2008. doi: 10.1056/NEJMoa1911303

52. Heerspink HJ, Stefánsson BV, Correa-Rotter R, Chertow GM, Greene T, Hou F-F, et al. Dapagliflozin in patients with chronic kidney disease. *New Engl J Med* (2020) 383 (15):1436–46. doi: 10.1056/NEJMoa2024816

53. Vallon V, Verma S. Effects of SGLT2 inhibitors on kidney and cardiovascular function. *Annu Rev Physiol* (2021) 83:503–28. doi: 10.1146/annurev-physiol-031620-095920

54. Filippatos TD, Liontos A, Papakitsou I, Elisaf MS. SGLT2 inhibitors and cardioprotection: a matter of debate and multiple hypotheses. *Postgraduate Med* (2019) 131(2):82–8. doi: 10.1080/00325481.2019.1581971

55. Javed Z, Papageorgiou M, Deshmukh H, Rigby AS, Qamar U, Abbas J, et al. Effects of empagliflozin on metabolic parameters in polycystic ovary syndrome: A randomized controlled study. *Clin Endocrinol (Oxf)* (2019) 90(6):805–13. doi: 10.1111/cen.13968

56. Elkind-Hirsch KE, Chappell N, Seidemann E, Storment J, Bellanger D. Exenatide, dapagliflozin, or Phentermine/Topiramate differentially affect metabolic profiles in polycystic ovary syndrome. *J Clin Endocrinol Metab* (2021) 106(10):3019–33. doi: 10.1210/clinem/dgab408

57. Tan S, Ignatenko S, Wagner F, Dokras A, Seufert J, Zwanziger D, et al. Licogliflozin versus placebo in women with polycystic ovary syndrome: A randomized, double-blind, phase 2 trial. *Diabetes Obes Metab* (2021) 23(11):2595–9. doi: 10.1111/dom.14495

58. Bays HE, Kozlovski P, Shao Q, Proot P, Keefe D. Licogliflozin, a novel SGLT1 and 2 inhibitor: body weight effects in a randomized trial in adults with overweight or obesity. *Obesity* (2020) 28(5):870–81. doi: 10.1002/oby.22764

59. Yilmaz M, Bukan N, Gk A, Karakoç A, Toruner F, Çakir N, et al. The effects of rosiglitazone and metformin on oxidative stress and homocysteine levels in lean patients with polycystic ovary syndrome. *Hum Reproduction* (2005) 20(12):3333–40. doi: 10.1093/humrep/dei258

60. Micic D, Popovic V, Nesovic M, Sumarac M, Dragasevic M, Kendereski A, et al. Androgen levels during sequential insulin euglycemic clamp studies in patients with polycystic ovary disease. *J Steroid Biochem* (1988) 31(6):995–9. doi: 10.1016/0022-4731(88)90344-5

61. Nestler JE. Insulin regulation of human ovarian androgens. *Hum Reprod* (1997) 12 (suppl_1):53–62. doi: 10.1093/humrep/12.suppl_1.53

62. Swerdloff RS, Dudley RE, Page ST, Wang C, Salameh WA. Dihydrotestosterone: biochemistry, physiology, and clinical implications of elevated blood levels. *Endocrine Rev* (2017) 38(3):220–54. doi: 10.1210/er.2016-1067

63. Diamond MP, Grainger D, Diamond MC, Sherwin RS, DeFronzo RA. Effects of methyltestosterone on insulin secretion and sensitivity in women. *J Clin Endocrinol Metab* (1998) 83(12):4420–5. doi: 10.1210/jc.83.12.4420

64. Manneras L, Cajander S, Holmaüng A, Seleskovic Z, Lystig T, Loünn M, et al. A new rat model exhibiting both ovarian and metabolic characteristics of polycystic ovary syndrome. *Endocrinology* (2007) 148(8):3781–91. doi: 10.1210/en.2007-0168

65. Yanes LL, Romero DG, Moulana M, Lima R, Davis DD, Zhang H, et al. Cardiovascular-renal and metabolic characterization of a rat model of polycystic ovary syndrome. *Gender Med* (2011) 8(2):103–15. doi: 10.1016/j.genm.2010.11.013

66. McCartney CR, Marshall JCCLINICAL PRACTICE. Polycystic ovary syndrome. *N Engl J Med* (2016) 375(1):54–64. doi: 10.1056/NEJMcp1514916

67. Association AD. 7. approaches to glycemic treatment. *Diabetes Care* (2015) 38 (Supplement 1):S41–S8. doi: 10.2337/dc15-S010

68. Guan Y, Wang D, Bu H, Zhao T, Wang H. The effect of metformin on polycystic ovary syndrome in overweight women: a systematic review and meta-analysis of randomized controlled trials. *Int J Endocrinol* (2020) 2020:5150684. doi: 10.1155/2020/5150684

69. Diamanti-Kandarakis E, Kouli C, Tsianateli T, Bergiele A. Therapeutic effects of metformin on insulin resistance and hyperandrogenism in polycystic ovary syndrome. *Eur J Endocrinology* (1998) 138(3):269–74. doi: 10.1530/eje.0.1380269

70. Jensterle M, Kocjan T, Kravos NA, Pfeifer M, Janez A. Short-term intervention with liraglutide improved eating behavior in obese women with polycystic ovary syndrome. *Endocrine Res* (2015) 40(3):133–8. doi: 10.3109/07435800.2014.966385

71. Han Y, Li Y, He B. GLP-1 receptor agonists versus metformin in PCOS: a systematic review and meta-analysis. *Reprod Biomed Online* (2019) 39(2):332–42. doi: 10.1016/j.rbmo.2019.04.017

72. Dalmasso C, Maranon R, Patil C, Bui E, Moulana M, Zhang H, et al. Cardiometabolic effects of chronic hyperandrogenemia in a new model of postmenopausal polycystic ovary syndrome. *Endocrinology* (2016) 157(7):2920–7. doi: 10.1210/en.2015-1617

73. 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. *Endocrine Rev* (2020) 41(4):bnaa010. doi: 10.1210/endrev/bnaa010

74. Stener-Victorin E. Update on animal models of polycystic ovary syndrome. *Endocrinology* (2022) 163(12):bqac164. doi: 10.1210/endocr/bqac164

75. Holte J, Gennarelli G, Berne C, Bergh T, Lithell H. Elevated ambulatory day-time blood pressure in women with polycystic ovary syndrome: A sign of a pre-hypertensive state? *Hum Reprod* (1996) 11(1):23–8. doi: 10.1093/oxfordjournals.humrep.a019028

76. Mellembakken JR, Mahmoudan A, Mørkrid L, Sundström-Poromaa I, Morin-Papunen L, Tapanainen JS, et al. Higher blood pressure in normal weight women with PCOS compared to controls. *Endoc Connect* (2021) 10(2):154–63. doi: 10.1530/EC-20-0527

77. Diamanti-Kandarakis E, Economou FN, Livadas S, Tantalaki E, Piperi C, Papavassiliou AG, et al. Hyperreninemia characterizing women with polycystic ovary syndrome improves after metformin therapy. *Kidney Blood Press Res* (2009) 32(1):24–31. doi: 10.1159/000201791

78. Jensterle M, Janez A, Vrtovec B, Meden-Vrtovec H, Pfeifer M, Prezelj J, et al. Decreased androgen levels and improved menstrual pattern after angiotensin II receptor antagonist telmisartan treatment in four hypertensive patients with polycystic ovary syndrome: Case series. *Croat Med J* (2007) 48(6):864–70. doi: 10.3325/cmj.2007.6.864

79. Hall JE. Control of blood pressure by the renin-angiotensin-aldosterone system. *Clin Cardiol* (1991) 14(S4):6–21. doi: 10.1002/clc.4960141802

80. Hall J. *Guyton and Hall textbook of medical physiology. 13th ed.* Philadelphia, Pennsylvania: Elsevier (2016).

81. Chappell MC. Non-classical renin-angiotensin system and renal function. *Compr Physiol* (2012) 2(4):2733. doi: 10.1002/cphy.c120002

82. Muñoz MC, Giani JF, Burghi V, Mayer MA, Carranza A, Taira CA, et al. The Mas receptor mediates modulation of insulin signaling by angiotensin-(1–7). *Regul peptides* (2012) 177(1-3):1–11. doi: 10.1016/j.regpep.2012.04.001

83. Nehme A, Zouein FA, Deris Zayeri Z, Zibara K. An update on the tissue renin angiotensin system and its role in physiology and pathology. *J Cardiovasc Dev Dis* (2019) 6(2):14. doi: 10.3390/jcdd6020014

84. Chen J, Williams S, Ho S, Loraine H, Hagan D, Whaley JM, et al. Quantitative PCR tissue expression profiling of the human SGLT2 gene and related family members. *Diabetes Ther* (2010) 1(2):57–92. doi: 10.1007/s13300-010-0006-4

85. Miyata N, Park F, Li XF, Cowley AWJr. Distribution of angiotensin AT1 and AT2 receptor subtypes in the rat kidney. *Am J Physiology-Renal Physiol* (1999) 277(3):F437–46. doi: 10.1152/ajprenal.1999.277.3.F437

86. Navar LG, Kobori H, Prieto MC, Gonzalez-Villalobos RA. Intratubular renin-angiotensin system in hypertension. *Hypertension* (2011) 57(3):355–62. doi: 10.1161/HYPERTENSIONAHA.110.163519

87. Chen M, Harris M, Rose D, Smart A, He X, Kretzler M, et al. Renin and renin mRNA in proximal tubules of the rat kidney. *J Clin Invest* (1994) 94(1):237–43. doi: 10.1172/JCI117312

88. Moe OW, Ujiie K, Star RA, Miller RT, Widell J, Alpern RJ, et al. Renin expression in renal proximal tubule. *J Clin Invest* (1993) 91(3):774–9. doi: 10.1172/JCI116296

89. Prieto-Carrasquero MC, Harrison-Bernard LM, Kobori H, Ozawa Y, Hering-Smith KS, Hamm LL, et al. Enhancement of collecting duct renin in angiotensin II–dependent hypertensive rats. *Hypertension* (2004) 44(2):223–9. doi: 10.1161/01.HYP.0000135678.20725.54

90. Mezzano S, Droguett A, Burgos ME, Ardiles LG, Flores CA, Aros CA, et al. Renin-angiotensin system activation and interstitial inflammation in human diabetic nephropathy. *Kidney Int* (2003) 64:S64–70. doi: 10.1046/j.1523-1755.64.s86.12.x

91. Komlosi P, Fuson AL, Fintha A, Peti-Peterdi J, Rosivall L, Warnock DG, et al. Angiotensin I conversion to angiotensin II stimulates cortical collecting duct sodium transport. *Hypertension* (2003) 42(2):195–9. doi: 10.1161/01.HYP.0000081221.36703.01

92. Sun P, Yue P, Wang W-H. Angiotensin II stimulates epithelial sodium channels in the cortical collecting duct of the rat kidney. *Am J Physiology-Renal Physiol* (2012) 302(6):F679–F87. doi: 10.1152/ajprenal.00368.2011

93. Baker EH. Ion channels and the control of blood pressure. *Br J Clin Pharmacol* (2000) 49(3):185–98. doi: 10.1046/j.1365-2125.2000.00159.x

94. Moin ASM, Sathyapalan T, Atkin SL, Butler AE. Renin-angiotensin system overactivation in polycystic ovary syndrome, a risk for SARS-CoV-2 infection? *Metab Open* (2020) 7:100052. doi: 10.1016/j.metop.2020.100052

95. Alreja G, Joseph J. Renin and cardiovascular disease: Worn-out path, or new direction. *World J Cardiol* (2011) 3(3):72–83. doi: 10.4330/wjc.v3.i3.72

96. Torres Fernandez ED, Huffman AM, Syed M, Romero DG, Yanes Cardozo LL. Effect of GLP-1 receptor agonists in the cardiometabolic complications in a rat model of postmenopausal PCOS. *Endocrinology* (2019) 160(12):2787–99. doi: 10.1210/en.2019-00450

97. Pruett JE, Torres Fernandez ED, Everman SJ, Vinson RM, Davenport K, Logan MK, et al. Impact of SGLT-2 inhibition on cardiometabolic abnormalities in a rat model of polycystic ovary syndrome. *Int J Mol Sci* (2021) 22(5):2576. doi: 10.3390/ijms22052576

98. Cooper WO, Hernandez-Diaz S, Arbogast PG, Dudley JA, Dyer S, Gideon PS, et al. Major congenital malformations after first-trimester exposure to ACE inhibitors. *New Engl J Med* (2006) 354(23):2443–51. doi: 10.1056/NEJMoa055202

99. Saji H, Yamanaka M, Hagiwara A, Ijiri R. Losartan and fetal toxic effects. *Lancet* (2001) 357(9253):363. doi: 10.1016/S0140-6736(00)03648-5

100. Martinovic J, Benachi A, Laurent N, Daïkha-Dahmane F, Gubler MC. Fetal toxic effects and angiotensin-II-receptor antagonists. *Lancet* (2001) 358(9277):241–2. doi: 10.1016/S0140-6736(01)05426-5

101. Sawamura T, Karashima S, Nagase S, Nambo H, Shimizu E, Higashitani T, et al. Effect of sodium–glucose cotransporter-2 inhibitors on aldosterone-to-renin ratio in diabetic patients with hypertension: A retrospective observational study. *BMC Endocrine Disord* (2020) 20(1):1–8. doi: 10.1186/s12902-020-00656-8

102. Kidokoro K, Cherney DZ, Bozovic A, Nagasu H, Satoh M, Kanda E, et al. Evaluation of glomerular hemodynamic function by empagliflozin in diabetic mice using *in vivo* imaging. *Circulation* (2019) 140(4):303–15. doi: 10.1161/CIRCULATIONAHA.118.037418

103. Schork A, Saynisch J, Vosseler A, Jaghutriz BA, Heyne N, Peter A, et al. Effect of SGLT2 inhibitors on body composition, fluid status and renin–angiotensin–aldosterone system in type 2 diabetes: A prospective study using bioimpedance spectroscopy. *Cardiovasc Diabetol* (2019) 18(1):1–12. doi: 10.1186/s12933-019-0852-y

104. Masuda T, Muto S, Fukuda K, Watanabe M, Ohara K, Koepsell H, et al. Osmotic diuresis by SGLT2 inhibition stimulates vasopressin-induced water reabsorption to maintain body fluid volume. *Physiol Rep* (2020) 8(2):e14360. doi: 10.14814/phy2.14360

105. Woods TC, Satou R, Miyata K, Katsurada A, Dugas CM, Klingenberg NC, et al. Canagliflozin prevents intrarenal angiotensinogen augmentation and mitigates kidney injury and hypertension in mouse model of type 2 diabetes mellitus. *Am J Nephrol* (2019) 49(4):331–42. doi: 10.1159/000499597

106. Shin SJ, Chung S, Kim SJ, Lee EM, Yoo YH, Kim JW, et al. Effect of sodium-glucose Co-transporter 2 inhibitor, dapagliflozin, on renal renin-angiotensin system in an animal model of type 2 diabetes. *PLoS One* (2016) 11(11):e0165703. doi: 10.1371/journal.pone.0165703

107. Kojima N, Williams JM, Slaughter TN, Kato S, Takahashi T, Miyata N, et al. Renoprotective effects of combined SGLT2 and ACE inhibitor therapy in diabetic Dahl s rats. *Physiol Rep* (2015) 3(7):e12436. doi: 10.14814/phy2.12436

108. Bautista R, Manning R, Martinez F, Avila-Casado Mdel C, Soto V, Medina A, et al. Angiotensin II-dependent increased expression of na+-glucose cotransporter in hypertension. *Am J Physiol Renal Physiol* (2004) 286(1):F127–33. doi: 10.1152/ajprenal.00113.2003

109. Yildirir A, Aybar F, Kabakci G, Yarali H, Oto A. Heart rate variability in young women with polycystic ovary syndrome. *Ann Noninvasive Electrocardiology* (2006) 11 (4):306–12. doi: 10.1111/j.1542-474X.2006.00122.x

110. Maranon R, Lima R, Spradley FT, do Carmo JM, Zhang H, Smith AD, et al. Roles for the sympathetic nervous system, renal nerves, and CNS melanocortin-4 receptor in the elevated blood pressure in hyperandrogenemic female rats. *Am J Physiology-Regulatory Integr Comp Physiol* (2015) 308(8):R708–R13. doi: 10.1152/ajpregu.00411.2014

111. Schlaich MP, Straznicky N, Grima M, Ika-Sari C, Dawood T, Mahfoud F, et al. Renal denervation: A potential new treatment modality for polycystic ovary syndrome? *J Hypertens* (2011) 29(5):991–6. doi: 10.1097/HJH.0b013e328344db3a

112. Yildizhan R, Ilhan GA, Yildizhan B, Kolusari A, Adali E, Bugdayci G. Serum retinol-binding protein 4, leptin, and plasma asymmetric dimethylarginine levels in obese and nonobese young women with polycystic ovary syndrome. *Fertil Steril* (2011) 96 (1):246–50. doi: 10.1016/j.fertnstert.2011.04.073

113. Hall JE, do Carmo JM, da Silva AA, Wang Z, Hall ME. Obesity, kidney dysfunction and hypertension: Mechanistic links. *Nat Rev Nephrology* (2019) 15 (6):367–85. doi: 10.1038/s41581-019-0145-4

114. da Silva AA, do Carmo JM, Wang Z, Hall JE. Melanocortin-4 receptors and sympathetic nervous system activation in hypertension. *Curr Hypertension Rep* (2019) 21 (6):1–7. doi: 10.1007/s11906-019-0951-x

115. Maddox TM, Januzzi JLJr., Allen LA, Breathett K, Butler J, Davis LL, et al. 2021 Update to the 2017 ACC expert consensus decision pathway for optimization of heart failure treatment: answers to 10 pivotal issues about heart failure with reduced ejection fraction: A report of the American college of cardiology solution set oversight committee. *J Am Coll Cardiol* (2021) 77(6):772–810. doi: 10.1016/j.jacc.2020.11.022

116. MRC Working Party. Medical research council trial of treatment of hypertension in older adults: Principal results. *BMJ* (1992) 304(6824):405–12. doi: 10.1136/bmj.304.6824.405

117. Messerli FH. Implications of discontinuation of doxazosin arm of ALLHAT. *Lancet* (2000) 355(9207):863–4. doi: 10.1016/S0140-6736(00)00092-1

118. Nojima T, Matsubayashi Y, Yoshida A, Suganami H, Abe T, Ishizawa M, et al. Influence of an SGLT2 inhibitor, tofogliflozin, on the resting heart rate in relation to adipose tissue insulin resistance. *Diabetic Med* (2020) 37(8):1316–25. doi: 10.1111/dme.14279

119. Sano M, Chen S, Imazeki H, Ochiai H, Seino Y. Changes in heart rate in patients with type 2 diabetes mellitus after treatment with luseogliflozin: Subanalysis of placebo-controlled, double-blind clinical trials. *J Diabetes Invest* (2018) 9(3):638–41. doi: 10.1111/jdi.12726

120. Herat LY, Magno AL, Rudnicka C, Hricova J, Carnagarin R, Ward NC, et al. SGLT2 inhibitor–induced sympathoinhibition: a novel mechanism for cardiorenal protection. *JACC: Basic to Trans Science* (2020) 5(2):169–79. doi: 10.1016/j.jacbts.2019.11.007

121. Wu P, Wen W, Li J, Xu J, Zhao M, Chen H, et al. Systematic review and meta-analysis of randomized controlled trials on the effect of SGLT2 inhibitor on blood leptin and adiponectin level in patients with type 2 diabetes. *Hormone Metab Res* (2019) 51 (08):487–94. doi: 10.1055/a-0958-2441

122. Pieczenik SR, Neustadt J. Mitochondrial dysfunction and molecular pathways of disease. *Exp Mol Pathol* (2007) 83(1):84–92. doi: 10.1016/j.yexmp.2006.09.008

123. Nicolson GL. Mitochondrial dysfunction and chronic disease: Treatment with natural supplements. *Integr Med (Encinitas)* (2014) 13(4):35.

124. Katerji M, Filippova M, Duerksen-Hughes P. Approaches and methods to measure oxidative stress in clinical samples: Research applications in the cancer field. *Oxid Med Cell Longevity* (2019) 2019:1279250. doi: 10.1155/2019/1279250

125. Bhatti JS, Bhatti GK, Reddy PH. Mitochondrial dysfunction and oxidative stress in metabolic disorders–a step towards mitochondria based therapeutic strategies. *Biochim Biophys Acta Basis Dis* (2017) 1863(5):1066–77. doi: 10.1016/j.bbadis.2016.11.010

126. Zhou B, Tian R. Mitochondrial dysfunction in pathophysiology of heart failure. *J Clin Invest* (2018) 128(9):3716–26. doi: 10.1172/JCI120849

127. Zhang X, Agborbesong E, Li X. The role of mitochondria in acute kidney injury and chronic kidney disease and its therapeutic potential. *Int J Mol Sci* (2021) 22 (20):11253. doi: 10.3390/ijms222011253

128. Zhang J, Bao Y, Zhou X, Zheng L. Polycystic ovary syndrome and mitochondrial dysfunction. *Reprod Biol Endocrinol* (2019) 17(1):1–15. doi: 10.1186/s12958-019-0509-4

129. Lee S-H, Chung D-J, Lee H-S, Kim T-J, Kim M-H, Jeong HJ, et al. Mitochondrial DNA copy number in peripheral blood in polycystic ovary syndrome. *Metabolism* (2011) 60(12):1677–82. doi: 10.1016/j.metabol.2011.04.010

130. Reddy TV, Govatati S, Deenadayal M, Sisinthy S, Bhanoori M. Impact of mitochondrial DNA copy number and displacement loop alterations on polycystic ovary syndrome risk in south Indian women. *Mitochondrion* (2019) 44:35–40. doi: 10.1016/j.mito.2017.12.010

131. Chappell NR, Zhou B, Schutt AK, Gibbons WE, Blesson CS. Prenatal androgen induced lean PCOS impairs mitochondria and mRNA profiles in oocytes. *Endocrine connections* (2020) 9(3):261–70. doi: 10.1530/EC-19-0553

132. Okuno Y, Fukuhara A, Hashimoto E, Kobayashi H, Kobayashi S, Otsuki M, et al. Oxidative stress inhibits healthy adipose expansion through suppression of SREBF1-mediated lipogenic pathway. *Diabetes* (2018) 67(6):1113–27. doi: 10.2337/db17-1032

133. Mouton AJ, Li X, Hall ME, Hall JE. Obesity, hypertension, and cardiac dysfunction: Novel roles of immunometabolism in macrophage activation and inflammation. *Circ Res* (2020) 126(6):789–806. doi: 10.1161/CIRCRESAHA.119.312321

134. Corona JC, Duchen MR. PPARγ as a therapeutic target to rescue mitochondrial function in neurological disease. *Free Radical Biol Med* (2016) 100:153–63. doi: 10.1016/j.freeradbiomed.2016.06.023

135. Croteau D, Luptak I, Chambers JM, Hobai I, Panagia M, Pimentel DR, et al. Effects of sodium-glucose linked transporter 2 inhibition with ertugliflozin on mitochondrial function, energetics, and metabolic gene expression in the presence and absence of diabetes mellitus in mice. *J Am Heart Assoc* (2021) 10(13):e019995. doi: 10.1161/JAHA.120.019995

136. Shao Q, Meng L, Lee S, Tse G, Gong M, Zhang Z, et al. Empagliflozin, a sodium glucose co-transporter-2 inhibitor, alleviates atrial remodeling and improves mitochondrial function in high-fat diet/streptozotocin-induced diabetic rats. *Cardiovasc Diabetol* (2019) 18(1):1–14. doi: 10.1186/s12933-019-0964-4

137. Takagi S, Li J, Takagaki Y, Kitada M, Nitta K, Takasu T, et al. Ipragliflozin improves mitochondrial abnormalities in renal tubules induced by a high-fat diet. *J Diabetes Invest* (2018) 9(5):1025–32. doi: 10.1111/jdi.12802

138. Wei D, Liao L, Wang H, Zhang W, Wang T, Xu Z. Canagliflozin ameliorates obesity by improving mitochondrial function and fatty acid oxidation *via* PPARα *in vivo* and in vitro. *Life Sci* (2020) 247:117414. doi: 10.1016/j.lfs.2020.117414

139. Pruett JE, Everman SJ, Hoang NH, Salau F, Taylor LC, Edwards KS, et al. Mitochondrial function and oxidative stress in white adipose tissue in a rat model of PCOS: Effect of SGLT2 inhibition. *Biol Sex Differ* (2022) 13(1):45. doi: 10.1186/s13293-022-00455-x

140. Reckelhoff JF, Shawky NM, Romero DG, Yanes Cardozo LL. Polycystic ovary syndrome: Insights from preclinical research. *Kidney360* (2022) 3(8):1449–57. doi: 10.34067/KID.0002052022

141. Zahid S, Khan MZ, Gowda S, Faza NN, Honigberg MC, Vaught A, et al. Trends, predictors, and outcomes of cardiovascular complications associated with polycystic ovary syndrome during delivery hospitalizations: A national inpatient sample analysis (2002–2019). *J Am Heart Assoc* (2022) 11(16):e025839. doi: 10.1161/JAHA.121.025839

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 24 May 2023
DOI 10.3389/fendo.2023.1022272

OPEN ACCESS

EDITED BY
Yanting Wu,
Fudan University, China

REVIEWED BY
Jiangfeng Ye,
Agency for Science, Technology and Research (A*STAR), Singapore
M. Ishaq Geer,
University of Kashmir, India

*CORRESPONDENCE
Nirmin F. Juber
nirmin.juber@nyu.edu

RECEIVED 18 August 2022
ACCEPTED 10 May 2023
PUBLISHED 24 May 2023

CITATION
Juber NF, Abdulle A, AlJunaibi A, AlNaeemi A, Ahmad A, Leinberger-Jabari A, Al Dhaheri AS, AlZaabi E, Mezhal F, Al-Maskari F, Alanouti F, Alsafar H, Alkaabi J, Wareth LA, Aljaber M, Kazim M, Weitzman M, Al-Houqani M, Hag-Ali M, Oumeziane N, El-Shahawy O, Sherman S, Shah SM, Loney T, Almahmeed W, Idaghdour Y and Ali R (2023) Association between pediatric asthma and adult polycystic ovarian syndrome (PCOS): a cross-sectional analysis of the UAE healthy future Study (UAEHFS).
Front. Endocrinol. 14:1022272.
doi: 10.3389/fendo.2023.1022272

Association between pediatric asthma and adult polycystic ovarian syndrome (PCOS): a cross-sectional analysis of the UAE healthy future Study (UAEHFS)

Nirmin F. Juber[1]*, Abdishakur Abdulle[1], Abdulla AlJunaibi[2], Abdulla AlNaeemi[3], Amar Ahmad[1], Andrea Leinberger-Jabari[1], Ayesha S. Al Dhaheri[4], Eiman AlZaabi[5], Fatima Mezhal[1], Fatma Al-Maskari[6,7], Fatme Alanouti[8], Habiba Alsafar[9,10,11], Juma Alkaabi[12], Laila Abdel Wareth[13], Mai Aljaber[14], Marina Kazim[15], Michael Weitzman[16], Mohammed Al-Houqani[17], Mohammed Hag-Ali[18], Naima Oumeziane[15], Omar El-Shahawy[19], Scott Sherman[19], Syed M. Shah[6], Tom Loney[20], Wael Almahmeed[21], Youssef Idaghdour[1] and Raghib Ali[1,22]

[1]Public Health Research Center, New York University Abu Dhabi, Abu Dhabi, United Arab Emirates, [2]Department of Pediatrics, Zayed Military Hospital, Abu Dhabi, United Arab Emirates, [3]Department of Cardiology, Zayed Military Hospital, Abu Dhabi, United Arab Emirates, [4]Department of Nutrition and Health, College of Medicine and Health Sciences; United Arab Emirates University, Al-Ain, United Arab Emirates, [5]Department of Pathology, Sheikh Shakhbout Medical City, Abu Dhabi, United Arab Emirates, [6]Institute of Public Health, College of Medicine and Health Sciences, United Arab Emirates University, Al-Ain, United Arab Emirates, [7]Zayed Center for Health Sciences, United Arab Emirates University, Al-Ain, United Arab Emirates, [8]College of Natural and Health Sciences, Zayed University, Abu Dhabi, United Arab Emirates, [9]Center for Biotechnology, Khalifa University of Science and Technology, Abu Dhabi, United Arab Emirates, [10]Department of Genetics and Molecular Biology, Khalifa University of Science and Technology, Abu Dhabi, United Arab Emirates, [11]Department of Biomedical Engineering, Khalifa University of Science and Technology, Abu Dhabi, United Arab Emirates, [12]Department of Internal Medicine, College of Medicine and Health Sciences, United Arab Emirates University, Al-Ain, United Arab Emirates, [13]Department of Laboratory Medicine, National Reference Laboratory, Abu Dhabi, United Arab Emirates, [14]Healthpoint Hospital, Abu Dhabi, United Arab Emirates, [15]Abu Dhabi Blood Bank Services, SEHA, Abu Dhabi & Al-Ain, United Arab Emirates, [16]Department of Environmental Medicine, New York University of Medicine, New York, United States, [17]Department of Medicine, College of Medicine and Health Sciences, United Arab Emirates University, Al-Ain, United Arab Emirates, [18]Faculty of Health Sciences, Higher Colleges of Technology, Abu Dhabi, United Arab Emirates, [19]Department of Population Health, New York University School of Medicine, New York, United States, [20]College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, United Arab Emirates, [21]Heart and Vascular Institute, Cleveland Clinic Abu Dhabi, Abu Dhabi, United Arab Emirates, [22]Medical Research Council (MRC) Epidemiology Unit, University of Cambridge, Cambridge, United Kingdom

Introduction: Asthma and polycystic ovarian syndrome (PCOS) are linked in several possible ways. To date, there has been no study evaluating whether pediatric asthma is an independent risk factor for adult PCOS. Our study aimed to examine the association between pediatric asthma (diagnosed at 0-19 years) and adult PCOS (diagnosed at ≥20 years). We further assessed whether the aforementioned association differed in two phenotypes of adult PCOS which were diagnosed at 20-25 years (young adult PCOS), and at >25 years (older adult

10.3389/fendo.2023.1022272

PCOS). We also evaluated whether the age of asthma diagnosis (0-10 vs 11-19 years) modified the association between pediatric asthma and adult PCOS.

Material and methods: This is a retrospective cross-sectional analysis using the United Arab Emirates Healthy Future Study (UAEHFS) collected from February 2016 to April 2022 involving 1334 Emirati females aged 18-49 years. We fitted a Poisson regression model to estimate the risk ratio (RR) and its 95% confidence interval (95% CI) to assess the association between pediatric asthma and adult PCOS adjusting for age, urbanicity at birth, and parental smoking at birth.

Results: After adjusting for confounding factors and comparing to non-asthmatic counterparts, we found that females with pediatric asthma had a statistically significant association with adult PCOS diagnosed at ≥20 years (RR=1.56, 95% CI: 1.02-2.41), with a stronger magnitude of the association found in the older adult PCOS phenotype diagnosed at >25 years (RR=2.06, 95% CI: 1.16-3.65). Further, we also found females reported thinner childhood body size had a two-fold to three-fold increased risk of adult PCOS diagnosed at ≥20 years in main analysis and stratified analyses by age of asthma and PCOS diagnoses (RR=2.06, 95% CI: 1.08-3.93 in main analysis; RR=2.74, 95% CI: 1.22-6.15 among those diagnosed with PCOS > 25 years; and RR=3.50, 95% CI: 1.38-8.43 among those diagnosed with asthma at 11-19 years).

Conclusions: Pediatric asthma was found to be an independent risk factor for adult PCOS. More targeted surveillance for those at risk of adult PCOS among pediatric asthmatics may prevent or delay PCOS in this at-risk group. Future studies with robust longitudinal designs aimed to elucidate the exact mechanism between pediatric asthma and PCOS are warranted.

KEYWORDS

asthma, pediatric asthma, polycystic ovarian syndrome, PCOS, epidemiology, risk factors, public health

1 Introduction

Asthma is a multifactorial respiratory disease defined by reversible airway hyperactivity and a wide range of symptoms (1). Inflammation is a key factor in the pathology of asthma, and cross-communication between the airways and inflammatory mediators leads to inflammation that is not only confined to the local airways but also tends to be systemic (2). Asthma is a common pediatric disease with more than 80% of first asthma episodes happening in the first six years of life (3). The main risk factors associated with pediatric asthma include genetic predisposition, viral respiratory infections, and female sex hormones (4). In addition, pediatric asthma has long-term health consequences and is known to be associated with adult non-communicable diseases such as hypertension and diabetes (5).

Polycystic Ovarian Syndrome (PCOS) is a complex endocrine disorder affecting 5-10% of females of reproductive age (6). PCOS is a multifactorial disorder and risk factors associated with PCOS include genetic predisposition, hormonal factors, as well as maternal environmental factors (e.g. metabolic disturbance during pregnancy) (7, 8). Previous studies found that females with PCOS have an increased risk of developing subsequent metabolic disorders, such as cardiovascular disease, hypertension, and diabetes (7, 9, 10). The etiology of PCOS is not exactly known, however, chronic systemic inflammation has been proposed as one of the possible mechanisms (8, 11). In addition, PCOS may have its early-life origins through exposure to excess androgens at any stage from fetal development to childhood period (7, 8).

Asthma and PCOS are linked in several possible ways. Previous studies have established the association between PCOS and subsequent asthma among reproductive-aged females (12–14). There is clinical overlap between asthma and PCOS (15), including alterations in gut microbiota (16–18), menstrual cycle abnormalities (19–21), infertility (22, 23), obesity (24–26), and insulin resistance that was found in asthmatics as well as females with PCOS (27, 28). Previous epidemiological studies have also found an association between PCOS and subsequent asthma (13, 29). However, there is a limited epidemiological study on another possible direction of the association between asthma and subsequent PCOS, including our previous work examining the

association between asthma diagnosed at <25 years with subsequent PCOS diagnosed at ≥25 years (30). Asthma may be associated with subsequent PCOS as asthma and PCOS are multifactorial complex diseases and they shared pathophysiological mechanisms, including female hormonal disturbance, systemic/low-grade inflammation, as well as obesity and, metabolic syndrome (11–14, 31). We thus predict that pediatric asthma might be associated with adult PCOS, independent of other relevant risk factors found in our dataset.

Our study aimed to examine the association between pediatric asthma (diagnosed at 0-19 years of age) and adult PCOS (overall adult PCOS: diagnosed at ≥20 years, young adult PCOS: diagnosed at 20-25 years, and older adult PCOS: diagnosed at 25-49 years). We also assessed whether the age of asthma diagnosis (childhood asthma: diagnosed at 0-10 years, and adolescent asthma: diagnosed at 11-19 years) modified the association between pediatric asthma and adult PCOS. Finally, in the main analysis and stratified models, we performed restriction analysis by body size at 10 years old and health status up to 10 years old, to better address the potential mediating effect of childhood body size and childhood health on the association between pediatric asthma and adult PCOS.

2 Material and methods

2.1 Study design, participants, and setting

This is a retrospective cross-sectional study using the United Arab Emirates Healthy Future Study (UAEHFS) collected from February 2016 to April 2022. We included all 1334 females aged 18-49 years who had complete information on the age of asthma and PCOS diagnosis (Figure 1). The study design, questionnaire, and methodologies of the UAEHFS are described elsewhere (32). In brief, the UAEHFS is an ongoing population-based prospective cohort study among Emirati nationals aged 18 years or above. A convenience sample of Emirati individuals was invited to participate from across the UAE. Multiple recruitment centers were set up across the country where participants filled out the questionnaire and had some physical measurements. Due to the COVID-19 pandemic, the recruitment shifted to online-based starting in April 2020, and an online questionnaire was introduced to the new participants. Physical measurements, such as body mass index (BMI), were taken in the participating centers for new participants that filled out and returned the online questionnaire.

2.2 Ethical approval

The study and its procedures have been reviewed and approved by the Institutional Review Board at New York University Abu Dhabi, Dubai Health Authority, Ministry of Health and Prevention in the UAE, and Health Research and Technology Committee, reference number DOH/HQD/2020/516. Written consent was obtained from participants at the centers or by filling out an online consent form before data collection started.

2.3 Measurements

We analyzed self-reported physician diagnoses for asthma based on the questionnaire response to: "Has a doctor ever told you that you had asthma?". The age at asthma diagnosis was extracted from the questionnaire response to: "What was your age when asthma was first diagnosed?", and pediatric asthma was defined as asthma first diagnosed at 19 years of age or younger (pediatric population) (5, 33). Similarly, self-reported physician diagnosis for PCOS was used based on the questionnaire response to: "Has a doctor ever told you that you had PCOS?". The age at PCOS diagnosis was extracted from the questionnaire response to: "How old were you when the doctor first told you that you had PCOS?", and adult PCOS was defined as PCOS first diagnosed at 20 years of age or above (34). Overall health up to 10 years old was determined based on the questionnaire response to: "In general,

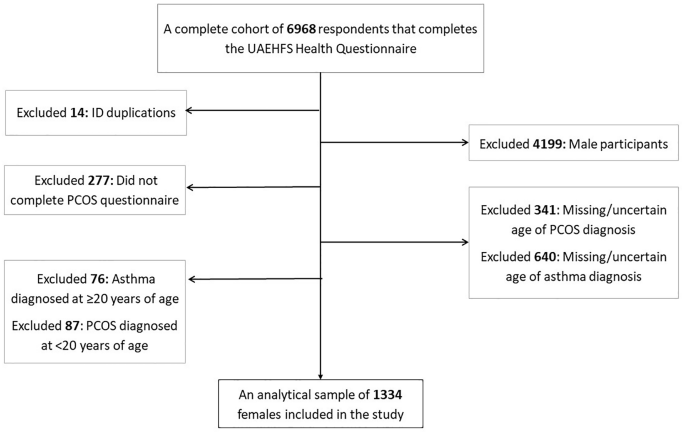


FIGURE 1
Flowchart of the final analytical sample included in the study.

10.3389/fendo.2023.1022272

how was your health in childhood (less than 10 years old)?", we then categorized childhood health status variable into two categories: poor or fair and good or excellent based on the responses. Body size at 10 years old was determined based on the questionnaire responses to: "When you were 10 years old, compared to average, would you describe yourself as: about average, thinner, or plumper?", we then classified body size at 10 years old into four categories of around average, average, thinner, and plumper. Age was constructed based on the questionnaire response to "What is your date of birth", and we kept its continuous form in our analysis. Urbanicity at birth and current urbanicity were determined based on the questionnaire response to: "Where do you and your family live around the time of your birth?", and "Where do you and your family live now?", respectively. We then categorized the city as an urban area and non-cities (villages, deserts, islands, and others) as rural or other non-urban. Parental smoking (at birth) was constructed based on the questionnaire response to "Did your mother or father smoke regularly around the time when you were born?" (no/yes). Parental education and education attainment were constructed based on the questionnaire response to: "What level of education did your father or mother complete?", and "What is the highest level of education that you have completed?", respectively. We categorized education levels into three categories (≤6 years, >6 to 12 years, and 12+ years of schooling). Birthweight which was determined based on the questionnaire item: "What was your birth weight (in kg)?", was categorized later into low birthweight (<2.5 kg) and normal birthweight (2.5+ kg). BMI was calculated using the Tanita MC 780 by nurses at the recruitment centers, and the values for each participant were recorded and its continuous form was used in our analysis.

2.4 Statistical analysis

Characteristics of the study participants were evaluated using numbers with percentage (n, %) for each categorical variable and mean with standard deviation (mean ± SD) for each continuous variable (Table 1). To establish asthma as an exposure preceding PCOS as an outcome, we relied on age at disease diagnosis and excluded those with adult asthma (diagnosed at >19 years of age) and adolescent PCOS (diagnosed at <20 years of age). We then fitted a Poisson regression model with robust variance to estimate the risk ratio (RR) and its 95% confidence interval (95% CI) to assess the association between pediatric asthma (diagnosed at 0-19 years of age) and adult PCOS (diagnosed at ≥20 years of age) (Tables 2-4) (35). We examined the RR and its 95% CI in the crude and adjusted models, adjusting for age (5, 36), urbanicity at birth (37, 38), and parental smoking (37, 39). Due to a high amount of missing values (>50%) on parental education (40, 41) and birthweight (42, 43), we excluded these potential confounding factors in the regression analysis even though these are important confounding factors in this study. In addition, the missing indicator method was used to handle uncertain values from missing, prefer not to answer (PFA), and do not know (DN) responses. Analyses were carried out using STATA 17.0 (StataCorp, TX). *P* values <0.05 were considered statistically significant.

3 Results

Table 1 summarizes the descriptive characteristics of study participants based on their pediatric asthma status. Out of 1334 females aged 18 to 49 years, 130 females reported ever being diagnosed with asthma when they were 0-19 years of age (pediatric asthma prevalence = 9.75%). Compared to non-asthmatics, females with pediatric asthma were older (24.8 ± 6.4 vs 23.8 ± 6.1 years), had a higher BMI (28.5 ± 7.6 vs 23.2 ± 4.7 kg/m^2), a greater proportion resided in urban areas (89.2% vs 83.2%), a greater proportion had >12 years of schooling (high level of education) (53.9% vs 48.1%), and a higher proportion diagnosed with PCOS (14.6% vs 8.5%). In terms of early-life characteristics, females with pediatric asthma had a higher proportion reporting poorer overall health up to 10 years old (25.4% vs 11.0%), had a higher proportion having plumper body size at 10 years old (16.2% vs 8.4%), had a higher proportion of parental smoking at birth (29.2% vs 22.4%), and had a higher proportion of lower birthweight (26.2% vs 15.7%), compared to non-asthmatic females.

Table 2 shows the risk ratios (RRs) of the associations between pediatric asthma (diagnosed at 0-19 years of age) and adult PCOS (diagnosed at ≥20 years of age). In the crude and fully adjusted model, compared to non-asthmatics, pediatric asthma was significantly associated with adult PCOS (RR=1.73, 95% CI: 1.10-2.72 in the crude model; and RR=1.56, 95% CI: 1.02-2.41 in the adjusted model). In the restriction analysis by early-life risk factors, namely, body size at 10 years old and childhood health status up to 10 years old, the significance diminished among those who had about average and plumper body size, and those who reported excellent or good childhood health. Meanwhile, marginal significance was observed among those who reported poor or fair childhood health. Lastly, the statistical significance persisted among those with thinner body size at 10 years old (RR=2.00, 95% CI: 1.01-3.99 in the crude model; and RR=2.06, 95% CI: 1.08-3.93 in the adjusted model).

Table 3 shows the risk ratios (RRs) of the associations between pediatric asthma (diagnosed at 0-19 years of age) and adult PCOS (diagnosed at ≥20 years of age), stratified by the age of adult PCOS diagnosis (20-25 years vs >25 years at PCOS diagnosis), in the main analysis and restricted by early-life risk factors, namely, body size at 10 years old and childhood health status up to 10 years old. In the young adult PCOS diagnosis stratum (diagnosed at 20-25 years), pediatric asthma was not associated with adult PCOS. On the contrary, in the older adult PCOS diagnosis stratum (diagnosed at >25 years) and after adjusting for confounding, we found that pediatric asthma had a significantly increased risk for adult PCOS (RR=2.06, 95% CI: 1.16-3.65). The statistical significance was still observed among those with thinner and about average body size at 10 years old in the older adult PCOS group even after adjusting for confounding factors (RR=2.74, 95% CI: 1.22-6.15 among thinner childhood body size category; and RR=3.40, 95% CI: 1.49-7.73 among about average body size category).

Table 4 presents the risk ratios (RRs) of the associations between pediatric asthma (diagnosed at 0-19 years of age) and adult PCOS (diagnosed at ≥20 years of age), stratified by the age of asthma diagnosis (0-10 vs 11-19 years at asthma diagnosis), in main

10.3389/fendo.2023.1022272

TABLE 1 Characteristics of female study participants based on pediatric asthma status, missing or uncertain vales are not shown (N=1334).

Characteristics	Non-asthmatics (N= 1204)	With pediatric asthma (N= 130)
Age, year (mean ± SD)	23.8 ± 6.1	24.8 ± 6.4
BMI, kg/m2 (mean ± SD)	23.2 ± 4.7	28.5 ± 7.6
Marital status, n (%)		
Single	914 (75.9)	100 (76.9)
Married	235 (19.5)	26 (20.0)
Divorced or separated	38 (3.2)	4 (3.1)
Widow or widower	17 (1.4)	0 (0)
Urbanicity at questionnaire, n (%)		
Rural or other non-urbans	173 (14.4)	14 (10.8)
Urban (city)	1002 (83.2)	116 (89.2)
Urbanicity at birth, n (%)		
Rural or other non-urbans	233 (19.4)	21 (16.2)
Urban (city)	899 (74.6)	102 (78.5)
Education attainment, n (%)		
6 years of schooling or below	48 (4.0)	2 (1.5)
>6-12 years of schooling	577 (47.9)	58 (44.6)
>12 years of schooling	579 (48.1)	70 (53.9)
Parental education attainment, n (%)		
6 years of schooling or below	51 (4.3)	5 (3.9)
>6-12 years of schooling	150 (12.5)	18 (13.9)
>12 years of schooling	183 (15.2)	28 (21.5)
Overall health up to 10 years, n (%)		
Poor or fair	132 (11.0)	33 (25.4)
Good or excellent	1072 (89.0)	97 (74.6)
Body size at 10 years, n (%)		
About average	436 (36.2)	54 (41.5)
Thinner	518 (43.0)	46 (35.4)
Plumper	101 (8.4)	21 (16.2)
Parental smoking at birth, n (%)		
No	819 (68.0)	82 (63.1)
Yes	270 (22.4)	38 (29.2)
Birthweight, n (%)		
<2.5 kg	189 (15.7)	34 (26.2)
2.5+ kg	288 (23.9)	40 (30.8)
With PCOS diagnosed at> 20 years, n (%)		
No	1102 (91.5)	111 (85.4)
Yes	102 (8.5)	19 (14.6)

10.3389/fendo.2023.1022272

TABLE 2 Modified Poisson regression analysis between females with pediatric asthma history (diagnosed at 0-19 years of age) and adult PCOS (diagnosed at ≥20 years of age), in total population and restricted by potential mediators (N=1334).

	Crude Model			Adjusted Model[a]		
	RR	[95% CI]	*P*	RR	[95% CI]	*P*
Main analysis						
Non-asthmatics (N=1204)	(Reference)			(Reference)		
Pediatric asthmatics (N=130)	1.73	1.10-2.72	0.019	1.56	1.02 -2.41	0.040
Restriction analysis by body size at 10 years old						
About average						
Non-asthmatics (N= 436)	(Reference)			(Reference)		
Pediatric asthmatics (N= 54)	1.66	0.78-3.57	0.192	1.39	0.73 -2.66	0.323
Thinner						
Non-asthmatics (N=518)	(Reference)			(Reference)		
Pediatric asthmatics (N=46)	2.00	1.01-3.99	0.048	2.06	1.08-3.93	0.028
Plumper						
Non-asthmatics (N=101)	(Reference)			(Reference)		
Pediatric asthmatics (N=21)	1.03	0.32-3.29	0.959	1.13	0.27 -4.69	0.871
Restriction analysis by childhood health status up to 10 years old						
Poor or fair						
Non-asthmatics (N=132)	(Reference)			(Reference)		
Pediatric asthmatics (N=33)	2.00	0.73-5.47	0.177	2.41	0.95 -6.10	0.063
Excellent or good						
Non-asthmatics (N=1072)	(Reference)			(Reference)		
Pediatric asthmatics (N=97)	1.68	0.99-2.84	*0.051*	1.44	0.88-2.38	0.149

[a]Adjusted for age (continuous), urbanicity at birth (rural/urban), and parental smoking at birth (no/yes).

analysis and restricted by early-life risk factors, namely, body size at 10 years old and childhood health status up to 10 years old. We did not find any statistically significant associations in the main analysis for both strata. However, in the 0-10 years of age of asthma diagnosis and after adjusting for confounding, the significance was observed among those with poor or fair childhood health status (RR=2.54, 95% CI: 1.03-6.30). Meanwhile, in the 11-19 years of age of asthma diagnosis stratum and after adjusting for confounding factors, we found that those diagnosed with asthma at 11-19 years had a significantly increased risk of adult PCOS among those reported thinner childhood body size and those reported excellent or good childhood health status (RR=3.50, 95% CI: 1.38-8.43 among those reported thinner childhood body size, and RR=2.13, 95% CI: 1.01-4.50 among those reported excellent or good childhood health status).

4 Discussion

To our knowledge, this is the first population-based study evaluating the association between pediatric asthma diagnosed at 0-19 years and adult PCOS diagnosed at 20 years of age or above. We are able to demonstrate temporality as there is a long interval time between pediatric asthma and adult PCOS diagnoses in this analysis. In both crude and adjusted models and compared to non-asthmatic females, we found that pediatric asthma was significantly positively associated with adult PCOS (Table 2). Previous studies have found that PCOS was an independent risk factor for asthma among reproductive-aged females and suggested a strong correlation between PCOS and chronic systemic inflammation such as asthma (12–14, 31). However, our study provided a new perspective on how asthma and PCOS might be linked in the opposite direction. Our recent bi-directional study examining asthma and PCOS found a significant association between asthma diagnosed at <25 years and adult PCOS diagnosed at ≥25 years, independent of age and BMI (30). Female hormonal disturbance, metabolic syndrome, and obesity have been suggested as overlapping mechanisms that link asthma and PCOS (11–14, 31). A previous study has shown that obesity may worsen hormone dysregulation (25), and although there is no exact explanation linking asthma and metabolic syndrome, there were various known risk factors, including obesity or high BMI and dyslipidemia (44). Compared to non-asthmatics, those with pediatric asthma are known to be more susceptible to subsequent

10.3389/fendo.2023.1022272

TABLE 3 Modified Poisson regression analysis between females with pediatric asthma and adult PCOS, stratified by the age of PCOS diagnosis (20-25 years vs >25 years), in total population and restricted by potential mediators (N=1334).

	Age of PCOS diagnosis: 20-25 years of age						Age of PCOS diagnosis: > 25 years of age					
	Crude Model			Adjusted Model[a]			Crude Model			Adjusted Model[a]		
	RR	[95% CI]	*P*	RR	[95% CI]	*P*	RR	[95% CI]	*P*	RR	[95% CI]	*P*
Main analysis												
Non-asthmatics	(Reference)			(Reference)			(Reference)			(Reference)		
Pediatric asthmatics	1.32	0.65 -2.70	0.443	1.33	0.65 -2.72	0.442	2.40	1.27 -4.53	0.007	2.06	1.16 -3.65	0.013
Restriction analysis by body size at 10 years old												
About average												
Non-asthmatics	(Reference)			(Reference)			(Reference)			(Reference)		
Pediatric asthmatics	0.40	0.06-2.92	0.367	0.39	0.06-2.79	0.350	3.91	1.53-9.98	0.004	3.40	1.49-7.73	0.004
Thinner												
Non-asthmatics	(Reference)			(Reference)			(Reference)			(Reference)		
Pediatric asthmatics	1.46	0.46-4.63	0.523	1.55	0.49-4.94	0.454	2.87	1.13-7.26	0.026	2.74	1.22-6.15	0.015
Plumper												
Non-asthmatics	(Reference)			(Reference)			(Reference)			(Reference)		
Pediatric asthmatics	1.92	0.54-6.85	0.316	2.55	0.65-9.96	0.180	N/A			N/A		
Restriction analysis by childhood health status up to 10 years old												
Poor or fair												
Non-asthmatics	(Reference)			(Reference)			(Reference)			(Reference)		
Pediatric asthmatics	1.42	0.30-6.73	0.657	1.63	0.37-7.10	0.517	3.05	0.72-13.0	0.132	3.11	0.61-15.7	0.170
Excellent or good												
Non-asthmatics	(Reference)			(Reference)			(Reference)			(Reference)		
Pediatric asthmatics	1.31	0.58-2.97	0.512	1.25	0.55-2.82	0.599	2.30	1.11-4.77	0.026	1.87	0.94-3.69	0.073

[a]Adjusted for age (continuous), urbanicity at birth (rural/urban), and parental smoking at birth (no/yes). N/A reflects insufficient sample size.

chronic diseases due to immune system impairment and persistent systemic inflammation (45, 46). In addition, compared to their healthy counterparts, pediatric asthmatics are known to have lower sympathetic nervous activity, and thereby a lower metabolic rate which may subsequently affect their vital biological process such as growth and reproduction (47–49). A previous study that linked pediatric asthma and reproductive health found pediatric asthma to be significantly associated with an earlier age at menarche (37). Therefore, we believe there are possible mechanisms involving biological factors in the association between pediatric asthma and adult PCOS since PCOS has been recognized as a chronic metabolic condition beyond a merely reproductive disorder (50).

We stratified by the age of PCOS diagnosis (Table 3) to better understand the association between pediatric asthma and adult PCOS phenotypes; young adult PCOS (diagnosed at 20-25 years), and older adult PCOS (diagnosed at >25 years). Compared to non-asthmatics, we found pediatric asthma was significantly associated with older adult PCOS. Most PCOS-related studies have involved adult populations (mean age > 25 years), however, PCOS can also be present in adolescence and young adulthood (aged ≤25 years) (51, 52). To our knowledge, there is no study on the association between pediatric asthma and any PCOS phenotypes (young adult PCOS or older adult PCOS) to compare to our study findings, however, several possible mechanisms may explain the observed findings. The expression of PCOS in early adulthood may differ from and does not necessarily resemble that of clinical and endocrinological features observed in later adulthood (52). Elevated sex hormone of adrenal androgen was observed in females with PCOS (8), and a previous study found that a greater decrease in adrenal androgen secretion happens between the ages of 20 to 25 years (53). In addition, inflammation is known to be a key factor in the pathology of asthma (2), and a previous study has shown that inflammation affects the level of female sex hormones (11). We believe possible mechanisms involving chronic inflammation and endocrinological features or sex hormones may explain the observed association between pediatric asthma and

10.3389/fendo.2023.1022272

older adult PCOS phenotype in our study. Future studies to elucidate the exact mechanism of the association between pediatric asthma and older adult PCOS are warranted.

We further stratified the analysis by the age of asthma diagnosis (Table 4) to separate two distinct asthma phenotypes of childhood asthma (diagnosed at 0-10 years of age) and adolescent asthma (diagnosed at 11-19 years of age) and its association with adult PCOS. Childhood asthma (asthma diagnosed at 0-10 years) and adolescent asthma (asthma diagnosed at 11-19 years) were shown to be not significantly associated with adult PCOS in the crude and adjusted models. Asthma is known to be a uniquely diverse disorder with many clinical expressions throughout childhood and adolescence period (3). Childhood asthma and adolescent asthma are shown to be distinct in several ways, including their risk factors. The main risk factor for childhood asthma is a genetic predisposition (4), whereas the main risk factor for adolescent asthma is related to sex hormones (3). Our significant association between pediatric asthma and adult PCOS was only observed among those diagnosed with asthma at 11-19 years in the crude analysis, but the significant association disappeared after adjusting for confounding factors. Adolescence is a transitional stage of physical and psychological development, which is marked by the puberty period in which changes in reproductive hormones occur, and body weight gain following menarche is suggested to mediate the association between pediatric asthma and adult PCOS (54). Compared to healthier counterparts, pediatric asthmatics tend to have a lower sympathetic activity, hence a lower metabolic rate that may affect fat storage and may lead to overweight or obesity (47, 48). In addition, weight gain at puberty has been shown to be a significant risk factor for adult PCOS (52). The involvement of weight gain during puberty in the association between adolescent asthma and adult PCOS may be worth further investigation.

We also performed restriction analysis by body size at 10 years old and childhood health status up to 10 years old in all analyses (Table 2-4) to further explore the role of obesity in the association between pediatric asthma and adult PCOS since we could not rule out the possibility that childhood obesity and childhood health status may mediate the association between pediatric asthma and adult PCOS. Further analysis showed that childhood obesity or childhood health status alone was unlikely to explain the

TABLE 4 Modified Poisson regression analysis between females with pediatric asthma and adult PCOS, stratified by the age of asthma diagnosis (0-10 vs >11-19 years), in total population and restricted by potential mediators (N=1334).

	Age of asthma diagnosis: 0-10 years						Age of asthma diagnosis: 11-19 years					
	Crude Model			Adjusted Model[a]			Crude Model			Adjusted Model[a]		
	RR	[95% CI]	*P*	RR	[95% CI]	*P*	RR	[95% CI]	*P*	RR	[95% CI]	*P*
Main analysis												
Non-asthmatics	(Reference)			(Reference)			(Reference)			(Reference)		
Pediatric asthmatics	1.59	0.94-2.68	0.082	1.46	0.89-2.39	0.130	2.27	1.01-5.10	0.047	1.99	0.93-4.23	0.075
Restriction analysis by body size at 10 years old												
About average												
Non-asthmatics	(Reference)			(Reference)			(Reference)			(Reference)		
Pediatric asthmatics	1.46	0.60-3.54	0.405	1.18	0.56-2.49	0.657	2.57	0.71-9.25	0.150	2.39	0.80-7.19	0.120
Thinner												
Non-asthmatics	(Reference)			(Reference)			(Reference)			(Reference)		
Pediatric asthmatics	1.69	0.72-4.00	0.229	1.68	0.76-374	0.202	2.88	1.04-7.98	0.042	3.50	1.38-8.43	0.008
Plumper												
Non-asthmatics	(Reference)			(Reference)			(Reference)			(Reference)		
Pediatric asthmatics	1.20	0.38-3.78	0.753	1.36	0.36-5.17	0.649	N/A			N/A		
Restriction analysis by childhood health status up to 10 years old												
Poor or fair												
Non-asthmatics	(Reference)			(Reference)			(Reference)			(Reference)		
Pediatric asthmatics	2.28	0.83-6.18	0.106	2.54	1.03-6.30	0.044	N/A			N/A		
Excellent or good												
Non-asthmatics	(Reference)			(Reference)			(Reference)			(Reference)		
Pediatric asthmatics	1.39	0.74-2.66	0.307	1.23	0.66-2.28	0.511	2.65	1.20-5.87	0.016	2.13	1.01-4.50	0.047

[a]Adjusted for age (continuous), urbanicity at birth (rural/urban), and parental smoking at birth (no/yes). N/A reflects insufficient sample size.

mechanism between pediatric asthma and adult PCOS, as the observed significance disappeared when the analysis was restricted to those with average or plumper body size at 10 years old, as well as to those with poorer/fair or excellent/good childhood health status up to 10 years old (Table 2). Childhood body size (obesity or overweight) may be related to pediatric asthma and adult PCOS through the following mechanisms. In addition to their lower metabolic rate as previously mentioned (47, 48), pediatric asthmatics are known to have poorer childhood health and may limit their physical activities due to their asthma conditions, hence, more susceptible to subsequent childhood overweight or obesity, compared to their healthy counterparts (37, 55). However, due to the design of this study, we could not further evaluate whether childhood obesity or childhood health status indeed mediated the association between pediatric asthma and adult PCOS.

4.1 Strengths and limitations

To our knowledge, our study is unique because it is the first population-based study examining the association between pediatric asthma and adult PCOS. We were able to assess the association with clear temporality and were able to control for relevant confounding factors. In addition, the large sample size in this study was suitable to perform stratification and/or restriction analysis to better assess and address the potential mediating effect on the association between pediatric asthma and adult PCOS. Epidemiological study of PCOS during different life stages is still limited and this study might guide future research.

Despite the strength of our study, we acknowledge some limitations. One major limitation pertains to the self-reported diagnosis of asthma and PCOS which might raise the concern about disease ascertainment accuracy. However, previous studies have shown self-reported asthma and PCOS diagnoses to be reliable (56–58). Self-reported age of asthma diagnosis was found to be accurate and had a low variability across categories of demographic and health-related characteristics (56), and self-reported PCOS was found to have high sensitivity in predicting PCOS (78%) compared to the sensitivity of PCOS diagnosis using the Rotterdam criteria (89%) (59). We have also adjusted for age in our adjusted analysis to further address the recall error for all self-reported variables. Furthermore, our study was also prone to misclassification, especially regarding childhood variables such as childhood body size, childhood health status, and parental smoking. However, we believe asthmatics and non-asthmatics in this study reported childhood variables in a similar fashion, resulting in non-differential misclassification. With regards to the convenience sampling design employed in our study, we have attempted to improve the representativeness of the sample by inviting the entire eligible population of the UAE to participate and operating multiple recruitment centers in different regions across the country to ensure ease of access. Another limitation is that we did not have information on the severity of asthma and PCOS to examine the associations involving disease severity (31). Our study may be prone to survivorship bias since we only included those who were alive at the questionnaire time; however, childhood mortality rates are low in the UAE and this is likely to have had a minimal effect on the findings (60). Our study also a had low sample size and statistical power in certain stratification analyses, such as in certain strata in the childhood body size and childhood health status (with N/A reflecting insufficient sample size). Lastly, our study may have been subjected to unmeasured confounding factors, including those factors with significant missing values such as parental education (40, 41) and birthweight (42, 43). However, our sensitivity analysis revealed similar results with or without the involvement of parental education and birthweight in the adjusted model (data not shown).

4.2 Conclusions

Our data demonstrated that pediatric asthma was an independent risk factor for adult PCOS. More targeted surveillance for those at risk of adult PCOS among pediatric asthmatics, may prevent or delay adult PCOS occurrence in this at-risk group. Future population-based studies with robust longitudinal designs aimed to elucidate the exact mechanism between pediatric asthma and PCOS are warranted.

Institutional review board statement

The study and its procedures have been reviewed and approved by the Institutional Review Board at New York University Abu Dhabi, Dubai Health Authority, Ministry of Health and Prevention in the UAE, and Health Research and Technology Committee, reference number DOH/HQD/2020/516.

Informed consent statement

Written consent was obtained from participants at the centers or by filling out an online consent form before data collection started.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by The study and its procedures have been reviewed and approved by the Institutional Review Board at New York University Abu Dhabi, Dubai Health Authority, Ministry of Health and Prevention in the UAE, and Health Research and Technology Committee, reference number DOH/HQD/2020/516. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization, NJ, RA. Formal analysis, NJ. Data curation, NJ. Writing—original draft preparation, NJ. Writing—review and editing, AAb, AAh, AL-J, ASA., EA, FM, FA-M, FA, HA, JA, LW, MA, MK, MW, MA-H, MH-A, NO, OE-S, SS, SMS, TL, WA, YI, RA. Funding acquisition, RA All authors contributed to the article and approved the submitted version.

Funding

This publication is supported by Tamkeen under Research Institute Grant No. G1206.

Acknowledgments

The authors would like to thank all members of the Public Health Research Center at New York University Abu Dhabi and those who are actively involved in the UAE Healthy Future Study (UAEHFS). This study would not have been possible without their collective effort.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Miranda C, Busacker A, Balzar S, Trudeau J, Wenzel SE. Distinguishing severe asthma phenotypes: role of age at onset and eosinophilic inflammation. *J Allergy Clin Immunol* (2004) 113(1):101–8. doi: 10.1016/j.jaci.2003.10.041

2. Bjermer L. Time for a paradigm shift in asthma treatment: from relieving bronchospasm to controlling systemic inflammation. *J Allergy Clin Immunol* (2007) 120(6):1269–75. doi: 10.1016/j.jaci.2007.09.017

3. Trivedi M, Denton E. Asthma in children and adults-what are the differences and what can they tell us about asthma? *Front Pediatr* (2019) 7:256. doi: 10.3389/fped.2019.00256

4. Bisgaard H, Bønnelykke K. Long-term studies of the natural history of asthma in childhood. *J Allergy Clin Immunol* (2010) 126(2):187–97. doi: 10.1016/j.jaci.2010.07.011

5. Juber NF, Lee CC, Pan WC, Liu JJ. Associations between pediatric asthma and adult non-communicable diseases. *Pediatr Allergy Immunol* (2021) 32(2):314–21. doi: 10.1111/pai.13395

6. De Leo V, Musacchio M, Cappelli V, Massaro M, Morgante G, Petraglia F. Genetic, hormonal and metabolic aspects of PCOS: an update. *Reprod Biol Endocrinol* (2016) 14(1):1–17. doi: 10.1186/s12958-016-0173-x

7. Franks S, McCarthy MI, Hardy K. Development of polycystic ovary syndrome: involvement of genetic and environmental factors. *Int J Androl* (2006) 29(1):278–85. doi: 10.1111/j.1365-2605.2005.00623.x

8. Sanchez-Garrido MA, Tena-Sempere M. Metabolic dysfunction in polycystic ovary syndrome: pathogenic role of androgen excess and potential therapeutic strategies. *Mol Metab* (2020) 35:100937. doi: 10.1016/j.molmet.2020.01.001

9. Talbott EO, Zborowski JV, Rager JR, Kip KE, Xu X, Orchard TJ. Polycystic ovarian syndrome (PCOS): a significant contributor to the overall burden of type 2 diabetes in women. *J Womens Health* (2007) 16(2):191–7. doi: 10.1089/jwh.2006.0098

10. Bednarska S, Siejka A. The pathogenesis and treatment of polycystic ovary syndrome: what's new? *Adv Clin Exp Med* (2017) 26(2):359–67. doi: 10.17219/acem/59380

11. Zierau L, Gade EJ, Lindenberg S, Backer V, Thomsen SF. Coexistence of asthma and polycystic ovary syndrome: a concise review. *Respir Med* (2016) 119:155–9. doi: 10.1016/j.rmed.2016.08.025

12. Htet TD, Teede HJ, De Courten B, Loxton D, Real FG, Moran LJ, et al. Asthma in reproductive-aged women with polycystic ovary syndrome and association with obesity. *Eur Respir J* (2017) 49(5):1601334. doi: 10.1183/13993003.01334-2016

13. Sun H, Li D, Jiao J, Liu Q, Bian J, Wang X. A potential link between polycystic ovary syndrome and ssthma: a meta-analysis. *Reprod Sci* (2022) 29(1):312–9. doi: 10.1007/s43032-021-00662-8

14. Grieger JA, Hodge A, Mishra G, Joham AE, Moran LJ. The association between dietary intake, asthma, and PCOS in women from the Australian longitudinal study on women's health. *J Clin Med* (2020) 9(1):233. doi: 10.3390/jcm9010233

15. Guarnieri G, Iervolino M, Cavallone S, Unfer V, Vianello A. The "Asthma-polycystic ovary overlap syndrome" and the therapeutic role of yo-inositol. *Int J Mol Sci* (2023) 24(8):6959. doi: 10.3390/ijms24086959

16. Tremellen K, Pearce K. Dysbiosis of gut microbiota (DOGMA)–a novel theory for the development of polycystic ovarian syndrome. *Med Hypotheses* (2012) 79(1):104–12. doi: 10.1016/j.mehy.2012.04.016

17. Molyneaux PL, Cox MJ, Wells AU, Kim HC, Ji W, Cookson WO, et al. Changes in the respiratory microbiome during acute exacerbations of idiopathic pulmonary fibrosis. *Respir Res* (2017) 18:1–6. doi: 10.1186/s12931-017-0511-3

18. Ramírez-Labrada AG, Isla D, Artal A, Arias M, Rezusta A, Pardo J, et al. The influence of lung microbiota on lung carcinogenesis, immunity, and immunotherapy. *Trends Cancer* (2020) 6(2):86–97. doi: 10.1016/j.trecan.2019.12.007

19. Hart R, Doherty DA. The potential implications of a PCOS diagnosis on a woman's long-term health using data linkage. *J Clin Endocrinol Metab* (2015) 100(3):911–9. doi: 10.1210/jc.2014-3886

20. Sánchez-Ramos JL, Pereira-Vega AR, Alvarado-Gómez F, Maldonado-Pérez JA, Svanes C, Gómez-Real F. Risk factors for premenstrual asthma: a systematic review and meta-analysis. *Expert Rev Respir Med* (2017) 11(1):57–72. doi: 10.1080/17476348.2017.1270762

21. Becerra-Diaz M, Song M, Heller N. Androgen and androgen receptors as regulators of monocyte and macrophage biology in the healthy and diseased lung. *Front Immunol* (2020) 11:1698. doi: 10.3389/fimmu.2020.01698

22. Collée J, Mawet M, Tebache L, Nisolle M, Brichant G. Polycystic ovarian syndrome and infertility: overview and insights of the putative treatments. *Gynecol Endocrinol* (2021) 37(10):869–74. doi: 10.1080/09513590.2021.1958310

23. Gade E, Thomsen S, Lindenberg S, Backer V. Female asthma has a negative effect on fertility: what is the connection? *Allergy* (2014) 2014:131092. doi: 10.1155/2014/131092

24. Assad N, Qualls C, Smith LJ, Arynchyn A, Thyagarajan B, Schuyler M, et al. Body mass index is a stronger predictor than the metabolic syndrome for future asthma in women. the longitudinal CARDIA study. *Am J Respir Crit Care Med* (2013) 188(3):319–26. doi: 10.1164/rccm.201303-0457OC

25. Glueck CJ, Goldenberg N. Characteristics of obesity in polycystic ovary syndrome: etiology, treatment, and genetics. *Metab* (2019) 92:108–20. doi: 10.1016/j.metabol.2018.11.002

26. Naderpoor N, Shorakae S, Joham A, Boyle J, De Courten B, Teede HJ. Obesity and polycystic ovary syndrome. *Minerva Endocrinol* (2014) 40(1):37–51.

27. Barber TM, Franks S. Obesity and polycystic ovary syndrome. *Clin Endocrinol* (2021) 95(4):531–41. doi: 10.1111/cen.14421

28. Carpaij OA, Van den Berge M. The asthma–obesity relationship: underlying mechanisms and treatment implications. *Curr Opin Pulm Med* (2018) 24(1):42–9. doi: 10.1097/MCP.0000000000000446

29. Zierau L, Meteran H, Backer V, Lindenberg S, Skytthe A, Thomsen SF. The risk of asthma is increased among women with polycystic ovary syndrome: a twin study. *ERJ Open Res* (2019) 5(3):00018-2018. doi: 10.1183/23120541.00018-2018

30. Juber NF, Abdulle A, AlJunaibi A, AlNaeemi A, Ahmad A, Leinberger-Jabari A, et al. Association between self-reported polycystic ovary syndrome with chronic diseases among emiratis: a cross-sectional analysis from the UAE healthy future study. *Int J Women's Health* (2023) 15:289–98. doi: 10.2147/IJWH.S398651

31. Xu Y, Zhou Z-Y, Pan J-X, Huang H-F. Associations between asthma and polycystic ovary syndrome: current perspectives. *Front Endocrinol* (2022) 13:936948. doi: 10.3389/fendo.2022.936948

32. Abdulle A, Alnaeemi A, Aljunaibi A, Al Ali A, Al Saedi K, Al Zaabi E, et al. The UAE healthy future study: a pilot for a prospective cohort study of 20,000 united Arab Emirates nationals. *BMC Public Health* (2018) 18(1):1–9. doi: 10.1186/s12889-017-5012-2

33. Furu K, Skurtveit S, Langhammer A, Nafstad P. Use of anti-asthmatic medications as a proxy for prevalence of asthma in children and adolescents in Norway: a nationwide prescription database analysis. *Eur J Clin Pharmacol* (2007) 63 (7):693–8. doi: 10.1007/s00228-007-0301-9

34. Carmina E, Campagna AM, Lobo RA. A 20-year follow-up of young women with polycystic ovary syndrome. *Obstet Gynecol* (2012) 119(2):263–9. doi: 10.1097/AOG.0b013e31823f7135

35. Barros AJ, Hirakata VN. Alternatives for logistic regression in cross-sectional studies: an empirical comparison of models that directly estimate the prevalence ratio. *BMC Med Res Methodol* (2003) 3(1):1–13. doi: 10.1186/1471-2288-3-21

36. Bennett CJ, Mansfield DR, Mo L, Joham AE, Cain SW, Blumfield ML, et al. Sleep disturbances may influence lifestyle behaviours in women with self-reported polycystic ovary syndrome. *Br J Nutr* (2022) 127(9):1395–403. doi: 10.1017/S0007114521002361

37. Juber NF, Waits A, Dlamini LP, Nguyen T, Masango BZ. Associations between pediatric asthma and age at menarche: evidence from the Indonesian family life survey. *J Asthma* (2022) 60:1–10. doi: 10.1080/02770903.2022.2030750

38. Balaji S, Amadi C, Prasad S, Bala Kasav J, Upadhyay V, Singh AK, et al. Urban rural comparisons of polycystic ovary syndrome burden among adolescent girls in a hospital setting in India. *BioMed Res Int* (2015) 2015:158951. doi: 10.1155/2015/158951

39. Gollenberg AL, Addo OY, Zhang Z, Hediger ML, Himes JH, Lee PA. In utero exposure to cigarette smoking, environmental tobacco smoke and reproductive hormones in US girls approaching puberty. *Horm Res Paediatr* (2015) 83(1):36–44. doi: 10.1159/000369168

40. Celedón JC, Soto-Quiros ME, Silverman EK, Hanson LA, Weiss ST. Risk factors for childhood asthma in Costa Rica. *Chest* (2001) 120(3):785–90. doi: 10.1378/chest.120.3.785

41. Merkin SS, Azziz R, Seeman T, Calderon-Margalit R, Daviglus M, Kiefe C, et al. Socioeconomic status and polycystic ovary syndrome. *J Womens Health* (2011) 20 (3):413–9. doi: 10.1089/jwh.2010.2303

42. Xu X-F, Li Y-J, Sheng Y-J, Liu J-L, Tang L-F, Chen Z-M. Effect of low birth weight on childhood asthma: a meta-analysis. *BMC Pediatr* (2014) 14(1):1–8. doi: 10.1186/1471-2431-14-275

43. Sadrzadeh S, Hui E, Schoonmade L, Painter R, Lambalk C. Birthweight and PCOS: systematic review and meta-analysis. *Hum Reprod Open* (2017) 2:10. doi: 10.1093/hropen/hox010

44. Serafino-Agrusa L, Spatafora M, Scichilone N. Asthma and metabolic syndrome: current knowledge and future perspectives. *World J Clin cases* (2015) 3(3):285. doi: 10.12998/wjcc.v3.i3.285

45. Juhn YJ. Influence of asthma epidemiology on the risk for other diseases. *Allergy Asthma Immunol Res* (2012) 4(3):122–31. doi: 10.4168/aair.2012.4.3.122

46. Sumino K, O'Brian K, Bartle B, Au DH, Castro M, Lee TA. Coexisting chronic conditions associated with mortality and morbidity in adult patients with asthma. *J Asthma* (2014) 51(3):306–14. doi: 10.3109/02770903.2013.879881

47. Wolf JM, Nicholls E, Chen E. Chronic stress, salivary cortisol, and α-amylase in children with asthma and healthy children. *Biol Psychol* (2008) 78(1):20–8. doi: 10.1016/j.biopsycho.2007.12.004

48. Spraul M, Ravussin E, Fontvieille AM, Rising R, Larson DE, Anderson EA. Reduced sympathetic nervous activity. a potential mechanism predisposing to body weight gain. *J Clin Investig* (1993) 92(4):1730–5. doi: 10.1172/JCI116760

49. Glazier DS. Is metabolic rate a universal 'pacemaker'for biological processes. *Biol Rev* (2015) 90(2):377–407. doi: 10.1111/brv.12115

50. El Hayek S, Bitar L, Hamdar LH, Mirza FG, Daoud G. Polycystic ovarian syndrome: an updated overview. *Front Physiol* (2016) 7:124. doi: 10.3389/fphys.2016.00124

51. Jena SK, Mishra L, Naik SS, Khan S. Awareness and opinion about polycystic ovarian syndrome (PCOS) among young women: a developing country perspective. *Int J Adolesc Med Health* (2021) 33(3):123–6. doi: 10.1515/ijamh-2018-0166

52. Michelmore KF. Polycystic ovary syndrome in adolescence and early adulthood. *Hum Fertil* (2000) 3(2):96–100. doi: 10.1080/1464727002000198771

53. Davison SL, Bell R, Donath S, Montalto JG, Davis SR. Androgen levels in adult females: changes with age, menopause, and oophorectomy. *J Clin Endocrinol Metab* (2005) 90(7):3847–53. doi: 10.1210/jc.2005-0212

54. Abraham S, Boyd C, Lal M, Luscombe G, Taylor A. Time since menarche, weight gain and body image awareness among adolescent girls: onset of eating disorders? *J Psychosom Obstet Gynaecol* (2009) 30(2):89–94. doi: 10.1080/01674820902950553

55. Alqahtani N, Scott J, Ullah S. Physical activity and sedentary behaviors as risk factors of obesity among rural adolescents. *J Child Adolesc Behav* (2015) 3:1. doi: 10.4172/2375-4494.1000185

56. Mirabelli MC, Beavers SF, Flanders WD, Chatterjee AB. Reliability in reporting asthma history and age at asthma onset. *J Asthma* (2014) 51(9):956–63. doi: 10.3109/02770903.2014.930480

57. Day F, Karaderi T, Jones MR, Meun C, He C, Drong A, et al. Large-Scale genome-wide meta-analysis of polycystic ovary syndrome suggests shared genetic architecture for different diagnosis criteria. *PloS Genet* (2018) 14(12):e1007813. doi: 10.1371/journal.pgen.1007813

58. Toren K, Brisman J, Järvholm B. Asthma and asthma-like symptoms in adults assessed by questionnaires: a literature review. *Chest* (1993) 104(2):600–8. doi: 10.1378/chest.104.2.600

59. Elting MW, Korsen TJ, Rekers-Mombarg LT, Schoemaker J. Women with polycystic ovary syndrome gain regular menstrual cycles when ageing. *Hum Reprod* (2000) 15(1):24–8. doi: 10.1093/humrep/15.1.24

60. Sagynbekov K. Childhood and maternal health in the middle East and north Africa. *Milken Institute* (2018), 1–26.

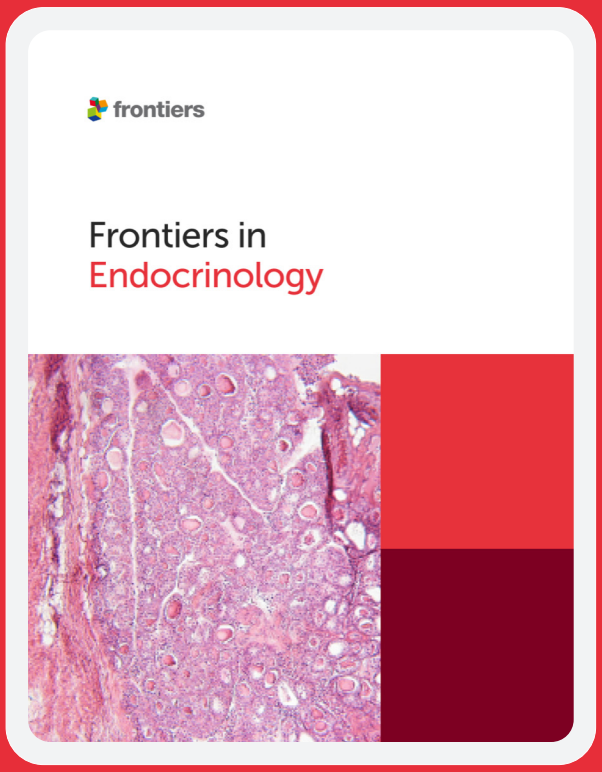
Frontiers in Endocrinology

Explores the endocrine system to find new therapies for key health issues

The second most-cited endocrinology and metabolism journal, which advances our understanding of the endocrine system. It uncovers new therapies for prevalent health issues such as obesity, diabetes, reproduction, and aging.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact