

Exenatide Improves Endometrial Glands in PCOS Rats through AMPK α -SIRT1

Junxian He

Third Affiliated Hospital of Sun Yat-Sen University

Lisi Cai

Sun Yat-sen University First Affiliated Hospital

Jing Li

Third Affiliated Hospital of Sun Yat-Sen University <https://orcid.org/0000-0002-7485-8582>

Yunhui Li

Third Affiliated Hospital of Sun Yat-Sen University

Xin Tao

Third Affiliated Hospital of Sun Yat-Sen University

Yu Zhang (✉ zhangyu6@mail.sysu.edu.cn)

Department of Gynecology, the Third Affiliated Hospital, Sun-Yet Sen University, People's Republic of China <https://orcid.org/0000-0002-5632-9791>

Research

Keywords: Polycystic ovary syndrome (PCOS), AMPK, SIRT1, Exenatide

Posted Date: May 13th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-28388/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Exenatide can contribute to the therapeutic effect for PCOS patients in restoring menstrual cycles. To exploring endometrial tissue change in PCOS rats and the effects of exenatide on endometrial tissue, we carried out in vivo study of PCOS rat models. Method: PCOS rat models were obtained after DHEA treatment, and the corresponding parameters were measured to confirm the establishment of PCOS models. Hematoxylin-eosin (H&E) staining was performed to observe endometrium morphological change, and western blot and RT-PCR were performed to identify the alteration AMP-activated protein kinase (AMPK α) and SIRT1 proteins and the relative expression of SIRT1 mRNA in endometrial cellular after the intervention of exenatide (EX). Results: The endometrium of PCOS rats appeared to not only the gland number increased but also the gland size enlarged. When the PCOS rats underwent EX treatment, the gland number decreased and the gland size narrowed, the expression of AMPK α and SIRT1 protein increased, and the expression of SIRT1 mRNA level augmented. Conclusion: EX could decrease gland number and narrow gland size in PCOS rat endometrium which may be partly via AMPK α -SIRT1 pathway.

Introduction

Endometrial hyperplasia (EH) is a non-physiological and non-invasive endometrial hyperplasia. The change of glandular structure (size and shape), glandular and interstitial ratio (greater than 1:1) leads to the increase of endometrial volume[1]. The risk of EH without atypia progressing to endometrial cancer is 1%~3%, and the risk of EH with atypia progressing to endometrial cancer is 25%~40%[2]. It is a common endometrial lesion causing abnormal uterus bleeding (AUB)[3], and we found that EH (including endometrial hyperplasia without atypia; mild, moderate and severe atypia) accounted for 45% of AUB cases[4]. Polycystic ovary syndrome (PCOS) is characterized by the ultrasonographic appearance of polycystic ovaries, ovulatory dysfunction, and hyperandrogenism, resulting in approximately 8.3%~9.13% of adolescent and reproductive-aged women to infertility[5]. Metabolic dysfunction characterized by insulin resistance was evident in the vast majority of affected individuals [2]. There was increasing studies indicated that the application of insulin sensitizers, such as metformin (MET), can improve the reproductive function of PCOS patients [2, 3]. And SIRT1 agonist such as exenatide (EX) appeared to be superior to MET in restoring menstrual cycles and regulating metabolic disorders[6]. In our previous study[7, 8], insulin resistance in PCOS rats was associated with the AMPK α -SIRT1 pathway. Furthermore, in our newly study showed that both EX and MET could improve the reproductive and endocrine functions of PCOS mice via the AMPK α -SIRT1 pathway, which may be the molecular mechanism for IR in PCOS and could possibly serve as a therapeutic target [9].

And what is the endometrium morphological images in PCOS rats and what is the endometrium changes after EX treatment? So in this study [9], we have also observed the endometrium change and investigated whether their protective effects were correlated to the pathway of AMPK α -SIRT1.

Materials And Methods

PCOS rat models

Fifty female SD rats (25-day-old) were obtained from The Animal Experimental Center of Sun Yat-sen University Medical College (SCXK (GuangDong) 2011–0029). The average body weight of these rats was 79.79 ± 4.18 g. They were randomly divided into two groups: PCOS model group (n = 37) and normal control group (n = 13). The PCOS group rats were subcutaneously injected for 20 days with dehydroepiandrosterone (DHEA 6 mg/(100 g·d)) (Millipore (252805)) and 0.2 ml injectable soybean oil; while the NC group rats were subcutaneously injected with only 0.2 ml of injectable soybean oil. The rats' weight was recorded daily. After ten days of injections, the rats in both groups were vaginally swabbed daily and the discharge was observed under the microscope throughout three estrous cycles. After the estrous cycle of the PCOS group disappeared or irregular, the PCOS model were considered to have been successfully established. Eight rats were randomly selected (3 from the control group and 5 from the PCOS group) for the fasting blood glucose, serum testosterone and fasting insulin tests, as well as for histological examination of their ovarian issues to further evaluate the efficiency of model establishment.

The rest of 32 PCOS rats were randomly divided into 3 groups: PCOS-NS group (n = 10), PCOS-EX group (n = 11), PCOS-MET group (n = 11), and the remaining normal control group rats were for the normal control group (Ctrl group, n = 8). PCOS-EX group was subcutaneously injected EX 10 μ g/(kg·d) daily, dissolved in 0.2 ml of sterile distilled water. PCOS-MET group was administered MET 300 mg (kg·d) daily, dissolved in 0.2 ml sterile distilled water. The PCOS-NS group and Ctrl group were subcutaneously injected with only 0.2 ml of sterile distilled water every day. All injections lasted for 4 weeks.

Hematoxylin-eosin (H&E) staining

The endometrium tissue collection and the hematoxylin-eosin (H&E) staining was done as previously described[7]. Endometrium tissue were placed in xylene and deparaffinized. After hydrating the tissue, hematoxylin and eosin were used to stain them. In the microscopic examination of endometrium tissue, 3 fields were randomly selected in every pathological section for observation and gland number in glandular epithelium were calculated under high power microscope fields (HPF) (200X), then we calculated the percentage of gland (diameter > 50 μ m) in total glands to evaluate the gland size.

Western blot assays (WB)

The primary antibody was purchased from Cell Signaling Technology (CST): anti-AMPK α (5832S), anti-pAMPK α 1/2 (2535S), anti-SIRT1 (8469S). All corresponding secondary antibodies were purchased from Sino Biological (China, Beijing).

Quantitative real-time polymerase chain reaction (qPCR)

Total RNA from endometrium tissues were extracted using Trizol reagent (Invitrogen), and cDNA was generated using a reverse transcription kit (Takara (RR047A)). The RT-PCR kit was purchased from Takara (RR820A). Primer sequences were as follows: SIRT1: 5'-TCGGCTACCGAGGTCCATA – 3'(forward), 5'-ACAATCTGCCACAGCGTCAT – 3'(reverse); control GAPDH: 5'-AGTGCCAGCCTCGTCTCATA – 3'(forward), 5'-ATGAAGGGGTCGTTGATGGC – 3'(reverse).

Statistical analysis

Data statistics and analysis were performed using GraphPad Prism version 8.0.0 for Mac OS GraphPad Software (San Diego, California USA, www.graphpad.com). The results were expressed in mean \pm standard deviation (SD) or median and interquartile ranges. The non-parametric test was used for homogeneity of variance. Unpaired t test with two tails was carried out to evaluate two groups. One-way ANOVA was carried out when multiple comparisons were evaluated.

Results

Construction of PCOS model and its corresponding parameters

After DHEA pretreatment, the PCOS models were constructed successfully. Details of construction were comprehensively presented in previous paper [9].

Endometrium morphologic changes after DHEA pretreatment

To investigate the effect of exenatide and metformin in mice with PCOS, we performed hematoxylin-eosin (H&E) staining. From the morphological images, there were slightly larger size of uterus tissue in PCOS groups (Fig. 1b, c, d) comparing to that in the normal control group (Fig. 1a). Additionally, we found increased endometrial gland numbers and enlarged gland size after DHEA treatment (comparing PCOS-NS group to Ctrl group, $P=0.0058$). And comparing to PCOS-NS group, the gland number decreased (EX: $P=0.0324$, MET: $P=0.0465$, Fig. 1e, f) and the gland size diminished (EX: $P=0.0007$, MET: $P=0.0119$, Fig. 1e, f) in PCOS-EX group and PCOS-MET group. These results implied that EX or MET treatment may reverse the PCOS rat's endometrial gland number and gland size.

AMPK α and SIRT1 protein in rat endometrial tissues after EX or MET intervention

The results of Western blot detecting the proteins (AMPK α and SIRT1) (Fig. 2a) and RT-PCR detecting the gene of SIRT1 (Fig. 2e) were applied to investigate whether EX or MET could reverse PCOS endometrial glands via AMPK α -SIRT1 pathway. After EX or MET treatment, the expression of P-AMPK α (EX: $P=0.0194$, MET: $P=0.0012$, Fig. 2a & 2b) and SIRT1 protein increased comparing to those in PCOS-NS

group, but the increased SIRT1 protein was not significant higher than that in PCOS-NS group (Fig. 2a & 2d). The expression of SIRT1 mRNA level augmented in PCOS-EX group ($P = 0.0442$, Fig. 2e) comparing to that in PCOS-NS group, but there was no remarkable change in MET group (Fig. 2e) comparing to that in PCOS-NS group. These results revealed that EX or MET may exert positive effect to PCOS rat endometrium via AMPK α -SIRT1 pathway.

Discussion

PCOS is known as metabolic dysfunction disease including hyperandrogenism, ovulatory dysfunction and insulin resistance (IR), which endangers the health of woman worldwide. Moreover, the PCOS patients are always suffer from its following diseases including type 2 diabetes, cardiovascular disease, endometrial hyperplasia or endometrial cancer, and so on[10]. It has been confirmed that MET and EX, extensively applied in metabolic diseases such as T2DM, metabolic syndrome, cardiovascular disease and PCOS, improved patients' metabolic imbalance and protect patients from corresponding injury. We have investigated the interrelationship between these two drugs and AMPK-SIRT1 pathway in PCOS rat model ovaries, the improved ovarian reservation function has been seen in PCOS rats when intervened by EX or MET [9]. And now, this paper just focused on another side of the same study, the effect of EX on the endometrium of PCOS rat models.

According to previous study [11], the endometrium of PCOS patients does not undergo the consecutive circles that result in normal endometrial glands proliferation. The endometrial proliferation in woman with PCOS are often regulated by androgens, insulin, and unopposed estrogens. Without the protection of adequate progesterone, the endometrium does not undergo secretory period and tends to overgrowth or hyperplasia even cause cancer. Thus, it is high risk for PCOS patients to suffer from endometrial hyperplasia even cancer.

In our study, the endometrial glands of PCOS rats appeared to overgrowth even hyperplasia, not only the gland number increased but also the gland size enlarged. And when the rats underwent EX or MET treatment, the gland number decreased and the gland size narrowed, which inferred that either EX or MET could improve the endometrial hyperplasia that is a common pathological state in PCOS, and MET may have therapeutic benefits for PCOS patients in endometrial hyperplasia, and some studies have already prompted or confirmed[12, 13]. We have investigated that EX or MET influenced the ovaries of PCOS rats, not only in the ovarian morphology but also in the ovarian reservation function [8, 9], and similarly, in the endometrium of PCOS mice models, we also found EX or MET were able to exert positive effect on endometrial glands in PCOS rats.

MET, known as an AMPK activator, is a classic drug that is widely used for type 2 diabetes and have been applied extensively in PCOS patients as adjuvant medication, and has been widely accepted to improve IR, glucose tolerance, liver metabolism and so on [14]. It is also reported that MET ameliorated the glucose metabolism and reduced the related complications by improving the PCOS rats' IR and normalizing their

serum insulin concentration[15]. Hui Guo et al have reported that MET can inhibited the overgrowth endometrium in endometrial cancer and the anti-tumor effect was greater in obese rats than in lean rats[16]. EX is a GLP-1 receptor agonist, which has been used as newly emerging drug for type 2 diabetes as well as other metabolic diseases. The effect of EX improved IR in *ob/ob* mice and decreases hepatic lipid storage[17], additionally, it could reduce fasting plasma glucose, body weight, and liver fat in type 2 diabetes patients[18], and EX could protect PCOS's endometrial and ovarian environment against oxidative stress [19]. Jinmi Lee investigated that exenatide improves steatohepatitis by enhancing the expression of SIRT1 protein and phosphor-AMPK α in HepG2 cells of obese mice[20]. SIRT1 is also another metabolic regulation enzyme that can regulates the ratio of NAD⁺ and NADH and maintain the balance of them. Altogether, AMPK and SIRT1 play main roles in metabolic regulation. Carles Cantó et al demonstrated that AMPK regulates the deacetylation of SIRT1 by increasing NAD⁺ level in cellular, and it have coordinated closely with SIRT1 to control the modulation of the activity of downstream targets[21]. Moreover, we also found that EX could attenuate the growth of endometrial cancer Ishikawa xenografts in nude mice, and AMPK may be the target of the mechanism[22]. So, in this study, we also found EX improved endometrial hyperplasia in PCOS rats at least partially via AMPK α -SIRT1 activation, which was line with the ovary mechanism [9].

In conclusion, exenatide-the synthetic GLP-1 drug-could protect PCOS rat models endometrium against gland number increasing and gland size enlarging at least partially via AMPK α -SIRT1 activation, which would give future clinical trials a foundational theory, and clinical studies are needed to further investigate the efficacy and the underlying mechanism of exenatide to treat endometrial hyperplasia in PCOS.

Declarations

Ethics approval and consent to participate

Ethical approval All procedures involving mice were operated under strict criteria based on the Guide for Care and Use of Laboratory Animals of Sun-yet sen University, and the protocols were approved by The Institutional Animal Care and Use Committee of Sun-yet sen University.

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests

Funding

This study was supported by Science and Technology Planning Project of Guangdong Province of China (2017A020215020).

Authors' contributions

JX He: Manuscript writing, Data analysis, Project development

LS Cai: Data management, Project development

J Li: Data collection, Project development

YH Li: Data collection

X Tao: Manuscript revising

Y Zhang: Protocol development, Manuscript revising

Acknowledgements

Not applicable

References

1. Practice CoG. Society of Gynecologic Oncology. The American College of Obstetricians and Gynecologists Committee opinion no.631 Endometrial intraepithelial neoplasia. *Obstet Gynecol.* 2015;125:1272–8.
2. Sanderson PA, Critchley HO, Williams AR. New concepts for an old problem: the diagnosis of endometrial hyperplasia. *Hum Reprod Update.* 2017;23:232–54.
3. Rao S, Sundaram S, Narasimhan R. Biological behavior of preneoplastic conditions of the endometrium: A retrospective 16-year study in south India Indian. *J Med Paediatr Oncol.* 2009;30:1311–35.
4. Zhang Y, Cheng J, Ye Q, Li X. (2019) Pathological Characteristics of Endometrium in Abnormal Uterine Bleeding and the Relationship between Pathology and Obesity. *JOURNAL OF SUN YAT-SEN UNIVERSITY (MEDICAL SCIENCES)* 40 (2):316–320. doi:10.13471/j.cnki.j.sun.yat-sen.univ(med.sci).2019.0045.
5. Sirmans SM, Pate KA. Epidemiology, diagnosis, and management of polycystic ovary syndrome. *Clin Epidemiol.* 2013;6:1–13.

6. Elkind-Hirsch K, Marrioneaux O, Bhushan M, Vernor D, Bhushan R. Comparison of single and combined treatment with exenatide and metformin on menstrual cyclicity in overweight women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2008;93(7):2670–8. doi:<https://doi.org/10.1210/jc.2008-0115>.
7. Tao X, Chen L, Cai L, Ge S, Deng X. (2017) Regulatory effects of the AMPK α -SIRT1 molecular pathway on insulin resistance in PCOS mice: an in vitro and in vivo study. *Biochem Biophys Res Commun* 292:615–20. doi: <https://doi.org/10.1016/j.bbrc.2017.09.154>.
8. Tao X, Zhang X, Ge SQ, Zhang EH, Zhang B. Expression of SIRT1 in the ovaries of rats with polycystic ovary syndrome before and after therapeutic intervention with exenatide. *nt J Clin Exp Pathol.* 2015;8(7):8276–83.
9. Tao X, Cai L, Chen L, Ge S, Deng X. Effects of metformin and Exenatide on insulin resistance and AMPK α -SIRT1 molecular pathway in PCOS rats. *J Ovarian Res.* 2019;12(1):86. doi:10.1186/s13048-019-0555-8.
10. Azziz R, Carmina E, Chen Z, Dunaif A, Laven JS, Legro RS, Lizneva D, Natterson-Horowitz B, Teede HJ, Yildiz BO. Polycystic ovary syndrome. *Nat Rev Dis Primers.* 2016;2:16057. doi:10.1038/nrdp.2016.57.
11. Giudice LC. Endometrium in PCOS: Implantation and predisposition to endocrine CA. *Best Pract Res Clin Endocrinol Metab.* 2006;20:235–44. doi:10.1016/j.beem.2006.03.005.
12. Wang T, Zhang J, Hu M, Zhang Y, Cui P, Li X, Li J, Edwin V, Mats B, Linus RS, Håkan B. 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.
13. Clement NS, Oliver TR, Shiwani H, Sanner JR, Mulvaney CA, Atiomo W. Metformin for endometrial hyperplasia. *Cochrane Database Syst Rev.* 2017;10:CD012214. doi:10.1002/14651858.CD012214.pub2.
14. JAGER JD, KOOTY A, LEHERT P, WULFFELE MG, BETSD, TEERLINK T, SCHEFFER PG, SCHALKWIJK CG, DONKER AJM, STEHOUWER CDA (2005) Effects of short-term treatment with metformin on markers of endothelial function and inflammatory activity in type 2 diabetes mellitus- a randomized, placebo-controlled trial. *J Intern Med* 257:100–9. doi:10.1111 / j.1365-2796.2004.01420.x.
15. Di Pietro M, Parborell F, Irusta G, Pascuali N, Bas D, Bianchi MS, Tesone M, Abramovich D. Metformin Regulates Ovarian Angiogenesis and Follicular Development in a Female Polycystic Ovary Syndrome Rat Model. *Endocrinology.* 2015;156(4):1453–63. doi:10.1210/en.2014-1765.
16. Guo, Hui K, Weimin Z, Lu, Han, Jianjun, Leslie HC, Yin, Yajie F, Ziwei S, Wenchuan W, Jiandong, Timothy PG, Douglas L, Liza M, Chunxiao Z, Victoria LB-J. Reversal of obesity-driven aggressiveness of endometrial cancer by metformin. *Am J Cancer Res.* 2019;9(10):2170–93.
17. Ding X, Saxena NK, Lin S, Gupta NA, Anania FA. Exendin-4, a glucagon-like protein-1 (GLP-1) receptor agonist, reverses hepatic steatosis in ob/ob mice. *Hepatology.* 2006;43(1):173–81. doi:10.1002/hep.21006.

18. Tushuizen ME, Bunck MC, Pouweis PJ, vW JHT, Heine MD RJ. Incretin mimetics as a novel therapeutic option for hepatic steatosis. *Liver International*. 2006;26:1015–7.
19. Merhi Z. Advanced glycation end products and their relevance in female reproduction. *Hum Reprod*. 2014;29(1):135–45. doi:10.1093/humrep/det383.
20. Ji Hun Choi, Ji Cheol Bae, Se Eun Park, Eun-Jung Rhee, Cheol-Young Park, Ki-Won Oh, Sung-Woo Park, Sun-Woo Kin, Won-Young Lee
10.1371/journal.pone.0031394
Jinmi Lee S-WH, Chae SW, Kim DH, Ji Hun Choi, Ji Cheol Bae, Se Eun Park, Eun-Jung Rhee, Cheol-Young Park, Ki-Won Oh, Sung-Woo Park, Sun-Woo Kin, Won-Young Lee (2012) Exendin-4 improves steatohepatitis by increasing sirt1 expression in high-fat diet-induced obese C57BL/6J mice. *PLoS ONE* 7 (2):e31394. doi:10.1371/journal.pone.0031394.
21. Carles C, Zachary G-H, Jerome NF, Marie L, Liliana N, Jill CM, Peter JE, Pere P, Johan A. AMPK regulates energy expenditure by modulating NAD + metabolism and SIRT1 activity. *Nature*. 2009;458(7241):1056–60. doi:10.1038/nature07813.
22. Zhang Y, Xu F, Liang H, Cai M, Wen X, Li, Xiaomao Weng, Jianping. Exenatide inhibits the growth of endometrial cancer Ishikawa xenografts in nude mice. *Oncol Rep*. 2016;35(5):1340–8. doi:10.3892/or.2015.4476.

Figures

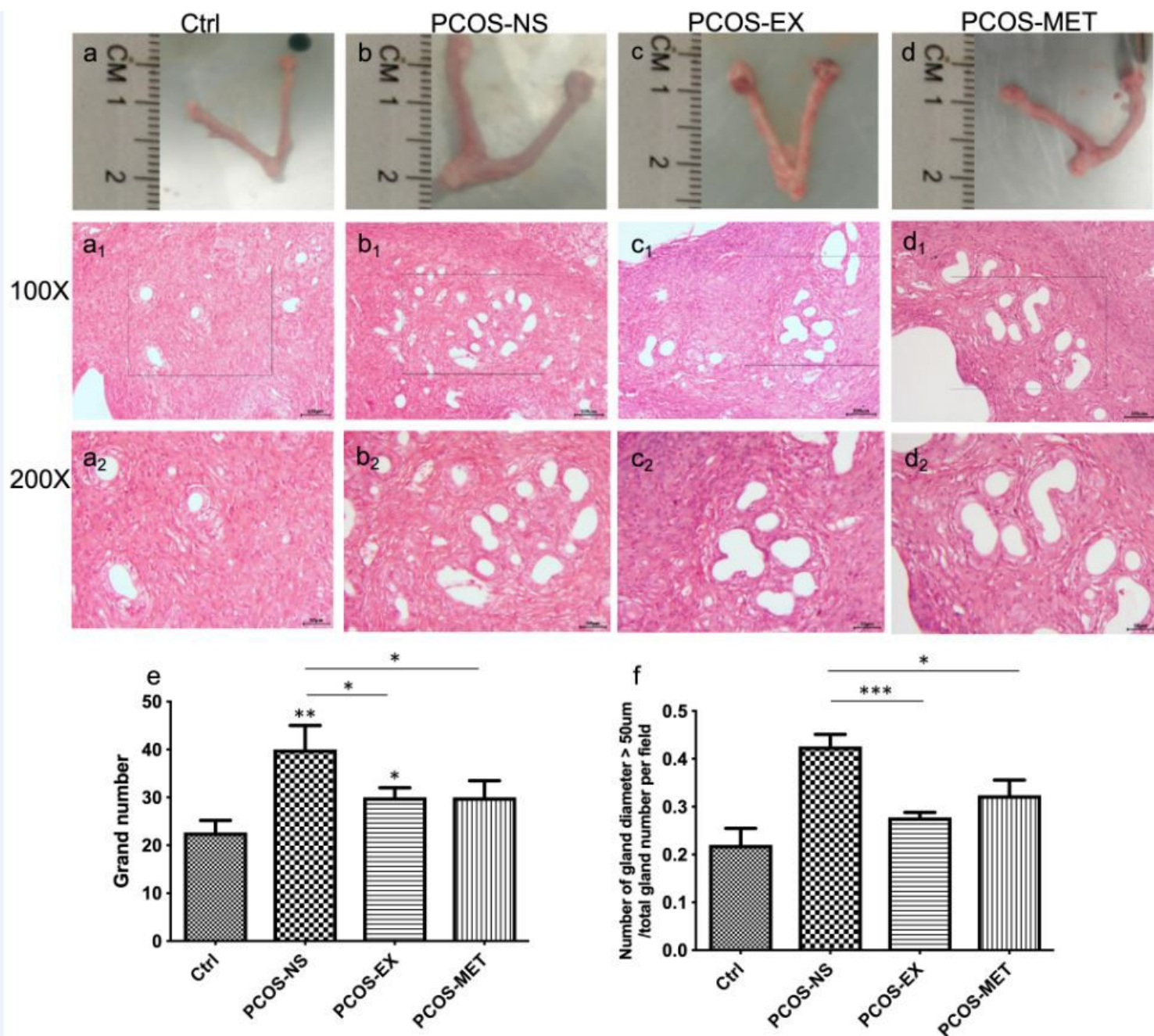


Figure 1

HE staining of the endometrial tissues of rats. a, b, c, d Representative morphological images of rat uterus with DHEA treatment and following exenatide or metformin treatment. The endometrial tissues of the normal control group rats: a1 (100X), a2 (200X); PCOS-NS group: b1 (100X), b2 (200X); PCOS-EX group: c1 (100X), c2 (200X); PCOS- MET group: d1 (100X), d2 (200X); e: Gland number of each groups. f: The ratio of the number of gland diameter>50um/total gland number per field. EX: exenatide; MET: metformin.

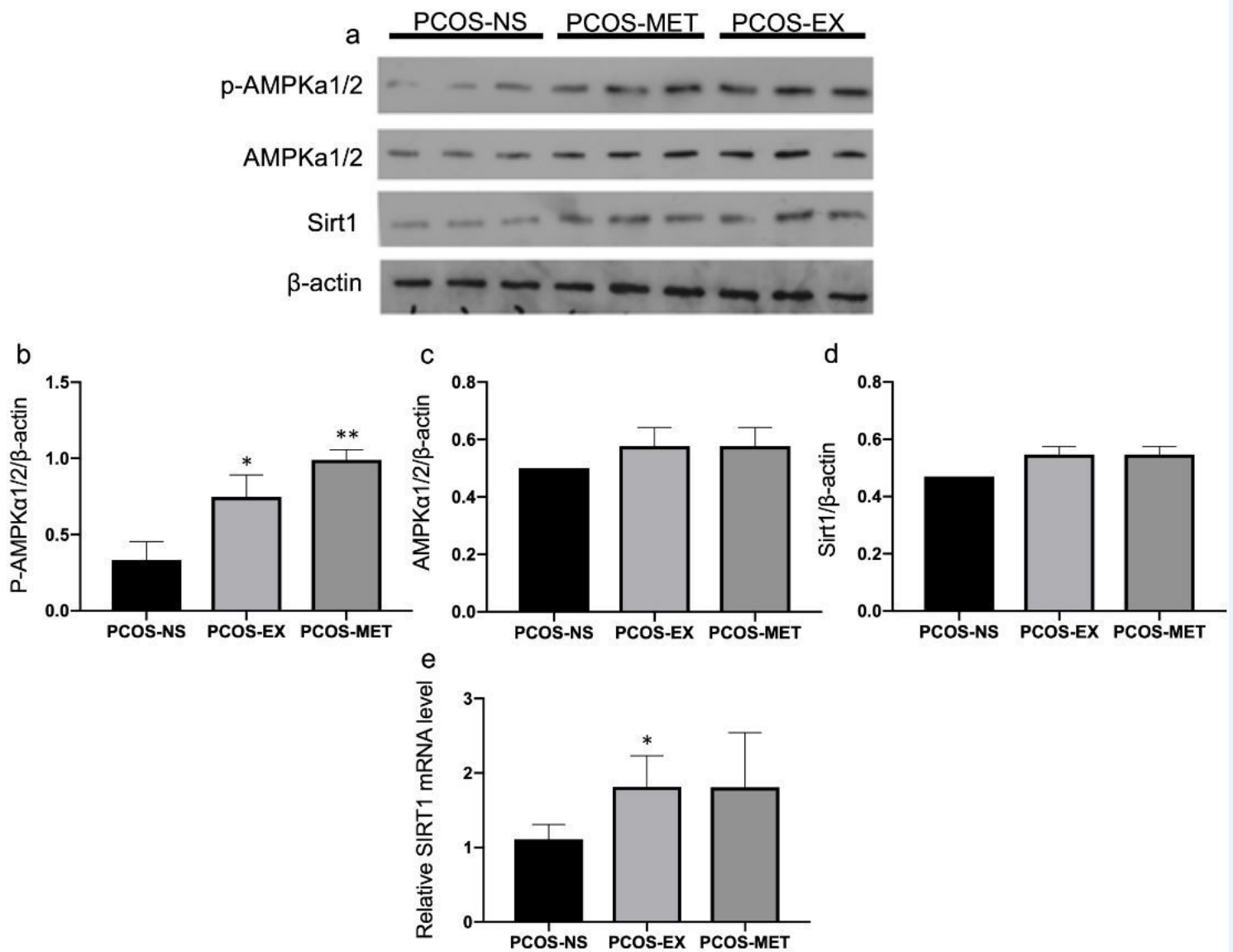


Figure 2

a, b, c, d: The result of Western blot of the expression of AMPKα and SIRT1 between three PCOS groups. e: The results of RT-PCR of the expression of SIRT1 between three PCOS groups. *P<0.05, **P<0.01, ***P<0.001. EX: exenatide; MET: metformin.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Conflictsofintereststatement.docx](#)
- [Informedconsentstatement.docx](#)
- [Authorsscontribution.docx](#)