

Effect of Sleeve Gastrectomy on Kisspeptin Expression in the Hypothalamus of Rats with Polycystic Ovary Syndrome

Lingying Wen ^{1,2}, Wei Lin ¹, Qian Li ¹, Gang Chen ¹, and Junping Wen ¹

Objectives: The purpose of this study was to determine changes in the expression levels of kisspeptin-1 (Kiss1) in the hypothalamus during the development of polycystic ovary syndrome (PCOS) and after treatment with sleeve gastrectomy (SG).

Methods: This study used chronic dehydroepiandrosterone (DHEA) alone and DHEA plus a high-fat diet (HFD) to generate a PCOS rat model. Subsequently, SG was performed in the animals with PCOS and the effects on glucose tolerance, insulin sensitivity, sex hormones, estrous cyclicity, adiponectin, and Kiss1 expression in the hypothalamus were investigated.

Results: Impaired glucose tolerance, decreased insulin sensitivity, reduced adiponectin levels, disrupted estrous cyclicity, and elevated sex hormone levels associated with PCOS models were restored to normal following SG. In addition, SG was able to restore the increase in the expression of Kiss1 mRNA and Kiss1-positive neurons in the arcuate nucleus of rats with PCOS. Interestingly, although SG did not result in a significant loss of body weight in rats administered DHEA under a chow diet, it resulted in comparable metabolic improvements and Kiss1 expression in rats that had been administered DHEA along with an HFD.

Conclusions: The recovery of normal levels of Kiss1 expression in the hypothalamus after SG in this study suggests that Kiss1 might play an important role in the development of PCOS and its improvement by SG.

Obesity (2020) **28**, 1117–1128.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine and metabolic disorders in women of reproductive age, with a prevalence of 9% to 18% (1). Until now, the cause of PCOS was not clear. However, insulin resistance and compensatory hyperinsulinism enhancing ovarian (and adrenal) androgen production are considered to be central to its pathogenesis (2). The syndrome is also associated with persistent and rapid gonadotropin-releasing hormone (GnRH) pulses, excess of luteinizing hormone (LH), and insufficient follicle-stimulating hormone (FSH) secretion, which may contribute to excessive androgen production in the ovary and ovulatory dysfunction.

In addition to these classical endocrine hormones, abnormalities in some neurotransmitters of the central nervous system are implicated in the pathogenesis of PCOS. Kisspeptin-1

Study Importance

What is already known?

- ▶ Peripheral mechanisms, including insulin resistance and hyperandrogenism, are pathogenic causes of polycystic ovary syndrome (PCOS).
- ▶ The expression of central peptides, such as adiponectin (APN) and kisspeptin-1 (Kiss1), are modulated during the development and treatment of PCOS.
- ▶ Sleeve gastrectomy (SG) is an effective way to treat PCOS in women with obesity. However, its impact on the hypothalamic neuroendocrine network remains unknown.

What does this study add?

- ▶ SG surgery increased serum APN levels, reduced Kiss1 expression in the hypothalamus, improved metabolic parameters, and improved cyclicity and follicular structure of the ovary in PCOS animal models.
- ▶ These improvements were also observed in chow-fed animals with PCOS that did not show significant body weight loss after surgery.

How might these results change the direction of research or the focus of clinical practice?

- ▶ The impact of SG on serum kisspeptin levels in women with PCOS needs to be assessed.
- ▶ Our study may have clinical implications for predicting the recovery of reproductive phenotypes in patients with PCOS and obesity at an earlier stage following SG.

¹ Department of Endocrinology, Key Laboratory of Endocrinology, Fujian Provincial Hospital, Shengli Clinical Medical College of Fujian Medical University, Fuzhou, China. Correspondence: Gang Chen (chengangfj@163.com) and Junping Wen (junpingwen@163.com) ² Department of Neonatology, The First Affiliated Hospital of Fujian Medical University, Longyan, China.

© 2020 The Authors. *Obesity* published by Wiley Periodicals, Inc. on behalf of The Obesity Society (TOS).

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

Received: 2 July 2019; Accepted: 3 March 2020; Published online 29 April 2020. doi:10.1002/oby.22795

(Kiss1), encoded by *KISS1* (KiSS-1 metastasis suppressor), is a major regulator of GnRH neurosecretion and ovulation (3). Wang et al. (4) found that kisspeptin levels are positively correlated with LH levels, suggesting that kisspeptin may be involved in PCOS development through the regulation of the gonadal axis and ovulation. There is evidence (5) that the hypothalamic Kiss1 system is sensitive to the regulatory effects of sex steroids, which partly regulate GnRH/gonadotropin secretion via modulation of Kiss1 neurons through feedback effects. In rodents, Kiss1 neurons are mainly distributed in the hypothalamic arcuate nucleus (ARC) and the anterior ventricular nucleus (AVPV), whereas in humans and other mammals, Kiss1 is mainly located in the ARC (6). Kiss1 neurons in the ARC are considered to participate in negative feedback, whereas the Kiss1 neurons in the AVPV appear to play a role in mediating the positive feedback effects of estradiol (E₂) (5). Other studies (7-9) indicate that Kiss1 may be involved in the disturbance of the hypothalamic-pituitary-ovarian axis in PCOS rat models.

Lifestyle intervention is the first line of treatment in patients with PCOS (10). For the patients with PCOS and overweight or obesity, weight loss by changing diet and physical activity decreases serum insulin and androgen levels, improves reproductive function, and reduces the risk of developing glucose intolerance and type 2 diabetes. However, there are patients who fail to follow this intensive intervention (10).

Bariatric surgery has become a popular weight loss method, as it can lead to substantial and sustained loss of weight (11). Several studies (12-16) have shown that bariatric surgery yields significant benefits to individuals with PCOS both on metabolic and reproductive levels. There are various hypotheses regarding the mechanism by which bariatric surgery relieves PCOS. Some studies found that long-term and significant weight loss through surgery is an important mechanism for relieving PCOS. However, in other studies, normalized menstruation is observed in patients shortly after surgery (within 4 weeks) even when the weight loss does not become significant (11,15). It is assumed that there are other mechanisms that contribute to recovery from PCOS, independent of weight loss, after bariatric surgery in patients with PCOS. Improvements in the secretion of gut hormones, inflammation markers, and adipokines after gastric bypass surgery are partly responsible for the weight loss-independent mechanism of PCOS treatment (16,17).

Adiponectin (APN) is the key adipokine protein that is expressed exclusively in adipose tissue. Two endogenous APN receptors (ADIPOR), ADIPOR1 and ADIPOR2, are expressed in the hypothalamus and pituitary (18). A meta-analysis revealed that serum APN levels are lower in women with PCOS than in BMI-matched healthy controls (19). APN protects against the development of metabolic disturbances in mouse models of PCOS (20), and systemic APN administration could reverse PCOS-like features in the animal model (21). Furthermore, our previous results have shown that APN inhibits GnRH release (22) and Kiss1 expression in the hypothalamus (23), suggesting their potential impact on the regulation of the central reproductive endocrine axis. However, the underlying mechanism that contributes to the treatment of PCOS by bariatric surgery needs to be further determined, in particular regarding the association with the APN and Kiss1 expression levels.

The aim of this study was to investigate whether sleeve gastrectomy (SG) can reverse the metabolic and reproductive disorders in PCOS rat models. We used dehydroepiandrosterone (DHEA) to induce PCOS in

lean rats and DHEA accompanied with a high-fat diet (HFD) to induce PCOS in rats with obesity. Then we assessed the effects of DHEA and HFD on metabolic parameters and reproductive function. Following SG intervention, we investigated whether the metabolic and reproductive disorders in the two groups of PCOS rats were ameliorated. It was hypothesized that SG may reduce the expression of hypothalamic Kiss1 and the number of Kiss1-positive neurons in the PCOS models both with and without obesity.

Methods

Animals

All procedures for rat use were provided by the Shanghai Laboratory Animals Center Co., Ltd. (Shanghai, China). A total of 73 young (20-24 days old), female Sprague-Dawley rats (50-55 g) were individually housed and maintained in a room with a 12/12-hour light/dark cycle at 22°C to 24°C and 60% to 65% humidity. All rats were given access to water ad libitum. The care of the rats and all experimental procedures used in these experiments were approved by the Committee on Animal Experiments of the Fujian Medical University (Fujian, China). The rats were divided randomly into three groups as follows: a control group ($n=15$), DHEA group ($n=29$), and DHEA + 60% HFD (DH) group ($n=29$). The PCOS model was generated by subcutaneous injection of DHEA (6 mg/100 g of body weight dissolved in 0.2 mL of tea oil for 20 consecutive days, given daily). The control rats were administered 0.2 mL of tea oil and were fed a normal rodent diet containing 10% fat (D12450J; Research Diets, New Brunswick, New Jersey; 3.85 kcal/g). The DHEA group was fed a normal rodent diet containing 10% fat (D12450J, Research Diets). The DH group received the same DHEA injection but was fed a diet containing 60% fat (D12492, Research Diets; 5.24 kcal/g). After 20 days, nine rats in each group were randomly selected and euthanized to analyze PCOS-related parameters. The rest of the rats were divided randomly into the following five groups for surgery: (a) control + sham group ($n=6$), (b) DHEA + sham (DSh) group ($n=6$), (c) DHEA + SG (DSg) group ($n=14$), (d) DH + sham (DHSh) group ($n=6$), and (e) DH + SG (DHSg) group ($n=14$). We continued to administer DHEA to the rats after surgery until they were euthanized, 3 weeks after the surgery. The study design is depicted in Supporting Information Figures S1 and S2.

Gastric surgery

Rats fasted for 20 hours with ad libitum access to water before surgery. SG was conducted in accordance with a previously described method (24). Anesthesia was performed with 10% chloral hydrochloride at 0.3 mL/100 g using intraperitoneal (i.p.) injection. A 4-cm midline epigastric incision was made, and the stomach was carefully removed from the abdominal cavity. Two vascular forceps were placed along the greater curvature from the antrum to the fundus across the forestomach and glandular stomach. After excision, a 6/0 silk suture was used to stitch the wound from the fundus to the antrum with a double line of intermittent sutures. Then the peritoneum, muscular layer, and skin were sutured with a 4/0 silk suture. All incisions, and the dissociation of the perigastric tissue and blood vessels, were consistent in the sham group. After dissociation, a 1-cm incision was made at the junction of the anterior stomach and glandular stomach on the side of the great bend of the stomach. The gastric tissue was sutured *in situ* without resection after disinfection. Other operating procedures were the same as those for the experimental group.

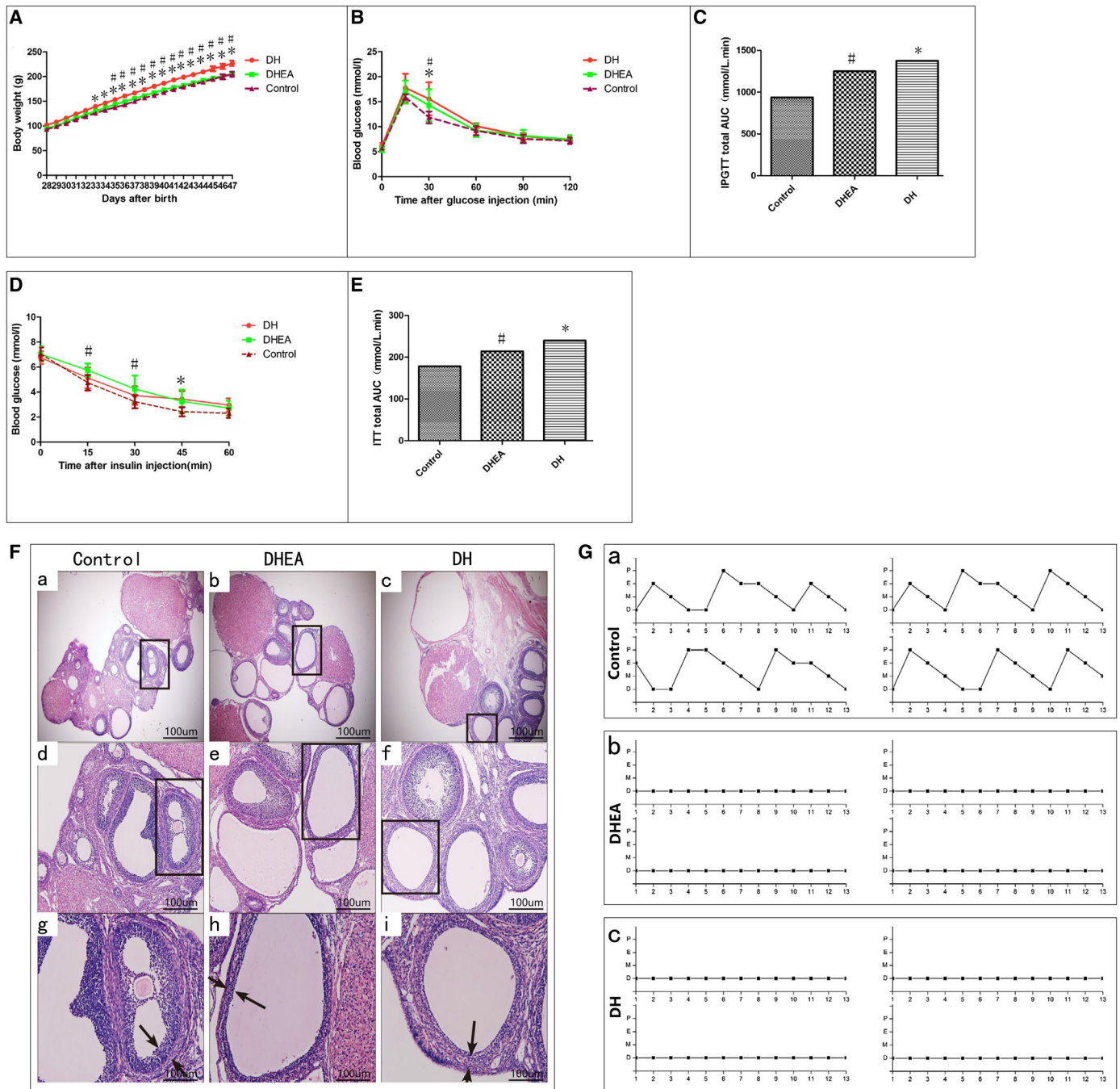


Figure 1 Metabolic and reproductive manifestations of DHEA- and DH-induced PCOS in rats. (A) Body weight changes. (B) Intraperitoneal GTT in DHEA and DH rats. (C) Total AUC of GTT in DHEA and DH rats. (D) ITT in DHEA and DH rats. (E) Total AUC of ITT in DHEA and DH rats. (F) H&E staining of the ovarian structure in DHEA and DH rats (scale bars, 100 μ m). Short arrow represents follicular wall. Long arrow represents granular cell layer. (G) E cycle in DHEA and DH rats. $P < 0.05$, DH vs. control; $\#P < 0.05$, DHEA vs. control ($n = 9$ for each group). AUC, area under the curve; D, diestrus; DH, DHEA+60% HFD; DHEA, dehydroepiandrosterone; E, estrus; GTT, glucose tolerance test; HFD, high-fat diet; H&E, hematoxylin and eosin; ITT, insulin tolerance test; M, metestrus; P, proestrus; PCOS, polycystic ovary syndrome. [Color figure can be viewed at wileyonlinelibrary.com]

Postsurgical care

During the first 24 hours, the rats were fasted without food and water to accelerate the healing of the suture wound. Twenty-four hours after

surgery, the rats were given a liquid diet. A small dose of solid feed was given on the third day after surgery. Standard animal feed was given on the fifth day after surgery.

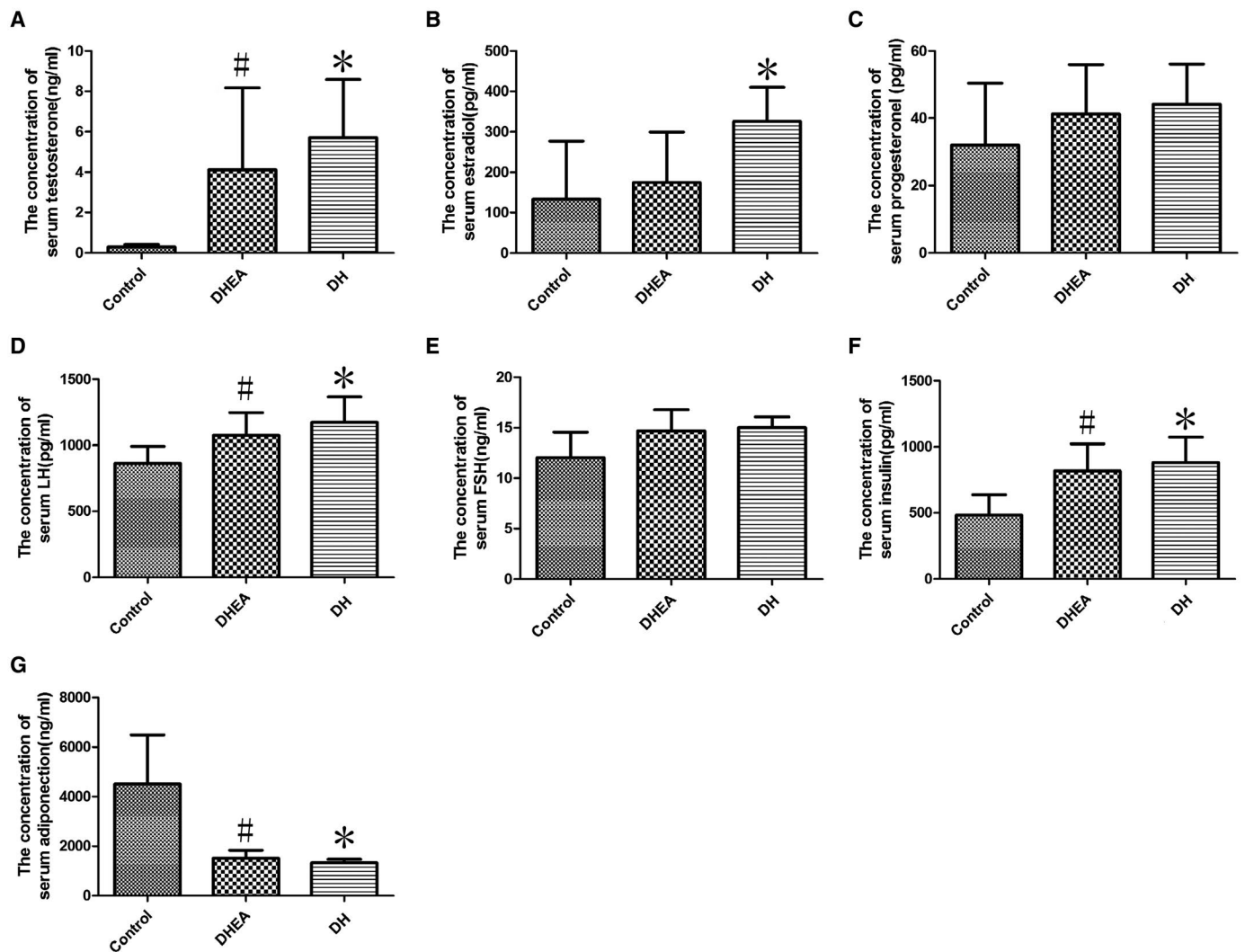


Figure 2 Serum hormone levels in DHEA- and DH-induced PCOS in rats. (A) Total testosterone levels, (B) estradiol levels, (C) progesterone levels, (D) LH levels, (E) FSH levels, (F) insulin levels, and (G) adiponectin levels. $^*P < 0.05$, DH vs. control; $^{\#}P < 0.05$, DHEA vs. control ($n = 9$ for each group). DH, DHEA + 60% HFD; DHEA, dehydroepiandrosterone; FSH, follicle-stimulating hormone; HFD, high-fat diet; PCOS, polycystic ovary syndrome; LH, luteinizing hormone.

Assessment of body weight and estrous cyclicity

Body weight was measured daily for all rats, starting at postnatal day 28, until they were euthanized. We started monitoring the estrous cycle from the eighth day after the surgery. Vaginal-smear cells from each animal were viewed and determined as diestrus, proestrus, or estrus, based on the shape and density of the cells, as previously described (25).

Glucose tolerance and insulin tolerance tests and area under curve

For the glucose tolerance test (GTT) and insulin tolerance test (ITT), we used the protocols according to a previously described method (26). Then, the calculation of the areas under the curve (AUCs) for glucose tolerance and insulin tolerance were accomplished with GraphPad Prism version 5 (San Diego, California).

Collection of blood and soft tissue samples

All rats were euthanized with i.p. injection of 10% chloral hydrochloride at 0.3 mL/100 g of body weight at diestrus. Blood samples were immediately collected from the heart through needle aspiration. For the detection of preoperative levels, blood samples were collected from the inner canthus vein. Serum was isolated by centrifugation at 3,000g for 15 minutes and stored at -80°C until measurement. After perfusion with 150 mL of normal saline and 150 mL of 4% paraformaldehyde, the brain and ovaries were removed. Brains were soaked in 4% paraformaldehyde+diethyl pyrocarbonate (DEPC; 1:1,000) overnight and then transferred in turn to 15% sucrose+DEPC (1:1,000), 20% sucrose+DEPC (1:1,000), and 30% sucrose+DEPC (1:1,000). Coronal sections (40 μm in thickness), including the ARC and AVPV, were sectioned using a cryostat, collected in cryoprotectant solution, and stored at -20°C until the performance of immunohistochemistry.

(IHC). Ovaries were fixed in 10% formalin and embedded in paraffin for hematoxylin and eosin staining.

Hormonal measurements

The concentrations of E₂, progesterone, testosterone, and APN were measured using enzyme-linked immunosorbent assay kits from R&D Systems (Minneapolis, Minnesota) in accordance with the manufacturer's instructions. Serum samples were assayed for the presence of three cytokines (LH, FSH, insulin) using the LUMINEX 200 (Luminex, Austin, Texas) in accordance with the manufacturer's instructions.

IHC of Kiss1

Six free-floating sections of the brain corresponding to the ARC and AVPV were rinsed six times (5 minutes each) in 0.01 M PBS and incubated in 3% H₂O₂ for 20 minutes. The sections were rinsed and treated with a blocking solution (sheep serum) at room temperature for 1 hour. Then they were incubated at 4°C for 24 hours with anti-KISS1 rabbit polyclonal primary antibody (1:50; ABBIOTEC, Inc., Escondido,

California), and were subsequently incubated with biotinylated goat anti-rabbit secondary antibody (Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China) for 1 hour. Next, the sections were stained with 3,3'-diaminobenzidine for 10 to 15 seconds and with hematoxylin for 10 minutes and sequentially rinsed twice with PBS (5 minutes each). Finally, the sections were placed on polylysine-treated glass slides and sealed with neutral gum.

Fluorescence *in situ* hybridization

Brains were cut into 20- μ m sections through the AVPV and ARC. We performed fluorescence *in situ* hybridization to detect Kiss1 mRNA in the AVPV and ARC, as described previously (7). First, the sections were washed with distilled water, treated with 100 μ L/mL of proteinase K, and then washed with PBS. Multiple oligonucleotide probes against the rat Kiss1 gene (5'-ATG CCT GGC AAA AGG GCC CGC GGT ATG CAG AGA GC-3'; 5'-ACC AGC GGC CCC CGT GTG CCA CCC GCA GTC GCC TG-3'; 5'-GCT ACG GCA GGA GGC AGG TGG CGC GGG CGG CAC GG-3') were used for the detection of Kiss1 mRNA (Wuhan Boster Biological Technology., Ltd.,

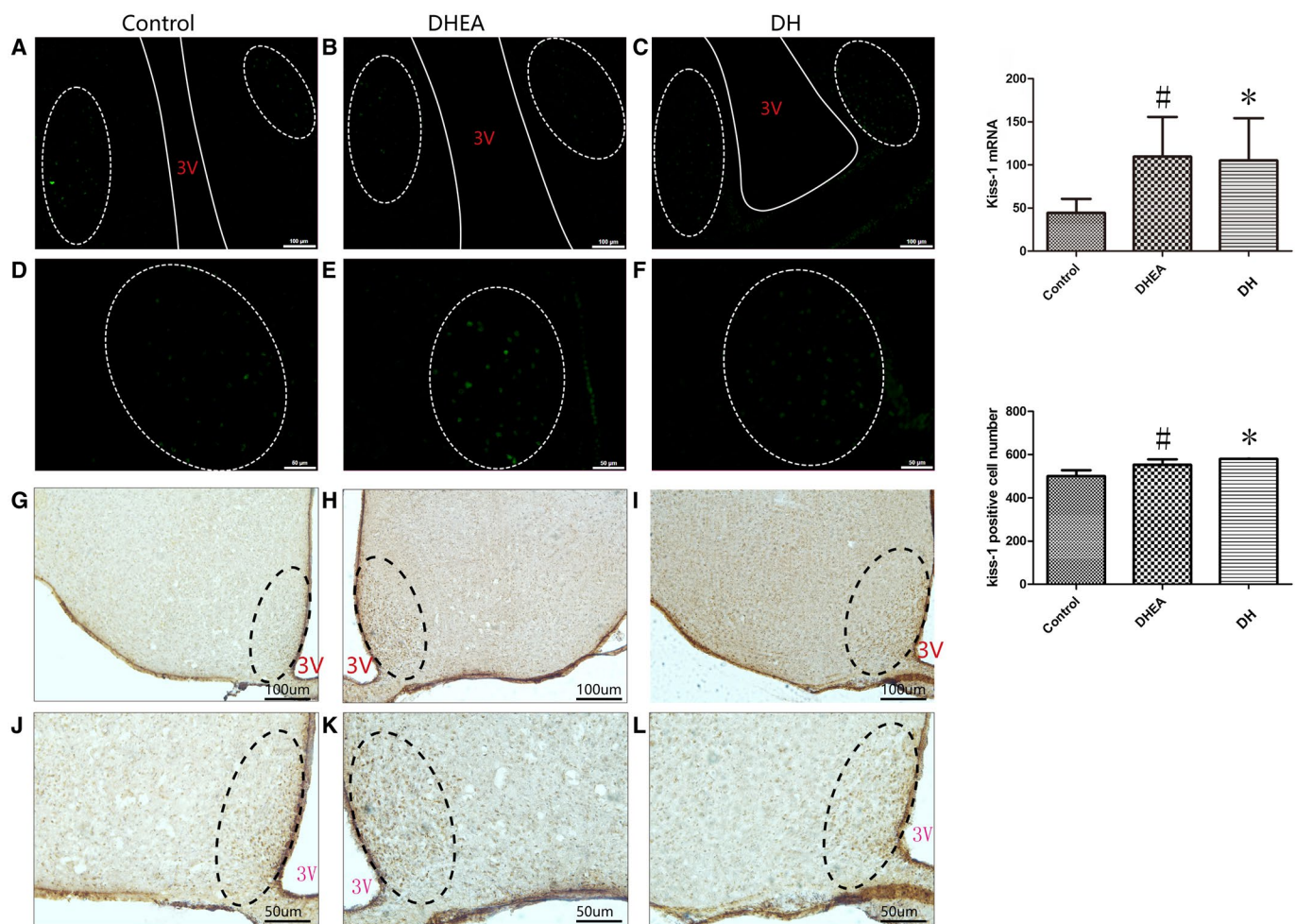


Figure 3 Kiss1 mRNA levels and number of Kiss1-positive neurons in the ARC of DHEA and DH rats. (A-C, G-I: scale bars, 100 μ m; D-F, J-L: scale bars, 50 μ m.) [#]*P*<0.05, DH vs. control; ^{*}*P*<0.05, DHEA vs. control (*n*=9 for each group). 3V, third ventricle; ARC, arcuate nucleus; DH, DHEA+60% HFD; DHEA, dehydroepiandrosterone; HFD, high-fat diet; Kiss1, kisspeptin-1. [Color figure can be viewed at wileyonlinelibrary.com]

Wuhan, China). The sections were washed with 2× saline-sodium citrate (SSC), 0.5× SSC, and 0.2× SSC for 15 minutes and then immersed in a blocking solution for 30 minutes at 37°C. Following these steps, the sections were incubated with a biotinylated anti-digoxigenin antibody for 60 minutes at 37°C. Finally, the sections were treated with fluorescein isothiocyanate until a visible signal was detected. Sections were mounted and examined by fluorescence microscopy (BX43; Olympus, Tokyo, Japan). Three visual fields were randomly selected for each sample under 200× magnification and counted for statistical analysis.

Statistics

Data were analyzed using GraphPad Prism. All data were analyzed using one-way or two-way ANOVA. When we measured serum hormone levels and Kiss1 expression, we used one-way ANOVA, as there was only one factor affecting the readout. However, when we assessed body weight, glucose tolerance, and insulin sensitivity among groups, we used two-way ANOVA, as there were two factors, multiple time points, and different treatments, which may have influenced the endpoints. When appropriate, Tukey post hoc comparisons were used to determine pair-wise differences between groups. All results are expressed

as the mean ± SD. $P < 0.05$ was considered significant for each of these analyses.

Results

Both DHEA alone and DH models resembled metabolic features of PCOS

To induce PCOS in the rat model, one group of rats was administered i.p. injections of DHEA alone, and another group of rats was administered i.p. injections of DHEA and challenged with an HFD. During the 20-day experiment, we found that there was no difference in body weight between the DHEA-treated rats and the control group. The body weight of rats significantly increased from day 6 in the DH-treated rats compared with controls and, from day 12, compared with the DHEA group (Figure 1A). At 7 weeks of age, both DHEA and DH groups showed impaired glucose tolerance compared with controls (Figures 1B-1C). Further, the two groups showed damaged insulin sensitivity in an ITT (Figures 1D-1E). In addition, DHEA and DH rats showed higher insulin levels than the control group, supporting their insulin resistance features (Figure 2F). These results indicated that

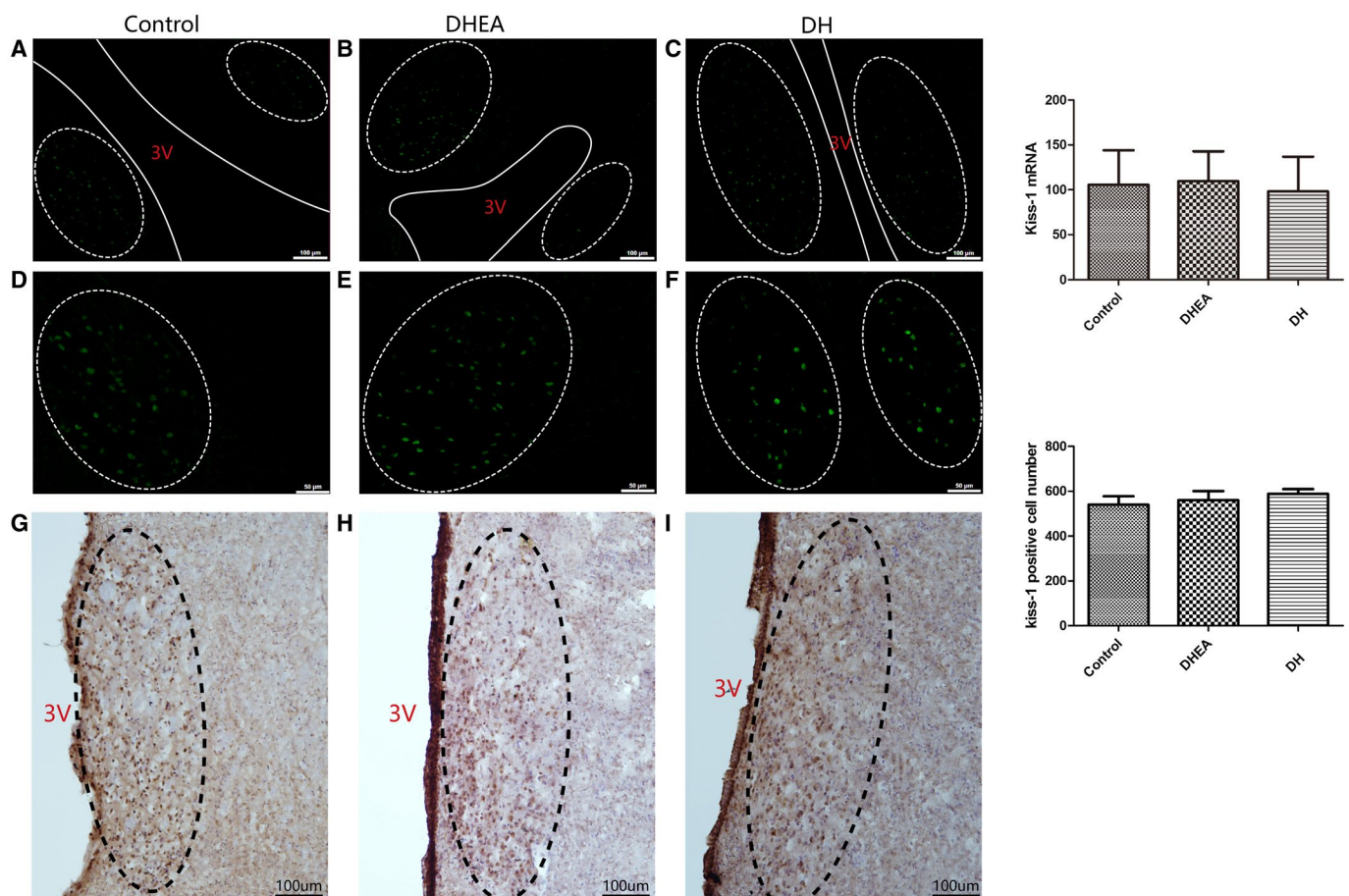


Figure 4 Kiss1 mRNA levels and number of Kiss1-positive neurons in the AVPV of DHEA- and DH-induced PCOS in rats. (A-C: scale bars, 100 µm; D-I: scale bars, 50 µm.) ($n = 9$ for each group.) 3V, third ventricle; AVPV, anterior ventricular nucleus; DH, DHEA + 60% HFD; DHEA, dehydroepiandrosterone; HFD, high-fat diet; Kiss1, kisspeptin-1; PCOS, polycystic ovary syndrome. [Color figure can be viewed at wileyonlinelibrary.com]

these two models recapitulated the metabolic features of PCOS in humans, such as insulin resistance and polycystic ovary structure.

Both DHEA alone and DH models recapitulated hyperandrogenism of PCOS

Histological analysis revealed that the number of cystic follicles characterized by thickened follicular walls and diminished granulosa cell layers was significantly increased in the DHEA as well as DH groups (Figure 1F). Importantly, the estrous cycles in DHEA and DH rats—as detected by vaginal smears—were thoroughly absent, whereas the control rats showed a constant diestrus (Figure 1G). DHEA injection, with or without the 60% HFD, induced a significant increase in serum

testosterone and LH levels, but no significant change was observed in serum FSH and progesterone levels (Figures 2A, 2C–2E). DH induced a significant increase in serum E₂ levels relative to that in the DHEA and control groups (Figure 2B). Interestingly, we also observed decreased APN levels in the two groups as compared with the controls (Figure 2G). These results indicated that the two models also recapitulated the polycystic ovary–like morphological changes and hyperandrogenism associated with PCOS, accompanied by the reduced secretion of APN.

Both DHEA alone and DH treatment promoted Kiss1 expression in ARC

To explore the change of hypothalamic Kiss1 expression and the number of Kiss1-positive neurons, we detected the hypothalamus using

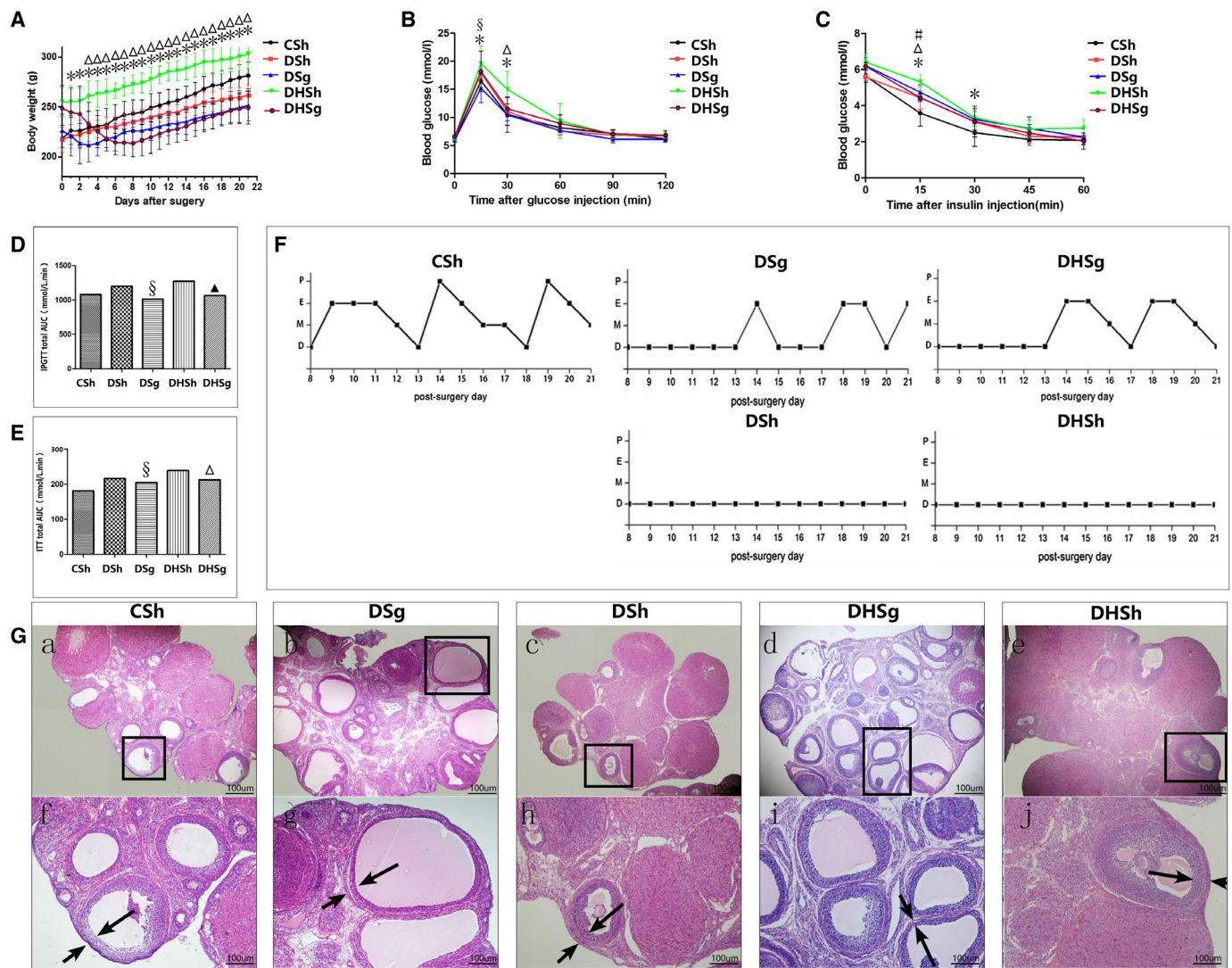


Figure 5 Changes in the metabolic and reproductive disorders in rats with PCOS treated with SG. (A) Change in the body weight of the DHEA and DH rats after surgery. (B) Intraperitoneal GTT in the DHEA and DH rats after surgery. (C) ITT in the DHEA and DH rats after surgery. (D) Total AUC of GTT in the DHEA and DH rats after surgery. (E) Total AUC of ITT in the DHEA and DH rats after surgery. (F) Changes in E cyclicity in the DHEA and DH rats after surgery. (G) Changes in ovary structure in the DHEA and DH rats after surgery. Short arrow represents follicular wall. Long arrow represents granular cell layer. (Scale bars, 100 μ m.) [#]*P* < 0.05, DHS vs. CSh; ^Δ*P* < 0.05, DHSg vs. DHS; ^{*}*P* < 0.05, DSh vs. CSh; [§]*P* < 0.05, DSg vs. DSh (*n* = 6 for CSh, DSh, DSg, and DHS and *n* = 7 for DHSg). AUC, area under the curve; CSh, control + sham; D, diestrus; DH, DHEA + 60% HFD; DHEA, dehydroepiandrosterone; DHSg, DH + SG; DHS, DH + sham; DSg, DHEA + SG; DSh, DHEA + sham; E, estrus; GTT, glucose tolerance test; HFD, high-fat diet; ITT, insulin tolerance test; M, metestrus; P, proestrus; PCOS, polycystic ovary syndrome; SG, sleeve gastrectomy. [Color figure can be viewed at wileyonlinelibrary.com]

fluorescence *in situ* hybridization and IHC methods respectively. We observed that the Kiss1 mRNA levels and the number of Kiss1-positive neurons in the ARC of DHEA and DH rats were significantly higher than those in the control group (Figure 3). However, there were no significant changes in the expression level of Kiss1 in the AVPV among the groups (Figure 4). These results suggested that increased Kiss1 in the ARC may be involved in the development of PCOS.

SG attenuated metabolic and reproductive disorders in the PCOS models

To test the role of SG in PCOS animal models, we performed SG in the DHEA (DSg) and DH (DHSg) groups. We found that there was a marked loss in body weight in the DHSg rats compared with that in the sham (DHS_h) controls (Figure 5A). However, there was no significant body weight loss in DSg rats compared with that in the sham (DS_h) controls during the entire experiment (Figure 5A). Moreover, *i.p.* GTT and ITT tests, glucose tolerance, and insulin sensitivity were dramatically improved in the DHSg group and even in the DSg group when compared with their corresponding controls (Figures 5B–5E). Both PCOS models, which were acyclic prior to the surgery, began to

regain normal cyclicity from 2 weeks after SG, and their cyclicity was almost completely restored at the end of the intervention (Figure 5F). In addition, an examination of the representative hematoxylin and eosin-stained ovaries of the surgery group showed a normal number and structure of corpora lutea and follicles at various developmental stages (Figure 5G). Notably, even though there was no noticeable loss in body weight in DSg rats, the metabolic and reproductive disorders were successfully attenuated, which suggested that SG may treat PCOS in a manner independent of, at least in part, its effect on body weight.

SG improved sex hormone and APN levels

In the third week after surgery, the concentrations of serum testosterone, LH, and FSH were significantly reduced to their normal levels in DSg and DHSg rats (Figures 6A, 6D–6E). There was no significant difference in E₂, progesterone, or insulin levels between the surgery and sham groups (Figures 6B, 6C, 6F). Importantly, serum APN levels showed a significant rebounding increase in DSg rats but showed only an increasing trend in DHSg rats compared with their corresponding sham controls (Figure 6G). These results indicated that the improvement of serum APN levels by SG may have occurred before the loss of body weight.

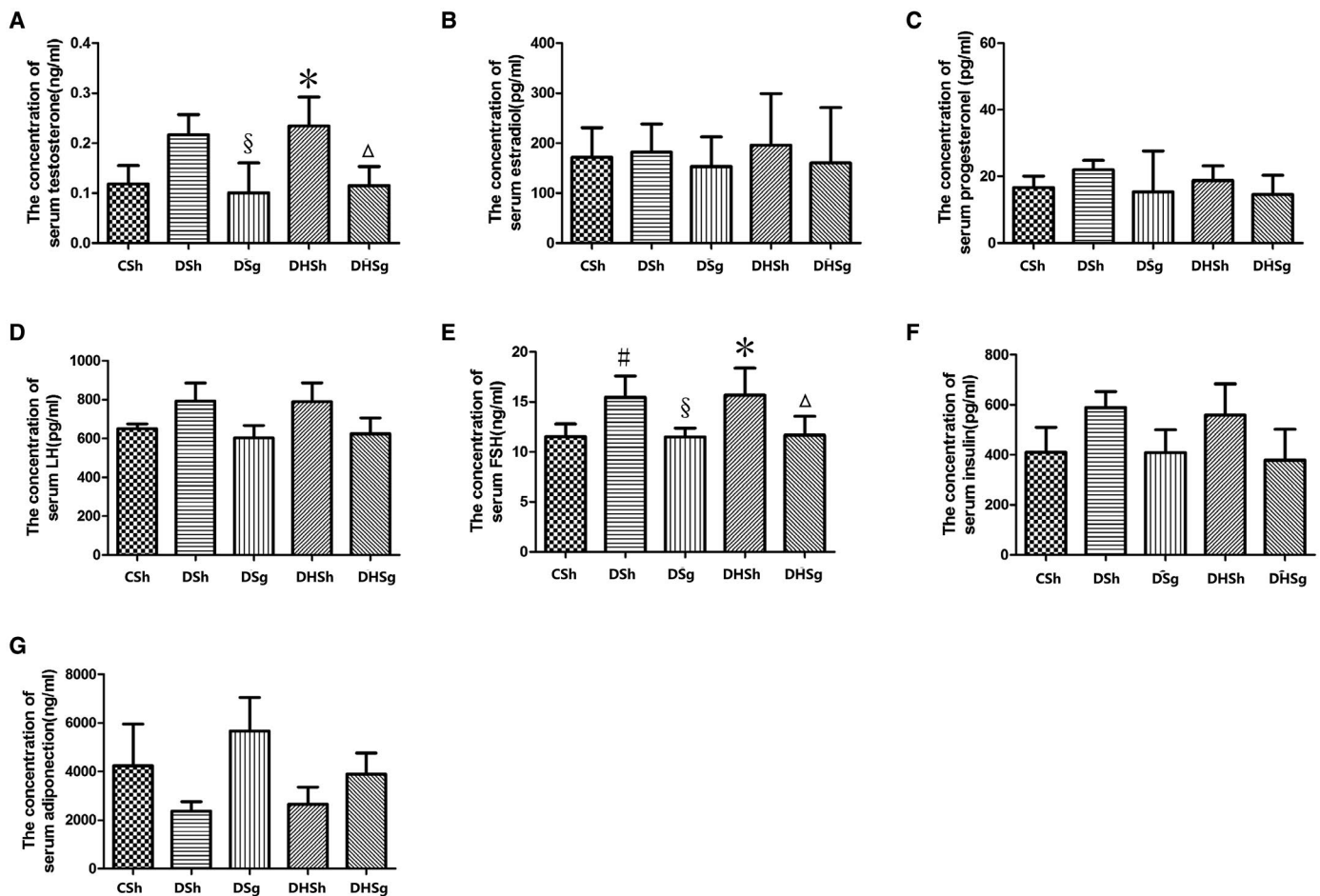


Figure 6 Changes in serum hormone levels in PCOS rats treated with SG. (A) Total testosterone levels, (B) estradiol levels, (C) progesterone levels, (D) LH levels, (E) FSH levels, (F) insulin levels, and (G) adiponectin levels. * $P < 0.05$, DHSg vs. CSh; ^Δ $P < 0.05$, DHSg vs. DHSg; [#] $P < 0.05$, DSh vs. CSh; [§] $P < 0.05$, DSg vs. DSh ($n = 6$ for CSh, DSh, DSg, and DHSg, and $n = 7$ for DHSg). CSh, control+sham; DHEA, dehydroepiandrosterone; DHSg, DHEA+60% HFD+SG; DHSg, DHEA+60% HFD+sham; DSg, DHEA+SG; DSg, DHEA+sham; FSH, follicle-stimulating hormone; HFD, high-fat diet; LH, luteinizing hormone.

SG restored Kiss1 expression and Kiss1-positive neurons in ARC

Further, we evaluated the expression of Kiss1 mRNA and immunoreactivity in the ARC and AVPV of SG and sham rats. Both PCOS models in the DSh and DHSg groups maintained a significantly higher expression of Kiss1 mRNA in the ARC than the control+sham rats (Figure 7). Three weeks after SG, a significant rebounding decrease in Kiss1 mRNA and immunoreactivity was observed in the ARC of the DSg and DHSg rats compared with their controls (Figure 7). There were no significant differences in the Kiss1 mRNA and immunoreactivity in the AVPV between the surgery and sham groups (Figure 8). These results indicated that Kiss1 expression in the ARC might contribute to the improvement of PCOS by SG.

Discussion

PCOS is characterized by a hypothalamic-pituitary-ovary axis dysfunction, anovulation, and androgen excess. It is considered to be a heterogeneous disease, in terms of physiological pathology as well as the severity of clinical consequences, including a high risk for metabolic

syndrome. Recently, new insights into the regulation of hormones and cytokines in gut and fat tissue support the concept that PCOS is a systemic syndrome (2). Treatment of PCOS should be proposed not only to mitigate hyperandrogenic symptoms and induce ovulation for those who wish to conceive but also to prevent the occurrence of long-term complications, such as type 2 diabetes.

Obesity and insulin resistance are tightly correlated with PCOS. Currently, lifestyle management targeting weight loss is the first-line treatment for PCOS (27). However, lifestyle changes along with structured weight loss programs are usually ineffective because the majority of patients are unable to maintain more than 5% weight loss for the long term (10). Patients with PCOS can consume oral contraceptive pills to control symptoms of hyperandrogenism or take insulin-sensitizing drugs, such as metformin or pioglitazone, to alleviate insulin resistance; however, these drugs can effectively improve insulin sensitivity without significant weight loss or even with body weight gain (28), indicating the crucial roles of improving insulin sensitivity in the treatment of PCOS. Bariatric surgery has been shown to yield a robust and sustained effect on weight loss and improvement of the metabolic and reproductive phenotypes in PCOS with obesity. However, the mechanism by which bariatric surgery improves PCOS

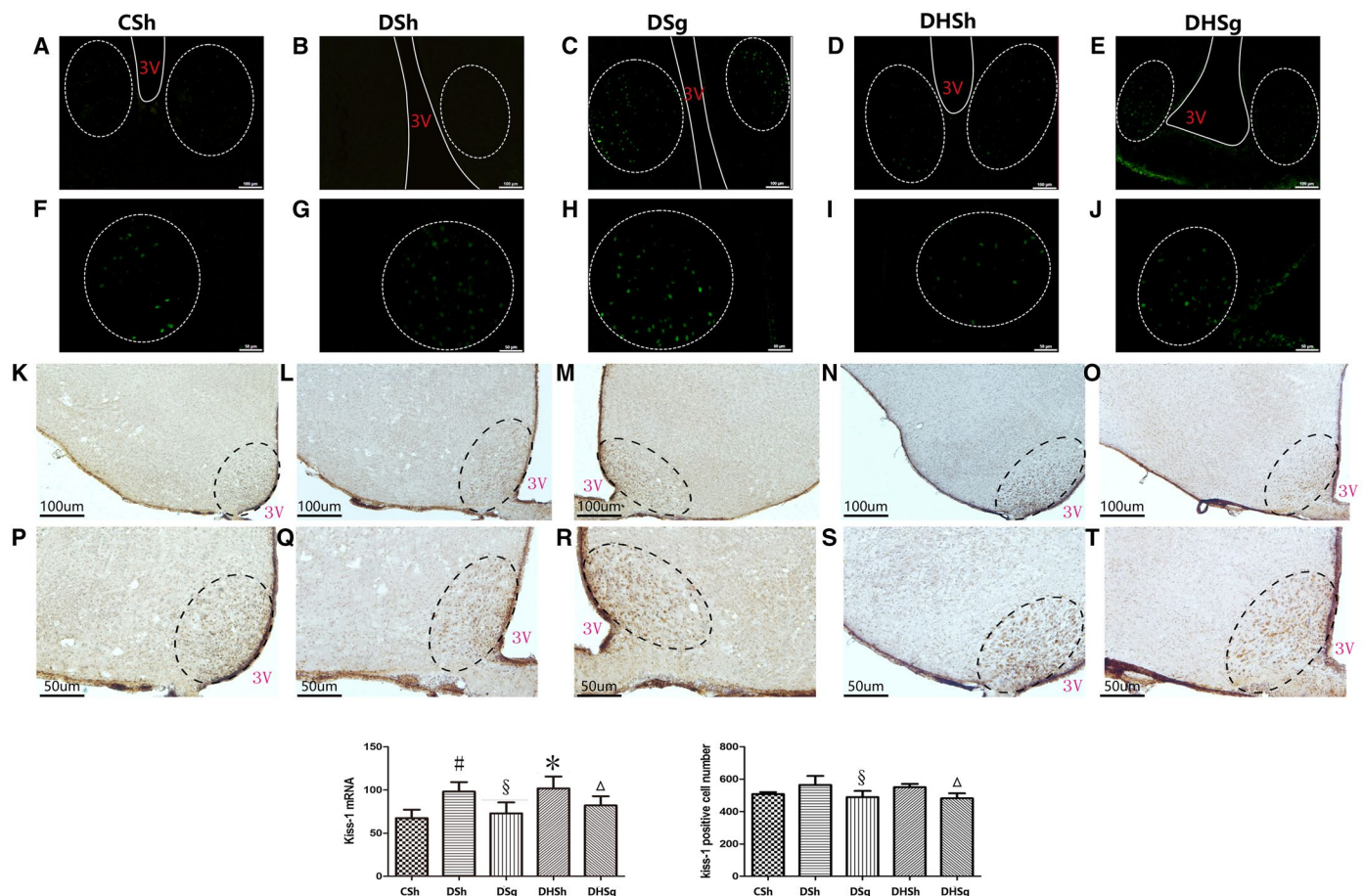


Figure 7 Changes in Kiss1 mRNA levels and number of Kiss1-positive neurons in the ARC of PCOS rats treated with SG. (A-E, K-O: scale bars, 100 μ m; F-J, P-T: scale bars, 50 μ m.) [#] $P < 0.05$, DHSg vs. CSh; ^Δ $P < 0.05$, DHSg vs. DHSg; [#] $P < 0.05$, DSh vs. CSh; [§] $P < 0.05$, DSg vs. DSh ($n = 6$ for CSh, DSh, DSg, and DHSg, and $n = 7$ for DHSg). 3V, third ventricle; ARC, arcuate nucleus; CSh, control+sham; DHEA, dehydroepiandrosterone; DHSg, DHEA+60% HFD+SG; DHSh, DHEA+60% HFD+sham; DSg, DHEA+SG; DSh, DHEA+sham; HFD, high-fat diet; Kiss1, kisspeptin-1; PCOS, polycystic ovary syndrome; SG, sleeve gastrectomy. [Color figure can be viewed at wileyonlinelibrary.com]

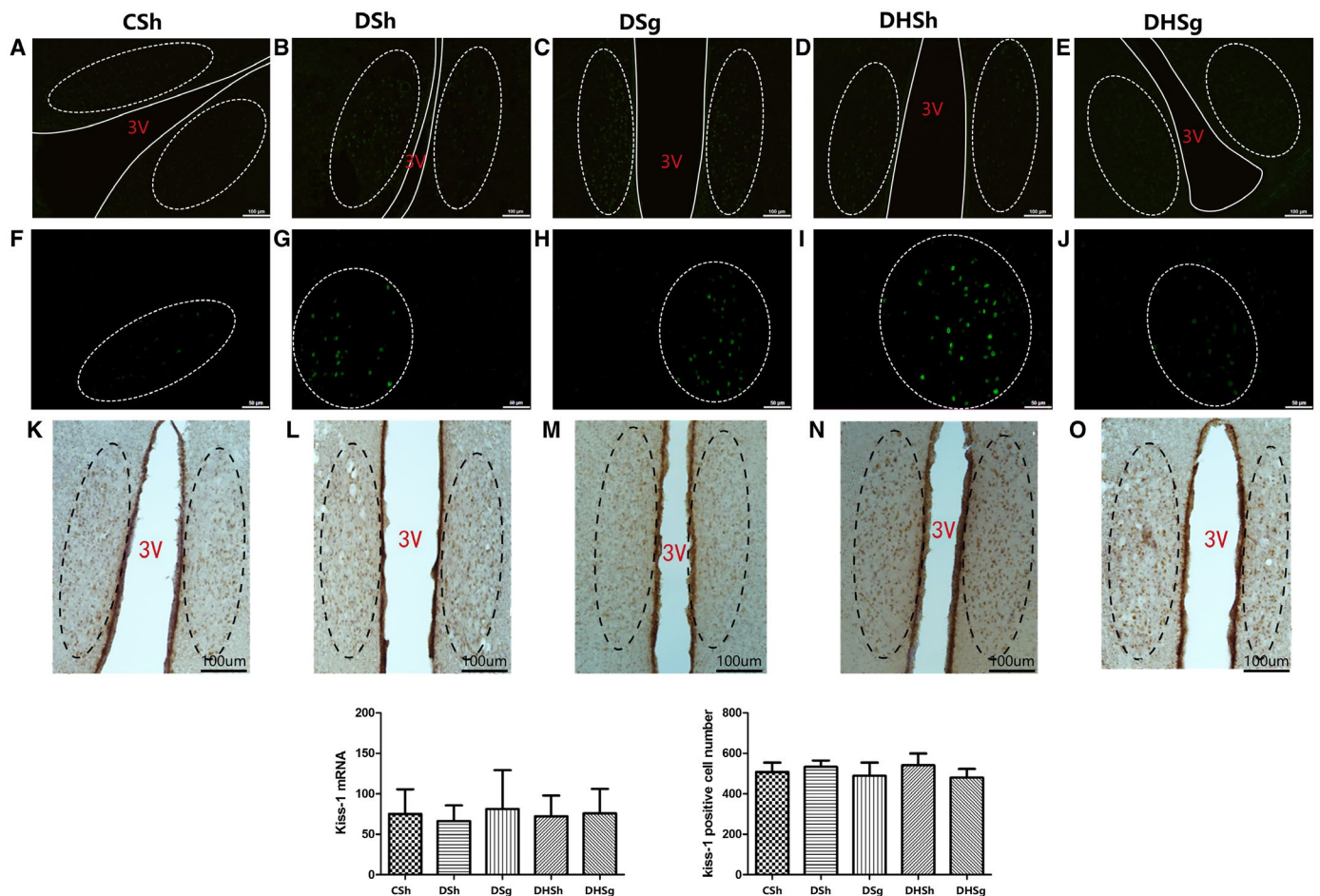


Figure 8 Changes in Kiss1 mRNA levels and number of Kiss1-positive neurons in the AVPV of rats with PCOS treated with SG. (A–E: scale bars, 100 μm; K–O: scale bars, 50 μm.) (n = 6 for CSh, DSh, DSg, and DHSh, and n = 7 for DHSg.) 3V, third ventricle; AVPV, anterior ventricular nucleus; CSh, control + sham; DHEA, dehydroepiandrosterone; DHSg, DHEA + 60% HFD + SG; DHSh, DHEA + 60% HFD + sham; DSg, DHEA + SG; DSh, DHEA + sham; HFD, high-fat diet; Kiss1, kisspeptin-1; PCOS, polycystic ovary syndrome; SG, sleeve gastrectomy. [Color figure can be viewed at wileyonlinelibrary.com]

is still not completely clear. Recently, several studies (11,13) suggested that treatment of PCOS symptoms is related to weight loss and the improvement of insulin resistance after surgery. However, other studies have argued that even without significant weight loss in the first weeks after surgery, certain PCOS symptoms also show a clinical improvement, such as menstruation recovery, suggesting that the mechanisms of the improvement of PCOS symptoms by bariatric surgery may be attributed to increased insulin sensitivity, rather than body weight loss per se, at least in the early stage after surgery (15).

Women with PCOS usually exhibit a high level of LH impulse secretion, and their LH/FSH ratio is higher than one, indicating disturbed hypothalamic GnRH secretion. Especially, patients with PCOS and without obesity have less insulin resistance with relatively higher LH levels (29,30). Although a series of studies with experimental and clinical evidence illustrated the impact of androgen and insulin levels on the neuroendocrine pathogenesis of PCOS, the detailed mechanism underlying the neuron-endocrine-hormone network remains unknown. As an important upstream regulatory factor of GnRH neurons, Kiss1-positive neurons play an indispensable role with respect

to reproduction, including ovulation and fertility (6,31–33). Previous studies have found that the Kiss1 in the ARC regulates energy balance in addition to GnRH secretion (34–36). Metabolic dysfunction and reproductive dysfunction in patients with PCOS are tightly related, and some studies reported that APN, an adipokine secreted by adipose tissues, can function on the hypothalamus to regulate metabolism and reproduction in the hypothalamus (37). Our previous study has also confirmed that APN significantly inhibits GnRH secretion in GT1-7 neurons derived from the hypothalamus (22). Furthermore, we showed that APN inhibits Kiss1 expression *in vitro* and *in vivo* through the adenosine monophosphate-activated protein kinase pathway (23). These studies suggest that serum APN elevation may inhibit Kiss1 expression and Kiss1 neuronal activities in the hypothalamus, which has also been observed in SG surgery models.

Here, we demonstrated that administrating DHEA and DH to rats resulted in metabolic and reproductive disorders that properly recapitulate most aspects of human PCOS (38,39). Moreover, we found that the expression of Kiss1 mRNA and immunoreactivity were significantly increased in the ARC of both models. These results are similar

to those showing an increase of Kiss1 neurons in the ARC of progesterone antagonist RU486-induced (8) and letrozole-induced PCOS rats (9) but are in opposition to those showing a decrease of Kiss1 neurons in the ARC of dihydrotestosterone-induced PCOS rats (7). These findings provide solid evidence indicating possible involvement of Kiss1 neurons in the hypothalamic ARC during the development of PCOS. The discrepancy observed in these independent investigations might be due to differences in the experimental approaches (e.g., rat genetic background, ages, induction drugs, and even antibodies with various specificities) (7-9). Thus, additional data are required for clarifying the mechanism underlying PCOS development in these models. Of note, several studies have identified the existence of a leptin-kisspeptin-GnRH pathway, in which leptin regulates the GnRH neurons via modulation of kisspeptin afferents, thereby allowing normal maturation and function of the hypothalamic-pituitary-gonadal axis under conditions of energy (leptin) abundance (4). Given that our previous studies have found that APN, which is also affected by energy states, regulates GnRH secretion in the hypothalamus (22,23), we proposed that a disturbance of the APN-kisspeptin-GnRH pathway may additionally be present in DHEA-induced PCOS models, which is recovered after SG.

The physiological parameters, including reduced insulin sensitivity and disrupted estrous cyclicity, were restored to normal levels in both PCOS models by SG intervention. These findings are consistent with the result of a previous study that used dihydrotestosterone together with HFD-induced PCOS rats (40). The increases in Kiss1 mRNA expression and the number of Kiss1-positive neurons in the ARC of PCOS rats were also recovered to their normal levels with SG. Of note, in our study, SG intervention recovered the reduced serum APN in DHEA models but not in DH models, in parallel with sustained weight loss in DH rats with obesity but no change in DHEA rats with normal weight. This indicated that SG can achieve therapeutic effects, not only by reducing body weight but also through other weight loss-independent ways. Interestingly, there was a significant increase in APN levels in the DHEA group and only an increasing trend (without statistical significance) in the DH group after SG surgery, which, together with the biological effects of APN on Kiss1 neurons, further supported the hypothesis. Additional evidence to characterize the treatment of APN in patients with PCOS will be necessary in the future, and this evidence could be gathered through means such as randomized controlled trials and studies of genetic-deficiency carriers.

Our study has some limitations, including the lack of Kiss1- and APN-knockout animal models and antagonism experiments (Kiss1-neutralizing antibody) to demonstrate the possible role of reduced ARC Kiss1 expression in the improvement of PCOS symptoms. Besides, although our rat model fully recapitulated the clinical manifestations of patients with PCOS, we could only observe outcomes 3 weeks after surgery and thus have no long-term data to support the benefits of SG on PCOS symptoms.

Conclusion

Our results have raised the possibility that hypothalamic Kiss1 plays an essential role in the development of PCOS and that SG might reduce the expression of Kiss1, at least in part independently of the weight loss effect, to improve metabolic and reproductive disorders. In the future, more studies are needed to confirm the central mechanism of metabolic surgery in patients with PCOS. **O**

Acknowledgments

The authors thank Dr. Jing Zhang for her expert advice and statistical help with this study. This study was presented at the American Endocrine Society annual meeting in Chicago, Illinois, March 17-20, 2018.

Funding agencies: This research was funded by the Natural Science Foundation of China (81770848 and 81170774) and the National Key Research and Development Program of China (2018YFC2001100-5). Additional funding was provided by the Natural Science Foundation of Fujian Province (2015Y0008, 2016Y9006, and 2019J01175) and the Fujian Provincial Health System via the Youth Personnel Training Project financing plan (2018-ZQN-1).

Disclosure: The authors declared no conflict of interest.

Author contributions: LW, WL, and QL contributed to the study equally. LW, WL, and QL performed the research; contributed to the conception and design of the study, the acquisition of data, and analysis and interpretation of data; drafted all versions of the article; and approved the final version for publication. GC contributed to the conception and design of the study, all revisions, and the final approval for publication. JW contributed to the conception and design of the study, the analysis and interpretation of data, all revisions, and the final approval for publication.

Supporting information: Additional Supporting Information may be found in the online version of this article.

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;25:544-551.
2. Barber TM, Hanson P, Weickert MO, Franks S. Obesity and polycystic ovary syndrome: implications for pathogenesis and novel management strategies. *Clin Med Insights Reprod Health* 2019;13:1179558119874042. doi:10.1177/1179558119874042
3. Harter CJL, Kavanagh GS, Smith JT. The role of kisspeptin neurons in reproduction and metabolism. *J Endocrinol* 2018;238:R173-R183.
4. Wang T, Han S, Tian W, Zhao M, Zhang H. Effects of kisspeptin on pathogenesis and energy metabolism in polycystic ovarian syndrome (PCOS). *Gynecol Endocrinol* 2019;35:807-810.
5. Garcia-Galiano D, Pinilla L, Tena-Sempere M. Sex steroids and the control of the Kiss1 system: developmental roles and major regulatory actions. *J Neuroendocrinol* 2012;24:22-33.
6. Oakley AE, Clifton DK, Steiner RA. Kisspeptin signaling in the brain. *Endocr Rev* 2009;30:713-743.
7. Brown RE, Wilkinson DA, Imran SA, Caraty A, Wilkinson M. Hypothalamic kiss1 mRNA and kisspeptin immunoreactivity are reduced in a rat model of polycystic ovary syndrome (PCOS). *Brain Res* 2012;1467:1-9.
8. Kondo M, Osuka S, Iwase A, et al. Increase of kisspeptin-positive cells in the hypothalamus of a rat model of polycystic ovary syndrome. *Metab Brain Dis* 2016;31:673-681.
9. Aliabadi E, Namavar MR, Mortezaee K, et al. Kisspeptin expression features in the arcuate and anteroventral periventricular nuclei of hypothalamus of letrozole-induced polycystic ovarian syndrome in rats. *Arch Gynecol Obstet* 2017;296:957-963.
10. Domecq JP, Prutsky G, Mullan RJ, et al. Lifestyle modification programs in polycystic ovary syndrome: systematic review and meta-analysis. *J Clin Endocrinol Metab* 2013;98:4655-4663.
11. Jamal M, Gunay Y, Capper A, Eid A, Heitshusen D, Samuel I. Roux-en-y gastric bypass ameliorates polycystic ovary syndrome and dramatically improves conception rates: a 9-year analysis. *Surg Obes Relat Dis* 2012;8:440-444.
12. Turkmen S, Andreen L, Cengiz Y. Effects of Roux-en-Y gastric bypass surgery on eating behaviour and allopregnanolone levels in obese women with polycystic ovary syndrome. *Gynecol Endocrinol* 2015;31:301-305.
13. Skubleny D, Switzer NJ, Gill RS, et al. The impact of bariatric surgery on polycystic ovary syndrome: a systematic review and meta-analysis. *Obes Surg* 2016;26:169-176.
14. Escobar-Morreale HF, Santacruz E, Luque-Ramirez M, Botella Carretero JJ. Prevalence of 'obesity-associated gonadal dysfunction' in severely obese men and women and its resolution after bariatric surgery: a systematic review and meta-analysis. *Hum Reprod Update* 2017;23:390-408.
15. Eid GM, McCloskey C, Titchner R, et al. Changes in hormones and biomarkers in polycystic ovarian syndrome treated with gastric bypass. *Surg Obes Relat Dis* 2014;10:787-791.
16. Casimiro I, Sam S, Brady MJ. Endocrine implications of bariatric surgery: a review on the intersection between incretins, bone, and sex hormones. *Physiol Rep* 2019;7:e14111. doi:10.14814/phy2.14111
17. Abiad F, Khalife D, Safadi B, et al. The effect of bariatric surgery on inflammatory markers in women with polycystic ovarian syndrome. *Diabetes Metab Syndr* 2018;12:999-1005.
18. Kubota N, Yano W, Kubota T, et al. Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. *Cell Metab* 2007;6:55-68.
19. Toulis KA, Goulis DG, Farmakiotis D, et al. Adiponectin levels in women with polycystic ovary syndrome: a systematic review and a meta-analysis. *Hum Reprod Update* 2009;15:297-307.

20. Benrick A, Chanclón B, Micallef P, et al. Adiponectin protects against development of metabolic disturbances in a PCOS mouse model. *Proc Natl Acad Sci U S A* 2017;114:E7187-E7196.
21. Singh A, Bora P, Krishna A. Systemic adiponectin treatment reverses polycystic ovary syndrome-like features in an animal model. *Reprod Fertil Dev* 2018;30:571-584.
22. Wen JP, Lv WS, Yang J, et al. Globular adiponectin inhibits GnRH secretion from GT1-7 hypothalamic GnRH neurons by induction of hyperpolarization of membrane potential. *Biochem Biophys Res Commun* 2008;371:756-761.
23. Wen JP, Liu C, Bi WK, et al. Adiponectin inhibits KISS1 gene transcription through AMPK and specificity protein-1 in the hypothalamic GT1-7 neurons. *J Endocrinol* 2012;214:177-189.
24. Stefater MA, Perez-Tilve D, Chambers AP, et al. Sleeve gastrectomy induces loss of weight and fat mass in obese rats, but does not affect leptin sensitivity. *Gastroenterology* 2010;138:2426-2436, 2436.e1-3.
25. Byers SL, Wiles MV, Dunn SL, Taft RA. Mouse estrous cycle identification tool and images. *PLoS One* 2012;7:e35538. doi:10.1371/journal.pone.0035538
26. Yuan X, Hu T, Zhao H, et al. Brown adipose tissue transplantation ameliorates polycystic ovary syndrome. *Proc Natl Acad Sci U S A* 2016;113:2708-2713.
27. Domecq JP, Prutsky G, Mullan RJ, et al. Lifestyle modification programs in polycystic ovary syndrome: systematic review and meta-analysis. *J Clin Endocrinol Metab* 2013;98:4655-4663.
28. Legro RS, Arslanian SA, Ehrmann DA, et al. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2013;98:4565-4592.
29. Grulet H, Hecart AC, Delemer B, et al. Roles of LH and insulin resistance in lean and obese polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 1993;38:621-626.
30. Anttila L, Koskinen P, Jaatinen TA, Erkkola R, Irjala K, Ruutiainen K. Insulin hypersecretion together with high luteinizing hormone concentration augments androgen secretion in oral glucose tolerance test in women with polycystic ovarian disease. *Hum Reprod* 1993;8:1179-1183.
31. Navarro VM, Tena-Sempere M. Neuroendocrine control by kisspeptins: role in metabolic regulation of fertility. *Nat Rev Endocrinol* 2011;8:40-53.
32. Roa J, Aguilar E, Dieguez C, Pinilla L, Tena-Sempere M. New frontiers in kisspeptin/GPR54 physiology as fundamental gatekeepers of reproductive function. *Front Neuroendocrinol* 2008;29:48-69.
33. d'Anglemont de Tassigny X, Colledge WH. The role of kisspeptin signaling in reproduction. *Physiology (Bethesda)* 2010;25:207-217.
34. Tolson KP, Garcia C, Yen S, et al. Impaired kisspeptin signaling decreases metabolism and promotes glucose intolerance and obesity. *J Clin Invest* 2014;124:3075-3079.
35. Stengel A, Wang L, Goebel-Stengel M, Tache Y. Centrally injected kisspeptin reduces food intake by increasing meal intervals in mice. *Neuroreport* 2011;22:253-257.
36. Fu LY, van den Pol AN. Kisspeptin directly excites anorexigenic proopiomelanocortin neurons but inhibits orexigenic neuropeptide Y cells by an indirect synaptic mechanism. *J Neurosci* 2010;30:10205-10219.
37. Comminos AN, Jayasena CN, Dhillon WS. The relationship between gut and adipose hormones, and reproduction. *Hum Reprod Update* 2014;20:153-174.
38. Lai Hao, Jia Xiao, Qiuxiao Yu, et al. High-fat diet induces significant metabolic disorders in a mouse model of polycystic ovary syndrome. *Biol Reprod* 2014;91:127. doi:10.1095/biolreprod.114.120063
39. Zhang Haolin, Yi Ming, Zhang Yan, et al. High-fat diets exaggerate endocrine and metabolic phenotypes in a rat model of DHEA-induced PCOS. *Reproduction* 2016;151:431-441.
40. Ressler IB, Grayson BE, Seeley RJ. Metabolic, behavioral, and reproductive effects of vertical sleeve gastrectomy in an obese rat model of polycystic ovary syndrome. *Obes Surg* 2014;24:866-876.