Japanese Journal of Veterinary Research 68(1): 55-62, 2020

JJVR

REGULAR PAPER Experimental Research

The effect of temperature acclimation on torpor expression pattern in mice

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Received for publication, August 1, 2019; accepted, October 25, 2019

Abstract

Torpor is a hibernation like status which drops core body temperature, and continues for several hours. Although torpor is observed among a wide range of mammals, most of the body mass of torpor expressing species is less than 100 g, suggesting the size of the body would be a key factor of torpor expression. In the small animals, continuous heat production is essential to keep body temperature within a certain range. Thus, we hypothesized that torpor expression pattern would be affected by the capacity of heat production. We prepared mice with different capacities of shivering and non-shivering thermogenesis, two major heat producing systems in mice. To modulate the capacity of both thermogenesis, mice were acclimated to cool (18 °C) or warm (28 °C) ambient temperature. Mice deficient in uncoupling protein 1 (UCP1), a protein that mediates non-shivering thermogenesis, were acclimated to cool ambient temperature to enhance capacity of shivering, but not non-shivering thermogenesis. By food deprivation, all mice in all groups expressed torpor. Although the time for induction and duration of torpor did not show any differences among the groups, the minimum body temperature during torpor in warm-acclimated mice was lower than that in cool-acclimated UCP1-knockout mice. Cool-acclimated UCP1-knockout mice did not show any significant difference in torpor expression pattern compared to cool-acclimated wild-type mice, indicating a minor role of non-shivering thermogenesis in torpor expression in our experiment condition.

Key Words: mouse, thermogenesis, torpor, ucp1

Introduction

Torpor is a short-term hibernation like state with low metabolic rate. Some mammals and birds exhibit torpor when faced with harsh environment such as food shortage condition, suggesting torpor expression can be advantageous to survive by saving energy. It has been shown that torpor is observed in mammalian species of 14 orders among 27 orders so far[4]. In addition, torpor is observed in a wide range of climate zone from Tropic to Frigid Zone. Therefore, this physiological phenomenon could be regarded as a universal phenotype of mammals. The common characteristics among the torpor expressing animals is small body mass. The body masses of such animals are generally below 100 g[5]. In small animals, the ratio of body mass to surface area is small, causing a large proportion of heat dissipation. Therefore, continuous heat

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doi: 10.14943/jjvr.68.1.51

production is essential for keeping a constant body temperature. Once heat production stops, the body temperature would rapidly decline. Thus the heat producing capacity would play a critical role in torpor expression patterns.

Heat production system in mammals consists of two distinct mechanisms, shivering and non-shivering thermogenesis[2]. Shivering thermogenesis occurs in skeletal muscle, whereas non-shivering thermogenesis is mainly due to the activity of brown adipose tissue (BAT), which contains high density of mitochondria expressing uncoupling protein 1 (UCP1). UCP1 is a key protein to generate heat by leaking hydrogen molecule through mitochondria membrane. The capacity of both types of thermogenesis drastically changes depending on environmental temperature. For example, acclimation of mice to cold temperature potentiates the capacity of non-shivering thermogenesis by increasing UCP1 expression in BAT and also the number of brown adipocyte itself[2]. UCP1-knockout mice are unable to maintain body temperature at 4°C because of the lack of non-shivering thermogenesis[3]. However, when acclimated to 18°C, UCP1-knockout mice sustain their normal body temperature at 4°C due to the induction of compensatory thermogenic mechanism including shivering[11].

Although torpor expression has been observed universally among mammals, there are torpor expression species and non-expression species even in small body size species. It is still unknown what makes such species difference. One plausible reason for the species difference is the degree of heat producing ability; low heat producing animals may easily enter torpor whereas high heat producing animal may not. For instance, Lesser hedgehog tenrec (*Echinops telfairi*), which lacks brown adipose tissue, continuously express torpor except reproductive season[7]. Although the effect of thermogenesis ability on torpor expression has been examined, these studies had been done by comparisons between different season under natural condition[9]. No study has examined the torpor expression pattern between animals possessing different heat producing capacity, including shivering and nonshivering thermogenesis, under controlled condition.

In this study, we prepared a few groups of mice having different thermogenic abilities to examine the effect on torpor expression pattern. As a control group, the mice were kept at 18 °C, which is below thermoneutral zone, suggesting that both shivering and non-shivering thermogenesis were active[6]. The second group was acclimated to warm environment (28 °C), thus thermogenic ability of both type of thermogenesis would be downregulated. The third group was UCP1-knockout mice kept at 18 °C, resulting in the activation of shivering due to the lack of non-shivering thermogenesis in BAT. By using combination of thermal adaptation and UCP1-knockout mice, it was possible to examine the role of two types of heat producing mechanism in torpor expression. Such comparison would provide an insight into the contribution of each heat producing abilities on torpor expression.

Materials and Methods

Animals. We used mice with a background of C57BL/6J. Wild-type mice were purchased from Clea Japan (Tokyo, Japan). UCP1-knockout (*Ucp1*$^{-/-}$) mice on a congenic background of C57BL/6J were generated by backcross mating of heterozygous (+/-) mice on a mixed 129/SvPas and C57BL/6J background with C57BL/6J mice for 15 generations, and kindly given by Dr. L. Kozak (Pennington Biomedical Research Center, Baton Rouge, LA, USA). Because it is thought that female would more easily enter a torpor than male when fasted12), only female mice were used for the experiments. Mice were raised in plastic cages placed in an air-conditioned room at 24 °C with a 12:12-h light-dark cycle (lights on at 07:00-19:00) and given free access to laboratory chow (MF: Oriental Yeast, Tokyo, Japan) and tap water. All mice were housed singly throughout

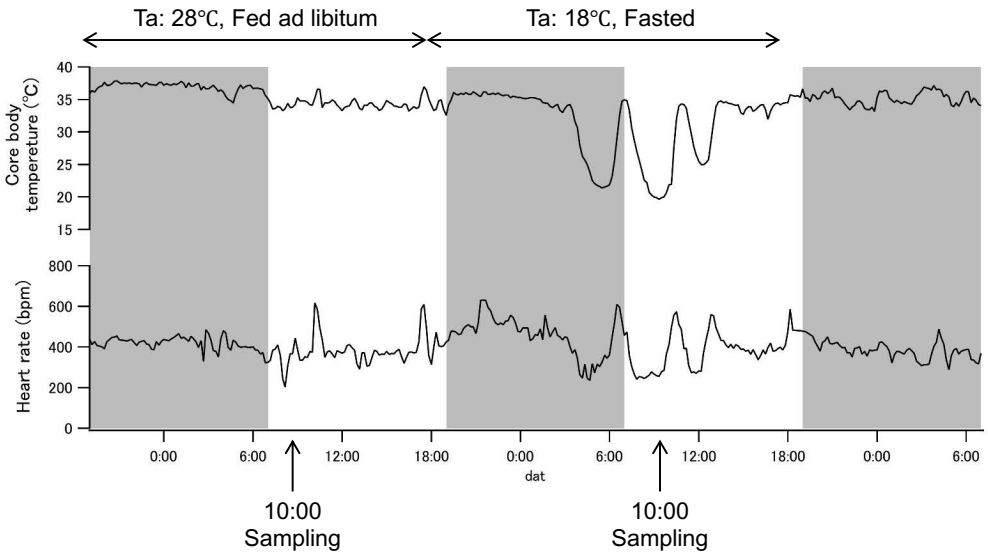

Fig. 1
An example of core body temperature and heart rate trace of warm-acclimated mouse. On the day before torpor induction, mice were kept at the ambient temperature (Ta) of 28 °C with ad libitum feeding. At 18:00, foods were removed and the ambient temperature was changed to be 18 °C. The mouse started to express torpor in the later part of dark phase and the core body temperature decreased. We conducted two types of the experiments: biotelemetry and tissue sampling. The body temperature profile was obtained by biotelemetry experiment. For tissue sampling, mice were sacrificed at the time mentioned above. Brown adipose tissues were sampled for mRNA expression analysis at 10:00 on the day before torpor induction and the day of torpor induction. The background colors indicate the light (white) and dark (grey) phases.

the entire study period. Experimental procedures and animal care were performed in accordance with the Guidelines of Animal Care and Use from Hokkaido University and were approved by the University Committee for the Care and Use of Laboratory Animals (protocol number 14016).

Biotelemetry. In this study, two types of the experiments were performed, biotelemetry and tissue sampling. For each purpose, different mice were used. For the biotelemetry experiment to monitor core body temperature, mice were implanted with a telemeter capable of measuring temperature, heart rate, and locomotor activity (G2-HR E-Mitter, Starr Life Sciences Corp, Oakmont, PA, USA) at the age of 8 weeks. In this study, the parameters of temperature and heart rate were used to monitor the condition of mice during torpor. Unfortunately, we failed to measure locomotor activity with the devices. The transmitters weigh 1.3 g. For implantation, the mice were anesthetized with 3% isoflurane, followed by maintenance with 1.8% isoflurane. The telemeter was implanted in abdominal cavity, and ECG leads were placed subcutaneously, approximating a Lead II configuration. Carprofen (4 mg/kg b.w./day, sc) was administered for 3 days after surgery to alleviate pain. Following surgery, mice were maintained at 24 °C for a week to recovery. The signal emitted by the transmitter was received and recorded by TR 3000 receiver plate with VitalView software (Starr Life Sciences Corp). The signals were measured each min and 10-min averages were used for further analysis to eliminate short term fluctuation of the signals.

Torpor induction. At the age of 9 weeks (after a week of recovery from surgery), wild-type mice were randomly assigned to two groups to be acclimated to different room temperatures. One group was kept at 18 °C (cool acclimated; CA). The other group was kept at 28 °C (warm acclimated; WA), which is within the range of thermoneutral zone of mice and heat producing capacity of both shivering and non-shivering thermogenesis is expected to be reduced. For UCP1-knockout mice, the mice were kept at 18 °C (KO) to enhance shivering, but not non-shivering, thermogenic

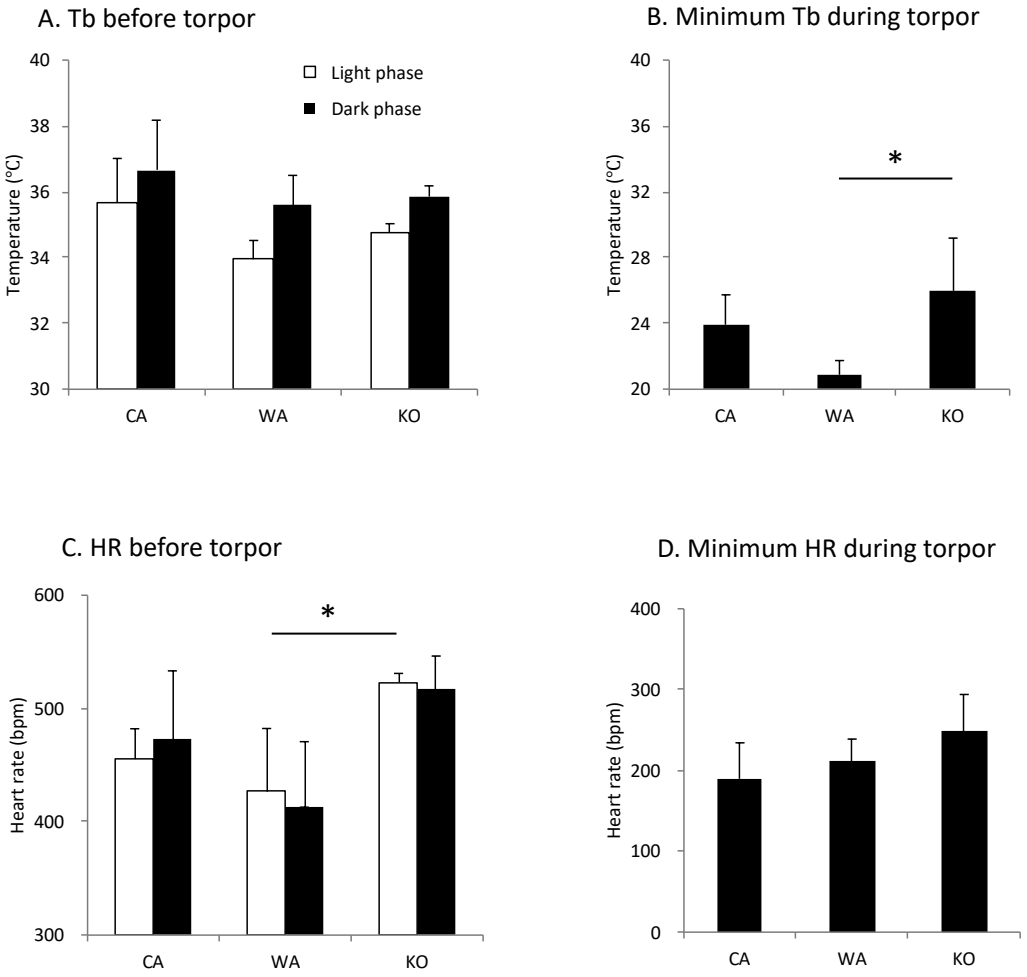

Fig. 2
Core body temperature and heart rate of mice before and during torpor. (A) The core body temperature (Tb) on the day before torpor induction. (B) The minimum core body temperature during torpor. (C) The heart rate (HR) on the day before torpor induction. (D) The minimum heart rate during torpor. Cool-acclimated wild-type (CA, n=4), warm-acclimated wild-type (WA, n=4) and cool-acclimated UCP1-knockout (KO, n=3) mice were examined. In A and C, the data in the light (white bar) and dark (black bar) phases are shown. Data are shown as means ± SD. $*P < 0.05$, Tukey's HSD test.

ability. As total, we set three experimental groups with different thermal acclimation conditions for this study (WA, CA and KO).

Mice were maintained at the respective acclimation temperatures for 3 weeks for temperature acclimation before the onset of torpor trial. At the onset of torpor trial, all mice, aged 3 months, were kept at 28 °C for 24 hours started from 18:00, followed by the induction of torpor. For torpor induction, mice were deprived food with free access to water from 18:00 and kept at 18 °C for 24 hours. Mice were considered to be in torpor if the core body temperature was below 32 °C, which is often used as the threshold temperature below which an animal is called torpid1). After the trial, mice were refed at the ambient temperature of 28 °C, and confirmed that mice kept normothermia.

Tissue Sampling. Independent mice were used for tissue sampling from the biotelemetry experiment. The same procedure of torpor induction was conducted on the mice for tissue sampling except transmitter implantation. BAT were collected at 10:00 on the day before the torpor induction, and of the torpor induction (Fig. 1). Mice were sacrificed and interscapular

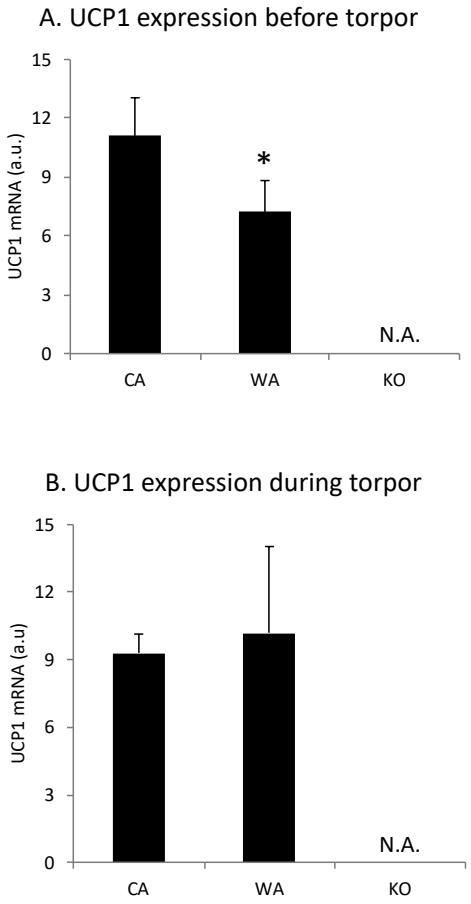

Fig. 3
UCP1 mRNA expression in brown adipose tissue. (A) The expression levels on the day before torpor induction in cool-acclimated (CA, n=4) and warm-acclimated (WA, n=4) wild-type mice. (B) The expression levels during torpor induction in CA (n=5) and WA (n=4). The level of UCP1-knockout (KO) mice was not analyzed. Data are shown as means ± SD. *$P <$ 0.05, ANOVA.

BAT were collected. Collected BAT were sunk in RNAlater (Thermo Fisher Scientific, Waltham, MA, USA), frozen by liquid nitrogen and stored at -80 °C until RNA analysis.

Total RNA was extracted from the tissue using the RNAiso reagent (Takara Bio, Shiga, Japan) according to the manufacturer's protocol. Total RNA (1 μg) was reverse transcribed using a 15-mer oligo(dT) adaptor primer and M-MLV reverse transcriptase (Life Technologies Inc., Carlsbad, CA, USA). Real-time PCR was performed on a fluorescence thermal cycler (LightCycler system; Roche Diagnostics, Mannheim, Germany) using FastStart Essential DNA Green Master (Roche Diagnostics). Absolute expression levels were determined using a standard curve method, with respective cDNA fragments as standards. The levels are expressed as relative value to ß-actin expression.

Statistical Analysis. Values are reported as means ± standard deviations. Statistical significance was determined by one-way ANOVA, followed by Tukey's HSD test for multiple groups comparison. Two tailed differences were considered significant at $P < 0.05$.

Results

All mice in all groups used in biotelemetry experiment successfully expressed torpor (n=11). At the day of the experiment, foods were removed at 18:00, followed by the room turned into dark at 19:00. The mice remained to be normothermia in the former half of the dark phase, then started to drop the body temperature in the latter part of dark phase (Fig. 1). The time passed from the food removal to the first torpor expression was 7.5±1.4 hours, which was no significant difference among groups. The torpor was characterized by the sequential temperature drop events. Once the temperature dropped into the bottom level, then increased to the level nearly normothermia, again dropped into hypothermia. This phenomenon was observed in all groups in a similar manner. The number of temperature drop events within a torpor bout was 5.1±2.1 times. The duration of torpor expression was 8.9±3.3 hours within a 24 hours of experiment. After the torpor expression, all mice recovered to normothermia, and behaved normally. The body mass has changed from 22.9±1.0 g to 18.6±0.9 g during the experiment of 24 hours fasting (20.5±1.4% of body mass reduction) with no significant difference among groups in terms of initial body mass, final body mass and percentage of body mass reduction.

We measured core body temperature on the day before torpor induction. Although the temperature in the light phase is slightly lower

than that of dark phase, there were no difference among groups on the day before the torpor induction (Fig. 2A). In contrast, the minimum core body temperature during torpor was different between groups. The temperature of CA, WA and KO were 23.9±1.8, 20.9±0.9 and 26.0±3.2 °C respectively (n=4, 4 and 3), with significant difference between WA and KO (Fig. 2B).

Heart rate during the light phase on the day before the torpor induction was different among the groups (Fig. 2C). The heart rate in the light phase of CA, WA and KO was 455±27, 426±56 and 523±8 beats per minutes (bpm), respectively (n=4, 4 and 3). The heart rate of WA was the lowest and significantly lower than that of KO. During the torpor, the heart rate was dramatically reduced to be less than half, ca 200 bpm (Fig. 2D). No significant difference was observed in the heart rate during torpor among groups.

To examine whether temperature acclimation was carried out successfully, we measured the expression levels of *Ucp1* mRNA. On the day before the torpor induction, the levels of mRNA expression of *Ucp1* were significantly higher in CA (n=4) than in WA (n=4), indicating *Ucp1* mRNA induction by successful cold acclimation in CA (Fig. 3A). When sampled at 10:00 on the day of torpor induction, the expression levels of *Ucp1* mRNA became at the same levels between CA (n=5) and WA (n=4) (Fig. 3B). The Ucp1 mRNA of KO was not examined as KO was a knockout strain of *Ucp1*.

Discussion

This study demonstrated that thermal acclimation had a significant impact on the minimum body temperature during torpor, whereas there was no significant effect on the time required to induce torpor, and torpor duration. Interestingly cool-acclimated UCP1-knockout mice (KO) did not show any difference from cool-acclimated wild-type mice (CA), suggesting that the existence of UCP1 itself does not have a significant effect on the determination of minimum temperature during torpor. During torpor, mice exhibit systematic cardiovascular adjustment: decreased heart rate and increased peripheral resistance with moderate blood pressure change[12]. High peripheral resistance, meaning vascular constriction in peripheral area, may inhibit blood flow into brown adipose tissue, resulting the modest level of thermogenesis. If such change occurs during torpor, it will be possible to explain why the existence of UCP1 have only a minor effect on torpor expression pattern. Another possibility would be that heat producing functions other than BAT were more activated in KO than CA, as KO was acclimated to cool ambient temperature without BAT thermogenesis.

Oelkrug et al. (2011) have compared the torpor expression pattern between wild-type and UCP1-knockout mice with the adaptation to warm room temperature (30 ºC) for three weeks[8]. When induced torpor at the ambient temperature of 18 °C, the minimum body temperature was not different between the groups (21.8 °C in wild-type and 21.2 °C and UCP1-knockout mice). Collectively, the existence of functional brown adipose tissue, that is, ability of non-shivering thermogenesis, is dispensable for the torpor expression. Interestingly, the minimum body temperature of warm-acclimated wild-type and UCP1-knockout mice in the study of Oelkrug et al. (2011) is similar to the temperature of WA in the present study, being 3°C lower than that of CA and KO[8].

The resting metabolic rate of cool-acclimated mice (18ºC) is 20% higher than that of warm-acclimated mice (30 ºC) at 30ºC[6]. In terms of energy metabolism, the difference of resting metabolic rate is particularly important when fasting. As an energy saving function, torpor would be more beneficial to cool-acclimated mice, although there was no clear difference between warm- and cool- acclimated mice.

During normothermia, the body temperatures of cool- and warm- acclimated mice are not

different, because the body temperature is adjusted to around the set point. When the body temperature is not adjusted within a certain range such as during torpor, the amount of resting metabolic rate may determine body temperature; the higher metabolic rate of cool-acclimated mice may cause higher body temperature during torpor. There is also a possibility that thermal acclimation modulates the set point during torpor, resulting the difference of the body temperature during torpor between the different thermal acclimated mice. This points should be clarified by further study.

The trend of the minimum body temperature was corresponding to that of heart rate in the day before torpor induction (Fig. 2C). The heart rates of KO were higher than WA. Cool acclimation process probably increased their heart rate though sympathetic nervous activity[10]. The relationship between the activities of shivering and heart rate would be an interesting issue and remains to be examined. In contrast to body temperature, the minimum heart rate during torpor was not different among groups. It is interesting that the difference of the heart rate among groups disappeared, once torpor was induced.

Heat producing system consists of shivering and non-shivering thermogenesis, however, non-shivering thermogenesis mediated by UCP-1 seemed to play only a minor role to characterize torpor expression in our experiment condition. Unfortunately, the direct measurement of shivering was not provided in this study to clarify the relationship between shivering and body temperature during torpor. Moreover, in this study, only a limited number of animals (n=3-4) were used for the experiment. Further study would be needed to examine the effect of heat producing system on torpor expression pattern.

Acknowledgments

We thank Mr. H. Shinkai and Ms. A. Tsubota for their technical assistance. We also would like to thank two anonymous reviewers. This study is supported by JSPS KAKENHI grant number 15K07758.

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