A single bout of vigorous exercise decreases subsequent non-exercise physical activity and body temperature

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Abstract

Exercise benefits the body and mind, but its weight loss effect is less than generally expected. Although this phenomenon is likely due to a decrease in non-exercise physical activity (NEPA) and thermogenesis following exercise, the underlying mechanisms and effects of exercise intensity remain unknown. Here we show the potential role of corticosterone dynamics in the decline in NEPA and heat production following vigorous exercise. Adult male C57BL/6J mice were categorized into three groups: sedentary, moderate exercise, and vigorous exercise, with exercise groups undergoing a 30 min treadmill session. Using an intraperitoneally implanted activity monitor, NEPA and body temperature (BT) were monitored for two days before and three days after exercise. The daily synchrony between the NEPA and BT was evaluated using a cross-correlation function. Plasma corticosterone was also detected at 6 and 24 h after exercise. Notably, only the vigorous exercise group exhibited a decline in both NEPA and BT, and increased body weight compared to the sedentary group, despite no observed changes in food intake. Furthermore, a distinct negative time lag was identified between the daily dynamics of NEPA and BT following vigorous exercise. A positive correlation was observed between plasma corticosterone levels and changes in NEPA before and after exercise across all exercise groups. Our findings provide evidence for vigorous exercise-specific reduction in NEPA, BT, and their synchrony, likely due to the disturbed circadian rhythm of corticosterone. Declined BT could imply lowered thermogenesis, providing novel insights into the role of exercise intensity in influencing the weight loss effects of exercise.

Introduction

Exercise has been widely recommended as a strategy for improving physiological and mental health (1–3). Given the growing prevalence of obesity, a leading cause of many health problems, optimal exercise prescriptions are required to promote a better energy balance and prevent obesity. However, it has been reported that the effect of exercise on body weight loss is often less than the theoretically predicted reduction based on exercise-induced energy expenditure (4–6). This might be because exercise-induced increases in energy expenditure reduce the energy spent on other physiological activities (7, 8). Therefore, we should better understand the physiological and behavioral changes in response to exercise to develop an effective exercise prescription for preventing obesity and promoting a healthy lifestyle.

Non-exercise physical activity (NEPA) is a crucial component of total energy expenditure and is closely associated with fat gain and the risk of obesity (9). Several studies have shown that a reduction in NEPA is likely a critical contributor to insufficient weight loss by exercise (10, 11). However, evidence of the effects of exercise on NEPA remains inconclusive in human studies (12, 13). In rodents, it has been reported that the introduction of a running wheel consistently decreases NEPA in mice in several studies (14–16), suggesting that voluntary exercise negatively affects NEPA. Furthermore, it was recently reported that a single bout of treadmill running decreased NEPA following exercise in mice (17). As described above, exercise certainly decreases NEPA, and this compensatory response may contribute to maintaining the total energy expenditure within a narrow range and insufficient exercise-induced weight loss. However, the effects of exercise type, intensity, and volume on compensatory reduction in NEPA induced by exercise are poorly understood and compensatory changes in other physiological activities after exercise have not been sufficiently investigated.

Although moderate-to vigorous-intensity physical activity is generally recommended for promoting overall health, the benefits of exercise vary depending on exercise intensity (18, 19). There is evidence that vigorous exercise increases stress-related physiological responses compared to moderate exercise intensity (20). Considering that vigorous exercise increases energy demands and contributes to the development of fatigue, it can be reasonably assumed that a substantial compensatory response is observed after vigorous exercise compared with moderate exercise.

However, it is unknown whether a compensatory reduction in NEPA and other physiological activities after exercise occurs in an exercise intensity-dependent manner. Body temperature is a physiological index that is increased by psychiatric stress and affected by metabolic rate (21, 22), whereas a reduction in body temperature is an adaptive mechanism to reduce energy expenditure when food availability is limited (23). Therefore, for a better

understanding of compensatory responses after exercise, it is important to investigate whether compensatory changes in body temperature also depend on exercise intensity.

Vigorous exercise activates the hypothalamic-pituitary-adrenal axis and increases corticosterone release depending on exercise intensity (24). Corticosterone exhibits circadian rhythms that typically rise in the late afternoon to early evening, just prior to the onset of a dark period in rodents (25, 26), which is synchronized with that of physical activity. In addition, corticosterone plays a key role in the regulation of stress responses, energy metabolism, and physical activity in rodents. For example, previous studies have reported that chronic corticosterone exposure causes obesity and decreases physical activity in mice (27, 28). Considering these findings showing a close link between corticosterone and physiological activities related to energy balance, exercise-induced changes in corticosterone are likely to be associated with compensatory responses in NEPA after exercise. However, whether corticosterone is associated with a compensatory reduction in NEPA after exercise remains unclear.

Here, we aimed to examine whether NEPA and body temperature decrease following exercise in an exercise intensity-dependent manner, and whether corticosterone is associated with decreased NEPA in mice. Furthermore, we attempted to investigate the relationship between daily changes in NEPA and body temperature following exercise using cross-correlation analysis to better understand the compensatory responses in NEPA and body temperature after exercise.

Materials and Methods

Animals

Male C57BL/6 mice (11 weeks old; n = 27) (SLC, Shizuoka, Japan) were used in these experiments. Three mice were excluded due to an accident during the experiment and the resulting change in group size. The mice were housed in standard laboratory cages (225 × 338 × 140 mm) under controlled temperature (22 ± 2 °C) and light (12:12-h light and dark cycle, light at 7:00–19:00). Food and water were provided ad libitum. All experimental protocols were conducted in accordance with the guidelines of the University of Tsukuba Animal Experiment Committee.

Experimental designs

A schematic of the experimental procedure is shown in Figure 1A. After 1-week habituation to the housing environment, mice underwent surgery to implant a device intraperitoneally to measure NEPA and body temperature, as described previously (29). After the 1-week of recovery, all mice were habituated to running on a treadmill (Natsume, Tokyo, Japan) for five sessions over 6 days for 30 min/day with incremental increases in speed (from 0 to 25 m/min). After the

habituation period for all mice, we randomly divided them into three groups: sedentary (SE), moderate (ME), and vigorous (VE) exercise, and exercised on a treadmill for 30 min at each intensity. Based on a previous study that determined the lactate threshold (LT) during treadmill running in mice (18), treadmill running was performed at SE (0 m/min), ME (sub-LT, 15 m/min), and VE (supra-LT, 25 m/min). The NEPA and body temperature were continuously measured in the home cage (Fig. 1B) from 2 days before to 3 days after the exercise intervention using an intraperitoneally implanted device. Three days after the first exercise intervention, the mice performed the same treadmill running again, and blood was sampled at 6 h and 24 h after exercise to measure plasma corticosterone.

Measuring NEPA and body temperature

NEPA and body temperature were measured using nanotag® (Kissei Comtec Ltd, Japan), a recently developed body-implantable device (29). Mice aged ten weeks were implanted with nanotag® intraperitoneally under anesthesia via the intraperitoneal injection of an anesthetic cocktail (hydrochloric acid medetomidine, 0.3 mg/kg; midazolam, 4 mg/kg; butorphanol tartrate, 5 mg/kg). After surgery, the mice were intraperitoneally administered atipamezole hydrochloride (an antagonist of medetomidine hydrochloride, 0.3 mg/kg) to recover from anesthesia, thereby preventing a sustained decline in body temperature. The mice underwent a 1-week recovery period after surgery because the negative effect on physical activity was confirmed to be nearly negligible after this period (29). Prior to nanotag® implantation, we set the device to start and stop recording automatically, according to the experimental procedure (Fig. 1A). The number of counts was recorded in the nanotag® at 5-minute intervals. After the experiment was completed, we removed the nanotag® from deeply anesthetized mice, and the data stored in the nanotag® were retrieved into a computer using the FeliCa® communication system.

Synchrony analysis

Nanotag® allows us to continuously measure NEPA and body temperature simultaneously in mice. These data were used to calculate the cross-correlation function (CCF) between daily changes in NEPA and body temperature on day 1 post-exercise, which can detect the strength of correlations and time lags among the temporal data. The time lag representing the peak CCF was obtained for each participant to investigate the causality between daily changes in NEPA and body temperature. A negative time lag indicates that the circadian rhythm of body temperature precedes that of NEPA.

Corticosterone

Corticosterone concentrations in mouse plasma samples were measured using the Corticosterone ELISA Kit (ARB, #K014-H1) according to the manufacturer's recommended protocols. The

absorbance was determined using a fluorescence microplate reader (Varioskan LUX multimode microplate reader, Thermo Fisher Scientific, MA, USA).

Statistical analysis

Daily changes in NEPA and body temperature were analyzed using a two-way (time × exercise-intervention) repeated-measures analysis of variance (ANOVA). Percent changes in NEPA were also analyzed using two-way (time × group) repeated-measures ANOVA. If a significant interaction or main effect was observed, Tukey's or Dunnett's multiple comparison tests were performed. CCF analysis was performed by an original program by Python 11. Body weight changes, plasma corticosterone levels, CCF, and lag were analyzed using ordinary one-way ANOVA. Pearson's correlation analysis (plasma corticosterone levels vs. percentage changes in physical activity) was also performed. All data are presented as mean ± standard error (SEM). The threshold for statistical significance was set at $P < 0.05$.

Results

Vigorous exercise decreases subsequent non-exercise physical activity and increases body weight without altering the food intake.

We first compared the daily changes in NEPA during pre-exercise with day 1 post-exercise within each group (Fig. 1C–E). Although there was no time × exercise-intervention interaction [$F(19, 95) = 0.925$, $P = 0.554$] in the SE group, the ME and VE groups showed time × exercise-intervention interaction [$F(19, 152) = 1.779$, $P < 0.05$; $F(19, 152) = 4.470$, $P < 0.0001$] (Fig. 1C). In ME, we observed a significant difference in NEPA at only one time point between pre-exercise and day 1 post-exercise, while VE exhibited significant differences at some time points (Fig. 1D, E).

In the analysis of percentage changes in NEPA from pre-exercise to days 1–3 post-exercise (Fig. 1F), two-way repeated-measures ANOVA revealed a time × group interaction [$F(6, 63) = 4.87$, $P < 0.001$]. In the post hoc analysis, a significant reduction from pre-exercise to day 1 post-exercise was observed only in the VE group ($P < 0.01$). Furthermore, the percent change on day 1 post-exercise from pre-exercise in the VE group was significantly lower than in the SE and ME groups ($P < 0.01$).

Body weight and food intake were measured weekly during the experiment. Changes in body weight after 24 h of exercise were greater in the VE group than in the SE group ($P < 0.01$, Fig. 1G). The average food intake per cage divided by the number of mice is shown in Figure 1H.

Vigorous exercise leads to a subsequent reduction in body temperature during the active dark period.

Daily changes in body temperature were compared between pre-exercise and day 1 post-exercise within each group (Fig. 2A–C). In the SE group, daily changes in body temperature did not differ between pre- and post-exercise [$F(19, 95) = 1.39$, $P = 0.152$]. In contrast, ME and VE showed a time × exercise-intervention interaction [$F(19, 152) = 4.14$, $P < 0.0001$; $F(19, 152) = 5.34$, $P < 0.0001$]. Post hoc analysis revealed a significant decline in body temperature during day 1 post-exercise compared to pre-exercise at some time points during the dark period in the ME and VE groups (Fig. 2B, C).

In the pre-exercise period, the average body temperature during the light and dark periods did not differ between the groups (Fig. 2D). However, on day 1 post-exercise, the average body temperature during the light period was not different among the groups, while a significant difference was observed during the dark period ($P < 0.01$). Post hoc analysis revealed that the body temperature in the VE group was significantly lower than that in the SE group ($P < 0.01$, Fig. 2E).

Vigorous exercise induces a distinct negative time lag between the daily dynamics of non-exercise physical activity and body temperature.

We showed typical data representing the daily changes in NEPA and body temperature during day 1 post-exercise in the ME and VE, respectively (CCF = 0.683, Lag = 10 min, Fig. 3A; CCF = 0.641, Lag = -10 min, Fig. 3B). There was no significant difference in the CCF values among the groups ($P = 0.391$, Fig. 3C). On the other hand, the lag time in VE was significantly shifted toward the negative direction compared to SE and ME ($P < 0.01$, $P < 0.05$, Fig. 3D).

Plasma corticosterone levels are positively correlated with changes in NEPA following exercise in all exercise groups.

No difference was found in plasma corticosterone levels 6 h after exercise among the groups ($P = 0.160$, Fig. 4A). There were also no differences in plasma corticosterone levels 24 h after exercise among the groups ($P = 0.138$). On the other hand, plasma corticosterone levels 6 h after exercise were significantly correlated with the percent changes in NEPA from pre-exercise to day 1 post-exercise in the ME ($r = 0.919$, $P < 0.05$) and VE ($r = 0.832$, $P < 0.05$), respectively (Fig. 4B, C).

Discussion

We examined whether a reduction in NEPA and body temperature following exercise depends on exercise intensity, and investigated the underlying mechanisms whereby NEPA decreases with exercise. Our study revealed that vigorous exercise specifically causes a substantial compensatory response, including a reduction in NEPA and body temperature, supporting the insufficient weight loss effect induced by exercise. Furthermore, delayed circadian rhythms of NEPA from that of body temperature induced by vigorous exercise and the lack of a rise in corticosterone before awakening may be associated with a compensatory reduction in NEPA following exercise.

Compensatory reduction in NEPA and body temperature following exercise

So far, there is growing evidence that the effect of exercise on body weight loss is less than expected. Pontzer et al. demonstrated that increasing physical activity does not simply add total energy expenditure in a dose-response manner (30). In addition, an animal study reported that the total energy expenditure does not increase despite the fact that the daily running distance considerably increases in mice (16). These findings suggest the possibility of exercise-induced compensatory responses related to the regulation of the energy balance. Although several studies have reported a compensatory reduction in NEPA after exercise (14, 15), whether the volume or intensity of exercise decreases NEPA is insufficiently understood. The present study demonstrated for the first time that NEPA decreases following vigorous exercise, but not moderate exercise (Fig. 1F), suggesting that a compensatory reduction in NEPA following exercise was specifically caused by vigorous exercise.

In addition, body weight gain was higher only in the vigorous exercise group than in the sedentary group, despite the fact that energy expenditure during exercise was the highest in the vigorous exercise group (Fig. 1G). This phenomenon might be attributed to a reduction in the NEPA. These findings indicate the possibility that vigorous exercise makes mice physically inactive following exercise, and thus, moderate exercise might be an optimal exercise program for promoting a physically active lifestyle and preventing obesity.

Body temperature is affected by whole-body metabolic activity, immune activity, and physical activity levels. Therefore, if exercise causes compensatory changes in physiological activity, the body temperature is likely to change. In addition, the circadian rhythm of resting energy expenditure parallels the changes in core body temperature (31). Body temperature represents the basal metabolic rate when mice are in a physically inactive phase. Our study demonstrated that moderate exercise decreased body temperature during the late dark period (Fig. 2B), whereas vigorous exercise decreased body temperature subsequent to both the early and late dark periods (Fig. 2C). During the early dark period, both NEPA and body temperature decreased following vigorous exercise, and the reduction in body temperature during this period was

considered to result from decreased NEPA.

However, the exercise-induced reduction in body temperature during the late dark period is likely to result from a decreased basal metabolic rate because NEPA did not decrease during this period. Taken together, exercise was likely to cause a compensatory reduction in the basal metabolic rate in mice, independent of exercise intensity. Furthermore, considering that a compensatory response was observed even following moderate exercise, this result may suggest that a reduction in the basal metabolic rate preferentially occurs as a compensatory response following exercise.

In this study, because there was no body weight reduction or increased food intake following exercise, a reduction in NEPA and body temperature following exercise certainly contributed to energy compensation. Considering that body weight gain was not increased in the moderate exercise group, decreased NEPA might largely contribute to body weight gain compared to the decreased basal metabolic rate. Altogether, we consider that exercise might cause a compensatory reduction in basal metabolic rate, and thereafter, a compensatory reduction in NEPA in response to increased exercise intensity, which may partly explain the background of Pontzer's constrained total energy expenditure model (30). However, we did not assess energy expenditure. For a better understanding of compensatory responses after exercise, further studies examining the effect of exercise on the metabolic rate assessed by respiratory analysis are required.

Investigation of synchrony analysis in circadian rhythms between NEPA and body temperature

NEPA and body temperature exhibit circadian rhythms that are highly synchronized; for example, an increase in these levels is observed during the early dark period. We attempted to investigate the synchrony in circadian rhythms between NEPA and body temperature as a possible underlying mechanism of the compensatory reduction in NEPA following exercise. Although the CCF between daily changes in NEPA and body temperature on day 1 post-exercise did not differ among groups (Fig. 3C), the time lag significantly shifted toward a negative direction in VE compared with SE and ME (Fig. 3D). This result indicates that the circadian rhythm of NEPA is different from that of body temperature following vigorous exercise.

If increased body temperature is partly responsible for the initiation of NEPA, there is a possibility that a reduction in the sensitivity of NEPA to a rise in body temperature contributes to compensatory reduction in NEPA following vigorous exercise. For a detailed investigation, NEPA and body temperature should be measured simultaneously at shorter intervals than those measured in this study. The synchrony between daily changes in NEPA and body temperature can potentially be used to investigate the underlying mechanism of the decline in NEPA following exercise.

The possible underlying molecular mechanism of compensatory reduction in NEPA following exercise

We first focused on corticosterone, a hormone that represents a circadian rhythm similar to that of physical activity and responds to a bout of exercise depending on its intensity (24, 26). In this study, plasma corticosterone was measured 6 h and 24 h after exercise, at which time points represent the late light period and the early light period, respectively. Plasma corticosterone commonly shows a higher value in the late light to early dark period, namely, before awakening (26). Consistent with previous studies, the average plasma corticosterone levels were higher at 6 h than at 24 h after exercise. Although plasma corticosterone levels were not different among the groups at either time point, plasma corticosterone levels at 6 h after exercise positively correlated with percent changes in NEPA in both the ME and VE groups (Fig. 4B, C). This result indicates that an increase in corticosterone levels before awakening is important to prevent decreased NEPA following exercise. Daily corticosterone release is regulated by many chronobiological mechanisms such as clock-related genes, neuronal activity in the suprachiasmatic nucleus, and hypocretin neurons in the paraventricular nucleus (32, 33). Therefore, daily changes in corticosterone represent a state of biological circadian rhythm. Compensatory changes in physiological activities, including NEPA, following exercise are also associated with impaired biological rhythms of the circadian clock.

In the present study, exercise intensity in the VE group (25 m/min) was probably higher than LT in mice (18). Considering that compensatory reduction in NEPA is specifically caused by vigorous exercise, compensatory reduction in NEPA following exercise might be associated with increased blood lactate levels. It was recently reported that the activation of lactate receptors, known as hydroxycarboxylic acid receptor 1, decreases spontaneous locomotor activity in mice (34). Therefore, it is worth investigating whether lactate signaling is associated with a reduction in NEPA following vigorous exercise. As described above, further studies featuring corticosterone, lactate, or other molecules are required to better understand the underlying mechanism of compensatory reduction in NEPA following exercise.

Conclusion

Our findings provide evidence for vigorous exercise-specific decreases in NEPA, body temperature, and their synchrony. These compensatory responses must be induced by the disturbed circadian rhythm of corticosterone. Adjusting exercise intensity not only dictates the effectiveness of the exercise protocol but also presents potential implications for post-exercise NEPA and thermogenesis. This novel insight paves the way for a comprehensive exercise strategy to address physical and mental health issues, and mitigate the challenges of overweight and obesity.

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Author contributions

D.F. and T.M. conceived and designed the study. D.F., S.D., and K.S. performed the experiments. D.F. and T.M. analyzed the data. D.F., S.D., K.S., H.S., T.N., and T.M. interpreted the results of the experiments. D.F. and T.M. prepared the figures. D.F. and T.M. drafted the manuscript. All authors edited and revised the manuscript, and read and approved the final version of this manuscript.

Declaration of interest

The authors have no conflicts of interest to declare.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figures

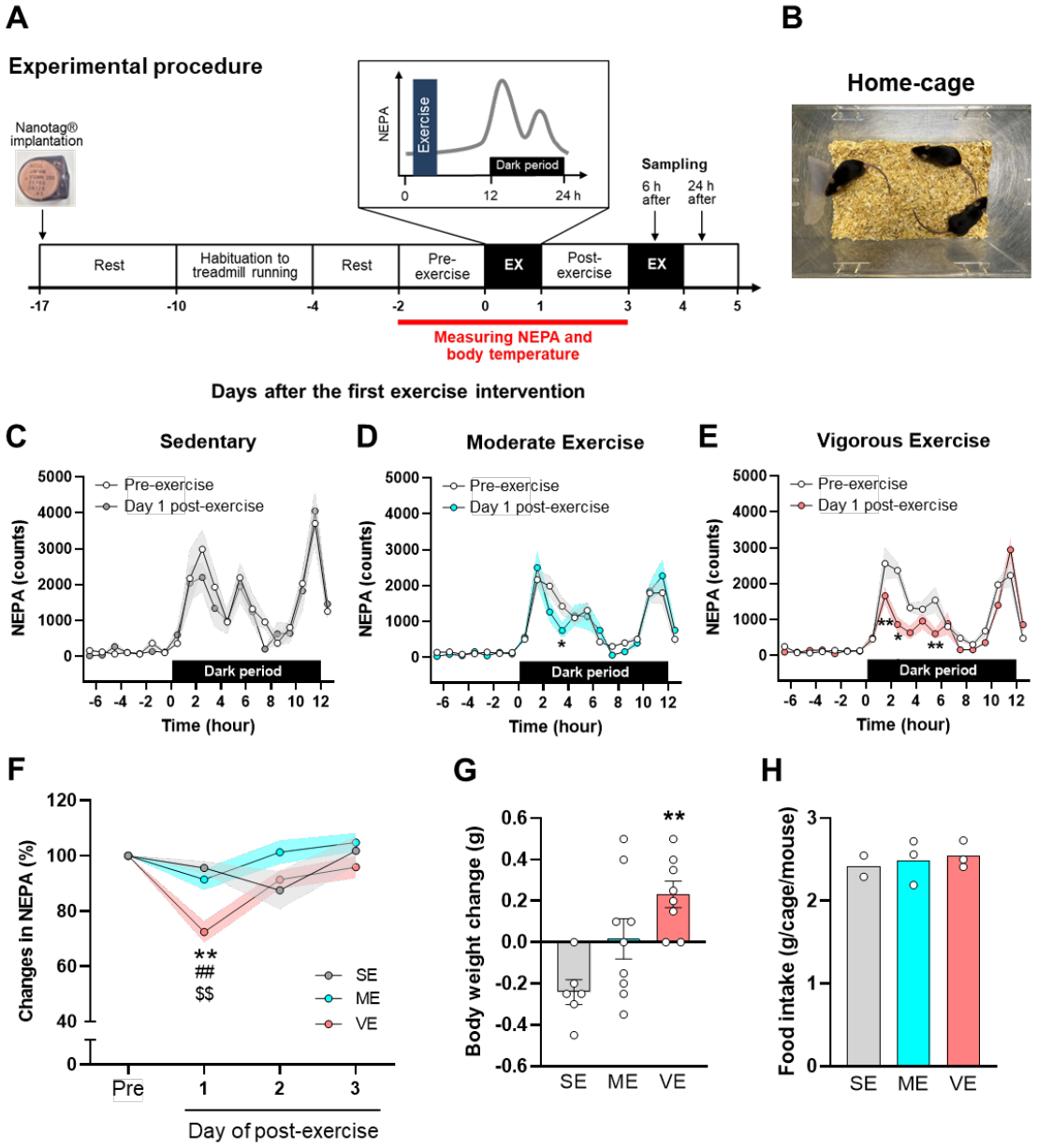

Figure 1 Vigorous exercise decreases subsequent non-exercise physical activity and increases body weight without altering food intake.

Schematic of experimental procedure (A). Exercise was performed on the day of exercise as an EX. Images of the home cage of mice (B). Daily changes in NEPA pre-exercise and on day 1 post-exercise in SE (C), ME (D), and VE (E). The pre-exercise was averaged for two days of NEPA before exercise. *P < 0.05, **P < 0.01 vs. pre-exercise. Percent changes in NEPA from pre-exercise to days 1–3 post-exercise (F). Body weight change (G) and food intake (H) after 24 h of exercise. **P < 0.01 vs. SE, ##P < 0.01 vs. ME, $$P < 0.01 vs. pre-exercise. Values represent mean ± SEM (n = 6–9 per group).

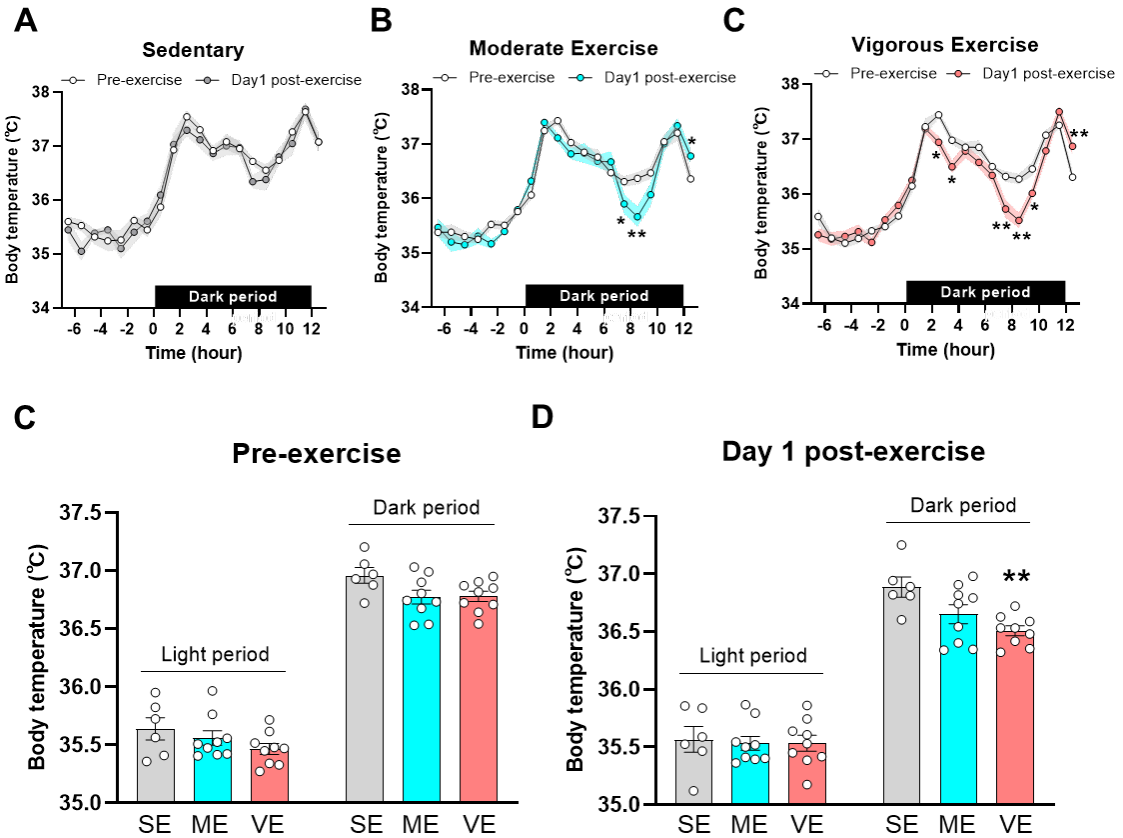

Figure 2 Vigorous exercise leads to a subsequent reduction in body temperature during the active dark period.

Daily changes in core body temperature pre-exercise and on day 1 post-exercise in SE (A), ME (B), and VE (C). The pre-exercise was averaged for two days of body temperature before exercise. *P < 0.05, **P < 0.01 vs. Pre-exercise. Average core body temperature during light and dark periods pre-exercise (D) and on day 1 post-exercise (E). **P < 0.01 vs. SE. Values represent mean ± SEM (n = 6–9 per group).

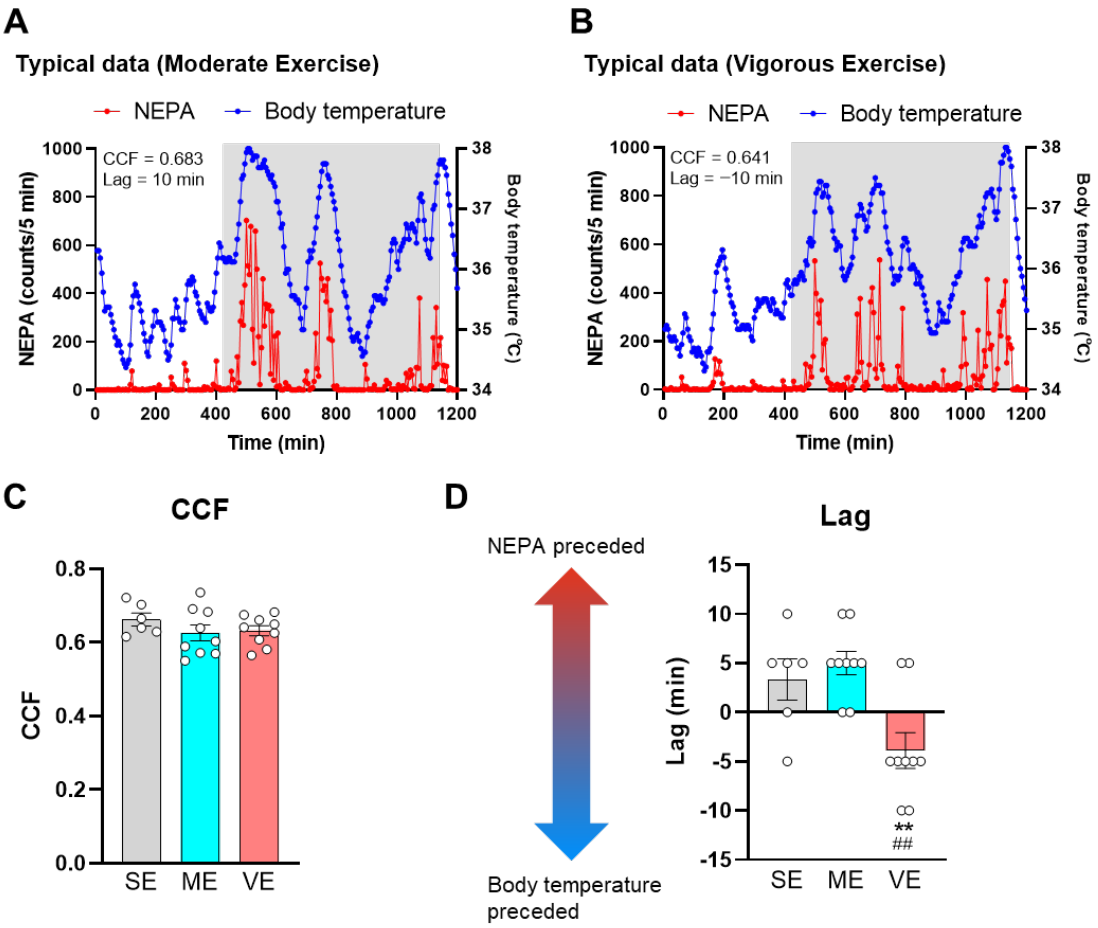

Figure 3 Vigorous exercise induces a distinct negative time lag between the daily dynamics of non-exercise physical activity and body temperature.

Typical data of daily changes in NEPA and body temperature during day 1 post-exercise in the ME (A) and VE (B). CCF (C) and lag time (D) of daily changes on day 1 post-exercise. A negative time lag means that circadian rhythms of body temperature precede that of NEPA. **P < 0.01 vs. SE, ##P < 0.01 vs. ME. Values represent mean ± SEM (n = 6–9 per group).

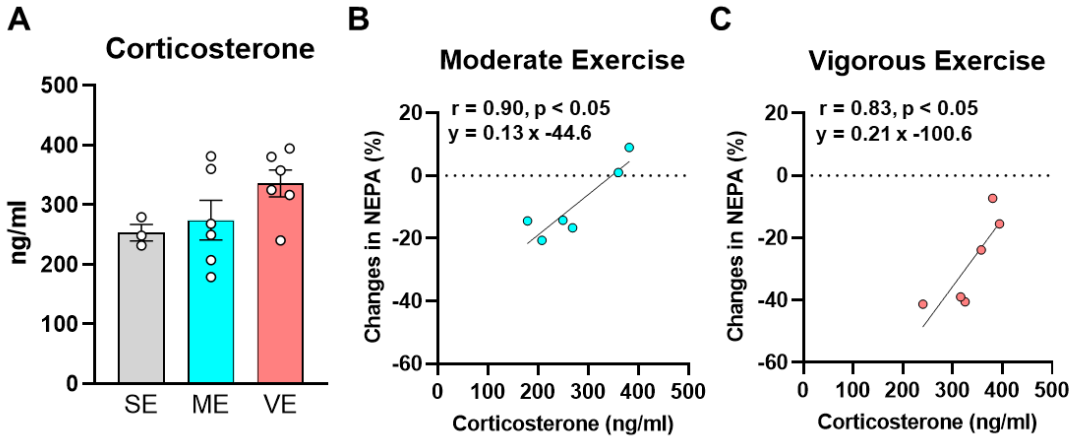

Figure 4 Plasma corticosterone levels positively correlated with changes in NEPA following exercise in all exercise groups.

Plasma corticosterone levels 6 h after exercise (A). Correlation between plasma corticosterone 6 h after exercise and percent changes in NEPA from pre-exercise to day 1 post-exercise in ME (B) and VE (C). Values represent mean ± SEM (n = 3–6 per group).