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# Daily Changes of Body Temperature and Heart Rate are Modulated after Estradiol Depletion in Female Rats

**Shuri Marui[1], Yuki Uchida[1] and Kei Nagashima[1,2]***

[1]*Body Temperature and Fluid Laboratory, Faculty of Human Sciences, Waseda University, Tokorozawa, Japan*
[2]*Institute of Applied Brain Sciences, Waseda University, Tokorozawa, Japan*

## Abstract

We assessed the influence of estradiol depletion on daily changes of core temperature, spontaneous activity, and heart rate in female rats. In addition, we evaluated the effects of estradiol depletion on β-adrenoreceptors (AR) in the heart and plasma norepinephrine. Rats were bilaterally ovariectomized and two tubes that either contained estradiol (E2) or were empty (C) were placed subcutaneously in each rat's abdominal cavity. The tubes were removed 10 days later. Core temperature and heart rates were continuously measured by biotelemetry before and 1, 7, and 21 days after removal of the tubes (PRE and Days 1, 7, and 21). Core temperature was higher in the E2 group than in the C group at 2330–0130 h on PRE and on Days 1–21. Core temperature exceeded the PRE level in the E2 group at 1430–1830 h on Days 1–21. Heart rates were lower in the E2 group than the C group throughout the day on PRE and on Days 1–21. β1-AR expression and plasma norepinephrine levels were lower in the E2 group than in the C group on PRE. Heart rates in the E2 group exceeded the PRE level on Days 1–21. We concluded that the depletion of plasma estradiol modulates daily changes of core temperature and heart rate, an effect that occurs immediately after the estradiol depletion. In addition, plasma norepinephrine and β-AR in the heart may at least partially affect heart rate.

**Keywords:** Female hormone; Hormone replacement therapy; β-adrenoreceptor; Norepinephrine; Menopause

## Introduction

Women entering menopause often experience a decrease in plasma female hormones (i.e., estradiol and progesterone) and changes in the rhythms of hormonal secretions [1]. In addition, some women experience physical and/or mental disorders (e.g., hot flashes, night sweats, and mood swings). Clinical data from patients who received surgical oophorectomy or hormone replacement therapy indicate that the disorders were caused by depletion of female sex hormones [2–5]. However, such disorders are not found during the pre-pubertal period and gradually disappear after menopause. Thus, it is speculated that the effect of estradiol depletion is transient.

Hot flashes [6–8] are a common physical disorder in menopausal women and are characterized by the sudden onset of hotness and palpitation. During hot flashes, skin blood flow increases, sweating occurs, and core temperature decreases [9]. Their incidence may be related to daily rhythm, as several studies have reported that hot flashes occur at a specific time of a day [10–12]. Thus, the depletion of female hormones appears to affect thermoregulation, cardiovascular function, and circadian rhythmicity. This speculation is supported to some extent by research that indicates circulating estradiol is involved in thermoregulation in female rats. Uchida et al. assessed the effect of estradiol on thermoregulation by comparing ovariectomized rats that did and did not receive estradiol replacement. They suggested that estradiol lessens the reduction in core temperature during cold exposure by affecting autonomic and behavioral thermoregulatory processes [13,14]. The influence of estradiol on heart rates has also been reported in female rats: they demonstrate tachycardia after the depletion of plasma estradiol [15–18]. Ovariectomy augments the expression of the β1-adrenoreceptors of the cardiomyocytes in rats [19–21], which may affect heart rates by increasing pacemaker rhythm of the heart (i.e., chronotropic action) [22].

Depletion of plasma estradiol and progesterone starts within an hour after surgical removal of the ovaries in rats [23]. To assess the influence of estradiol depletion, previous studies have compared ovariectomized animals to sham-operated/estradiol-dosed animals [13,14,24] or investigated animals with genetic depletion of estradiol or estradiol receptors [25]. However, these studies could not clarify the time-effect after the estradiol depletion. In addition, we do not know if the influence includes the circadian components of the core temperature and heart rate. Thus, it remains unclear i) how the depletion of estradiol is manifested through thermoregulation and cardiovascular function, and ii) whether the influence is observed throughout the day or at a specific time of day. The aims of the present study were to clarify whether the depletion of plasma estradiol affected daily changes of core temperature and heart rates and how the effects changed after depletion. We conducted 24-h measurement of core temperature and heart rates after surgical removal of the ovaries or stopping estradiol replacement in ovariectomized rats, for 21 days. We investigated the effect of the changes that altered plasma estradiol from high to low level, to replicate peri-menopause. In addition, we evaluated changes in plasma norepinephrine level and the expression of the β-adrenoreceptors of the cardiomyocytes.

## Materials and Methods

Adult virgin female Wistar rats (n = 65; body weight, 150–250 g;

***Corresponding author:** Kei Nagashima, Body Temperature and Fluid Laboratory (Laboratory of Integrative Physiology), Faculty of Human Sciences, Waseda University, Mikajima 2-579-15, Tokorozawa, Saitama 359-1192, Japan, Tel: +81-4-2947-6918; E-mail: k-nagashima@waseda.jp

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age, 7–9 w; Takasugi Experimental Animals Supply, Saitama, Japan) were used in the present study. They were individually housed in plastic cages (45 × 25 × 20 cm) at an ambient temperature of 25°C under a 12/12-h light/dark cycle (lights on at 0700 h). Food and water were available ad libitum. By obtaining vaginal smears every day for 10 days, we verified that the rats all had regular estrous cycles. All experimental procedures were approved by the Institutional Animal Care and Use Committee, Waseda University.

### Experiment 1 the effect of estradiol on core temperature, spontaneous activity, and heart rates in female rats

Rats (n = 14) underwent surgery under inhalation anesthesia with 2% isoflurane (Abbott Japan, Tokyo, Japan) and air. A radio transmitter device for the measurements of core temperature, spontaneous activity, and electrocardiogram (26 × 8 mm, 2.2 g; PDT-4000 HR E-Mitter, Mini Mitter Company, Bend, USA) was implanted in the abdominal cavity of each rat. The electrocardiogram was obtained from two electrode wires of the device that were placed under the chest skin. A bilateral ovariectomy was performed from the retroperitoneum. Penicillin G (1,000 U; Meiji Pharmaceutical, Tokyo, Japan) was subcutaneously injected to prevent post-surgical infection. Then, two silicon tubes (inner diameter 1.57 mm, outer diameter 3.18 mm, length 30 mm; Kaneka, Osaka, Japan) containing 17β-estradiol (E2; Sigma-Aldrich, St. Louis, USA) were subcutaneously placed in the rats of the treatment group (n = 7, E2 group), and two empty tubes were subcutaneously placed in the rats of the control group (n = 7, C group). The procedure for the preparation of the estradiol-containing tubes and the effect of these tubes on plasma estradiol level after placement were previously reported [14]. Briefly, each tube was filled with 50–60 mg of 17β-estradiol powder, and both ends were sealed with glue. The placement of the E2 tubes provided a constant plasma level of estradiol at least 9 days. In both groups, the tubes were removed 10 days after the surgery (Day 0) under anesthesia as previously described (Figure 1). Each rat's core temperature, spontaneous activity, and electrocardiogram information were recorded for 24 h before Day 0 (PRE) and on Days 1, 7, and 21. Heart rates were assessed form the R-R interval of the electrocardiogram.

### Experiment 2 the effect of estradiol on expression of β1- and β2-adrenoreceptors of the left cardiac ventricle

Rats (n = 30) were divided into two groups (n = 15 each in the E2 and C groups), as in Experiment 1 and underwent the same surgery and removal of the tubes on the same schedule (Figure 1). In this experiment, five rats in each group were killed by i.p. injection of overdose pentobarbital Na+ (Kyoritsu Seiyaku, Tokyo, Japan) at 1300 h on PRE and Days 7 and 21, respectively. The cardiac muscle of the left ventricle and the blood of each animal were sampled.

### Analyses of the cardiac muscle and blood

The sampled cardiac muscle (200 mg) was homogenized in RIPA buffer. After the supernatant was decanted, the proteins were collected in a sample buffer (4× Laemmli Sample Buffer, Bio-Rad, Hercules, USA) and denatured for 5 min at 95°C. The protein concentration was determined by the BCA method (Pierce BCA Protein Assay Kit, Thermo Scientific, Waltham, USA). A 60-μg sample of protein was separated by 8% polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulfate and transferred to a polyvinylidene difluoride membrane. After being incubated in blocking solution (0.3% skim milk), the membrane was allowed to react with a rabbit polyclonal primary antibody for β1- and β2-adrenoreceptors (ab3442 and 36956, respectively; 1:1200; Abcam, Cambridge, England) for 1 h at room temperature. The membrane was washed three times with Tween 20 in Tris-buffered saline and then allowed to react with the secondary antibody (horseradish peroxidase-linked donkey anti-rabbit IgG; 1:5000, NA934; GE Healthcare UK, Amersham, England). The signal was developed by applying the substrate (HRP substrate; Immobilon Western; Millipore, Billerica, USA) and detected by chemiluminescence (LAS3000, FUJIFILM, Tokyo, Japan). The membrane was then blotted with mouse primary antibody of β-actin (1:1000, ab8226; Abcam, Cambridge, England) and the secondary antibody (horseradish peroxidase-linked rabbit anti-mouse IgG; 1:5000, ab6728; Abcam, Cambridge, England). The washing procedure and the signal detection were conducted in the same manner for the β1- and β2-adrenoreceptors. The intensity of the protein signals was determined with Multi Gauge V3.0 software (FUJIFILM, Tokyo, Japan). The expression levels of the β1- and β2-adrenoreceptors were shown as the relative values to that of β-actin.

The blood was centrifuged at 4°C. The estradiol and norepinephrine levels in the plasma were determined by enzyme-linked immunosorbent assay (Estradiol EIA Kit; Cayman Chemical, Ann Arbor, USA; and Noradrenaline Research ELISA; LDN, Nordhorn, Germany, respectively). The detection limits of estradiol and norepinephrine were 20 pg/ml and 0.1 ng/ml, respectively. The coefficient of variation of estradiol and norepinephrine were <13% and <12%, respectively.

### Statistics

All values are shown as the means ± standard error (SE). Values for core temperature and spontaneous activity were averaged every 30 min. Heart rate was averaged over 24-h period. Differences between C and E2 groups in each day were determined by two-way analysis of variance (ANOVA; SPSS, Chicago, USA). Differences in daily changes among four treatment days were evaluated by two-way ANOVA with repeated measures with SPSS. Post -hoc tests were conducted by the Tukey method. Differences in heart rate among four treatment days were evaluated by one-way ANOVA with repeated measures with SPSS. Difference in heart rate between C and E2 groups was assessed by Student's t-test. The null hypothesis was rejected at $P < 0.05$.

## Results

Figure 2 shows the estradiol level in the plasma on PRE and Days 7 and 21 in the C and E2 groups. On PRE, the level was higher in the E2 group than in the C group (205.1 ± 20.6 and 36.7 ± 16.3 pg/ml, respectively). In the C group, plasma estradiol remained unchanged on PRE and on Days 7 and 21. On Days 7 and 21, the plasma estradiol

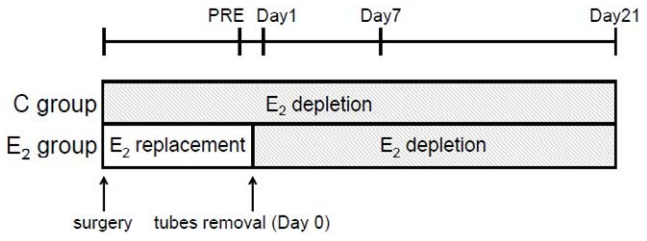


**Figure 1:** Experimental protocol. C group, ovariectomy without estradiol replacement; E2 group, ovariectomy with estradiol replacement, and the replacement was stopped one day before Day 1 (Day 0). PRE, one day before Day0; Days 1, 7, and 21, 1, 7, and 21 days after the tubes' removal. E2 depletion, the plasma estradiol level is similar to that in the diestrus phase in normal rats (shaded area).

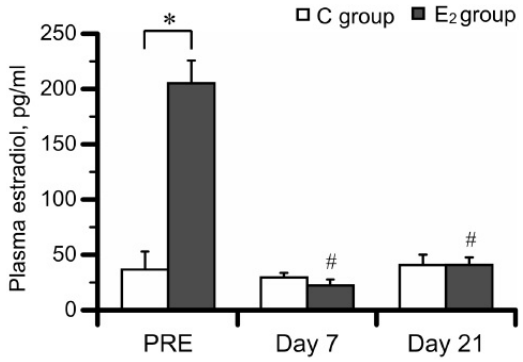


**Figure 2:** Plasma estradiol levels on PRE and Days 7 and 21 in the C and E2 groups. The values are the means ± SE (n = 5 on each day in the C and E2 groups, respectively). * Significant difference between the C and E2 groups, $p < 0.05$. # Significant difference from the value on PRE, $p < 0.05$. E2, 17β-estradiol.

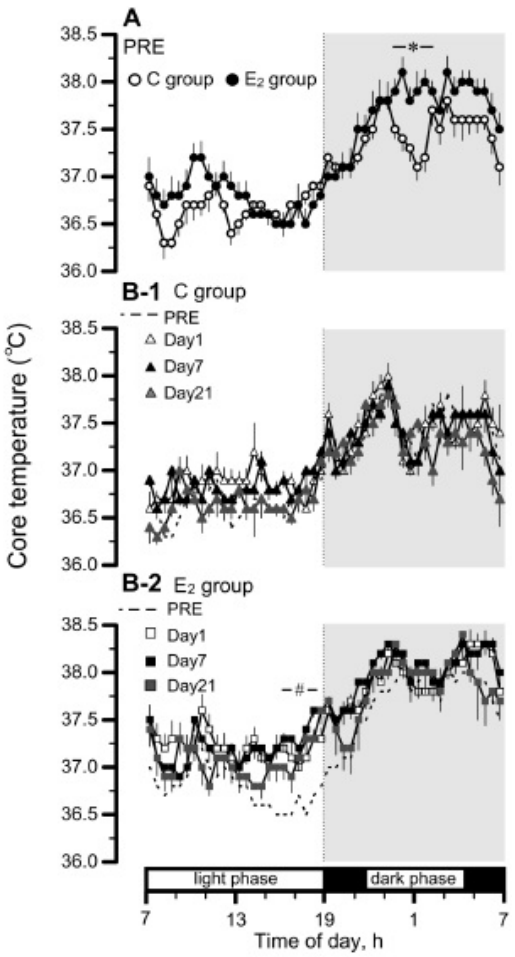


**Figure 3:** Daily change of core temperature on PRE and Days 1, 7, and 21 in the C and E2 groups. A) 24-h core temperature on PRE in the C and E2 groups. B-1 and B-2) the core temperature on Days 1, 7, and 21 in the C and E2 groups, respectively. Each value of PRE is superimposed as a dashed line. Each datum denotes a 30-min average. The values are the means ± SE (A, n = 7 in each group; B-1, n = 5; and B-2, n = 5) * Significant difference between the C and E2 groups, $p < 0.05$. # Significant difference from the value on PRE, $p < 0.05$.

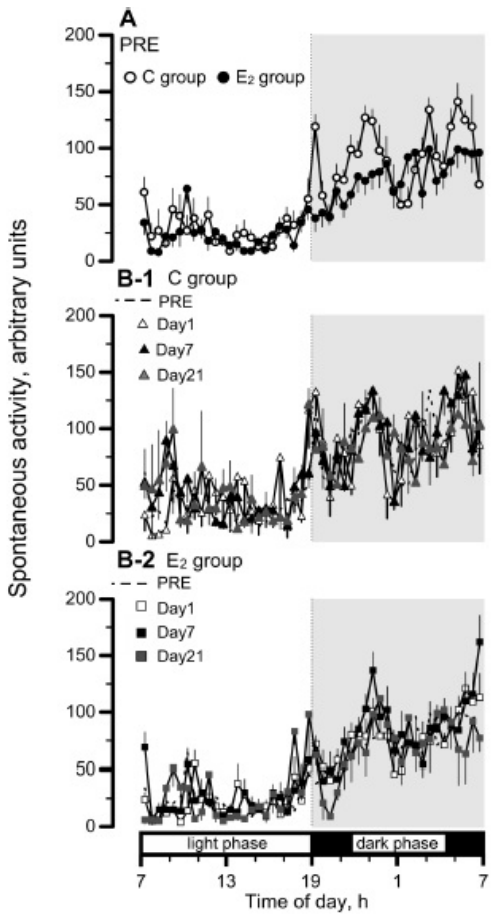


**Figure 4:** Daily change of spontaneous activity on PRE and Days 1, 7, and 21 in the C and E2 groups. A) 24-h spontaneous activity on PRE in the C and E2 groups. B-1 and B-2) the spontaneous activity on Days 1, 7, and 21 in the C and E2 groups, respectively. Each value of PRE is superimposed as a dashed line. Each datum denotes a 30-min average. The values are the means ± SE (A, n = 7 in each group; B-1, n = 5; and B-2, n = 5).

level decreased from the value on PRE in the E2 group. There were no differences in the plasma level between the two groups on Days 7 and 21.

## Experiment 1

Figure 3 shows the 24-h change in core temperature on PRE in the C and E2 groups (A), and on Days 1, 7, and 21 in the C and E2 groups (B-1 and B-2, respectively). The PRE value in each group was included in Figure 3B-1 and 2 to clarify the difference (dashed lines). On PRE, the core temperature was greater in the E2 group than in the C group at 2330–0130 h (Figure 3A). The difference became the greatest at 0100 h (0.8 ± 0.1°C).

There were no differences in core temperature among the values on PRE and on Days 1, 7, and 21 in the C group (Figure 3B-1). In the E2 group, core temperature was higher than the PRE value at 1600–1800 h on Days 1, 7, and 21; however, there was no difference among the values during the three days (Figure 3B-2). Core temperature throughout Day 7 in the E2 group was greater than that on PRE in the C group. In addition, core temperature on Day 21 in the E2 group was greater than that on Day 7 in the C group at 1000–1230, 2330–0130, and 0430–0630 h.

Figure 4 illustrates the 24-h change in spontaneous activity on PRE in the C and E2 groups (A), and on Days 1, 7, and 21 in the C and E2 groups (B-1 and B-2, respectively). The PRE value in each group was included in Figure 4B-1 and 2 to clarify the difference (dashed lines). There was no difference among the values for four days in each group (Figure 4B-1 and 2).

Figure 5 shows the average over 24-h period in heart rates on PRE in the C and E2 groups (A), and that on PRE, Days 1, 7, and 21 in the C and E2 groups (B-1 and B-2, respectively). The PRE value in each group was included in Figure 5B-1 and 2 to clarify the difference (dashed lines). On PRE, heart rates in the E2 group were smaller than those in the C group (419 ± 13 and 363 ± 13 beats/min on average, respectively; Figure 5A).

In the C group, heart rates on Days 1 and 7 remained unchanged from the values on PRE; however, those on Day 21 were lower than those of PRE and Day 1 (369 ± 16, 419 ± 13, and 409 ± 16 beats/min on average, respectively; Figure 5B-1). In the E2 group, heart rates on Day 7 exceeded those of PRE (406 ± 13 and 363 ± 13 beats/min on average, respectively; Figure 5B-2). Heart rates on Day 7 in the E2 group did not differ from those on PRE in the C group. In addition, heart rates on Day 21 in the E2 group also did not differ from those on Day 7 in the C group.

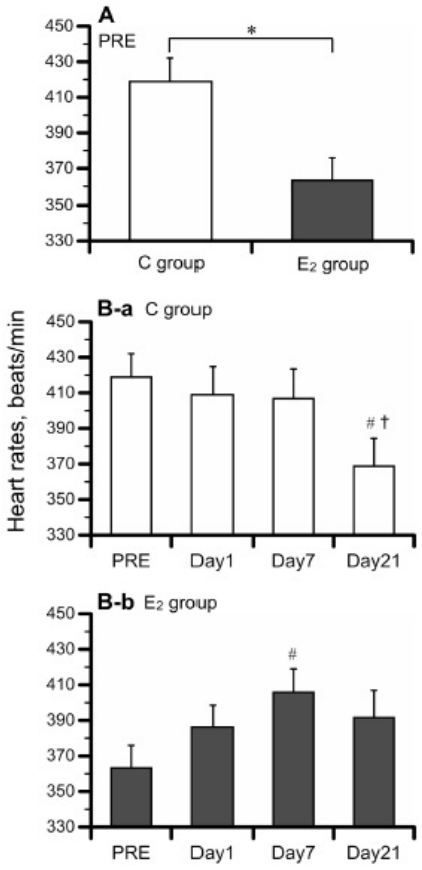


**Figure 5:** Average heart rate on PRE and Days 1, 7, and 21 in the C and E2 groups. A) average heart rate during 24-h on PRE in the C and E2 groups. B-1 and B-2) average heart rate during 24-h on PRE, Days 1, 7, and 21 in the C and E2 groups, respectively. The values are the means ± SE (A, n = 7 in each group; B-1, n = 5; and B-2, n = 5). * Significant difference between the C and E2 groups, $p < 0.05$. # Significant difference from the value on PRE, $p < 0.05$. † Significant difference between Day 1and Day 21, $p < 0.05$.

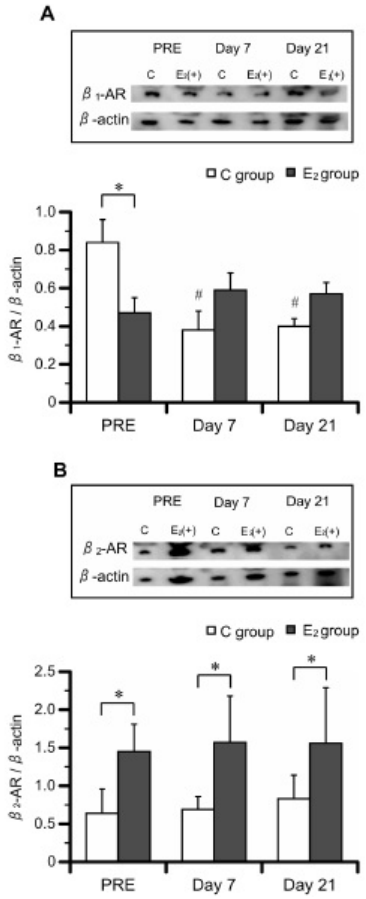


**Figure 6:** Western blotting analysis of the β1- and β2-adrenoreceptors (AR) of the left ventricle of the heart. The bands denote the majority of the results obtained from the blotting. The graphs show the relative values of the signal strength of the β1- and β2-ARs to β-actin expression. The values are the means ± SE (n = 5 in the C and E2 groups, respectively). * Significant difference between the C and E2 groups, $p < 0.05$. # Significant difference from the value on PRE, $p < 0.05$.

## Experiment 2

Figure 6 shows the protein expression of the β1- (A) and β2-adrenoreceptors (B) of the left ventricle. The photo images on the top of each graph denote western blotting signals from PRE and Days 7 and 21 in the C and E2 groups. The expression of the β1-AR was lower in the E2 group than in the C group on PRE (Figure 6A). In the C group, the expression levels on Days 7 and 21 were lower than on PRE. However, in the E2 group, no differences were observed on Days 7 and 21 from the value on PRE. In addition, there were no significant differences between the two groups on Days 7 and 21.

The expression of the β2-AR was greater in the E2 group than in the C group on PRE and on Days 7 and 21 (Figure 6B). The values on Days 7 and 21 did not differ from the value on PRE.

Figure 7 shows the norepinephrine level in the plasma. The plasma level was lower in the E2 group than in the C group on PRE. In the C group, the level on Day 7 fell below that of PRE, while the level on Day 21 exceeded the PRE level. In the E2 group, the levels on Days 7 and 21 were higher than on PRE. The value on Day 21 exceeded that of Day 7 in each group. On Days 7 and 21, there were no significant differences between the C and E2 groups.

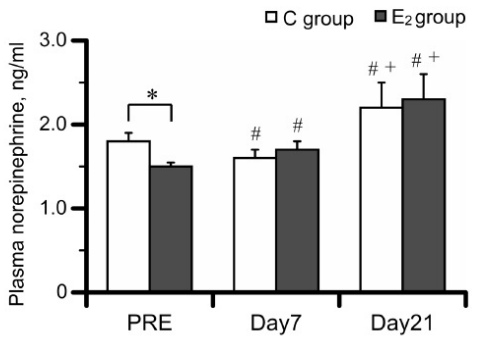


**Figure 7:** Plasma norepinephrine levels on PRE and Days 7 and 21 in the C and E2 groups. The values are the means ± SE (n = 5 on PRE, Day 7, and Day 21 in the C and E2 groups, respectively). * Significant difference between the C and E2 groups, $p < 0.05$. # Significant difference from the value on PRE, $p < 0.05$. + Significant difference between Day7 and Day 21, $p < 0.05$.

Table 1 summarizes the data for core temperature, heart rates, spontaneous activity, expression levels of β-AR, and plasma levels of norepinephrine and estradiol during the measurement period in Experiments 1 and 2.

## Discussion

In the present study, we studied whether estradiol depletion modulates daily changes of core temperature and heart rate. We evaluated the effect of the estradiol through two different comparisons: i) comparing the core temperature and heart rate rhythms in ovariectomized rats that did and did not receive estradiol replacement; and ii) comparing the core temperature and heart rate before and after estradiol withdrawal from ovariectomized rats that had been receiving estradiol replacement. The estradiol depletion affected both core temperature and heart rate in a different ways.

### Plasma estradiol level

On PRE, plasma estradiol levels in the E2 group rose to 6 times those in the C group. The level in the C group remained similar to that in the diestrus phase in normal rats (i.e., the lowest level in the estrus cycle) [14]. There were no differences among the values on PRE and Days 7 and 21 (Figure 2, Table 1) in the C group. The level in the E2 group was 2 times higher than that in the proestrus phase in normal rats (i.e., the highest level in the estrus cycle) [14]. A previous study which used the same method of estradiol replacement showed that the physiological data were similar with proestrus level [14]. The estradiol level in the E2 group on Days 7 and 21 approached that of the C group.

### Comparison of daily core temperature rhythm between the C and E2 groups

A difference in daily change of core temperature between the C and E2 groups was observed on PRE (Figure 3A, Table 1), although it was limited to the 2-h period in the middle of the dark phase. Previous studies reported that, during the dark phase, core temperature did not differ between ovariectomized rats that did and did not receive estradiol replacement [26,27]. However, these studies did not assess the daily change. Sanchez-Alavez et al. (2011) reported a similar decrease in core temperature during the dark phase, using ovariectomized mice [24].

Our data showed that spontaneous activity seemed to decrease, in association with the reduction of core temperature on PRE in the C group (Figure 4A). It has been reported that spontaneous activity decreases and paradoxical sleep during the dark phase increases after ovariectomy in rats [28–30]. As reported previously, estradiol shortened a period of the free-running activity rhythm of blind in rats [31] by changing the expression of clock-related genes [32]. Thus, the change in circadian core temperature rhythm may have partially reflected this spontaneous activity and disordered sleep rhythm.

Hosono et al. reported greater tail vasodilation in ovariectomized rats during heat exposure [33]. In women suffering hot flashes, core temperature becomes lower during a peak frequency of hot flashes than that in asymptomatic women [12]. A sudden reduction in core temperature in the C group was observed at the time when the daily core temperature rhythm peaked in the E2 group. Nagashima et al. showed that, in male rats, the tail surface temperature exceeds that of

| | | PRE (9 days after OVX) | Day 1 (11 days after OVX) | Day 7 (17 days after OVX) | Day 21 (31 days after OVX) |
|---|---|---|---|---|---|
| C group | core temperature | | no change from PRE | no change from PRE | no change from PRE |
| | heart rates | | no change from PRE | no change from PRE | lower than PRE and Day 1 |
| | spontaneous activity | | no change from PRE | no change from PRE | no change from PRE |
| | β1-AR expression | | | smaller than PRE | smaller than PRE |
| | β2-AR expression | | | no change from PRE | no change from PRE |
| | noradrenaline | | | lower than PRE | higher than PRE |
| | estradiol | | | no change from PRE | no change from PRE |
| E2 group | core temperature | greater than the C group at 2330-0130 h | greater than PRE at 1600-1800 h | greater than PRE at 16-1800 h<br>greater than PRE of the C group | greater than PRE at 1600-1800 h<br>greater than PRE of the C group at 1000-1230, 2330-0130, and 0430-0630 h |
| | heart rates | smaller than the C group | no change from PRE | greater than PRE<br>no change from PRE of the C group | no change from PRE<br>no change from Day 7 of the C group |
| | spontaneous activity | | no change from PRE | no change from PRE | no change from PRE |
| | β1-AR expression | smaller than the C group | | no change from PRE | no change from PRE |
| | β2-AR expression | greater than the C group | | greater than the C group | greater than the C group |
| | noradrenaline | lower than the C group | | higher than PRE | higher than PRE |
| | estradiol | higher than the C group | | lower than PRE | lower than PRE |

**Table 1:** Summary of the data of core temperature, heart rates, spontaneous activity, expressions of the β1 and 2-adrenoreceptors (AR), and plasma levels of norepinephrine and estradiol in Experiments 1 and 2. OVX, bilateral ovariectomy; C group, ovariectomy without estradiol replacement; E2 group, variectomy with estradiol replacement, and the replacement was stopped the day before Day 1.

the core temperature during dark phase [34], suggesting that tail skin blood flow is elevated. We did not assess the tail skin temperature in the present study. The results suggest that thermoregulatory skin vasodilation may have been augmented due to the circadian increase in core temperature, resulting in a decrease in core temperature.

### Changes in daily core temperature rhythm in the E2 group

The comparison of the core temperature rhythms between the C and E2 groups indicated that the depletion of plasma estradiol might be associated with the daily core temperature change in the C group. However, on Days 1, 7, and 21 in the E2 group, the core temperature did not fall below the value on PRE was observed during the dark phase. On the contrary, core temperature increased from the PRE level during the late light phase on all three dates (Figure 3B-2, Table 1). Spontaneous activity also did not change from the PRE level on Days 1, 7, and 21 (Figure 4B-2, Table 1). Thus, activity did not contribute to the change in daily core temperature rhythm in the E2 group. Therefore, the estimated effect of the estradiol depletion on daily core temperature rhythm differs substantially between the two study conditions.

A comparison of the rhythms on PRE in the C group and on Day 7 in the E2 group, (i.e., 9 days after ovariectomy and 7 days after estradiol withdrawal), revealed that core temperature in the E2 group exceeded that in the C group throughout the day (Table 1). In addition, the same comparison between Day 7 in the C group and Day 21 in the E2 group (17 days after the ovariectomy and 21 days after the estradiol withdrawal) indicated greater core temperature in the E2 group during several periods in both dark and light phases (Table 1). Previous report showed that postmenopausal women who discontinued hormone replacement therapy had vasomotor symptoms (e.g., hot flashes and night sweats) again [3]. These results suggest that hormonal state prior to estradiol depletion was reflected in a change of the core temperature rhythm. For example, greater estradiol level or presence of plasma progesterone may affect averaged level of daily core temperature change.

The effect of ovariectomy on daily core temperature rhythm remained unchanged on PRE and Days 1–21 in the C group. Moreover, on Day 7–21 in the E2 group, the core temperature rhythm did not change from that on Day 1 in the E2 group. Thus, the effect of estradiol depletion on the core temperature rhythm was evident immediately and lasted to Day 21.

### Comparison of heart rate between the C and E2 groups

The heart rates were higher in the C group than in the E2 group on PRE (Figure 5A, Table 1). However, the daily change of amplitude was similar between the two groups (153 ± 16 and 134 ± 9 beats/min in the C and E2 groups, respectively). The results clearly suggest that the effect of the estradiol depletion on heart rate (but not on core temperature) was similar throughout the day. In addition, estradiol depletion appears to affect core temperature and heart rate through different mechanisms. Previous studies reported that single injection and chronic replacement of estradiol decreased heart rates in ovariectomized rats [15–17]. The plasma norepinephrine level was higher in the C group than in the E2 group (Figure 7). He et al. [17] reported that ovariectomized rats had greater sympathetic nerve activity, a finding that supports our results. The change in heart rates may reflect greater sympathetic nerve activity throughout the course of the day, although it remains unknown whether plasma norepinephrine was also higher throughout the day.

Another mechanism for the increased heart rates in the C group may be the expression of β-adrenoreceptors. β1-AR is associated with the chronotropic action of the heart [22], which was greater in the C group than in the E2 group (Figure 6A). Previous studies have also reported that protein expression [19–21] and mRNA of the β1-AR in rats' hearts [21] increased after ovariectomy. The change of β1-AR expression from PRE to Day 7 suggests that ongoing effects of estradiol depletion (Figure 6A). On the contrary, β2-AR expression was lower in the C group than in the E2 group (Figure 6B). β2-AR is related to inotropic action of the heart. Several studies have shown that estradiol administration induces hypertension in ovariectomized rats [18, 35–39]. Thus, the lower levels of β2-AR expression may reduce the inotropic action of the heart, inducing a compensatory increase in heart rate to maintain cardiac output.

### Changes in heart rate in the E2 group

In the E2 group, the heart rates increased from those of PRE throughout Day 7 (Figure 5B-2, Table 1). Plasma norepinephrine increased from the PRE level on Days 7 and 21. However, there was no effect of estradiol depletion on the β-adrenoreceptors. Thus, estradiol depletion may increase heart rates via sympathetic activation, but not through changes in β-adrenoreceptors expression.

We compared the heart rate between PRE in the C group and Day 7 in the E2 group and between Day 7 in the C group and Day 21 in the E2 group. However, there were no differences in the heart rate. Thus, estradiol depletion increases heart rates despite the previous hormonal state.

Heart rate in the C group on Days 1 and 7 remained unchanged from the PRE level; however, they were lower on Day 21 (Figure 5B-1). A previous report also showed a reduction in heart rate one month after ovariectomy in rats [37]. Thus, the effect of the estradiol depletion on heart rate may extend only for a limited period.

## Conclusion

In summary, the depletion of plasma estradiol in female rats immediately affects daily changes of core temperature and heart rate. The variation of core temperature after depletion of estradiol may be influenced by plasma estradiol and/or progesterone levels prior to the depletion. The depletion of estradiol affects daily heart rate rhythm in a different manner from that of the core temperature. The increase of heart rate after loss of estradiol was transient, and also was related to the β-adrenoreceptors in the heart and the plasma norepinephrine level. Among the contributing factors to heart rates, the norepinephrine level may be more important.

These results suggest that core temperature and heart rate rhythms are also modulated in peri-menopausal women, whose estradiol levels decrease rapidly. We speculate that the modulated rhythms are associated with menopause syndromes such as hot flashes and palpitations.

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#### Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

#### Author Contributions

K.N. supervised the entire project. S.M. and K.N. designed the study and wrote the manuscript. S.M. and Y.U. performed experiments.

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