Effects of summer- and winter-like acclimation on the thermoregulatory behavior of fed and fasted desert hamsters, Phodopus roborovskii

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Abstract

1. Thermoregulatory behavior of fed and fasted desert hamsters (Phodopus roborovskii) acclimated to summer- [16 light (L):8 dark (D), ambient temperature ($T_a$) = 26.5 °C] and winter-like (8L:16D, $T_a$ = 10°C) conditions was studied. Body temperature ($T_b$), selected temperature and activity were measured in hamsters placed in a thermal gradient system for 48 h.

2. Acclimation to winter-like days led to the decrease in body mass by 14% and in body temperature by 1.1 °C. On the contrary, selected $T_a$ was higher by about 3.1 °C than in summer-like acclimated hamsters. Fasting affected only temperatures selected by night in winter-like acclimated hamsters.

3. Winter-like acclimation led to physiological changes, which may facilitate surviving long winter season. In the face of food shortage, behavioral thermoregulation may markedly reduce the energetic cost of normothermy.

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Keywords: Acclimation; Body mass; Body temperature; Desert hamster; Fasting; Phodopus roborovskii; Seasonal; Selected temperature; Thermoregulation

1. Introduction

The genus Phodopus consists of three different species: Phodopus sungorus, Phodopus campbelli and Phodopus roborovskii. P. sungorus, the Siberian hamster, is the most widely used in laboratory studies, either in chronobiological, thermophysiological or endocrinological research. P. campbelli, the Djungarian hamster, is less extensively used and P. roborovskii, the desert hamster, is almost unknown. It is the smallest of the dwarf hamsters, which inhabits mainly desert environment with sand dunes. P. roborovskii is mainly granivorous but insects are also included in its diet (Ross, 1994; Bao et al., 2002b). All three species: P. sungorus, P. campbelli and P. roborovskii inhabit Asian steppes, semi-deserts and desert ecosystems, facing similar environmental challenges like large seasonal and daily variations in an ambient temperature ($T_a$). To overcome harsh winter conditions, the best known species, the P. sungorus reduces body mass, lowers body temperature ($T_b$), increases its capacity for nonshivering thermogenesis (NST), changes pelage to a white one, regresses gonads, ceases reproduction and enters daily torpor. Daily torpor is restricted to the light phase of the day that allows for activity and foraging every night, even in a large distance from a burrow (Ruf and Heldmaier, 2000). P. campbelli does not enter torpor in winter but maintains high body mass and decreases metabolic rate (Ross, 1995; Weiner and Heldmaier, 1987). Unlike P. sungorus, which uses daily torpor in winter, P. roborovskii probably remains normothermic. However, its capacity for regulatory NST undergoes seasonal variations, which is much higher in autumn than in summer (Bao et al., 2002a).

I undertook this research to study thermoregulatory behavior of P. roborovskii acclimated to summer- and winter-like conditions. I hypothesized that acclimation to winter-like conditions leads to physiological adjustments lowering the energetic cost of normothermy. Based on the similarities between natural environments of P. sungorus and P. roborovskii as well as on previous experiments on P. sungorus (Weiner, 1987; Ross, 1994, 1998; Jefimow et al., 2004), I predicted that mean $T_b$ of winter-like acclimated
(WA) *P. roborovskii* would be lower by about 1 °C than *T* \(_b\) of summer-like acclimated (SA) hamsters and that their body mass (*m* \(_b\)) would decrease with the time of acclimation to winter-like days. Since selection of proper thermal environment may additionally reduce the cost of normothermy, I predicted that the difference between *T* \(_b\) and selected *T* \(_a\) would be smaller in winter- than in SA hamsters.

Based on the fact that in heterothermic animals food restriction affects torpor characteristics, leading to an increase in torpor bouts duration and its frequency as well as to a decrease in minimum *T* \(_b\) (Tannenbaum and Pivorun, 1987; Ruf et al., 1993), I predicted that food restriction would induce large daily variations in *T* \(_b\), at least in WA hamsters, or it may even elicit a shallow daily torpor.

To verify these predictions, I made four series of experiments in a thermal gradient system, where hamsters could select *T* \(_a\) freely. Experiments were done on SA and WA, fed and fasted *P. roborovskii*. Selected *T* \(_a\), *T* \(_b\) [measured as a brown adipose tissue (BAT) temperature] and activity were measured simultaneously and continuously during 48 h of measurements.

### 2. Materials and methods

#### 2.1. Animals and housing

Six male desert hamsters (*P. roborovskii*) obtained from commercial supplier were used in this study. The hamsters were housed individually in standard laboratory cages (33 cm × 20 cm × 18 cm) with sawdust as a bedding material. Food (seeds, dried vegetables, oat flakes, dried bread and a few slices of fresh carrot and apple once a week) and water were available *ad libitum*. Food restriction affects torpor characteristics, leading to an increase in torpor bouts duration and its frequency as well as to a decrease in minimum *T* \(_b\) (Tannenbaum and Pivorun, 1987; Ruf et al., 1993), I predicted that food restriction would induce large daily variations in *T* \(_b\), at least in WA hamsters, or it may even elicit a shallow daily torpor.

Experiments were done on SA and WA, fed and fasted *P. roborovskii*. Selected *T* \(_a\), *T* \(_b\) [measured as a brown adipose tissue (BAT) temperature] and activity were measured simultaneously and continuously during 48 h of measurements.

#### 2.2. Measurements of *T* \(_b\), selected *T* \(_a\) and activity

At least 3 days before an experiment, each hamster was implanted, under Sevorane anesthesia (Abbott Laboratories Ltd., UK), with a polyethylene cannula (φ 0.8 mm, 4 cm in length, SIMS Portex Ltd., England), which served as a guide for a copper–constantan thermocouple (φ 0.6 mm, W-TW-36 P2; Physitemp Instruments Inc., USA). The cannula was inserted under the skin, through a small incision (3 mm) at back of the neck, and then fixed with surgical thread and adhesive to the skin. After operation lasting for about 10 min, hamster recovered in a very short time on the same day.

Selected *T* \(_a\) was measured in a thermal gradient system that consisted of a long aluminum chamber (120 cm length × 10 cm height × 8 cm width), divided by half-width partitions into 16 compartments of the same size. The system was heated with circulating hot water at one end and cooled with refrigerating fluid at the other, resulting in a range of temperatures increasing linearly from 5 to 45 °C. The aluminum chamber was covered with transparent Perspex to permit light entry. A narrow slit in this lid allowed movement of the thermocouple that was suspended above the gradient by an elastic band. This design allowed hamsters to move freely inside the gradient without a load. Infrared photomitter–photodetector pairs placed in each compartment detected position of the hamster. Activity was measured using commercial ultrasound motion sensors that register hamsters’ movements as an all or nothing response and is presented as the percent of the time of experiment when hamsters were active.

*T* \(_b\), selected *T* \(_a\) and activity were automatically recorded at 1 s intervals and saved on disk. The apparatus was set up in the room, with the photoperiod reflecting the summer- or winter-like acclimation.

#### 2.3. Experimental design

Experiments were done on SA and WA hamsters, respectively. Each animal was placed in the thermal gradient chamber for 2 days. On the first day, the thermocouple wire was inserted into the cannula at the depth of interscapular region where large BAT deposits are present, and fixed with a small piece of adhesive tape. Then the hamster was left undisturbed to enable its habituation. While in the thermal gradient, fed hamsters were offered food and water *ad libitum*. To avoid the influence of food searching on temperature selection, six feeders were placed equidistantly along the gradient. Unfed (fasted) hamsters did not have access to any food during the experiment, only water was present *ad libitum*. If hamster chewed its thermocouple lead wires, the break was immediately repaired. During lead repair, hamster was handled for no more than 10 min.

#### 2.4. Data analysis

If a hamster chewed its lead, data collected for 1 h after the repair were discarded. The first day of each experiment was excluded from analysis. In each experiment, all data recorded within 24 h of measurements were averaged in 1-min intervals, and then mean *T* \(_b\), minimum *T* \(_b\) (*T* \(_b\)min), maximum *T* \(_b\) (*T* \(_b\)max), mean selected *T* \(_a\) and activity
during the day (light phase) and night (dark phase of the day) as well as $T_b - T_a$ difference [$\Delta(T_b - T_a)$] were calculated. Maximum and minimum temperatures were defined as the mean of the highest and the lowest readings recorded in each hamster during experiment, respectively.

The influence of acclimation (summer- or winter-like), time of day (day or night) and food availability (fed or fasted) on $T_b$, $T_{b\min}$, $T_{b\max}$, selected $T_a$, $\Delta(T_b - T_a)$ and activity as well as changes in body mass ($m_b$) were analyzed with repeated measures analysis of variance (ANOVA) followed by Tukey’s post hoc test.

Differences were considered statistically significant if $P < 0.05$. All values are presented as mean ± SE.

3. Results

3.1. Changes in body mass ($m_b$)

Body mass changed significantly with the time of acclimation ($F(9,45) = 6.06; P < 0.001$). Acclimation to winter-like days led to significant decrease in $m_b$ that reached its lowest (23.4 ± 1.3 g) after 6 weeks under short photoperiod and cold. Within next 7 weeks, $m_b$ was relatively constant and then started to increase gradually (Fig. 1).

Both, in summer- and winter-like conditions, 48 h of measurements did not alter $m_b$ in fed animals while fasting led to its significant decrease ($F(1,5) = 12.31; P < 0.05$; Fig. 2). In SA hamsters, mean $m_b$ before measurements was 26.1 ± 1.0 g. After 48 h in the thermal gradient, $m_b$’s of fed and fasted hamsters were 26.0 ± 1.2 and 22.0 ± 0.7 g, respectively ($P < 0.001$). In WA animals, $m_b$ before measurements in the thermal gradient was 25.8 ± 0.5 g.

3.2. Body temperature ($T_b$)

Acclimation to winter-like conditions led to the decrease in mean $T_b$ and its daily amplitude ($F(1,5) = 7.67; P < 0.05$; Figs. 3 and 4). Significant day–night differences were recorded in SA hamsters; in fed animals mean $T_b$’s by day and by night were 35.7 ± 0.1 and 36.7 ± 0.1 °C, respectively ($P < 0.05$). In fasted hamsters, the day–night difference was even larger (day 35.4 ± 1.0 °C, night 36.6 ± 1.0 °C; $P < 0.01$). In WA fed hamsters, $T_b$ did not vary significantly within 24 h of measurements, and averaged 35.2 ± 0.1 and 35.3 ± 0.3 °C, by day and by night, respectively. A small day–night difference was recorded in fasted animals (day 34.7 ± 0.1 °C, night 35.5 ± 0.2 °C; $P < 0.05$; Fig. 4).

Minimum $T_b$ ($T_{b\min}$) was lower in winter- than in SA hamsters, both fed and fasted ($F(1,5) = 7.82; P < 0.05$), while maximum $T_b$ ($T_{b\max}$) depended only on the time of day, being always higher by night than by day ($F(1,5) = 414.61; P < 0.001$, Fig. 5). Thus, ultradian variations ($T_{b\max} - T_{b\min}$) in fed and fasted hamsters were affected by acclimation and time of day ($F(1,5) = 15.42; P < 0.01$).

3.3. Selected $T_a$

Selected temperatures depended on the acclimation regime, food availability and time of day ($F(1,5) = 7.02; P < 0.05$; Figs. 3 and 5). In general, WA hamsters selected higher $T_a$ than SA animals ($P < 0.01$), except for the $T_a$
selected by fasted hamsters at night (30.0 ± 0.9 °C; Fig. 6). The day–night difference was found only in fasted WA animals (P < 0.01). In these hamsters, \( T_a \) selected by day was higher than in fasted SA animals (33.7 ± 0.9 and 31.4 ± 0.2 °C, respectively; P < 0.05) while \( T_a \) selected by night was slightly lower (NS).

3.4. \( (T_b - T_a) \) difference

As a consequence of changes in \( T_b \) and \( T_a \) related to the acclimation regime, food availability and time of day, the differences between \( T_b \) and \( T_a \) also varied (F(1,5) = 7.21; P < 0.05, Fig. 7). Daily variations in \( \Delta(T_b - T_a) \) were not observed, except for the WA fasted hamsters (P < 0.01). Fed SA hamsters maintained larger \( \Delta(T_b - T_a) \) both by day and by night than fed WA animals (P < 0.01). In the latter ones, \( (T_b - T_a) \) differences were the smallest (0.1 ± 0.9 °C by day and 1.4 ± 1.4 °C by night). Fasting affected \( \Delta(T_b - T_a) \) only in WA hamsters in which \( \Delta(T_b - T_a) \) by night
increased from 1.4 ± 1.4°C in fed to 5.7 ± 0.9°C in fasted individuals (P<0.01).

3.5. Activity

Independent of the acclimation regime or food availability, hamsters were always more active by night than by day (F(1,5) = 12.28; P<0.05; Figs. 3 and 8). Although fasted hamsters, either SA or WA, were less active by day than fed ones, the difference did not reach the level of significance (Fig. 8).

4. Discussion

The desert hamster (P. roborovskii) is one of the dwarf hamsters that has never received much scientific attention. The most extensively studied species are the Siberian hamster (P. sungorus) and then the Djungarian hamster (P. campbelli). Two papers by Bao et al. (2002a,b) concerned thermophysiology of the P. roborovskii living in its natural environment, the Kubuqi desert in the Ordos Plateau, Inner Mongolia. At the Ordos Plateau, climate is dry and windy, the summer is hot while the winter is very cold. In summer maximum T_a exceeds 40°C while minimum T_a in winter can decrease below −30°C (Jiang et al., 1995, Bao et al., 2002b). Hence, for small homeothermic mammals living in that environment, winter is an energetic challenge. In the cold, the larger gradient between body and ambient temperatures [Δ(T_b–T_a)] means the higher cost of normothermy because heat production must be increased to equal heat loss, which is necessary to maintain constant body temperature (T_b).

To cope with long and severe winters, small mammals living in the Holarctic Zone employ different strategies: they can change their body mass (m_b), body fat reserves, NST capacity, average T_b, metabolic rate (MR) and energy intake or can enter daily or seasonal torpor (Lyman et al., 1982; Heldmaier et al., 1989; Li et al., 2001; Merritt and Zegers, 2002; Li and Wang, 2005; Lovegrove, 2005; Zhao and Wang, 2006a, b).
Seasonal changes in \( m_b \) are crucial for surviving the winter. A highly photoperiodic species, the \( P. \) sungorus lowers its \( m_b \) towards winter, anticipating forthcoming cold season (Wade and Bartness, 1984; Mercer and Tups, 2003). These changes are controlled by a sliding set-point for \( m_b \) regulation (Klingenspor et al., 2000). Lower \( m_b \) translates into lower total energy requirements of an animal. In the present experiments, the highest \( m_b \) (27.1 ± 1.1 g) was recorded after summer series of experiments. In the natural environment, summer is a time of food abundance. Despite the high costs of reproduction, the energetic cost of normothermy is lower than in other seasons due to relatively high \( T_a \). After 4 months under long photoperiod, SA hamsters were probably in their “autumnal status” characterized by the highest \( m_b \). The hamsters could anticipate forthcoming winter and gain energy (fat) reserves, i.e. \( m_b \). With the time of acclimation to winter-like days, \( m_b \) of \( P. \) roborovskii gradually decreased and after 6 weeks reached the lowest level of 23.4 ± 1.3 g (Fig. 1). Until 13th week of acclimation, \( m_b \) was low but then started to increase. That increase can indicate the time of photorefractoriness, as in \( P. \) sungorus (Hoffmann, 1973; Lynch and Puchalski, 1986; Jefimow et al., 2004).

Similar seasonal changes in \( m_b \) were recorded in \( P. \) roborovskii living in their natural environment. Wild caught animals weighted ~13 g in spring, ~15 g in summer and ~19 g in autumn (Bao et al., 2002a). There are no records of winter \( m_b \), but based on the above data we can presume that \( m_b \) gradually declines as winter progresses. Even if seasonal changes in \( P. \) roborovskii are less profound than in \( P. \) sungorus, physiological meaning of \( m_b \) lowering, namely reduction in the total energy requirements, remains. Although heat loss from an organism increases with a decrease in \( m_b \), seasonal changes in fur insulation and average \( T_b \) as well as selection of proper thermal microenvironment can improve heat conservation leading to a significant energy savings throughout the winter.

During 48-h of measurements in the thermal gradient system, fed hamsters kept constant \( m_b \) while food restriction led to its significant decrease; by the end of experiment fasted hamsters were lighter by about 16%. Initial \( m_b \) was regained on the fifth or sixth day of refeeding, either in summer or winter (Fig. 2). In \( P. \) sungorus, food deprivation also results in significant decrease in \( m_b \). Body mass of Siberian hamsters fasted for 32 h is regained to baseline level on the first day of refeeding, while in fasted hamsters for 56 h \( m_b \) remains significantly lower until the third day postfast (Wood and Bartness, 1996). A long time needed for \( m_b \) regain in \( P. \) roborovskii suggests that for this smaller species 48 h of fasting is much more stressful than 56 h fasting for the larger \( P. \) sungorus. However, despite significant energy deficit, \( T_b \) of \( P. \) roborovskii did not decrease during the measurements and hamsters remained normothermic. \( T_b \) was measured in the interscapular BAT, the main site of regulatory NST in small placental mammals. Maintaining constant \( T_b \) despite the lack of energy supplies indicates a very high NST capacity. As far as we know, \( P. \) roborovskii neither hibernates nor enters daily torpor but its capacity for NST undergoes seasonal variations. The lowest capacity is recorded in summer, intermediate in spring and the highest in autumn (Bao et al., 2002a). Most probably NST capacity in winter would be highest. Puchalski et al. (1983) suggested that keeping high thermogenesis in the face of food shortage is an adaptation to harsh natural environment since in the \( P. \) sungorus 43 h of acute starvation does not affect basal metabolic rate (BMR) and NST capacity despite a decrease in \( m_b \) (Puchalski et al., 1983).

The animals used in this study were acclimated to summer-like days (16L:8D, \( T_a = 26.5 \) °C) and then transferred to winter-like conditions (8L:16D, \( T_a = 10 \) °C). I predicted that body temperature would be lower in WA hamsters than in SA ones. Indeed, independent of food availability, \( T_b \) was lower in WA than in SA hamsters (Figs. 3–5). Lower \( T_b \) reduces the energetic cost of normothermy in winter, when \( T_a \) is low. Clear daily variations in mean \( T_b \) were recorded only in animals acclimated to summer-like days. In WA hamsters only small difference was recorded in fasted individuals (Fig. 4), most probably due to lower \( T_b \) by day than in fed hamsters. During the day, ultradian variations \( \Delta(T_{b max} - T_{b min}) \) were similar in SA and WA animals, but during the night \( \Delta(T_{b max} - T_{b min}) \) was larger in WA than in SA hamsters (Fig. 5). Large ultradian variations can be related to more intense activity but can also ensure additional energy savings (Heldmaier et al., 1989), namely increased tolerance for \( T_b \) variations may result in lower threshold for regulatory thermogenesis.

Body temperature is regulated according to the actual level of set-point for \( T_b \) regulation and may be facilitated by behavioral thermoregulation, e.g. proper \( T_b \) selection (Gordon, 1993). During the rest phase of the day, normothermic animals selected \( T_b \) within their thermo-neutral zone (TNZ) which for \( P. \) roborovskii lies between 28 and 34 °C (Bao et al., 2002b). In summer, minimum MR is as high as 3 ml O₂ g⁻¹ h⁻¹ and is higher than minimum MR in \( P. \) sungorus or \( P. \) campbelli (Weiner and Heldmaier, 1987; Bao et al., 2002b; Chen et al., 2006). High MR in \( P. \) roborovskii contradicts the rule that RMR of animals living in arid and semi-arid environments is lower than predicted for body mass (Haim and Izhaki, 1993). Bao et al. (2002b) suggested that high MR and high upper critical temperature in \( P. \) roborovskii reflect thermal conditions in hamsters’ natural environment, especially long and cold winters.

In the present experiments, both fed and fasted SA hamsters selected \( T_a \) within TNZ from 30.8 ± 0.7 °C in fasted animals by night to 31.4 ± 0.2 °C in the same animals by day (Fig. 6). Acclimation to winter-like days led to increase in selected \( T_a \). The highest \( T_a \) (35.1 ± 0.9 °C) was recorded by day in fed individuals. Since metabolic measurements of TNZ were done in summer (Bao et al., 2002b) and WA animals select \( T_a \) out of TNZ, it its
reasonable to postulate that upper critical temperature is shifted up with season.

Both in summer and winter, fasting did not affect body temperature but the difference between $T_b$ and selected $T_a$ [$\Delta(T_b-T_a)$] was smaller in WA than in SA animals. Only in fasted WA hamsters, $\Delta(T_b-T_a)$ by night was the same as in SA individuals (Fig. 7). Most probably, this is a result of increased activity at that time that led to selection of lower $T_a$'s. Small $\Delta(T_b-T_a)$ in WA hamsters reduces the total costs of normothermy because the heat loss to the environment is smaller.

Present experiments were done after 16 weeks of acclimation to winter-like days, at the time when $m_b$ started to increase. Since this study is the first report on seasonal changes in thermoregulation of $P. \text{roborovskii}$ under laboratory conditions, I did not know the exact pattern of changes in $m_b$. I have expected that $m_b$ would decrease further and thus I was waiting with the commencement of the winter series of experiments. Immediately after $m_b$ started to increase, the experiments on WA animals were done. Thus, selection of higher $T_a$ in WA hamsters might result from the time of experiments. The hamsters were in the photorefractory period and could behave as in spring. At that time, $m_b$ of $P. \text{roborovskii}$ living in the natural environment is the lowest (Bao et al., 2002a) reflecting the smallest body (fat) reserves that were depleted during winter season. Thus, lowering of $\Delta(T_b-T_a)$ would be beneficial because small $\Delta(T_b-T_a)$ enables large energy savings (Studier, 1981; Webb et al., 1993; Wojciechowski et al., 2007).

Hamann (1987) reported that activity pattern of $P. \text{roborovskii}$ kept under seminatural conditions strongly depended on season. In the end of winter (February/March), activity reached the lowest level and animals were active for less than 10 min a day. In the present study, activity was always higher by night than by day, which was reflected in higher $T_{b\text{max}}$ at that time, both in fed and fasted individuals. Although significant differences in general activity between hamsters acclimated to summer- and winter-like days were not recorded (Fig. 8), I observed that fasted WA animals moved rapidly along the whole gradient, while fed animals were more quiet and moved within much narrower zone. This difference in the type of activity can explain large $\Delta(T_b-T_a)$ and relatively low selected $T_a$ by night in fasted hamsters in winter (Fig. 7). In the $P. \text{sungorus}$, food restriction induces increase in locomotor activity reflecting food-seeking behavior. Clear daily rhythm of activity is disrupted and animals are active both by day and by night (Masuda and Oishi, 1995). In summer, fasted hamsters were more quiet than in winter. They were heavier, had larger energy (fat) reserves, sufficient to maintain normothermy if staying in TNZ without a need for rapid food-seeking behavior. Additionally, larger $m_b$ decreases energy expenditure (Merritt and Zegers, 2002).

In conclusion, seasonal changes observed in $P. \text{roborovskii}$ differ from adaptive strategies of $P. \text{sungorus}$ and $P. \text{campbellii}$. In $P. \text{roborovskii}$, lowering of $m_b$ towards winter and lowering of $T_b$ together with improved capacity for regulatory NST is the most important adaptation to seasonally changing environment, especially to cold and long winter. Large capacity for NST maximizes survival in the cold even in the face of food shortage. Selection of proper $T_b$ in winter lowers the energetic cost of keeping high $T_b$ and facilitates maintaining normothermy due to smaller difference between body and ambient temperatures.

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