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Stress responses to bacterial and viral mimetics in polycystic ovary syndrome model rats

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ABSTRACT

Polycystic ovary syndrome (PCOS) is associated with an increased risk of psychological distress as well as enhanced responses to psychosocial stress. Recently, it was hypothesized that PCOS patients may be at high risk of novel COVID-19 infections and worse clinical presentations during such infections. Here, we evaluated the effects of PCOS on stress responses to bacterial and viral mimetics using dihydrotestosterone-induced PCOS model rats. Lipopolysaccharide (LPS; a bacterial mimetic) or polyinosinic-polycytidylic acid (Poly-IC; a viral mimetic) was injected into PCOS model rats (PCOS) and non-PCOS rats (control), and the rats' stress responses were evaluated. In the PCOS group, the rats' anorectic and febrile responses to LPS injection were enhanced, whereas their anorectic and febrile responses to Poly-IC injection were unaltered. The PCOS group also exhibited greater changes in peripheral cytokine levels in response to LPS, but not Poly-IC. On the contrary, after the injection of Poly-IC depressed locomotor activity was more evident in the PCOS group, whereas no such changes were observed after LPS injection. These findings indicate that although the stress responses of PCOS model rats to infection may be enhanced, the patterns of change in stress responses and their underlying mechanisms may differ between bacterial and viral infections.

1. Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age, with an estimated prevalence of 5–16% (Lauritsen et al., 2014; Rosenfield and Ehrmann, 2016; Ding et al., 2017). The main symptoms of PCOS are anovulation, a distinctive ovarian morphology, and hyperandrogenism, and it is commonly complicated with metabolic disorders, such as obesity, insulin resistance, and/or type 2 diabetes (Dunaif, 1997; Moran et al., 2010; Wekker et al., 2020). In addition to these physical problems, PCOS is associated with an increased risk of psychological conditions, such as anxiety, depression, or eating disorders (Dokras et al., 2018), as well as an enhanced response to psychosocial stress (Benson et al., 2009). For example, in women with PCOS the neuroendocrine system was hyperresponsive to psychological stressors (Benson et al., 2009; Gallinelli et al., 2000; Mezzullo et al., 2018), and these findings have been reproduced in PCOS model animals (Feng et al., 2011; Hu et al., 2015; Ressler et al., 2015; Manti et al., 2018). Thus, there is evidence that PCOS enhances psychosocial stress responses. However, as far as we know, no previous studies have examined whether PCOS changes the responses to other kinds of stress.

Although the etiology of PCOS remains unclear, hyperandrogenism is assumed to play a pivotal role in the onset and progression of the condition (Franks et al., 2006; Daan et al., 2014; Rosenfield and Ehrmann, 2016). The long-term administration of an androgen can reproduce PCOS-like phenotypes in rodents, and such animal models have been used to evaluate the etiology and pathophysiology of PCOS (Iwasa et al., 2018; Osuka et al., 2019). Although most studies using these PCOS

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models have focused on metabolic and reproductive functions, one study reported that dihydrotestosterone (DHT)-induced PCOS model rats showed anxiety-related behavior and an enhanced response to psychological stress (Ressler et al., 2015).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which was responsible for coronavirus disease 2019 (COVID-19), has spread around the world. Some emerging risk factors that increase the severity of COVID-19 have been proposed in epidemiological studies (Vaduganathan et al., 2020). These risk factors include male sex and PCOS, which have been suggested to be associated with a high risk of novel COVID-19 infections and worse clinical presentations during such infections (Jun et al., 2021; Subramanian et al., 2021; de Medeiros et al., 2022). In addition, it is hypothesized that androgens play pivotal roles in these increases in the risk and severity of COVID-19 in males and PCOS, e.g., women with hyperandrogenic PCOS tend to show pronounced symptoms (Cadegiani et al., 2020), and anti-androgens may have beneficial effects on severe COVID-19 symptoms (Kyrou et al., 2020; Mohamed et al., 2021). However, most of these findings are based on epidemiological and clinical data, and there has not been any basic research supporting these hypotheses. In addition, no previous studies have evaluated whether responses to bacterial infections are altered in PCOS.

In our previous studies, we proposed a novel PCOS model based on the long-term administration of low-dose DHT, which closely reproduces the reproductive and metabolic phenotypes of PCOS (Kamada et al., 2021; Yamamoto et al., 2022). In the present study, we examined the abovementioned hypothesis by evaluating the stress responses of these PCOS model rats to bacterial and viral mimetics; i.e., lipopolysaccharide (LPS) and polyinosinic-polycytidylic acid (Poly-IC), respectively. These two mimetics were selected for this study because LPS and Poly-IC have been used to evaluate stress responses in many previous studies, and their immunological mechanisms have been well established.

2. Materials and methods

2.1. Animals and treatment

Postnatal day 23 (PND23) female Wistar rats were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan), and housed in the animal laboratory of Tokushima University under controlled light (a 12-h light/dark cycle) and temperature (24 °C) conditions. In total, 108 rats were used in this study; 16 rats were used to confirm the phenotypes of the PCOS model, 35 rats were used for an experiment examining the effects of LPS or Poly-IC on body weight and food intake, 32 rats were used for an experiment investigating the effects of LPS or Poly-IC on circulating and hypothalamic cytokine levels, and another 25 rats ware used for an experiment evaluating the effects of LPS or Poly-IC on locomotor activity and body temperature. The surgical procedures and tissue sampling were carried out under sevoflurane-induced anesthesia. This study was approved by the institutional animal care and use committee of the University of Tokushima (No T2019-76), and all animal experiments were conducted in accordance with the relevant ethical standards.

The rats were divided into DHT-treated (PCOS, n = 54) and untreated (control, n = 54) groups. In the PCOS group, a silastic tube (As one Co., Ltd., Tokyo, Japan; inner diameter, 5 mm; outer diameter, 5 mm; length of the filled part, 10 mm) filled with diluted DHT (DHT [16 mg/mL] dissolved in a solution of 80% peanut oil and 20% ethanol) was implanted into each rat. In the control group, an empty tube was implanted into each rat. All surgery was done on PND26, and the rats were housed individually after the surgery.

Body weight and food intake were measured in representative rats (n = 8 in each group) every week after surgery. In addition, estrous cyclicity was checked during the 10-day period from PND48 to PND57 in these rats. A glass pipette filled with sterilized water was inserted into the vaginal orifice to a depth of 5 mm, and the vagina was flushed two or three times. Then, a small sample of the collected fluid was dropped onto a slide and dried in air. All of these slides were stained with Giemsa stain. Then, cytological examinations were performed, and the stages of the estrous cycle (proestrus, estrus, metestrus, and diestrus) were analyzed based on cell type and the relative numbers of each cell type in the vaginal smears. In the following experiments, immune stress was induced via the intraperitoneal (i.p.) injection of a septic dose (500 μg/kg) of LPS (0111:B4; Sigma, St. Louis, MO, USA) or via the i.p. injection of a septic dose (3 mg/kg) of Poly-IC (Sigma, St. Louis, MO, USA). These doses of LPS and Poly-IC were chosen based on the findings of previous studies (Iwasa et al., 2014; Cunningham et al., 2007).

2.2. Body weight and food intake after systemic LPS or Poly-IC injection

On PND58, LPS or Poly-IC was injected into the rats in the PCOS (n = 9 for LPS and n = 8 for Poly-IC) and control (n = 9 for each mimetic) groups. Body weight and food intake were measured for 72 h after the injection.

2.3. Circulating and hypothalamic cytokine levels after systemic LPS or Poly-IC injection

On PND58, LPS or Poly-IC was injected into the rats in the PCOS (n = 7 for LPS and n = 9 for Poly-IC) and control (n = 7 for LPS and n = 9 for Poly-IC) groups. Six hours after the injection, the rats were killed by decapitation, and then their blood, brains, and ovaries were collected. Whole blood was centrifuged at 3000 rpm for 20 min at 4 °C, and the serum was removed and stored at −40 °C, before being used for the subsequent analyses. The brains were stored at −80 °C, and the ovaries were fixed using formalin.

The serum levels of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) were measured using enzyme-linked immunosorbent assays (ELISA) (R&D Systems, Minneapolis, MN, USA). The serum leptin concentration was also measured using the mouse/rat leptin Quantikine® ELISA kit (R&D Systems Inc., Minneapolis, USA).

The ovarian samples were embedded in paraffin and then sliced into sections. Serial 4-μm-thick sections were stained with hematoxylin and eosin, and histological images were captured using the Olympus cellSens Standard software.

Hypothalamic explants were dissected out via an anterior coronal cut at the anterior border of the optic chiasm, a posterior cut at the posterior border of the mammillary bodies, parasagittal cuts along the hypothalamic fissures, and a dorsal cut 2.5 mm from the ventral surface. Total mRNA was isolated using a TRIzol reagent kit (Invitrogen Co., Carlsbad, CA, USA) and an RNeasy® mini kit (Qiagen GmbH, Hilden, Germany). cDNA was synthesized with oligo (deoxythymidine) primers at 50 °C using the SuperScript III first-strand synthesis system for real-time PCR (Invitrogen Co., Life Technologies Japan Ltd., Minato Ward, Tokyo, Japan). The polymerase chain reaction (PCR) analysis was performed using the StepOnePlus™ real-time PCR system (PE Applied Biosystems, Foster City, CA, USA) and Fast SYBR® green (Invitrogen Co.). The mRNA expression levels of IL-1β, TNF-α, and the leptin receptor (OBRb) were quantified. The mRNA expression levels of these molecules were normalized to that of GAPDH. Melting curve analysis was also performed for each gene at the end of the PCR. Each amplicon generated a single peak. The primer sequences and annealing temperatures are shown in Table 1. The PCR conditions were as follows: initial denaturation and enzyme activation were performed at 95 °C for 20 s, followed by 45 cycles of denaturation at 95 °C for 3 s, and annealing and extension for 30 s.

2.4. Locomotor activity and body temperature after systemic LPS or Poly-IC injection

Around PND50, pre-calibrated radio telemetry transmitters

Table 1
The primer sequences and annealing temperatures.

Primer	Sequence	Annealing temperature (°C)
IL-1β forward	GCT GTG GCA GCT ACC TAT GTC TTG	66
IL-1β reverse	AGG TCG TCA TCA TCC CAC GAG	
TNF-α forward	AGC CCT GGT ATG AGC CCA TGT	65.5
TNF-α reverse	CCG GAC TCC GTG ATG TCT AAG	
ObRb forward	GCA GCT ATG GTC TCA CTT CTT TTG	63
ObRb reverse	GTT CCC TGG GTG CTC TGA	
GAPDH forward	ATG GCA CAG TCA AGG CTG AGA	64
GAPDH reverse	CGC TCC TG GAA GAT GGT GAT	

(TA11TA-F10; Data Sciences International, New Brighton, MN, USA) were surgically implanted. After a recovery period of around one week, LPS or Poly-IC was injected into the rats in the PCOS (n = 7 for LPS and n = 6 for Poly-IC) and control (n = 6 for each mimetic) groups, and then their core body temperatures and locomotor activity levels were measured for 12 h. Locomotor activity was measured in this study because it has been established as a useful parameter for evaluating stress responses to LPS and Poly-IC (Gibney et al., 2013; Gong et al., 2019). In rats, the dark phase is the active phase, and body temperature is increased during this period (Ruby et al., 1999). Therefore, we decided that the light phase, which is the inactive phase, would be more suitable for obtaining these measurements. The radio-transmitter signals were recorded every 15 min and directly converted into body temperature and locomotor activity using the DATAQUEST software (Data Sciences). In the present study, the fever index was used to evaluate changes in body temperature over time due to drug administration. The fever index can be calculated by integrating the value obtained by subtracting the mean body temperature under normal conditions from the mean body temperature during the post-drug administration evaluation period. This method was developed in a previous study (Ledger et al., 1975).

2.5. Statistical analyses

Statistical analyses were performed via two-way factorial ANOVA when evaluating the effects of an intervention (i.e., the induction of PCOS by DHT administration), and the Student's t-test (parametric data) or Mann-Whitney *U* test (non-parametric data) was used for comparisons at each timepoint or of the fever index or mean locomotor activity. All results are expressed as mean plus standard error of the mean (SEM) values. To analyze the changes in body temperature, the post-injection fever index (°C × h) was calculated for the periods from 0 to 2 h and 8–10 h by summing the ΔBT (change from the baseline) at each timepoint.

3. Results

3.1. Body weight change, food intake, and ovarian morphology in the PCOS and control groups

The intervention had significant effects on body weight (two-way ANOVA; treatment: $F(1,70) = 17.68$, $P < 0.001$), and the mean body weight of the PCOS group was heavier than that of the control group at

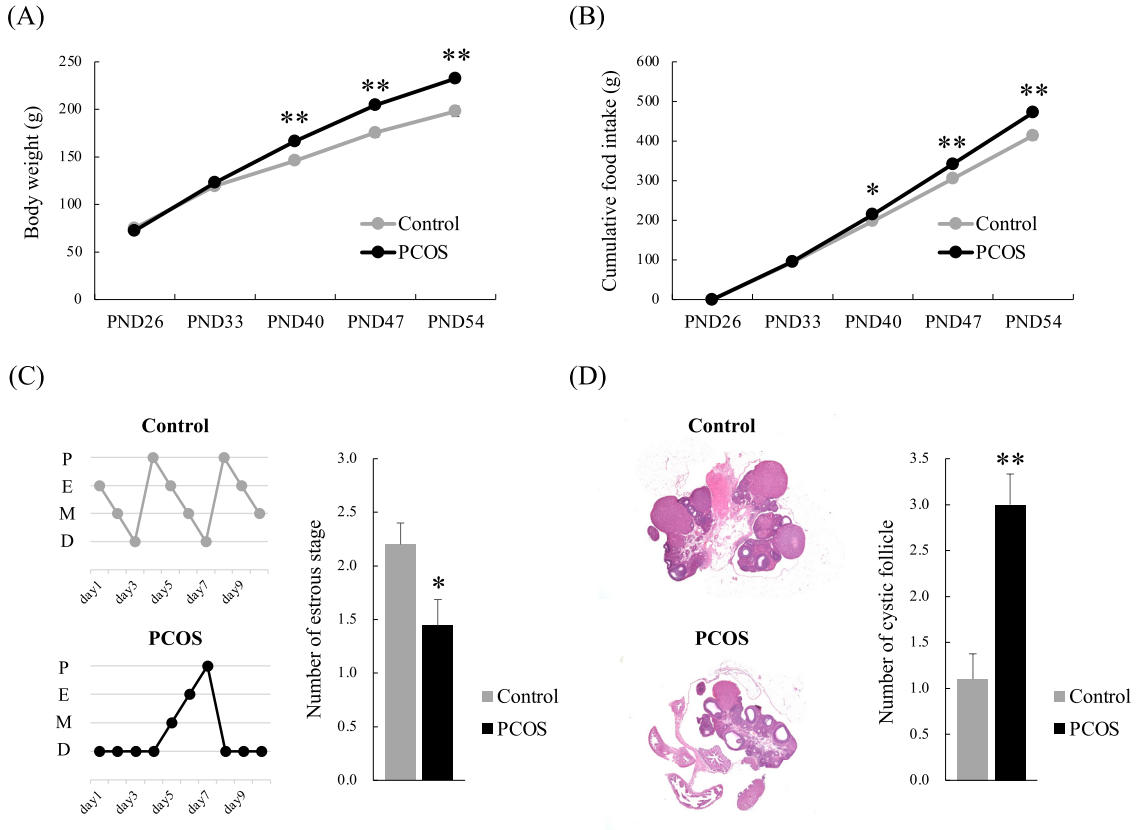

Fig. 1. Body weight change (A), cumulative food intake (B), representative pattern of estrous cyclicity and number of estrous stages (C), and representative ovarian morphology and number of cystic follicles (D) in DHT-induced PCOS model rats (PCOS) and control rats (control). Data are expressed as the mean ± SE. n = 8 in each group. *$P < 0.05$, **$P < 0.01$ vs. control group.

PND40, 47, and 54 (Student's t-test: $P < 0.01$) (Fig. 1A).

Similarly, the intervention had significant effects on food intake (two-way ANOVA; treatment: F (1,70) = 16.71, $P < 0.001$), and total food intake was greater in the PCOS group than in the control group at PND40, 47, and 54 (Student's t-test: $P < 0.05$ at PND40, $P < 0.01$ at PND47 and 54) (Fig. 1B).

Almost all rats in the control group showed regular estrous cycles, whereas a lot of the rats in the PCOS groups showed acyclic or irregular cycles. The number of estrous stages seen during the 10-day examination period was lower in the PCOS group than in the control group (Student's t-test: $P < 0.05$) (Fig. 1C).

The ovaries of the rats in the control group exhibited normal morphologies, whereas those of the rats in the PCOS group showed polycystic morphologies. The number of cystic follicles was significantly greater in the PCOS group than in the control group (Student's t-test: $P < 0.01$) (Fig. 1D).

3.2. Effects of systemic LPS or Poly-IC injection on body weight and food intake

The PCOS group exhibited significantly smaller body weight changes at 48 h (Student's t-test: $P < 0.05$) and 72 h (Student's t-test: $P < 0.05$) after the injection of LPS than the control group (Fig. 2A). Food intake during the period from 24 to 48 h after the injection of LPS was significantly lower in the PCOS group than in the control group (Student's t-test: $P < 0.05$) (Fig. 2B). The body weight changes seen after the injection of Poly-IC did not differ significantly between the PCOS and control groups (Fig. 2C). Food intake after the injection of Poly-IC did not differ significantly between the PCOS and control groups (Fig. 2D).

3.3. Effects of systemic LPS or Poly-IC injection on circulating and hypothalamic cytokine levels

In the LPS-injected rats, the serum IL-1β (Student's t-test: $P < 0.05$) and TNF-α (Student's t-test: $P < 0.05$) levels of the PCOS group were significantly higher than those of the control group (Fig. 3A). Serum leptin levels did not differ between the PCOS and control groups (Student's t-test: $P = 0.26$) (Fig. 3A). The hypothalamic mRNA expression levels of IL-1β (Mann-Whitney U test: $P = 0.75$), TNF-α (Mann-Whitney U test: $P = 0.14$), and OBRb (Mann-Whitney U test: $P = 0.40$) did not differ between the PCOS and control groups (Fig. 3A).

In the Poly–IC–injected rats, there were no significant differences between the PCOS and control groups with respect to serum IL-1β (Mann-Whitney U test: $P = 0.31$) or TNF-α (Mann-Whitney U test: $P = 0.09$) levels (Fig. 3B). Serum leptin levels did not differ significantly between the PCOS and control groups (Student's t-test: $P = 0.15$) (Fig. 3B). The hypothalamic mRNA expression levels of IL-1β (Mann-Whitney U test: $P = 0.31$), TNF-α (Mann-Whitney U test: $P = 0.09$), and OBRb (Mann-Whitney U test: $P = 0.45$) did not differ between the PCOS and control groups (Fig. 3B).

3.4. Effects of systemic LPS or Poly-IC injection on body temperature and locomotor activity

The intervention did not have significant effects on the response of body temperature to LPS (two-way ANOVA; treatment: F (1,480) = 0.091, $P = 0.91$) (Fig. 4A). However, the fever indices for the periods from 0 to 2 h and from 8 to 10 h after the injection of LPS were significantly lower (Student's t-test: $P < 0.05$) and significantly higher (Student's t-test: $P < 0.05$), respectively, in the PCOS group than in the control group (Fig. 4B). The intervention did not have significant effects on the response of body temperature to Poly-IC (two-way ANOVA; treatment: F (1,875) = 0.15, $P = 0.75$) (Fig. 4C). The fever indices for the periods from 0 to 2 h and from 3 to 5 h after the injection of Poly-IC did not differ between the PCOS and control groups (Fig. 4D).

The intervention did not have significant effects on the response of locomotor activity to LPS (two-way ANOVA; treatment: F (1,1167) = 0.38, $P = 0.84$) (Fig. 5A). The mean locomotor activity level after the injection of LPS did not differ between the PCOS and control groups (Fig. 5B). The intervention had significant effects on the response of locomotor activity to Poly-IC (two-way ANOVA; treatment: F (1,1008) = 14.96, $P < 0.01$) (Fig. 5C), and mean locomotor activity after the

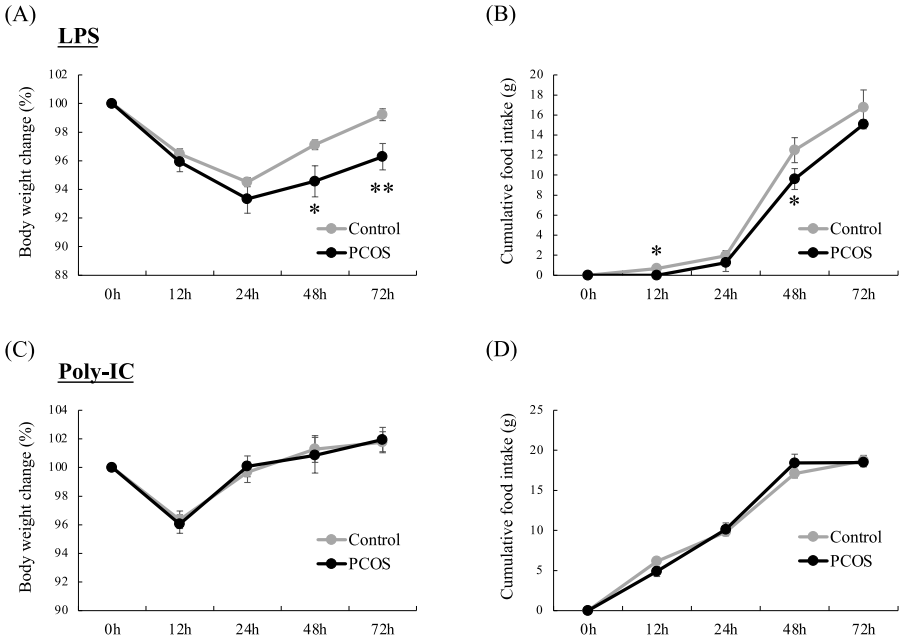

Fig. 2. Body weight change (% of body weight at injection) and cumulative food intake in the DHT-induced PCOS model rats (PCOS) and control rats (control) after the injection of LPS (A, B) or Poly-IC (C, D). Data are expressed as the mean ± SE. n = 9 for LPS and n = 8 for Poly-IC in the PCOS group, and n = 9 for each mimetic in the control group. *$P < 0.05$, **$P < 0.01$ vs. control group.

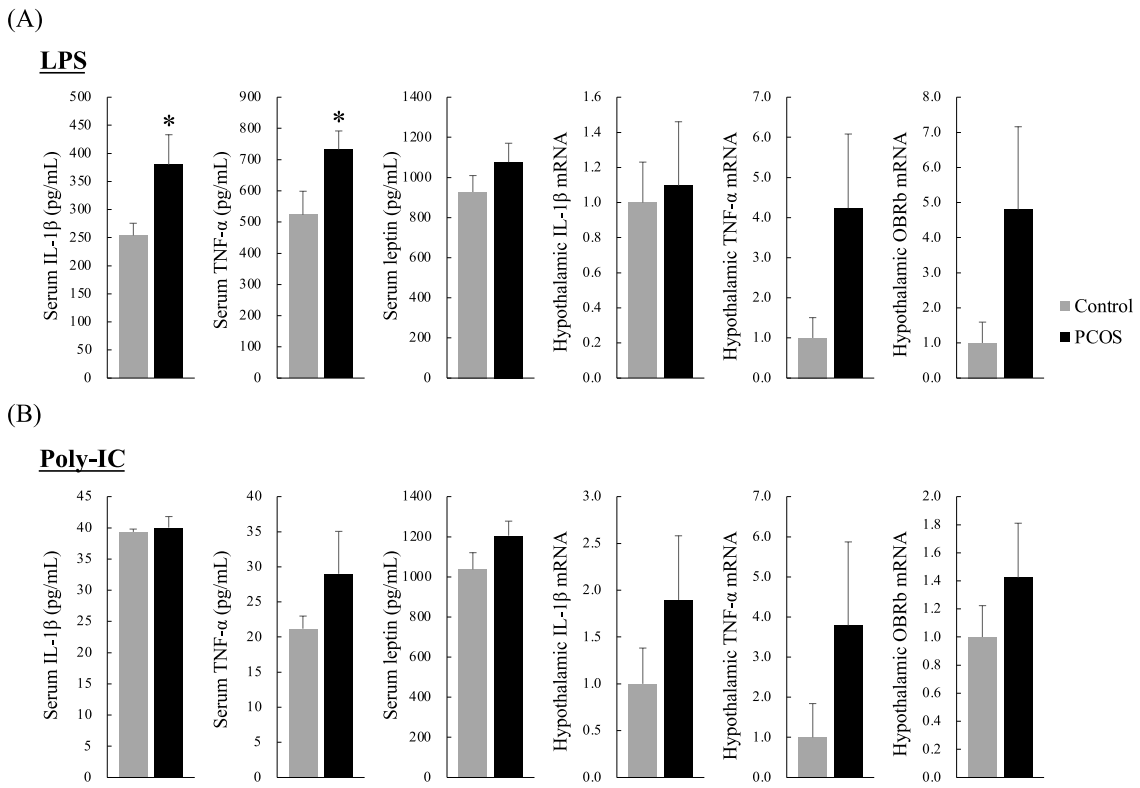

Fig. 3. Serum concentrations of IL-1β, TNF-α, and leptin and hypothalamic mRNA expression levels of IL-1β, TNF-α, and the leptin receptor (OBRb) in DHT-induced PCOS model rats (PCOS) and control rats (control) after the injection of LPS (A) or Poly-IC (B). Data are expressed as the mean ± SE. n = 7 for LPS and n = 9 for Poly-IC in the PCOS group, and n = 7 for LPS and n = 9 for Poly-IC in the control group. *$P < 0.05$ vs. control group.

injection of Poly-IC was significantly lower in the PCOS group than in the control group (Student's t-test: $P < 0.01$) (Fig. 5D).

4. Discussion

It has been revealed that women with PCOS are more likely to develop psychological stress-related disorders, such as depression, anxiety, bipolar disorder, and obsessive-compulsive disorder, and that the symptoms of these disorders are more severe in PCOS patients (Brutocao et al., 2018). In addition, in women with PCOS the hypothalamic-pituitary-adrenal axis (HPA axis) was found to be hyperresponsive to psychological stressors (Benson et al., 2009; Gallinelli et al., 2000; Mezzullo et al., 2018). These stress-related conditions have been reproduced in rodent PCOS models; i.e., the rats showed anxiety-like behaviors and HPA axis changes (Feng et al., 2011; Hu et al., 2015; Ressler et al., 2015; Manti et al., 2018). Furthermore, stress and stress-associated factors may cause and worsen PCOS symptoms (Basu et al., 2018). It has been shown that psychological stress and other kinds of stress share some common mechanisms. For example, the hypothalamic mRNA and serum levels of pro-inflammatory cytokines are increased by both psychological stress and immune stress (Sachot et al., 2004; Jankord et al., 2010; Voorhees et al.). These findings suggest that immune stress may also be increased in PCOS. Androgens may be one of the key factors that affect immune stress responses in PCOS. Women with PCOS and males are at higher risk of novel COVID-19 infections (Jun et al., 2021; Subramanian et al., 2021; de Medeiros et al., 2022). In addition, a previous study showed that prostate cancer patients receiving androgen suppression therapy had a 4-fold lower risk of contracting a novel COVID-19 infection than those not receiving such treatment (Montopoli et al., 2020). However, most of these findings were based on epidemiological and clinical data, and there has not been any basic research supporting these hypotheses. Moreover, no previous studies have evaluated whether stress responses to infection are altered in PCOS model animals.

Some animal models of PCOS have been established in laboratories, including ours, and interventional and basic studies of central and peripheral tissue samples from such models have also been performed. In this study, we used our novel PCOS model, which closely reproduces the features of PCOS (Kamada et al., 2021), to examine whether the responses to immune stress are altered in PCOS. Two kinds of immune inducers were used, LPS, which is a major component of the outer bacterial membrane and plays a key role in host-pathogen interactions in the innate immune system, and Poly-IC, which is a synthetic double-stranded RNA that is used to experimentally model viral infections in vivo. As a result, we found that the body weight changes and food intake seen after the injection of LPS were significantly lower in the PCOS group than in the non-PCOS (control) group, whereas the body weight changes and food intake observed after the injection of Poly-IC did not differ between the PCOS and control groups. Similarly, LPS-induced hypothermia and hyperthermia were more evident in the PCOS group than in the control group, whereas no differences in Poly–IC–induced febrile responses were seen between the PCOS and control groups. These findings indicate that febrile and anorectic responses to bacterial infections may be more marked in PCOS model rats, while their responses to viral infections may not be altered. It has been reported that inflammatory responses to LPS are enhanced in diet-induced obese rats and that greater increases in peripheral and central cytokine levels may mediate these alterations (Pohl et al., 2009). Similarly, prolonged fevers and a higher number of sickness symptoms were induced by LPS injection in diet-induced obese rats (Pohl et al., 2014). Although the

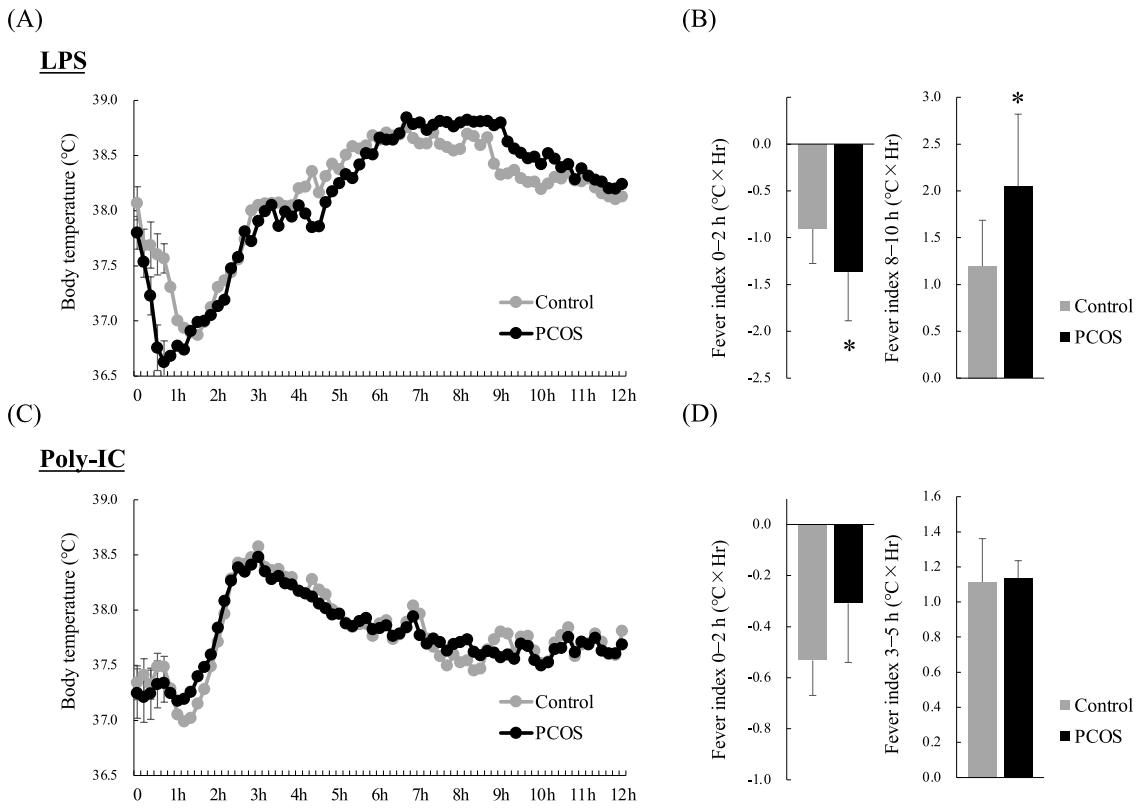

Fig. 4. Body temperature and fever index in DHT-induced PCOS model rats (PCOS) and control rats (control) after the injection of LPS (A, B) or Poly-IC (C, D). Data are expressed as the mean ± SE. n = 7 for LPS and n = 6 for Poly-IC in the PCOS group, and n = 6 for each mimetic in the control group. *$P < 0.05$ vs. control group.

underlying mechanisms responsible for changes in the stress responses to LPS and Poly-IC have not been clarified, it is assumed that obesity and adiposity specifically upregulated the stress responses to LPS in the PCOS group.

Our findings also indicate that the changes in peripheral cytokine levels seen in response to the systemic injection of LPS were more marked in the PCOS group than in the control group. IL-1β and TNF-α act as mediators and modulators of host defense responses to immune stress, such as tissue infection, tissue injury, and inflammation (Rothwell and Hopkins, 1995). These cytokines are synthesized locally, but also participate in brain-mediated responses, e.g., febrile and anorectic responses (Hopkins and Rothwell, 1995). In previous studies, the administration of LPS induced modest and transient changes in IL-1β and TNF-α levels (Michie et al., 1998; Cannon et al., 1990). Thus, the hyperresponsiveness of peripheral inflammatory cytokine levels seen under the LPS-injected conditions in the present study may have been the underlying mechanism through which febrile and anorectic responses to immune stress were enhanced in the PCOS group. On the contrary, under the LPS-injected conditions the central mRNA levels of IL-1β and TNF-α did not differ between the PCOS and control groups, suggesting that PCOS may mainly affect peripheral, rather than central, immune responses. Conversely, under the Poly–IC–injected conditions there were no differences in peripheral or central IL-1β or TNF-α levels between the PCOS and control groups, supporting our findings that there were no differences in febrile or anorectic responses between the two groups. In the present study, the serum levels of leptin and its receptor were also measured, as these factors may be involved in febrile and anorectic responses under immune stress conditions (Sachot et al., 2004). However, no differences in the levels of these factors were observed between the PCOS and control groups under the LPS- or Poly–IC–injected conditions.

In this study, under the Poly–IC–injected conditions locomotor activity was lower in the PCOS group than in the control group, whereas no such differences were observed under the LPS-injected conditions. As noted above, under the Poly–IC–injected conditions no differences in peripheral or central IL-1β, TNF-α, or leptin levels were seen between the PCOS and control groups, indicating that other factors may have been involved in the suppression of locomotor activity in the PCOS group.

This study had several limitations. First, the precise mechanism through which immune stress was increased in the PCOS model rats could not be clarified. Namely, it remains unclear whether the observed changes in immune responses were induced by hyperandrogenism itself or other indirect mechanisms. For example, it has been reported that diet-induced and ovariectomy-induced obesity may enhance immune responses in rodents and that upregulated cytokine responses are related to these alterations (Pohl et al., 2009; Iwasa et al., 2014). Thus, it is possible that the increases in body weight seen in the PCOS rats induced the alterations in stress responses in this study. Second, it is possible that the LPS-induced and Poly–IC–induced effects on the immune system seen in this study were not equal and that differences in stress strength may have contributed to the observed discrepancies in behavioral and febrile responses. Further precise examinations would be needed to clarify these points.

In summary, PCOS model rats showed enhanced anorectic and febrile responses to the injection of LPS, which was used to mimic bacterial infections. Furthermore, their peripheral, but not hypothalamic, cytokine levels increased markedly in response to the injection of LPS. In addition, the PCOS model rats showed markedly depressed locomotor activity after the injection of Poly-IC, which was used to mimic viral infections. However, the responses of peripheral and hypothalamic cytokine levels to Poly-IC were not altered in the PCOS model rats.

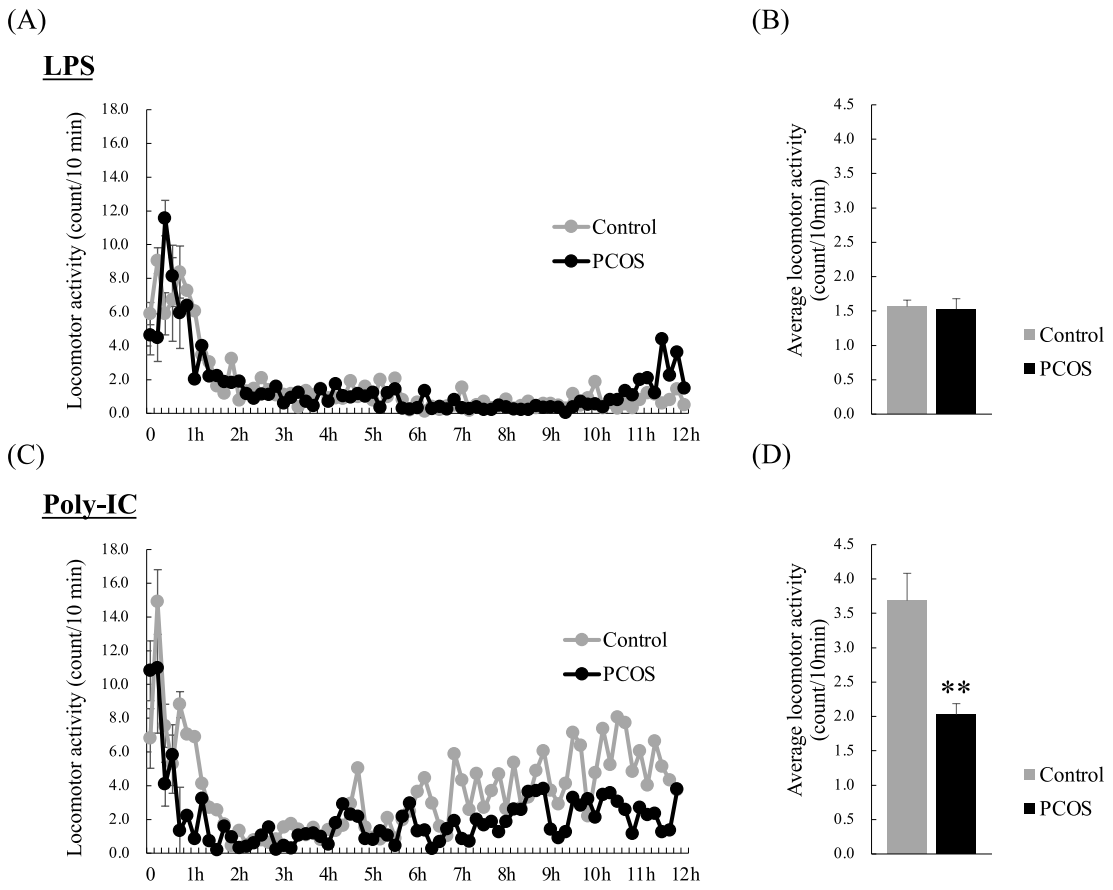

Fig. 5. Locomotor activity in DHT-induced PCOS model rats (PCOS) and control rats (control) after the injection of LPS (A, B) or Poly-IC (C, D). Data are expressed as the mean ± SE. n = 7 for LPS and n = 6 for Poly-IC in the PCOS group, and n = 6 for each mimetic in the control group. $^{**}P < 0.01$ vs. control group.

5. Conclusion

In conclusion, although stress responses to infection may be enhanced in PCOS, the patterns of such stress response enhancement and their underlying mechanisms may differ between bacterial and viral infections. Increases in pro-inflammatory cytokine expression may be related to the enhanced anorectic and febrile responses to bacterial infection seen in PCOS model rats, whereas the mechanisms responsible for the reductions in locomotor activity observed in such rats in response to viral infection have not been elucidated. These findings indicate that in addition to psychological stress, immune stress may also be increased in women with PCOS, and these alterations may exacerbate the symptoms of infections in PCOS.

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CRediT authorship contribution statement

Shuhei Kamada: Writing – review & editing, Writing – original draft, Methodology, Investigation. **Hiroki Noguchi:** Investigation, Formal analysis, Data curation. **Shota Yamamoto:** Supervision, Methodology, Investigation. **Kou Tamura:** Investigation. **Hidenori Aoki:** Investigation. **Asuka Takeda:** Investigation. **Maimi Uchishiba:** Project administration, Methodology. **Saki Minato:** Data curation. **Moeka Arata:** Methodology, Conceptualization. **Ryosuke Arakaki:** Visualization, Validation. **Hiroaki Inui:** Writing – review & editing, Methodology, Conceptualization. **Tomohiro Kagawa:** Investigation. **Takako Kawakita:** Validation, Project administration. **Atsuko Yoshida:** Methodology, Investigation. **Ayuka Mineda:** Writing – review & editing, Supervision. **Yuri Yamamoto:** Supervision. **Riyo Kinouchi:** Project administration, Conceptualization. **Kanako Yoshida:** Supervision. **Takashi Kaji:** Methodology, Data curation. **Masato Nishimura:** Project administration. **Takeshi Iwasa:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Data curation.

Declaration of competing interest

There are no conflict of interest among all authors.

Data availability

No data was used for the research described in the article.

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