

Thermal physiology and energetics in male desert hamsters (*Phodopus roborovskii*) during cold acclimation

Qing-Sheng Chi · De-Hua Wang

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Abstract The adjustments in thermal physiology and energetics were investigated in male desert hamsters (*Phodopus roborovskii*) which were acclimated to 5°C for 4 weeks. Mean core body temperature in cold acclimated animals decreased by 0.21°C compared with controls. Further analysis revealed that the decrease mainly occurred in the scotophase, while in the photophase core body temperature remained constant during the whole cold acclimation. Thermogenic capacity, represented by resting metabolic rate and nonshivering thermogenesis increased in cold acclimated hamsters from initial values of 1.38 ± 0.05 and 5.32 ± 0.30 to 1.77 ± 0.08 and 8.79 ± 0.31 mlO₂ g⁻¹ h⁻¹, respectively. After cold acclimation, desert hamsters maintained a relative stable body mass of 21.7 ± 0.1 g very similar to the controls kept at 23°C (21.8 ± 0.1 g). The mean values of food intake and digestible energy (metabolisable energy) in cold acclimated hamsters were 5.3 ± 0.1 g day⁻¹ and 76.3 ± 0.9 kJ day⁻¹ (74.8 ± 0.9), respectively, which were significantly elevated by 76.7 and 80.4% compared to

that in control group. The apparent digestibility was $81.0 \pm 0.3\%$ in cold acclimated animals which was also higher than the $79.7 \pm 0.2\%$ observed in controls. This increase corresponded with adaptive adjustments in morphology of digestive tracts with 20.2 and 36.8% increases in total length and wet mass, respectively. Body fat mass and serum leptin levels in cold acclimated hamsters decreased by 40.7 and 67.1%, respectively. The wheel running turns and the onset of wheel running remained unchanged. Our study indicated that desert hamsters remained very active during cold acclimation and displayed adaptive changes in thermal physiology and energy metabolism, such as enhanced thermogenic and energy processing capacities.

Keywords Desert hamster *Phodopus roborovskii* · Resting metabolic rate, RMR · Nonshivering thermogenesis, NST · Core body temperature · Energy intake · Wheel running · Cold acclimation

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Introduction

Desert hamsters (*Phodopus roborovskii*) mainly inhabit the desert region of the Zaysan Basin and the Tuvinskaya Autonomous Region (Tuva) in the Commonwealth of Independent States, Mongolia, Inner Mongolia and some northern provinces of China at elevations of 1,200–1,450 m (Ross 1994). Desert hamsters, like other small mammals inhabiting temperate and boreal regions, may face severe energetic challenges in the harsh winters typical of these areas. To maintain a high constant core body temperature is potentially very expensive and especially difficult when the ambient temperature is low and food resources are scarce (Speakman 2000; McNab 2002; Van Sant and Hammond 2008). Changes in behavioral and physiological traits are

expected to occur to reduce heat loss and generate more heat as the ambient temperature drops. For example, animals may stay in their nest to reduce the energy cost of foraging (Charnov 1976; Orians and Pearson 1979; Humphries et al. 2005; Zub et al. 2009), allow their body temperatures to fall (Geiser 2004) or huddle together in groups to conserve energy (Kaufman et al. 2003; Scantlebury et al. 2006). For nonhibernating small mammals, elevated thermogenic capacity and reduced thermal conductance are very important to survive the cold (McNab 2002). Thermogenic capacity can be measured as maximum metabolic rate, which is composed of resting metabolic rate (RMR), shivering thermogenesis (ST) and nonshivering thermogenesis (NST) (Heldmaier 1993; Bozinovic et al. 1990; Wunder and Gettinger 1996). NST is mainly produced in brown adipose tissue (BAT) through the function of uncoupling protein 1 (UCP1), which allows proton flux across the mitochondrial inner membrane uncoupled from ATP production to generate heat. NST plays a key role in the cold acclimatization of small mammals (Cannon and Nedergaard 2004; Wunder 1985).

During winter, small mammals may increase food intake to compensate for the increased energy demands. However, digestive efficiency may be compromised unless there are associated digestive changes in gut size, enzyme activity, nutrient uptake and/or food transit time (Sibly 1981; Dykstra and Karasov 1992; Bozinovic 1993; Bozinovic and Nespolo 1997). Siberian hamsters (*Phodopus sungorus*), a well studied *Phodopus* species, decrease body mass and food intake and increase thermogenic capacity (basal metabolic rate, BMR and NST) during winter acclimation (Heldmaier and Steinlechner 1981a; Heldmaier et al. 1982). Siberian hamsters can also experience shallow daily torpor in their diurnal resting phase during winter, primarily in response to changes of photoperiod (Heldmaier and Steinlechner 1981b). Leptin, a hormone primarily synthesized and secreted from adipose tissue, is known to regulate both food intake and body mass (Friedman and Halaas 1998). Decreased plasma leptin is accompanied by hyperphagia in cold-exposed rats (Bing et al. 1998). Freeman et al. (2004) found that although simply reducing leptin concentrations below a threshold value was insufficient for torpor initiation in Siberian hamsters, reduced leptin concentrations nevertheless appeared necessary for its occurrence.

Previous studies did not observe torpor behavior in desert hamsters (Feoktistova and Meschersky 2005; Jefimow 2007). Observations in the field showed that desert hamsters foraged actively at night throughout the whole year (Wan et al. 2007). Because of their small body mass of only around 20 g (Bao et al. 2002b; Wan et al. 2007; Zhan and Wang 2005) heat loss would be expected to be relatively large. So maintaining energy balance in cold will be

challenging for these small mammals. At present we do not yet understand their behavioral and physiological responses that allow them to survive in the cold while remaining continuously active.

In this study, we aimed to explore the potential effects of low ambient temperature on thermal physiology and energetics in desert hamsters. To achieve this, we raised male desert hamsters in the laboratory at an ambient temperature of 23°C and a long photoperiod of 16L:8D. Some animals were then transferred to 5°C for cold acclimation of 4 weeks. Others remained at 23°C as control animals. In both groups, the photoperiod was unchanged. The body mass and core body temperature were monitored throughout the experiment to show the consequence of energy balance and thermoregulation. Changes in thermal capacity were determined by RMR and NST measurement before and after cold acclimation. Wheel running activity was also recorded to reflect animals' activity level or foraging efforts (Mather 1981). Energy metabolism was examined by 3-day feeding trials throughout the experiment. Body composition and serum leptin level were also analyzed in the end of the experiment.

Materials and methods

Animals and housing

Sixteen adult male desert hamsters about 9 months old at the beginning of the experiment were used in this study. They are the first generation offspring of the desert hamsters live-trapped in Hunshandake sandy land (annual average ambient temperature 0–3°C) of Inner Mongolia in August 2007. These male hamsters were housed individually in standard plastic cages (30 cm × 15 cm × 20 cm) with paper towel as bedding (about 4 g in each cage) to facilitate collection of feces and food scraps. The photoperiod and ambient temperature were maintained at 16L:8D (light onset at 0400 h) and 23 ± 1°C, respectively. We provided the animals with rat and mouse maintenance pellet food (crude protein ≥ 18%; crude fat ≥ 4%; coarse fiber ≤ 5%; ashes ≤ 8%; moisture ≤ 10%) (Beijing HFK Bio-Technology Co. Ltd.) and water ad libitum throughout the study. The use of these animals in this experiment is permitted by the Animal Care and Use Committee of Institute of Zoology, the Chinese Academy of Sciences.

Surgery

About 3 weeks before the experiment, each of the 16 male hamsters was implanted intraperitoneally with a temperature transmitter (15.5 mm × 6.5 mm; 1.1 g) (Mini Mitter Model G2 E-Mitter). Transmitters and surgical apparatus were

sterilized prior to surgery by being immersed in a 75% by volume alcohol solution for 30 min. Animals were anaesthetized by injection of pentobarbital sodium (0.5%) with a dose of 30 mg kg⁻¹. After sterilizing the skin with iodophor (Nanjing modern sanitation & anti-epidemic products Co. Ltd), an incision was made on the midline abdominal skin and muscles to open the abdomen. The incision was about 1 cm below the diaphragm and not more than 1 cm in length. After a transmitter was placed in the abdomen gently, the wound was closed with absorbable PGA surgical suture (Jinhuan Model R413, 4/0) and sterilized with iodophor again. Usually the surgery took about 20 min. During the surgery, bags filled with warm water were used to keep animals from hypothermia until they came around from the anaesthetic about 2 h after the injection.

Experimental procedure

This experiment was started in October 2008 and lasted for 6 weeks. At the beginning, hamsters were randomly assigned into two groups with eight animals each. One was a control group in which hamsters were continuously maintained at an ambient temperature of 23 ± 1°C. Animals in the other group experienced a 4-week cold acclimation at 5 ± 1°C, after 2 weeks of baseline measurements of body mass, body temperature, food intake and metabolic rate at 23 ± 1°C. During the whole experiment, photoperiod 16L:8D was kept unchanged for both groups. We identified the day when cold acclimation started as day 1, and hence, the first and last days of the experiment were days -14 and 31, respectively.

Core body temperature and wheel running

Core body temperature and gross activity were recorded telemetrically from the transmitter implanted in the abdomen (Mini Mitter, Model G2 E-Mitter, to ±0.1°C in the temperature range of 33–41°C). Stainless steel 11.5 cm diameter running wheels were attached inside the 16 cages. Voluntary wheel running turns were readily monitored using magnetic reed switches which were connected to a data acquisition system (Mini Mitter, QA-4 Activity Input Module, DP-24 DataPort and a PCI Interface Card). Individual cages were placed on the receiver board (Mini Mitter, Model ER-4000). All receivers and the DP-24 DataPort were connected to a computer with the VitalView software. Data of core body temperature, gross activity and wheel running were collected at 6-min intervals throughout the experiment. Animals fully recovered from the surgery and were accustomed to wheels in about 3 weeks, and then the formal experiment started. Although wheels were available to all the 16 hamsters, only the wheel running data of cold acclimated animals were collected because we

only had one PCI card and could not collect data for the two groups simultaneously. It should be noted that gross activity counts and wheel turns were all expressed as counts per 0.1 h, unless specially indicated.

Body mass and feeding trials

Food trials starting from the first day of the experiment were always conducted at around 1700 h and lasted for 3 days each. At the beginning and end of each trial, body mass was measured with an electronic balance (Sartorius Model BL1500, to 0.1 g). Animals were provided a pre-weighed quantity of food in excess. Uneaten food together with feces was collected after 3 days, separated manually and oven-dried at 60°C to constant mass. Energy content of dry food and feces was determined using an oxygen bomb calorimeter (Parr Instrument, Parr 1281). Energy content of the dry food was 17.9 kJ g⁻¹. Energy intake, digestible energy, metabolizable energy and apparent digestibility (%) were calculated according to Drożdż (1975) and Song and Wang (2006). Metabolizable energy was estimated by assuming urinary loss was 2% of digestible energy (Drożdż 1975; Grodziński and Wunder 1975).

Resting metabolic rate and nonshivering thermogenesis

Metabolic rate was determined by oxygen consumption with an open flow system (Sable, FoxBox). The hamster was placed in a transparent plastic chamber (volume 860 ml, 11 cm × 11 cm × 7.6 cm) with small pieces of paper just enough to absorb animal wastes. An incubator (Yiheng Model LRH-250, Shanghai, China) was used to maintain the chamber at a constant ambient temperature of 30 ± 0.5°C (within the thermal neutral zone 25–33°C of desert hamsters, Zhan and Wang 2004). Fresh air from outside the building was dried using a column filled with DRIERITE desiccants (W. A. Hammond Drierite) then pumped through the chamber at a mass flow rate of 300–400 ml min⁻¹. Before entering the chamber, dry air was warmed by passage through a copper coil inside the cabinet. After passing through the chamber, the gas was subsampled at a flow rate of approximately 100 ml min⁻¹ and dried again using a no chemical gas drier (Sable, ND-2). Then the gas was submitted to oxygen analyzer for analysis (Sable Foxbox). Baseline air measurement was carried out twice at the beginning and end of each metabolic measurement, respectively. Oxygen consumption was calculated using the equation:

$$VO_2 = \frac{FR(FiO_2 - FeO_2)}{1 - (1 - RQ)FiO_2}$$

where FR is the flow rate (STP), FiO₂ is input fractional concentration of O₂ to the chamber, FeO₂ is excurrent

fractional concentration of O₂ from the chamber, and RQ is respiratory quotient (Arch et al. 2006). The RQ was assumed to be 0.85 in this study and the fractional error in VO₂ is 3% (Withers 1977).

Animals were not fasted except when they were in the chamber for the metabolic rate measurements. This has been called RMRt elsewhere to indicate resting metabolism measured in the thermoneutral zone, but in animals that are not necessarily post-absorptive and, therefore, not at basal metabolism levels (Speakman et al. 2004). RMRs were measured twice for each animal. The first was at the end of the second week (day 1), i.e., the day before cold acclimation for the cold acclimated group. The second measurement of RMRt was conducted at the last day of the experiment. Each RMRt measurement was lasting for 2 h and was followed by another 45 min for measurement of NST. The maximum capacity for NST was induced by a subcutaneous injection of noradrenaline (NA) solution (0.2 mg ml⁻¹) with a dosage (NE (mg kg⁻¹) = 2.53 M^{-0.4}) suggested by Wunder and Gettinger (1996). Body mass was weighed before and after each metabolic measurement. The means were used to calculate the mass-specific metabolic rates. We took 5-min least variable and lowest VO₂ average as RMRt (Duarte et al. 2009). Maximum NST was considered the highest 3-min average after NE injection (Heldmaier et al. 1982; Van Sant and Hammond 2008). Previous studies (Ross 1994; Jefimow 2007) and our observations showed that desert hamsters are nocturnal animals, and that time of day has an impact on NST determination (Haim et al. 1995) so all the metabolic measurements were completed between 0800 and 1700 h in the middle of the light phase.

Serum leptin assays

At the end of the experiment, all the 16 animals were sacrificed by inhaling overdose carbon dioxide between 0900 and 1200 h. Blood samples were collected, cooled with ice and centrifuged at 1,830g for 15 min. Serum leptin concentrations were assayed by radioimmunoassay (RIA) with the ¹²⁵I Multi-species Kit (Linco, Cat. No.XL-85K). Serum leptin values were determined in a single RIA. The lower and upper limits of the assay kit were 1 and 50 ng ml⁻¹, respectively. The intra-assay variability was <3.6% (Zhang and Wang 2006).

Body composition analysis

After the animals were sacrificed, interscapular BAT was removed and weighed. The entire gastrointestinal tract and vital organs were also removed from the body cavity. Wet and dry mass of the following organs were measured: heart,

lungs, liver, spleen, kidneys, testis, stomach, small intestine and large intestine and cecum (Mettler PB153, to 0.001 g). We also measured the length of stomach, small intestine and large intestine and cecum using a ruler to the nearest 1 mm as previously described by Liu and Wang (2007). Digesta mass was estimated by comparing the difference between the wet mass of digestive tract with and without contents. The eviscerated carcass (without interscapular BAT) and viscera were dried separately to constant weight at 60°C for determination of dry mass. Total body fat was extracted from the dried carcass in a Soxhlet apparatus using petroleum ether (Foss Model Soxtec Avanti 2050) (Li and Wang 2005).

Statistics

Data were analyzed using SPSS 13.0. Changes of body mass, food intake, energy intake, digestible energy and apparent digestibility over time were tested by repeated measures analysis of variance and LSD test when appropriate. Independent *t* tests were used to analyze the differences in body mass, core body temperature, gross activity counts, food intake, energy intake, digestible energy, apparent digestibility and serum leptin levels between two groups. When performing the body composition analysis, we also used independent *t* tests to detect the differences in focused parameters between two groups. Paired sample *t* tests were used to test the difference in wheel running turns before and after cold acclimation in animals of cold acclimated group. Differences in RMRt and NST between two successive measurements in same groups were also assessed by paired sample *t* test. Because there was no significant difference in body mass between groups, we did not use body mass as a covariate when above parameters were analyzed. Linear regression analysis was used to determine the statistical significance of a correlation between food intake, body fat mass and serum leptin level also between RMR and NST measured at same ambient temperature. Rhythm analysis of core body temperature, gross activity and wheel running was performed using software Chronos-Fit (version 1.05, Zuther and Lemmer 2005). Rhythm analysis was combination of a partial Fourier analysis and a stepwise regression technique. While conventional partial Fourier analysis included all harmonics in the fitted model, the rhythm analysis fitted each harmonic separately and checked significance by *F* test for each. The most significant harmonic was then included to the model if it improved the existing model significantly (*P* < 0.05). *F* statistics and %rhythm for the best model were given. %rhythm is chronobiological term for the coefficient of determination, i.e., the squared coefficient of correlation times 100 (%rhythm = *R*² × 100). It represents the percentage of variation in the data that is

explained by the fitted model (Manual for Chronos-Fit, Zuther and Lemmer 2005). Actograms of the wheel running data were obtained also by Chronos-Fit. All data were presented as mean values \pm SE. $P < 0.05$ was considered to be statistically significant.

Results

Core body temperature and gross activity

There was no significant difference in daily average core body temperature between control and cold acclimated groups during the first two experimental weeks. Significant differences were detected on day 16, day 17 and day 24 between the two groups ($t = 2.261$, $df = 14$, $P < 0.05$; $t = 2.962$, $df = 14$, $P < 0.05$; $t = 2.272$, $df = 14$, $P < 0.05$, respectively). Over the last 4 weeks of experiment, the mean value of daily average core body temperature of the control group was $36.30 \pm 0.01^\circ\text{C}$, while the cold acclimated animals were 0.21°C cooler, with a mean value of $36.09 \pm 0.01^\circ\text{C}$ over the four experimental weeks of cold exposure (Fig. 1).

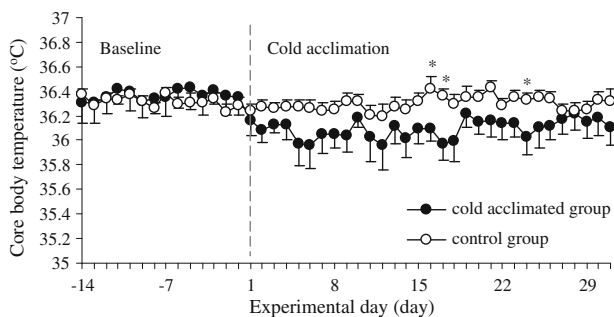
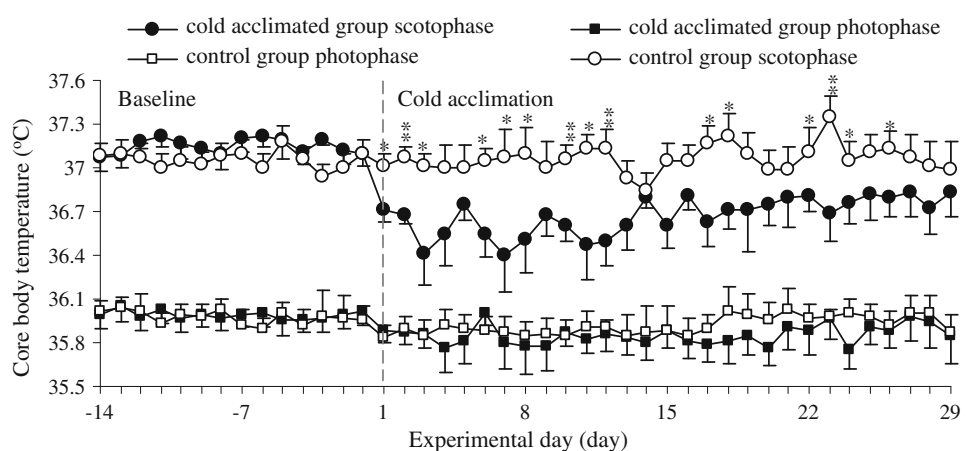


Fig. 1 Effects of cold acclimation on daily average core body temperature in male desert hamsters. An asterisk indicates significant difference between the two groups. The vertical gray dashed line indicates the day when cold acclimation began

Fig. 2 Effects of cold acclimation on photophase and scotophase core body temperature in male desert hamsters. One or two asterisks indicate significant or very significant difference between the two groups, respectively. The vertical gray dashed line indicates the day when cold acclimation began

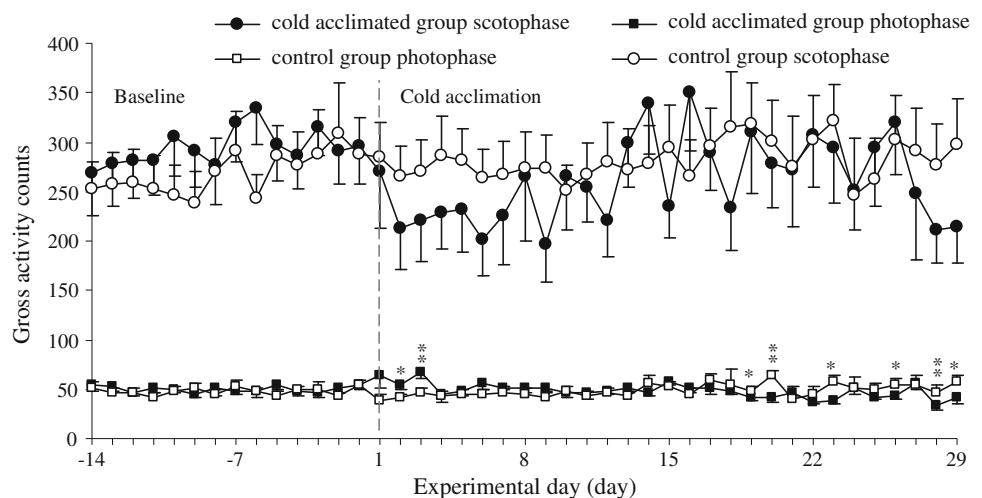


Desert hamsters are typical nocturnal mammals and showed dominating circadian rhythms in core body temperature, gross activity and wheel running behavior in all animals before and after cold acclimation (Period length of the most significant harmonic is 24 h). Rhythm analysis showed that the acrophases of core body temperature, gross activity and wheel running in a representative animal were at 2304, 2207 and 2212 h, respectively. The percentage of variations in the three parameters above that explained by fitted model are 65.22, 49.28 and 53.47%, respectively ($F = 1573.42$, $P < 0.001$; $F = 815.02$, $P < 0.001$ and $F = 964.02$, $P < 0.001$, respectively).

Because of the circadian rhythms in core body temperature and activity, we compared the core body temperature and gross activity between control and cold acclimated groups in the photophase and scotophase separately. There was no significant difference in daily average core body temperature between the two groups in either the photophase or scotophase in the first two experimental weeks (baseline). During the last 4 weeks of the experiment, there were significant differences between the two groups in the scotophase core body temperature from day 1 to day 26 (t values from 2.423 to 4.256, $df = 14$, $P < 0.05$) (Fig. 2). The mean value of the scotophase core body temperature in the cold acclimated group was $36.67 \pm 0.02^\circ\text{C}$, which was 0.39°C lower than the $37.06 \pm 0.02^\circ\text{C}$ in the control animals. No significant difference was detected in the photophase core body temperature between the two groups. The mean value of the photophase core body temperature in cold acclimated animals was $35.85 \pm 0.01^\circ\text{C}$, while a very similar value of $35.92 \pm 0.01^\circ\text{C}$ was observed in the controls (Fig. 2).

There was no significant difference in the scotophase gross activity counts between the two groups during the entire experiment. Over the last 4 weeks of experiment, the mean value of scotophase gross activity counts in the control group was 287.8 ± 2.5 , while it was 260.3 ± 7.8 in the cold acclimated group (Fig. 3). Significant differences

Fig. 3 Effects of cold acclimation on photophase and scotophase gross activity (expressed as average value of gross activity counts per 0.1 h) in male desert hamsters. *One or two asterisks* indicate significant or very significant difference between the two groups, respectively. The *vertical gray dashed line* indicates the day when cold acclimation began



were detected in the photophase gross activity between groups during the last 4 weeks but not in the first 2 weeks (t values from 2.382 to 4.772, $df = 14$, $P < 0.05$). However, the mean value of photophase gross activity counts in the two groups over the last 4 weeks were very close, 48.6 ± 1.4 in the control and 48.4 ± 1.2 in the cold acclimated group (Fig. 3).

Resting metabolism rate and nonshivering thermogenesis

RMRt and NST in control group did not change significantly throughout the experiment. The initial RMRt and NST in animals of the control group were 1.38 ± 0.08 and 5.53 ± 0.32 $\text{mlO}_2 \text{g}^{-1} \text{h}^{-1}$, which were measured at the end of the first two experimental weeks (baseline). While the final RMRt and NST were 1.40 ± 0.04 and 5.67 ± 0.23 $\text{mlO}_2 \text{g}^{-1} \text{h}^{-1}$, respectively. The RMRt and NST in the cold acclimated group were elevated by 28.2 and 65.2%, respectively, from initial value 1.38 ± 0.05 and 5.32 ± 0.30 to 1.77 ± 0.08 and 8.79 ± 0.31 $\text{mlO}_2 \text{g}^{-1} \text{h}^{-1}$ at the end of the experiment ($t_{\text{RMR}} = -8.026$, $df = 7$, $P < 0.01$; $t_{\text{NST}} = -8.945$, $df = 7$, $P < 0.01$) (Fig. 4).

There was no significant difference between the two groups in the initial RMRt and NST values. RMRt in the cold acclimated group increased significantly after 4-week cold acclimation compared to initial and final RMRt in the control group ($t = 3.499$, $df = 14$, $P < 0.01$; $t = 4.075$, $df = 14$, $P < 0.01$). The final NST in the cold acclimated group also increased significantly compared to initial and final NST in the control group ($t = 7.224$, $df = 14$, $P < 0.01$; $t = 8.044$, $df = 14$, $P < 0.01$) (Fig. 4).

There was a significant negative relationship between the RMRt and NST measurements at baseline when all the animals were housed at 23°C ($F_{1, 15} = 12.354$, $P < 0.01$). The linear regression equation was $\text{NST} = -3.3535$ (RMRt) + 10.167 ($R^2 = 0.4688$, $n = 16$) (Fig. 5).

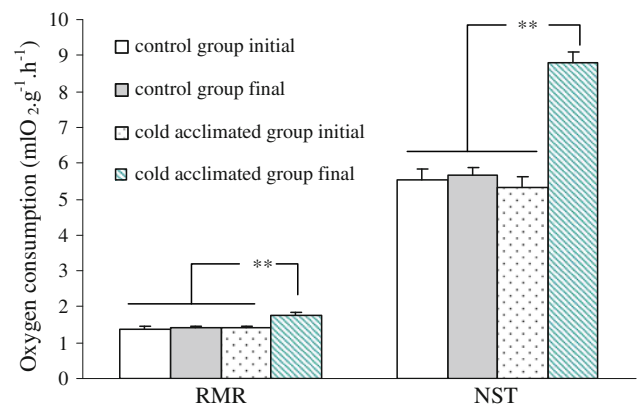


Fig. 4 Effects of cold acclimation on resting metabolism rate and nonshivering thermogenesis in male desert hamsters. *Two asterisks* indicate very significant difference in same parameter between different measurements

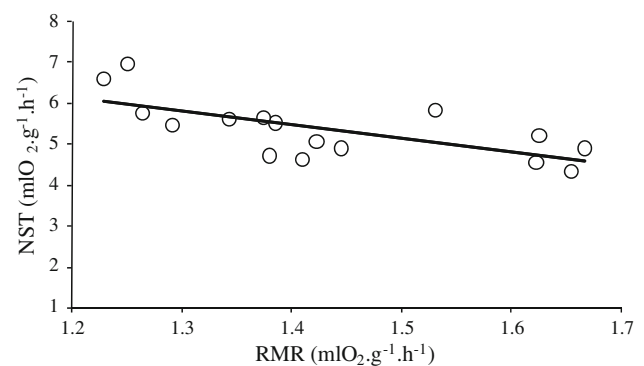


Fig. 5 Relation between RMRt and NST in male desert hamsters raised at 23°C

Body mass

Body mass in the control group remained constant throughout the experiment with a mean value of

21.8 ± 0.1 g ($F_{15,105} = 1.800$, $P > 0.05$, repeated measures ANOVA). No significant differences were detected in body mass between control and cold acclimated groups during the entire experiment. The mean value of body mass in cold acclimated animals was 21.7 ± 0.1 g over the last two experimental weeks ($F_{4,28} = 2.310$, $P > 0.05$, repeated measures ANOVA).

Energy metabolism

Food, energy and digestible energy intake (metabolizable energy intake) in the control group remained constant throughout the experiment with average values of 3.0 ± 0.0 g day⁻¹, 53.1 ± 0.4 and 42.3 ± 0.3 kJ day⁻¹ (41.4 ± 0.3), respectively ($F_{15,105} = 1.621$, $P > 0.05$, $F_{15,105} = 1.055$, $P > 0.05$, $F_{15,105} = 1.287$, $P > 0.05$, respectively; repeated measures ANOVA) (Fig. 6). Very significant differences were detected from day 3 to the end of the experiment between the two groups in food intake (t values from 6.408 to 10.075, $df = 14$, $P < 0.001$), energy intake (t values from 6.487 to 12.226, $df = 14$, $P < 0.001$) and digestible energy (metabolizable energy)

(t values from 6.663 to 12.922, $df = 14$, $P < 0.001$). On day 3, food intake, energy intake and digestible energy (metabolizable energy) in cold acclimated animals were 4.2 ± 0.2 g day⁻¹, 75.6 ± 2.3 and 61.9 ± 1.8 kJ day⁻¹ (60.7 ± 1.8), respectively. There were no significant differences among the measurements of food intake, energy intake, digestible energy (metabolizable energy) from day 6 to the end of the experiment in cold acclimated animals, with mean values of 5.3 ± 0.1 g day⁻¹, 94.2 ± 1.1 and 76.3 ± 0.9 kJ day⁻¹ (74.8 ± 0.9), respectively ($F_{8,56} = 0.532$, $P > 0.05$, $F_{8,56} = 0.681$, $P > 0.05$, $F_{8,56} = 0.633$, $P > 0.05$, respectively; repeated measures ANOVA). No significant difference was detected in apparent digestibility among different measurements in the control group animals with a mean value of 79.7 ± 0.2% ($F_{15,105} = 1.410$, $P > 0.05$, repeated measures ANOVA). Significant differences in apparent digestibility were detected between the two groups on day 3, day 6, day 21, day 24, day 27 and day 30 (t values from 2.313 to 2.720, $df = 14$, $P < 0.05$). There were no significant differences among the last four measurements in cold acclimated animals, the mean value of which was 81.0 ± 0.3% ($F_{3,21} = 1.222$, $P > 0.05$, repeated measures ANOVA) (Fig. 6).

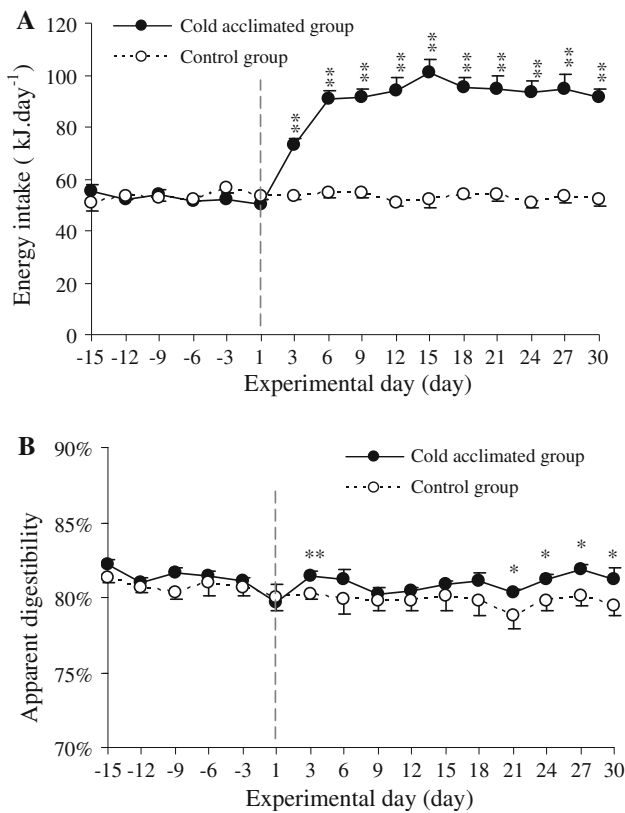


Fig. 6 Effects of cold acclimation on energy intake (a) and apparent digestibility (b) in male desert hamsters. One or two asterisks indicate significant or very significant difference between the two groups, respectively. The vertical gray dashed lines indicate the day when cold acclimation began

Wheel running behavior

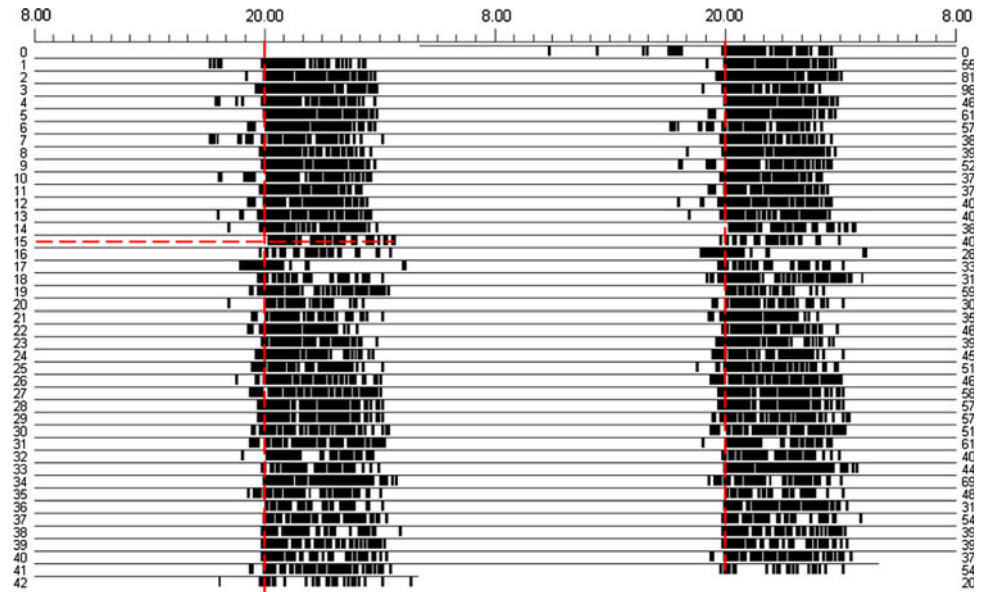
To determine the effects of cold acclimation on wheel running behavior, data of the wheel running turns in cold acclimated animals were pooled, averaged and expressed as turns per day over the first two experimental weeks and the last two experimental weeks separately. However, there was no significant difference in average wheel running turns before and after cold acclimation, with mean values of 9376.9 ± 1865.8 and 9176.4 ± 2098.6 turns day⁻¹, respectively ($t = 0.98$, $df = 7$, $P = 0.925$).

Wheel running data of cold acclimated hamsters over the entire six experimental weeks were used to generate actograms to show the changes in timing of above average wheel running behavior (Fig. 7). Cold acclimation had no effects on the onset of above average wheel running activity.

Body composition analysis and leptin assays

There was no significant difference in body mass, wet carcass mass, interscapular BAT mass, mass of vital organs like lungs, liver, spleen and kidneys, total digesta mass and dry mass of digestive tracts (total or different sections) between cold acclimated hamsters and those in control group at the end of experiment. However, body fat mass, dry carcass mass and wet and dry testicle mass in cold acclimated animals decreased significantly by 40.7, 17.1, 44.8 and 36.4%, respectively compared to that in control

Fig. 7 Double-plot actogram of wheel running turns in a representative cold acclimated male desert hamster. On top is the clock hour. On the left and right are the experimental day and the mean wheel running turns over the day, respectively. The black section in each line represents values above the mean of the day, while the white sections represent the values below the mean. Vertical dashed lines indicate the time when scotophase started. Horizontal dashed lines indicate the day when cold acclimation began



group, while wet and dry heart mass and total length and wet mass of digestive tracts in cold acclimated animals increased significantly by 21.3, 23.1, 20.2 and 36.8%, respectively (Table 1). Among different sections of the alimentary tracts of cold acclimated animals, the length of the stomach remain unchanged, while the length of small intestine, large intestine and cecum increased by 13.8, 34.6 and 38.4%, respectively. Wet mass of the stomach also remained stable, while in the small intestine, large intestine and cecum increased by 46.2, 50.0 and 38.5%, respectively, in the cold acclimated group (Table 1).

Serum leptin levels were $5.0 \pm 0.38 \text{ ng ml}^{-1}$ in cold acclimated desert hamsters, 67.1% lower than the control group which had a mean value of $15.2 \pm 2.8 \text{ ng ml}^{-1}$ ($t = 3.847$, $df = 14$, $P < 0.01$). There was a significant positive relationship between serum leptin levels and body fat mass ($F_{1,15} = 98.986$, $P < 0.001$). The fitted regression equation was leptin (ng ml^{-1}) = 9.2825 (fat mass, g) – 9.3003 ($R^2 = 0.8833$, $P < 0.001$, $n = 16$) (Fig. 8). Linear regression was also very significant between food intake and leptin levels ($F_{1,15} = 9.945$, $P < 0.01$). The regression equation is food intake (g) = -0.1181 (leptin, ng ml^{-1}) + 5.5109 ($R^2 = 0.4334$, $P < 0.01$, $n = 16$). There was also a significant linear relationship between the body fat mass and food intake ($F_{1,15} = 19.818$, $P < 0.01$). The regression equation is food intake (g) = -1.527 (fat mass, g) + 7.420 ($R^2 = 0.586$, $P < 0.01$, $n = 16$). Then we used multiple linear regression analysis to compare effects of body fat mass and serum leptin level on food intake. Although the F ratio ($F_{2,15} = 9.447$, $P = 0.003$) indicated that the model as a whole had statistically significant predictive capability, neither of the t ratios ($t_{\text{body fat}} = -1.866$, $P = 0.085$ or $t_{\text{leptin}} = 0.453$,

$P = 0.658$) reached a significant level. Anyway, the effect of body fat mass was greater than serum leptin level on food intake because $t_{\text{body fat}}$ was higher than t_{leptin} .

Discussion

Core body temperature and thermogenesis

Core body temperature in heterothermic endotherms during torpor usually falls from high normothermic values of ~ 32 to 42°C to values between -3 and 30°C (Geiser 2004). During the entire experiment, all desert hamsters maintained a high core body temperature almost always above 34°C . No signs of torpor were detected in any of the hamsters of the present study by continuous monitoring of core body temperature and gross activity. This suggests that desert hamsters may defend a relative high body temperature during cold exposure in the wild at least when without food shortage. However, it should be acknowledged that in Siberian hamsters the key factor that triggers torpor expression is reduced photoperiod, rather than reduced temperature alone (Heldmaier and Steinlechner 1981b). We cannot, therefore, completely rule out the possibility that desert hamsters may also show torpor if both temperature and photoperiod are simultaneously manipulated. Actually, we had found in another experiment that desert hamsters reared at 23°C could show reduced core body temperature below 30°C when food was deprived (unpublished data).

Theoretically, a small mammal could gain an energetic advantage by regulating core body temperature at a lower level during cold acclimation (Vaanholt et al. 2007).

Table 1 Effects of cold acclimation on body composition in male desert hamsters

	23°C (<i>n</i> = 8)	5°C (<i>n</i> = 8)	<i>P</i> value
Body mass (g)	22.0 ± 0.7	21.9 ± 0.7	0.92
Interscapular BAT (g)	0.050 ± 0.006	0.048 ± 0.005	0.72
Body fat mass (g)	2.7 ± 0.2	1.6 ± 0.1	<0.001**
Wet carcass mass (g)	15.5 ± 0.5	14.5 ± 0.43	0.15
Dry carcass mass (g)	7.6 ± 0.4	6.3 ± 0.2	0.010*
Vital organs			
Wet mass (g)			
Heart	0.155 ± 0.008	0.188 ± 0.008	0.010*
Lungs	0.201 ± 0.010	0.205 ± 0.013	0.802
Liver	0.918 ± 0.062	1.005 ± 0.051	0.294
Spleen	0.027 ± 0.003	0.025 ± 0.002	0.682
Kidneys	0.253 ± 0.007	0.280 ± 0.014	0.116
Testicles	0.688 ± 0.060	0.380 ± 0.051	0.001**
Dry mass (g)			
Heart	0.039 ± 0.002	0.048 ± 0.002	0.012*
Lungs	0.050 ± 0.002	0.050 ± 0.003	0.860
Liver	0.271 ± 0.017	0.298 ± 0.011	0.200
Spleen	0.007 ± 0.001	0.007 ± 0.001	0.729
Kidneys	0.063 ± 0.002	0.068 ± 0.003	0.182
Testicles	0.110 ± 0.008	0.070 ± 0.010	0.009**
Digestive tracts			
Length (mm)			
Stomach	27.0 ± 1.1	27.2 ± 1.6	0.891
Small intestine	278.5 ± 6.5	316.9 ± 12.8	0.021*
Large intestine	101.0 ± 3.6	135.9 ± 6.9	0.001**
Cecum	46.4 ± 5.3	64.2 ± 5.7	0.037*
Total	452.8 ± 12.8	544.2 ± 23.2	0.005**
Wet mass (g)			
Stomach	0.195 ± 0.005	0.215 ± 0.010	0.114
Small intestine	0.351 ± 0.059	0.513 ± 0.100	0.046*
Large intestine	0.122 ± 0.011	0.183 ± 0.012	0.002**
Cecum	0.156 ± 0.015	0.216 ± 0.017	0.018*
Total	0.824 ± 0.066	1.127 ± 0.116	0.045*
Dry mass (g)			
Stomach	0.045 ± 0.002	0.047 ± 0.002	0.45
Small intestine	0.059 ± 0.014	0.067 ± 0.019	0.726
Large intestine	0.025 ± 0.004	0.028 ± 0.004	0.665
Cecum	0.025 ± 0.002	0.030 ± 0.003	0.193
Total	0.154 ± 0.021	0.173 ± 0.025	0.576
Digesta (g)			
Stomach	0.543 ± 0.055	0.396 ± 0.035	0.037*
Small intestine	0.682 ± 0.101	0.836 ± 0.056	0.189
Large intestine	0.195 ± 0.026	0.199 ± 0.042	0.934
Cecum	0.482 ± 0.037	0.645 ± 0.043	0.013*
Total	1.901 ± 0.131	2.075 ± 0.095	0.291

Independent sample *t* test was used to detect the effects of cold acclimation

*** Significant or very significant difference between the two groups, respectively

Supporting this suggestion there was a 0.21°C decrease in average core body temperature in the cold acclimated desert hamsters. Such change in core body temperature

suggested that it was an energy saving adaptation. Mean core body temperature in Siberian hamsters was also lowered during cold exposure of 5°C in long photoperiod.

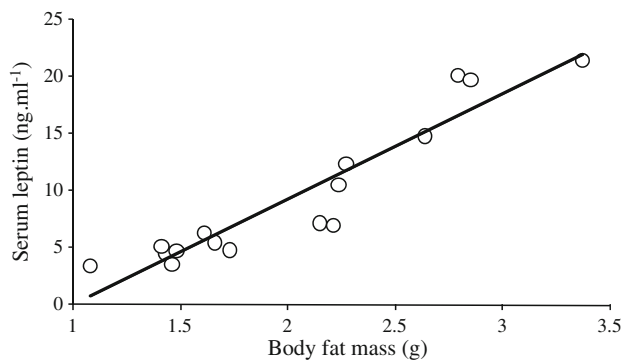


Fig. 8 Relation between body fat mass and serum leptin levels in male desert hamsters

In addition, there appeared larger regular ultradian fluctuation of core body temperature and gross activity in those hamsters (Heldmaier et al. 1989). This change in temporal organization allowed them to be active with a high level of body temperature and metabolism rate, but energy was saved during extended resting phase with body temperature adjusted to minimum levels (Heldmaier et al. 1989). However, such adaptive changes in temporal organization of body temperature and gross activity were not detected in desert hamsters, for dominating circadian rhythms of these variables were maintained throughout the experiment in cold acclimated animals. The decrease of body temperature in desert hamsters mainly occurred during the scotophase, when these typically nocturnal animals are active. The core body temperature in the scotophase of the cold acclimated animals was 0.39°C lower than that in the controls, while in the photophase the core body temperatures were not significantly different between the two groups. The photophase core body temperature in these hamsters was around 35.9°C, which was similar to the 36.2°C in *Peromyscus polionotus* (14 g) and 35.6°C *Peromyscus leucopus* (33 g) (Mortola and Lanthier 2004). These daily low values of core body temperature may be adapted to maximize energy saving while ensuring normal activity levels possible.

Basal metabolic rate predicted using equations suggested by McNab (2008) [for mammalia, $BMR (mlO_2 g^{-1} h^{-1}) = 3.50 W^{-0.279} (g)$ and for Rodentia, $BMR (mlO_2 g^{-1} h^{-1}) = 4.5 W^{-0.325} (g)$] in desert hamsters are 1.48 and 1.64 $mlO_2 g^{-1} h^{-1}$, respectively. RMRt in hamsters of the control group would be expected to exceed the BMR prediction and yet it was only 93.0 and 84.1% of the predicted BMR values. A lower RMRt should reduce the energy costs for maintenance in desert hamsters to facilitate survival. This is consistent with the observation that small mammals inhabiting desert environments often have a lower BMR than predicted (Haim and Izhaki 1993). This has been suggested to be an adaptation to minimize heat production in an environment where heat stress may

be a regular issue during summer (Speakman and Krol 2010). RMRt in cold acclimated animals increased by 28.2% which may reflect more efficient food processing, oxygen delivery systems and thermogenic tissue (Nespolo et al. 2001; Van Sant and Hammond 2008). This was still 14.1% lower however than the value in winter-acclimated (0–5°C) Siberian hamsters with a body mass of 25 g (Heldmaier and Steinlechner 1981a) and 21.2% lower than the RMRt observed in cold acclimated field voles (McDevitt and Speakman 1994b). Although an increase in RMRt after cold acclimation has been observed in many previous studies (Zhang and Wang 2006; Nespolo et al. 2001; McDevitt and Speakman 1994b), in some species BMR remains unchanged even after long periods of cold acclimation, for example in deer mice (*Peromyscus maniculatus*) (Van Sant and Hammond 2008). In some species, high BMR has been associated with increased over-winter survival (Jackson et al. 2001; Larivée et al. 2010) but this is not a universal finding (Sadowska et al. 2009).

Haim and Izhaki (1993) found a negative correlation between BMR and NST across more than 20 rodent species from arid and semi-arid environments and concluded that high NST is a compensation for the low BMR in those animals, even when not cold acclimated (at 25 or 28°C; photoperiod 12L:12D). A similar correlation was detected intra-specifically in the present study in desert hamsters when raised at 23°C. NST in cold acclimated desert hamsters was increased by 65.2% compared to that in the controls. This was even larger than the 40% increase that Van Sant and Hammond (2008) found in deer mouse (body mass, 24.0 ± 4.39 g) after similar cold acclimation. Bao et al. (2002a, b) also found that maximum NST was elevated by 92.3% during winter in wild desert hamsters. Hence, NST plays an important role during cold acclimation in desert mammals. Cold is an effective stimulus to enhance the NST capacity, however, nonthermal stimulus like photoperiod alone can also altered it as found in Siberian hamsters and wood mice (*Apodemus sylvaticus*) (Heldmaier and Steinlechner 1981a, b, 1982; Haim et al. 1995). Feist (1983) found thermogenesis enhanced in cold acclimated and winter acclimatized red-backed voles (*Clethrionomys rutilus*). Seasonal acclimatization to winter in red-backed voles appears to involve an increase in beta-adrenergic receptors in brown fat, but cold acclimation does not, which suggested quantitative and possibly qualitative differences in neural and hormonal stimulation of brown fat between cold acclimation and winter acclimatization (Feist 1983). This might be the case in desert hamster too. NST was determined at 30°C in the present study. About 5 min after the injection of NA, animals usually lied down on their abdomen to facilitate heat dissipation. Such a behavior was similar with what we observed in subsequent NA tests at 25°C ($n = 4$).

Although no abnormal behavior was detected in all animals during or after NA tests at both 25 and 30°C, we recorded a highest peak core body temperature of 40.29°C (body mass 26.0 g) among hamsters at 30°C, while at 25°C the highest peak value was only 38.64°C (body mass 21.5 g). Hence, 25°C is also an advisable choice to carry out the NST measurement in desert hamsters to avoid hyperthermia (Heldmaier et al. 1982).

Body mass, energy assimilation, digestive tracts and vital organs

To meet increased energy requirement and maintain the body mass, it took the hamsters about 6 days to increase their energy intake to levels that then remained significantly elevated for the rest of the cold exposure. This slow increase was consistent with the fact that during short term cold exposure of a single day small mammals may reduce their intake (McDevitt and Speakman 1994a) and short-tailed field voles (*Microtus agrestis*) took 10 days to reach their peak intake during longer term cold exposure (McDevitt and Speakman 1994b). The large increase in energy processing capacity during cold acclimation was probably achieved by distinct adaptive changes in the digestive tract (Hammond and Wunder 1991). However, in other species similar increases in food intake were not associated by such hypertrophic responses (McDevitt and Speakman 1994b). As summarized by Hammond and Wunder (1995), changes in length are more likely reflective of the ability of the tract to stretch and hold more digesta, while changes in mass of the tract reflect changes in uptake rate of nutrients and capacity as well as changes in volume of the digestive tracts. The total length and total wet mass of digestive tracts were increased significantly in cold acclimated desert hamsters, although there was no significant difference in total dry mass of digestive tracts and total digesta mass between groups. However, changes in wet mass of tissues of digestive tracts are also correlated with changes in uptake capacity of nutrients, without accompanying changes in dry mass (Hammond and Diamond 1994; Konarzewski and Diamond, 1994, Hammond and Wunder 1995). Mean retention time is the quotient of digestive volume and food intake ($MRT = \text{digestive volume}/\text{food intake}$) (Dykstra and Karasov 1992; Penry and Jumars 1987). As hamsters were sacrificed in the photophase, the similar digesta mass observed in two groups may reflect a shorter mean retention time in cold acclimated hamsters. Decrease of stomach digesta mass in cold acclimated hamsters by 37.1% may be a result of quick gastric emptying. Dry and wet heart mass in cold acclimated animals were increased significantly. In general, such changes in the vital organs are indicative of metabolic output (Hammond and Wunder 1995). Reproductive

activity might be suppressed in cold acclimated male desert hamsters, as suggested by the decrease in dry and wet mass of testicles.

Body fat, serum leptin and food intake

In the present study, desert hamsters maintained body mass when exposed to the cold, but decreased body fat content by 40.7%. The serum leptin level in cold acclimated animals also decreased significantly. The significant linear relationship between leptin levels and body fat mass was consistent with many previous studies showing secretion of leptin was positively correlated to body fatness (Friedman and Halaas 1998; Zhang and Wang 2006). It is known that lower serum leptin level in cold acclimated animals can act as a starvation signal and enable animals to increase energy intake (Bing et al. 1998; Korhonen and Saarela 2005; Li and Wang 2005; Zhang and Wang 2006). Lowered serum leptin level during lactation may act in a similar manner (Speakman 2008; Cui et al. 2010). However, in the present study this effect seemed weak in hamsters because when the effect of body fat mass was controlled, serum leptin level could not reach a significant level. Furthermore, body fat mass seemed a more powerful predictor of food intake than serum leptin level.

Wheel running behavior

There were two distinct previous suggestions regarding the significance of wheel running in captive animals. For a long time, this behavior was related to natural behaviors like exploratory migration (Mather 1981). In contrast, Sherwin (1998) considered it was an artifact of captive environment without any equivalent in natural behavior patterns. Here, we suggest wheel running in desert hamsters is probably a behavior related to foraging, based on the fact that rhythms of gross activity and wheel running were closely synchronous with onset of darkness. This timing of the wheel running behavior was consistent with the night foraging habit in hamsters observed in the wild (Wang et al. 2001).

A significant decrease in locomotive activity represented by wheel running turns was not detected as an expected energy saving strategy in cold acclimated animals. This was quite different to what Vaanholt et al. (2007) found in mice in which wheel running activity (distance run) was decreased by around 50% after animals were transferred from 20 to 10°C. During the cold acclimation, there was also no significant change in timing of the wheel running behavior in these hamsters. This suggested that hamsters would not change the onset time of foraging when facing similar cold temperature in the wild. In their natural habitat where the parents of these hamsters were caught, ambient temperature at night can drop below 10°C even in summer

(X. R. Wan, personal communication). Hence, hamsters should be adapted to the low ambient temperature and remain active in cold conditions. This was also consistent with the fact that desert hamsters display foraging behavior at all four seasons in their natural habitat (Wang et al. 2001, 2007).

Summary

Cold acclimation significantly enhanced thermogenic capacity (RMRt and NST levels) in male desert hamsters. Food intake and the digestible energy were also greatly increased and facilitated by morphological changes in their digestive tracts. These acclimatory changes allowed the hamsters to maintain a high body temperature and constant body mass in the cold. Desert hamsters remained active during the cold acclimation by maintaining a high level of wheel running activity. There was also no obvious change in the onset of wheel running. These finding was consistent with the fact that desert hamster usually forage in the cold night. Since short photoperiod had profound effects on energy balance and thermoregulation in small mammals (Heldmaier et al. 1989), the biology of cold acclimation in *P. roborovskii* might be even more complex than revealed in the present study.

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