Brown adipose tissue transplantation ameliorates polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS), which is characterized by anovulation, hyperandrogenism, and polycystic ovaries, is a complex endocrinopathy. Because the cause of PCOS at the molecular level is largely unknown, there is no cure or specific treatment for PCOS. Here, we show that transplantation of brown adipose tissue (BAT) reversed anovulation, hyperandrogenism, and polycystic ovaries in a dehydroepiandrosterone (DHEA)-induced PCOS rat. BAT transplantation into a PCOS rat significantly stabilized menstrual irregularity and improved systemic insulin sensitivity up to a normal level, which was not shown in a sham-operated or muscle-transplanted PCOS rat. Moreover, BAT transplantation, not sham operation or muscle transplantation, surprisingly improved fertility in PCOS rats. Interestingly, BAT transplantation activated endogenous BAT and thereby increased the circulating level of adiponectin, which plays a prominent role in whole-body energy metabolism and ovarian physiology. Consistent with BAT transplantation, administration of adiponectin protein dramatically rescued DHEA-induced PCOS phenotypes. These results highlight that endogenous BAT activity is closely related to the development of PCOS phenotypes and that BAT activation might be a promising therapeutic option for the treatment of PCOS.

brown adipose tissue | transplantation | ameliorates | PCOS | adiponectin

Polycystic ovary syndrome (PCOS) is now recognized as one of the most common endocrine diseases in women of reproductive age. The prevalence of PCOS ranges from 9% to 18%, depending on the criteria used for its definition and ethnicity (1, 2). The core feature of PCOS includes polycystic ovaries, hyperandrogenism, and chronic anovulation. Furthermore, PCOS is a complex and heterogeneous syndrome because it is associated with a high risk for the development of insulin resistance, type 2 diabetes (T2D), obesity, dyslipidemia, and cardiovascular disease (3–5). There are three different criteria used for the diagnosis of PCOS: androgen excess, irregular menstruation, and polycystic ovary appearance on ultrasound after excluding other causes of hyperandrogenism and anovulation (6). Because a single etiologic factor is not able to fully account for all of the clinical features in PCOS, the pathogenesis of PCOS is largely unknown. Several genetic and environmental factors may contribute to the development of PCOS; however, the underlying cellular mechanism of the induction and progression of PCOS remains to be elucidated.

Insulin resistance, which is common among PCOS patients, seems to be a key etiological characteristic, and about 85% of women with PCOS suffer from insulin resistance (7). Compensatory hyperinsulinemia can directly stimulate ovarian and adrenal secretion of androgen and decrease hepatic sex hormone binding globulin (*SHBG*) synthesis, resulting in an increased bioavailability of free testosterone levels (8, 9). Thus, insulin resistance and hyperandrogenism contribute to the key clinical presentation of PCOS. Because the clinical features are complex and vary among PCOS patients, it is hard to provide the first-line treatment of PCOS. Most treatment guidelines recommend that patients change lifestyles, including exercise and dietary modification. Patients can take oral contraceptive pills (OCPs) to control symptoms of hyperandrogenism or take insulin-sensitizing medicines such as metformin or pioglitazone when they have impaired glucose tolerance or features of a metabolic syndrome (10). However, there is a lack of effective treatment for PCOS at present.

It has been reported that the functional abnormality of adipose tissue in PCOS patients is primarily linked to insulin resistance, even in the absence of obesity (11, 12). In humans and other mammals, there are mainly two types of adipose tissue with opposing functions: white adipose tissue (WAT) and brown adipose tissue (BAT). The main function of WAT is to store excess energy in WAT as a form of triglycerides whereas BAT contains large numbers of mitochondria that uncouple large amounts of fuel for heat generation and the maintenance of body temperature (13). Recent studies using positron emission tomography (PET) have demonstrated that human adults also possess metabolically active BAT (14, 15) and that BAT activation inversely correlates with age and body mass index (BMI) (16). Therefore, increasing BAT mass and/or function is a promising strategy to

Significance

In the current study, we show that brown adipose tissue (BAT) activity is dramatically reduced in a dehydroepiandrosterone (DHEA)-induced polycystic ovary syndrome (PCOS) rat when compared with a normal control rat. Importantly, the key features of PCOS (such as insulin resistance and irregular estrous cycle) are alleviated after BAT transplantation. Mechanistically, transplanted BAT enhances endogenous BAT activity and thereby increases the circulating adiponectin level, which was lower in both the PCOS patient and PCOS rat model. Furthermore, exogenous adiponectin protein administration recapitulates beneficial effects from BAT transplantation in a PCOS rat. Taken together, these data highlight the important role of BAT in the development of PCOS and that BAT-induced adiponectin might open up a new way in the treatment of PCOS.

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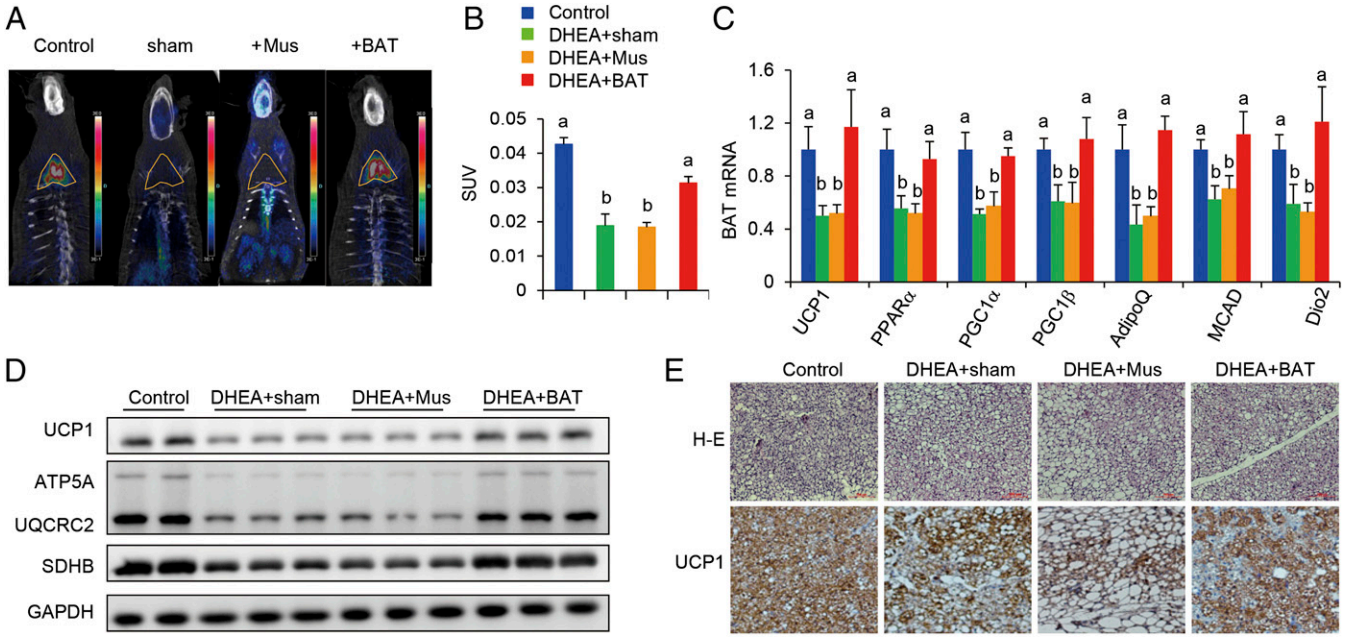

Fig. 1. BAT transplantation reverses PCOS BAT activity. BAT activity was assessed at the end of the experiment (3 wk after tissue transplantation) by using PET-CT. BAT transplantation could significantly increase endogenous BAT activity in the DHEA+BAT group compared with the DHEA+sham or DHEA+Mus groups (*A*). Yellow triangle indicates the anatomical site of the interscapular BAT. The activity of brown adipose tissue, expressed as the standard uptake values (SUVs), dramatically decreased in the DHEA+sham and DHEA+Mus groups compared with the control and BAT transplantation groups (*B*). Furthermore, BAT transplantation could significantly increase BAT-specific marker gene expression (*C*) and OXPHOS protein expression (*D*), as well as UCP1 expression (*E*), compared with the DHEA+sham or DHEA+Mus groups. Data were analyzed by one-way ANOVA with Tukey's post hoc test. $n = 8–10$ per group. Different lowercase letters indicate significant differences among groups (One-way ANOVA, with Tukey's post hoc test, $P < 0.05$).

treat obesity and metabolic diseases. Indeed, studies by our group and others have shown that BAT transplantation reverses metabolic disorders in various obese mouse models (17–19).

Given the several common features between PCOS and a metabolic syndrome, we aimed to investigate whether BAT possibly plays an important role in the development of PCOS

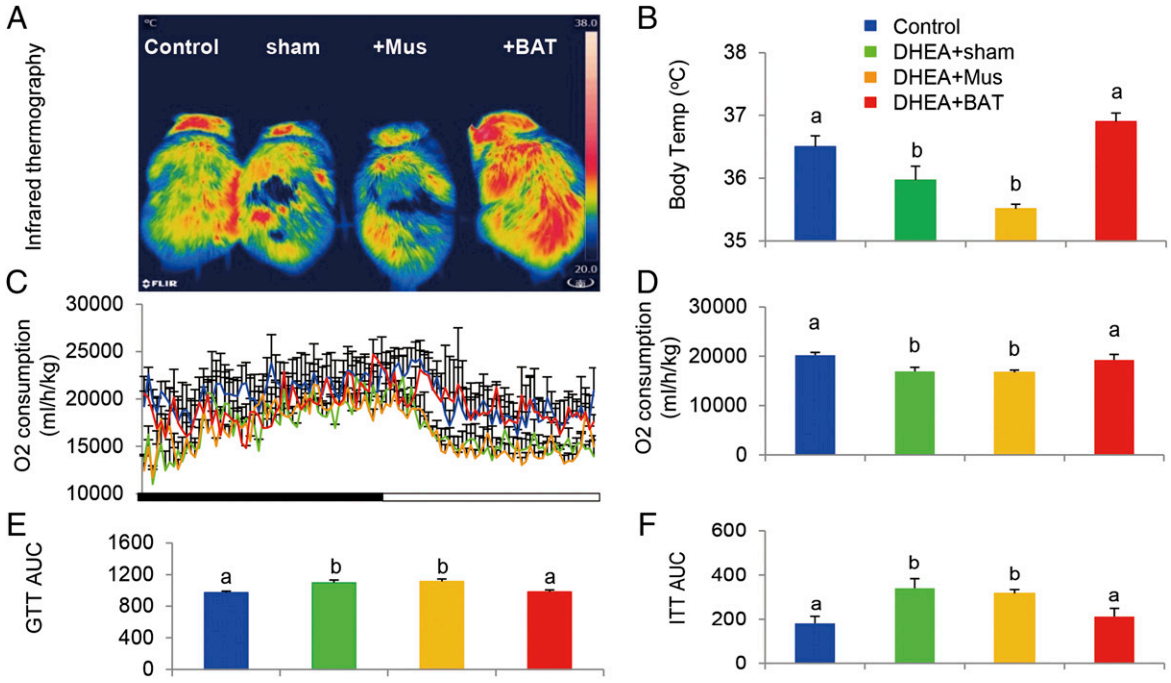

Fig. 2. BAT transplantation reverses PCOS metabolic abnormality. An infrared thermal image demonstrates that cold exposure significantly reduced body temperature of the DHEA+sham and DHEA+Mus groups whereas BAT transplantation significantly reversed DHEA-induced body temperature reduction (*A* and *B*). In addition, BAT transplantation significantly increased whole-body energy expenditure compared with the DHEA+sham or DHEA+Mus groups (*C* and *D*). Moreover, results from a glucose tolerance test (*E*) and insulin tolerance test (*F*) showed that BAT transplantation significantly reversed DHEA-induced glucose intolerance. Data were analyzed by one-way ANOVA with Tukey's post hoc test. $n = 8–10$ per group. (*A* and *B*) $P < 0.05$. Different lowercase letters indicate significant differences among groups (One-way ANOVA, with Tukey's post hoc test, $P < 0.05$).

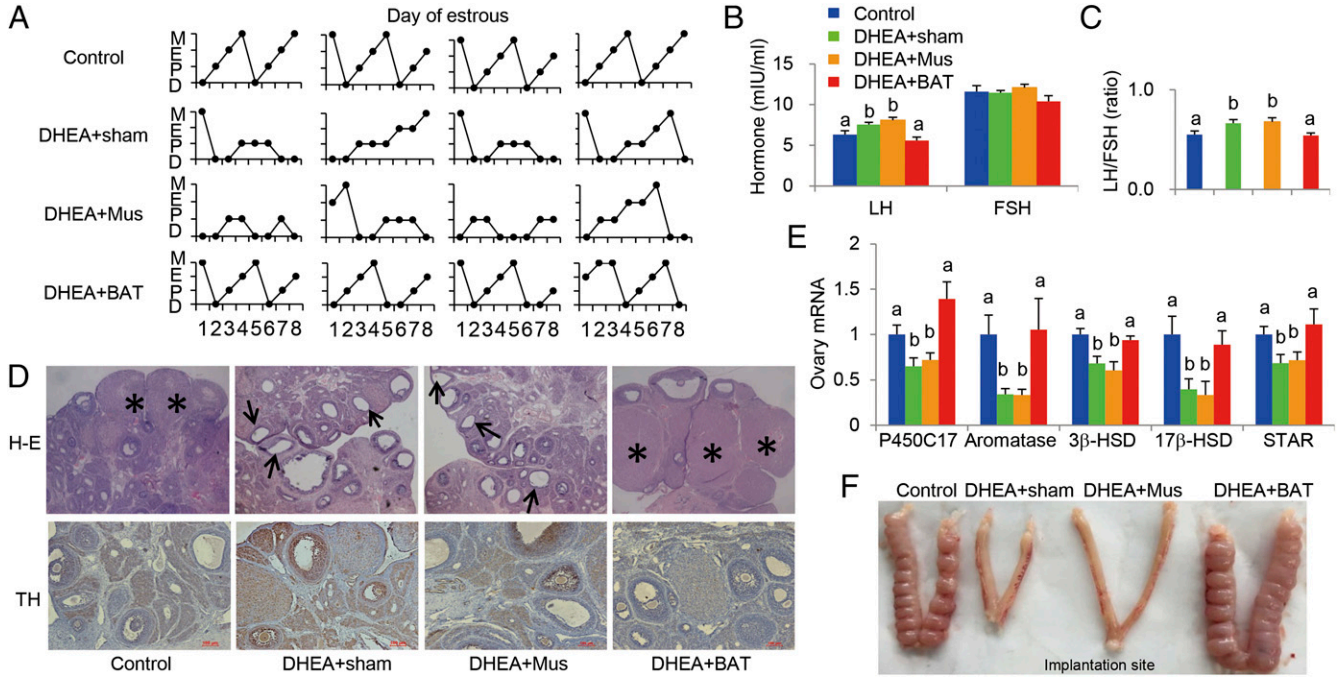

Fig. 3. BAT transplantation reverses PCOS acyclicity, ovarian phenotypes, and infertility. BAT transplantation could reverse abnormal estrous cycles in the PCOS rodent compared with abnormal estrous cycles in the DHEA+sham and/or DHEA+Mus groups (*A*). BAT transplantation further significantly reversed the concentrations of luteinizing hormone (LH) levels, as well as the LH/FSH ratio, to normal control levels compared with the DHEA+sham and DHEA+Mus groups (*B* and *C*). (*D*) Ovarian histology revealed that cystic follicles (arrow) appeared in the DHEA+sham and DHEA+Mus groups but not in the DHEA+BAT group. In addition, few corpora lutea (CL, asterisk) and low levels of TH expression were observed in the BAT transplantation group but not in the muscle transplantation group (*D*). Consistent with histology results, the expression of ovarian steroidogenic enzymes was dramatically reversed after BAT transplantation (*E*), and the DHEA+BAT group rats, but not the DHEA+sham and DHEA+Mus group rats, were also able to mate with proven stud males and produce a little (*F*). Data were analyzed by one-way ANOVA with Tukey's post hoc test. $n = 8$–10 per group. Different lowercase letters indicate significant differences among groups (One-way ANOVA, with Tukey's post hoc test, $P < 0.05$). D, diestrus; E, estrus; M, metestrus; P, proestrus.

phenotypes and the treatment of PCOS. In the current study, we show that BAT activity was dramatically reduced in a dehydroepiandrosterone (DHEA) (a precursor of androgen)-induced PCOS rat compared with a normal control rat. Notably, the key features of PCOS, such as insulin resistance, irregular estrous cycle, and low birth rate, were significantly improved after BAT transplantation in PCOS rats. Interestingly, transplanted BAT in PCOS rats enhanced endogenous BAT activity and thereby increased the circulating adiponectin level, which was lower in both PCOS patients and PCOS rats. In parallel, exogenous adiponectin protein administration in a PCOS rat recapitulated the effects that were seen in a BAT-transplanted PCOS rat. Taken together, these data suggest that BAT is one of the important organs regulating the features of PCOS and that the increase of BAT mass or its activity might provide a new therapeutic strategy for the treatment of PCOS.

Materials and Methods

All animal studies were conducted with the approval of the Institutional Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences. Tissue (0.5 g of BAT or muscle) transplantation experiments were performed in a DHEA-induced PCOS rat. Recombinant adiponectin [10 μg/kg body weight (BW)] was daily injected into a PCOS rat. Written informed consent was obtained from all participants and this study was approved by the Institutional Review Board of Reproductive Medicine of Shandong University. Please refer to *SI Materials and Methods* for detailed information.

Results

BAT Transplantation Reverses Reduced BAT Activity and Metabolic Abnormality in the PCOS Rat. Accumulating evidence indicates that insulin resistance is one of the most common clinical features in PCOS (20) and that insulin resistance is often accompanied with reduced BAT activity (21). Therefore, we hypothesized that BAT mass and/or its activity might be associated with PCOS phenotypes, including polycystic ovaries, hyperandrogenism, and chronic anovulation. To prove our hypothesis, a rat was daily injected with DHEA for 20 d and then the irregular estrous cycle was analyzed by vaginal smear check to confirm the development of PCOS. Next, we transplanted 0.5 g of BAT from an age- and sex-matched donor rat into a PCOS rat (DHEA+BAT), and three other groups—a PBS-treated (control) group, a sham-operated (DHEA+sham) group, or a skeletal muscle-transplanted (DHEA+Mus) group—served as control groups. At 3 wk after tissue transplantation, BAT activity was assessed with positron emission tomography–computed tomography (PET-CT). Results from PET-CT showed that BAT activity was significantly reduced in DHEA+sham and DHEA+Mus groups than in the control group; however, BAT transplantation into a DHEA-induced PCOS rat dramatically increased endogenous BAT activity up to the level of the control group (Fig. 1 *A* and *B*). Although obesity is a key feature of PCOS, there was no significant difference of body weight as well as food consumption among groups in the current study (Fig. S1). Uncoupling protein 1 (*UCP1*) is a BAT-specific protein that dissipates the proton electrochemical gradient in mitochondria to generate heat (22). Peroxisome proliferator activated receptor gamma coactivator 1 alpha (*PGC1α*) and peroxisome proliferator activated receptor gamma coactivator 1 beta (*PGC1β*) induce the expression of UCP1 and mitochondria thermogenesis-related genes (22). Peroxisome proliferator activated receptor alpha (*PPARα*) is a major regulator of lipid metabolism (23). Type II iodothyronine deiodinase (*Dio2*) is a marker gene of BAT activation (24). Therefore, we analyzed the gene expression levels of these genes to assess BAT thermogenic activity. In parallel to BAT activity results, BAT-specific gene expressions were significantly decreased in DHEA+sham and DHEA+Mus

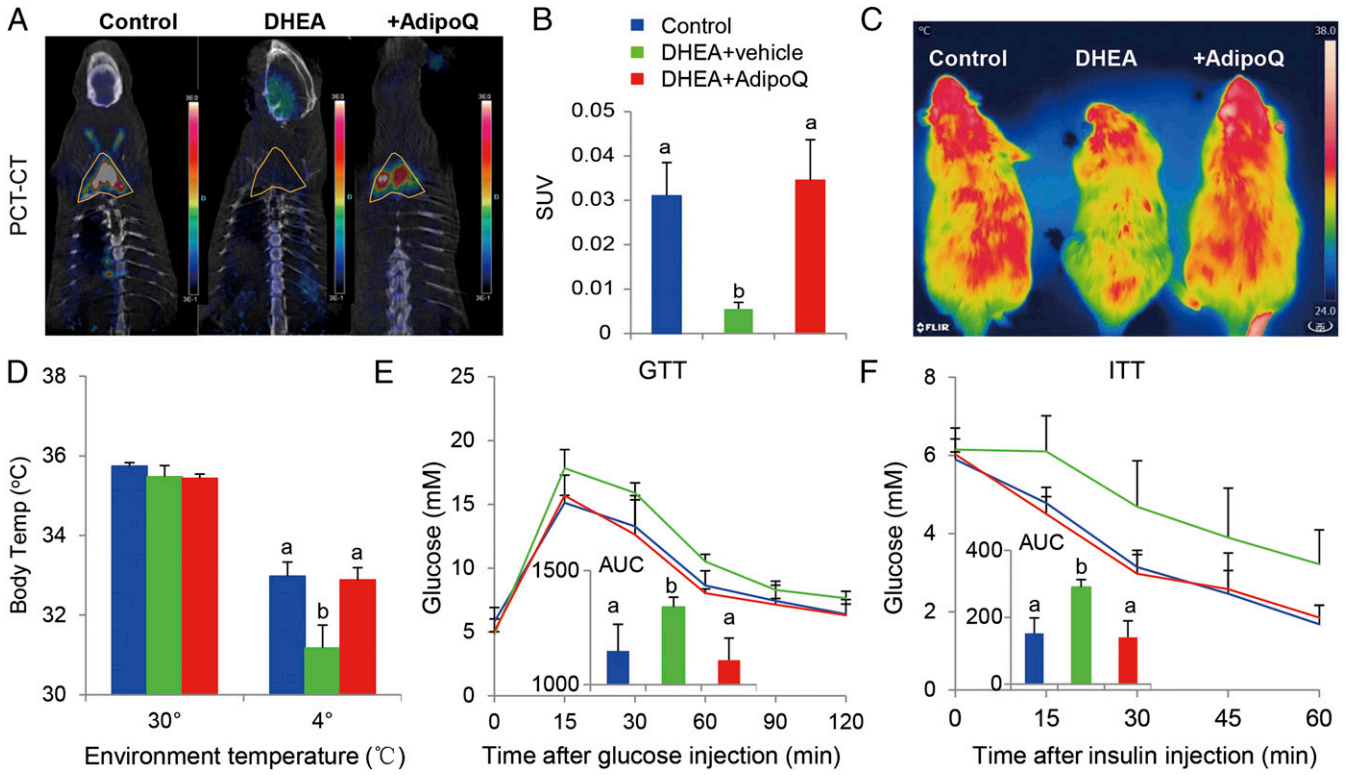

Fig. 4. Adiponectin recapitulates the beneficial effects of BAT transplantation. Adiponectin treatment could significantly increase endogenous BAT activity compared with DHEA groups as evidenced by PET-CT (*A* and *B*). Moreover, Infrared thermal images demonstrated that adiponectin treatment significantly reversed DHEA-mediated body temperature reduction (*C* and *D*). A glucose tolerance test (*E*) and insulin tolerance test (*F*) showed that adiponectin treatment significantly improved DHEA -induced insulin resistance (inner graph indicating area under the curve of GTT and ITT, respectively). Data were analyzed by one-way ANOVA with Tukey's post hoc test; $n = 6$ per group. Different lowercase letters indicate significant differences among groups (One-way ANOVA, with Tukey's post hoc test, $P < 0.05$).

groups compared with control and DHEA+BAT groups (Fig. 1*C*). Moreover, UCP1 and OXPHOS protein expressions were also increased in the DHEA+BAT group compared with DHEA+sham or DHEA+Mus groups (Fig. 1 *D* and *E*). It has been reported that postprandial thermogenesis is decreased in PCOS patients (25). In our PCOS rat model, body temperature after cold exposure was significantly decreased in DHEA+sham and DHEA+Mus groups whereas BAT transplantation significantly reversed DHEA-mediated body temperature reduction (Fig. 2 *A* and *B*). In addition, BAT transplantation, not sham operation or skeletal muscle transplantation, significantly improved energy expenditure in a DHEA-induced PCOS rat (Fig. 2 *C* and *D*). Consequently, glucose homeostasis and insulin sensitivity were dramatically improved in the DHEA+BAT group compared with DHEA+sham or DHEA+Mus groups (Fig. 2 *E* and *F* and Fig. S2). These results suggest that BAT transplantation reverses endogenous BAT activity and insulin resistance in the DHEA-induced PCOS rat.

BAT Transplantation Reverses PCOS Acyclicity. As mentioned above, irregular menstruation is one of the key criteria for the diagnosis of PCOS. We therefore investigated whether BAT transplantation could regulate the estrous cycle in a PCOS rat. After DHEA treatment, acyclicity detected by vaginal cytology was found in the DHEA+sham group and not in the control group, indicating that a rat PCOS model had been successfully developed (Fig. 3*A* and Table S1). Surprisingly, BAT transplantation normalized menstrual cyclicity in 7 out of 10 DHEA-induced PCOS rats, which was not found in the DHEA+Mus group (Fig. 3*A* and Table S1). These results highlighted that BAT transplantation could reverse abnormal estrous cycles in the PCOS rat. Abnormal estrous is accompanied with altered plasma gonadotropin concentration. Although plasma follicle-stimulating hormone (FSH) concentration was not altered among groups, plasma-luteinizing hormone (LH), as well as the LH/FSH ratio, which is one of the parameters for the diagnosis of PCOS in clinics, was significantly increased in DHEA+sham and DHEA+Mus groups compared with the control group. Notably, BAT transplantation reversed the plasma LH level and LH/FSH ratio to a normal level (Fig. 3 *B* and *C*). Additionally, the plasma testosterone (T) level was significantly attenuated after BAT transplantation in a DHEA-induced PCOS rat. Taken together, these results indicated that BAT transplantation reversed irregular estrous cyclicity in the PCOS rat.

PCOS Ovarian Phenotypes and Infertility Were Reversed by BAT Transplantation. Histologically, the number of corpora lutea (CL) was decreased and the thickness of the theca cell layer was increased in DHEA+sham and DHEA+Mus groups compared with the control group (Table S2). However, a normal layer of theca cells, mature follicles, and corpus luteum (CL) were observed in the ovary from the DHEA+BAT group (Fig. 3*D* and Table S2). Previous studies demonstrated that ovarian sympathetic tone was increased in women with PCOS (26, 27). Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the biosynthesis of norepinephrine (NE), and expression of TH in the ovary is highly restricted to sympathetic nerves. Thus, ovarian tissue sections from four groups were immunostained with an anti-TH antibody to detect sympathetic innervation. A large number of TH-positive sympathetic nerve fibers were found in ovaries from the DHEA+sham and DHEA+Mus groups whereas BAT transplantation significantly reduced the number of TH-expressing sympathetic nerve fibers in the ovaries (Fig. 3*D*). Consistent with immunostaining results, the expressions of ovarian steroidogenic enzymes, such as *P450C17*, *aromatase*, *3β-HSD, 17β-HSD*, and *STAR*, were significantly decreased in the DHEA+sham and DHEA+Mus groups compared with the control group, and BAT transplantation dramatically reversed their expressions

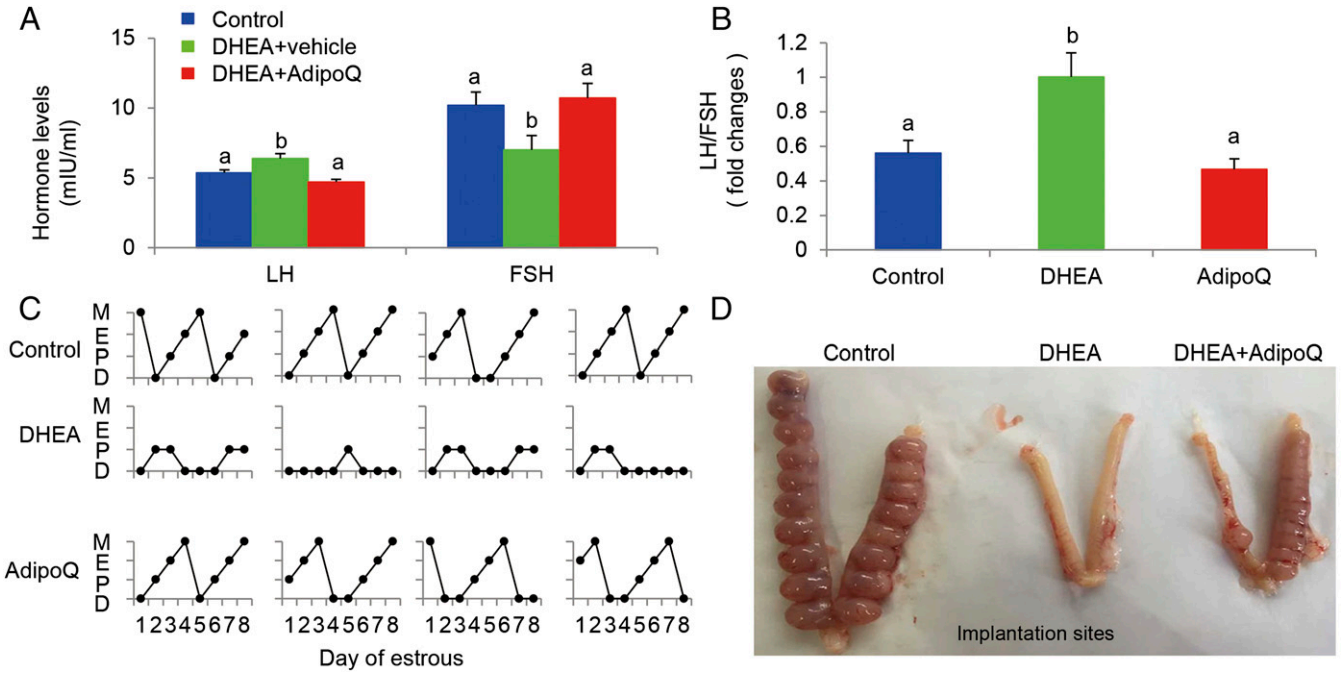

Fig. 5. Adiponectin reverses PCOS acyclicity, ovarian phenotypes, and infertility. The concentrations of luteinizing hormone (LH) and the LH/FSH ratio were significantly increased in the DHEA group compared with the control group, and it was reversed to a normal level after adiponectin treatment (*A* and *B*). In addition, adiponectin treatment could significantly reverse DHEA-induced acyclicity (*C*) and pregnant capacity in the PCOS rodent (*D*). Data were analyzed by one-way ANOVA with Tukey's post hoc test. $n = 6$ per group. Different lowercase letters indicate significant differences among groups (One-way ANOVA, with Tukey's post hoc test, $P < 0.05$).

up to normal levels (Fig. 3*E*). In particular, rats in the DHEA+sham and DHEA+Mus groups were infertile and unable to give birth to a litter; however, BAT transplantation enabled the PCOS rat to deliver a litter (Fig. 3*F* and Table S3). Collectively, these results indicate that BAT transplantation could significantly reverse infertility in the PCOS rat.

Administration of Adiponectin Recapitulates the Beneficial Effects of BAT Transplantation in the PCOS Rat. In our previous study, we showed that transplanted BAT activated endogenous BAT and increased the circulating adiponectin level in an obese mouse (17). Thus, we determined whether the adiponectin level is altered in the PCOS human and rat. Consistent with a previous report (28), the circulating adiponectin level was significantly decreased in both the PCOS patient and rat (Fig. S3 *A* and *B* and Table S4). Therefore, we reasoned that adiponectin might account, at least in part, for the beneficial effects of BAT transplantation in the PCOS rat. To address this question, a PCOS rat was daily injected with recombinant adiponectin protein (10 μg/kg BW) for 20 d. Results from PET-CT (Fig. 4 *A* and *B*), as well as cold-induced thermogenesis (Fig. 4 *C* and *D*), showed that administration of adiponectin in a PCOS rat significantly increased endogenous BAT activity up to the level of the control group. Similar to BAT transplantation, adiponectin treatment also increased energy expenditure and glucose homeostasis in the PCOS rat (Fig. 4 *E* and *F*). In addition, adiponectin treatment markedly attenuated the plasma LH/FSH ratio that was increased in the DHEA+sham group (Fig. 5 *A* and *B*). Interestingly, adiponectin treatment significantly reversed DHEA-induced acyclicity (Fig. 5*C* and Table S5), ovarian phenotypes (Table S5), and infertility in the PCOS rat (Fig. 5*D* and Table S5). These results highlight that the beneficial effects of BAT transplantation are partly mediated by an elevated circulating adiponectin level.

Discussion

In the current study, we showed that BAT activity was dramatically decreased in the PCOS rat and that BAT transplantation effectively ameliorated most of the symptoms found in the PCOS rat. In addition, we revealed that the beneficial effects of BAT transplantation in the PCOS rat were mediated by the increased circulating adiponectin level. To the best of our knowledge, this study is the first study showing that the activity of BAT is associated with clinical phenotypes of PCOS in an animal model. We believe that the current study points out BAT as a previously unidentified target organ for the treatment of PCOS.

Mice neonatally androgenized with testosterone that induces PCOS showed a significant decrease in energy expenditure (29). It has been speculated that this phenomenon could be due to the BAT hypofunction (30). In agreement with previous findings, BAT-specific thermogenic gene expression, UCP1, and mitochondrial OXPHOS protein expression and cold-induced thermogenic capacity, which are key factors accounting for the reduction of energy metabolism, were reduced in our PCOS rat (Fig. 1), indicating that the DHEA-induced PCOS rat had a significant defect in energy metabolism and BAT activity.

In parallel, it was also reported that women with PCOS show increased sympathetic tone (31). Consistently, we observed that sympathetic innervation, as evidenced by TH staining, was increased in the ovaries of the DHEA-treated PCOS rat (Fig. 2*C*). Sustained high sympathetic tone causes insensitivity of BAT and later influences disrupted whole-body energy metabolism in PCOS. Taken together, these results suggest that the attenuation of BAT activity might play a significant pathogenic role in PCOS.

It has been widely appreciated that women with PCOS show insulin resistance and glucose intolerance (32). On the other hand, BAT activity is often negatively associated with diabetes status but positively correlated with glucose uptake activity in humans (33). Recently, we demonstrated that BAT transplantation has a beneficial effect on the prevention and treatment of obesity in the HFD-induced obese mouse, as well as in the genetic obese Ob/Ob mouse (17, 18). In addition, we showed that BAT transplantation significantly improved glucose homeostasis in both diet-induced obesity and genetic obesity mice models (17, 18). In agreement with previous results, we observed that DHEA-induced glucose intolerance was significantly reversed by transplantation of BAT, but not muscle (Fig. 2 *E* and *F*).

These results again emphasize the important role of BAT in glucose homeostasis.

The remaining question we had was how the transplanted BAT displayed beneficial effects on PCOS. We speculated that the beneficial effects of BAT transplantation might be from activated endogenous BAT that might secret systemic brown adipose tissue-derived adipokine (batokine). In our previous report, we demonstrated that BAT transplantation in obese mice significantly increased the circulating adiponectin level (17), which is known to be attenuated in women with PCOS (34). Consistently, we also confirmed that there was a significant reduction of the circulating adiponectin level in both PCOS women and the DHEA-treated rats. Interestingly, we found that the adiponectin level was significantly reversed to normal level after BAT transplantation (Fig. S3 *A* and *B*). These results led us to investigate whether adiponectin administration recapitulates the beneficial effects of BAT transplantation in the PCOS rat. After adiponectin treatment, decreased BAT activity, metabolic abnormalities, acyclicity, and abnormal hormonal levels were surprisingly normalized up to normal levels in the PCOS rat. Based on recent publications, BAT also secretes a considerable number of adipokines, such as adiponectin, FGF21, NGF, NRG4, VEGF, and BMPs (16, 35). We have observed that there was no significant difference of FGF21 or NGF levels between groups (Table S6). Gunawardana et al. (36) reported that BAT transplantation can reverse type 1 diabetes in streptozotocin-treated mice without exogenous insulin treatment. Furthermore, we and other group have shown that BAT transplantation reversed metabolic disorders in various obese mouse models (17–19). These results further suggest that BAT secretes systemic mediators that could regulate in whole-body glucose homeostasis. It should be noted that we do not exclude other factors mentioned above that may be involved in the beneficial effects of BAT transplantation in the PCOS rat model. However, in our hands, we observed that adiponectin alone was enough to recapitulate the beneficial effects of BAT transplantation in the PCOS rat. Other mechanisms behind the adiponectin effect for the treatment of PCOS would be necessary to be revealed in the near future. Taken together, our findings highlight that systemic adiponectin treatment significantly improves PCOS phenotypes in an animal model.

In conclusion, we demonstrate here that BAT transplantation could significantly improve PCOS phenotypes, including disrupted energy metabolism, acyclicity, and infertility. In addition, these beneficial effects of BAT transplantation were at least in part mediated by systemic adiponectin. Because BAT transplantation is not easily applied to human beings, administration of batokines or drugs that enhance BAT activity will be alternative strategies for the treatment of PCOS.

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