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ORIGINAL PAPER

Hibernation patterns of Turkish hamsters: influence of sex and ambient temperature

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Abstract Turkish hamsters (*Mesocricetus brandti*) are a model organism for studies of hibernation, yet a detailed account of their torpor characteristics has not been undertaken. This study employed continuous telemetric monitoring of body temperature (T_b) in hibernating male and female Turkish hamsters at ambient temperatures (T_as) of 5 and 13 °C to precisely characterize torpor bout depth, duration, and frequency, as well as rates of entry into and arousal from torpor. Hamsters generated brief intervals of short (<12 h), shallow test bouts ($T_b > 20$ °C), followed by deep torpor bouts lasting 4–6 days at $T_a = 5$ °C and 2–3 days at $T_a = 13$ °C. Females at $T_a = 5$ °C had longer bouts than males, but maintained higher torpor T_b; there were no sex differences at $T_a = 13$ °C. Neither body mass loss nor food intake differed between the two T_as. Hamsters entered torpor primarily during the scotophase (subjective night), but timing of arousals was highly variable. Hamsters at both T_as generated short, shallow torpor bouts between deep bouts, suggesting that this species may be capable of both hibernation and daily torpor.

Keywords Turkish hamster · Hibernation · Torpor · Sex differences · Food intake

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Abbreviations

T_b	Body temperature
T_a	Ambient temperature
IBI	Interbout interval

Introduction

Mammalian torpor, characterized by a controlled reduction in body temperature (T_b) and metabolic rate, permits substantial energy savings under adverse environmental conditions (Lyman et al. 1982; Geiser and Ruf 1995; Heldmaier et al. 2004), and is an important survival strategy for a phylogenetically diverse array of species (Geiser and Ruf 1995; Lovegrove 2011). Detailed description of torpor patterns provides the foundation for comparative analyses, and is a prerequisite for elaborating the evolutionary and physiological underpinnings of hibernation and daily torpor (e.g., Geiser and Ruf 1995; Carey et al. 2003; Heldmaier et al. 2004; Lovegrove 2011). Moreover, baseline information about typical torpor behavior allows researchers to assess the impacts of changes in food availability, nutritional status, and/or climate change on hibernation (e.g., Lovegrove et al. 2001; Humphries et al. 2002; Humphries et al. 2003, Angilletta et al. 2010). Finally, because torpor affects other life history traits, including reproduction (e.g., Oxberry 1979; Barnes et al. 1986; Turbill et al. 2011) and longevity (e.g., Lyman et al. 1981; Turbill et al. 2011, 2012), specification of torpor patterns is essential for understanding seasonal adaptations of hibernators and provides a more comprehensive picture of a species' biology.

Turkish hamsters (*Mesocricetus brandti*) are of particular interest for studies of biological rhythms, including the circannual hibernation cycle, because they differ from virtually all other photoperiodic rodents. Most species

undergo gonadal regression—a prerequisite for hibernation in males—in response to a surge in melatonin secretion, which in turn is stimulated by exposure to short days; removal of the pineal gland, which disrupts melatonin secretion, prevents gonadal regression (referenced in Butler et al. 2008). In contrast, Turkish hamsters undergo gonadal regression not only in response to long duration melatonin signals, but also in response to very short duration melatonin signals or the complete absence of melatonin; these conditions occur upon removal of the pineal gland and/or exposure to very long days (>17L) or constant light (Carter et al. 1982; Hong et al. 1986; Butler et al. 2008; Jarjisian and Zucker 2011). Only one other species—the European hamster (*Cricetus cricetus*)—responds to pinealectomy in this manner (Masson-Pévet et al. 1987). In both species, suppression of melatonin secretion in nature is likely limited to intervals during which hamsters are torpid.

Turkish hamsters have been a model organism for studies of hibernation as it relates to longevity (Lyman et al. 1981), reproductive endocrinology (Hall and Goldman 1980; Hall et al. 1982; Hall and Goldman 1982; Goldman et al. 1986; Goldman and Darrow 1987), photoperiodism and melatonin (Hall and Goldman 1982; Hall et al. 1982; Darrow et al. 1986; Goldman et al. 1986; Goldman and Darrow 1987; Goldman 1989), diet (Bartness et al. 1991), and oxidative stress (Yigit et al. 2008). Despite extensive use of Turkish hamsters in hibernation research, basic aspects of their torpor behavior (e.g., bout length, depth, frequency, interbout intervals, etc.) remain to be established.

Past estimates of hibernation characteristics for this species—derived exclusively from daily observations of posture, respiration, displacement of sawdust or oats on the dorsum, and/or responsiveness to a puff of air (e.g., Hall and Goldman 1980; Hall et al. 1982; Lyman et al. 1983)—do not permit precise calculations of bout length, depth of torpor, or timing of entry into or arousal from torpor. Moreover, previous studies that maintained ambient temperature (T_a) as low as 3 °C (Bartness et al. 1991) and as high as 10 °C (Hall and Goldman 1980, 1982; Hall et al. 1982) reported a range of estimates for bout duration, which is known to vary inversely with torpor T_b in other hibernating rodents (e.g., Twente and Twente 1965; Geiser and Kenagy 1988; Buck and Barnes 2000). Some early studies of Turkish hamster hibernation conflated the effects of variable T_as and day lengths (e.g., Lyman et al. 1983), which complicates the evaluation of the relative contributions of these environmental factors to torpor patterns.

In the present study, Turkish hamsters were kept in a fixed short day length at one of two fixed T_as (5 and 13 °C). Continuous telemetric monitoring of T_b was employed to precisely characterize torpor behavior, and food intake was monitored as an index of energy consumption. In addition to providing detailed information on torpor characteristics, this study yields new insights into the effects of sex and ambient temperature on torpor behavior in this species.

Materials and methods

Animals

Male ($n = 12$) and female ($n = 12$) Turkish hamsters from the local breeding colony (See Butler et al. 2008 for details) aged 5–12 months were maintained from birth in 16L (16 h light/day, lights on at 0200 h) and 22 ± 2 °C. Hamsters were individually housed in 46 × 25 × 19 cm polypropylene cages containing Tek-Fresh Lab Animal Bedding, and provisioned with food (Harlan Teklad Rodent Diet 8664) and water ad libitum. All animal procedures were approved by the Animal Care and Use Committee of the University of California, Berkeley (institutional approval # R084-0911C) and conformed to the NIH Guide for the Care and Use of Laboratory Animals.

Prior to placement in cold chambers, hamsters were transferred to short days (10L; lights on at 0700 h) at 22 ± 2 °C. Females were held under these conditions for at least 4 weeks, and males for at least 6 weeks. SD exposures of these respective durations render females anovulatory and induce testicular regression in males (Stetson and Hamilton 1981; Hong et al. 1986; Hall et al. 1982); reproductive quiescence facilitates entry into hibernation in this species (Hall and Goldman 1980, 1982; Hall et al. 1982). Hamsters were subsequently moved to cold chambers maintained at either 5 ± 1 or 13 ± 1 °C (males, $n = 6$; females, $n = 6$ at each T_a), with the same 10L light cycle. Males and females were distributed evenly within each cold chamber. Animal monitoring was carried out between 0800 and 1000 h each day. Maximum and minimum cold chamber T_as were recorded daily to the nearest 0.1 °C with a calibrated digital thermometer. Hamsters remained in cold chambers until transmitter batteries failed, at which point they were returned to 16L and $T_a = 22 \pm 2$ °C.

Data from three 13 °C females were omitted from all analyses due to faulty transmitters (see below).

Recording of T_b

T_b was recorded telemetrically using radiotransmitters (model VM-FH; approx. 1.5 cm^3 and 3 g; MiniMitter, Sunriver, OR). Transmitters were coated in wax and calibrated using a water bath (30–38 °C) prior to implantation. Hamsters were deeply anesthetized using isoflurane vapors, and transmitters implanted intraperitoneally via a single midline incision, which was closed using sterile suture.

Hamsters received perioperative injections of 0.05 ml meloxicam (5 mg base/mL) and 0.3 mL of dilute buprenorphine (0.3 mg base/mL diluted 1:10 in sterile saline). The same postoperative doses of analgesics were administered 8 h later and every 8 h thereafter as needed. Hamsters recovered in 10L, 22 ± 2 °C conditions for at least 10 days before transfer to cold chambers. Once in the cold chambers, T_b data were collected every 10 min via receiver boards under each animal's cage. Data were transmitted and stored on a computer by the program Dataquest (St. Paul, MN, USA).

Torpor parameters

To account for differences among individual hamsters (Barclay et al. 2001), each hamster's mean normothermic T_b was calculated from data obtained during the first 72 h in the cold chamber. Torpor thresholds were set 1 °C below the lowest T_b exhibited by each individual during this interval. To identify the beginning and end of torpor bouts, T_b had to be at or below the threshold for three consecutive measurements (beginning of torpor), or above the threshold for three consecutive measurements (end of torpor). Torpor bout duration was calculated as the amount of time spent at or below the threshold. Interbout interval (IBI) was defined as the time between the end of one torpor bout and the onset of the next.

Test bouts were defined as those in which the T_b decrease did not achieve a stable value. Deep torpor bouts were those wherein T_b reached a plateau a few degrees above T_a; minimum T_b was measured at this nadir. The temperature difference between T_b and T_a ($T_b - T_a$) during deep torpor bouts was calculated using the minimum T_b and minimum T_a during each bout.

For test bout duration, minimum T_b, $T_b - T_a$, and IBI, mean values were calculated for each hamster; to avoid weighting data from individuals that generated higher than average numbers of bouts, these individual means were used in subsequent statistical analyses. Minimum and maximum values were used to generate individual ranges for test bout duration, test bout minimum T_b, and test/deep bout IBIs.

Hamsters at $T_a = 5$ °C were not disturbed by our presence in the cold chamber, but at $T_a = 13$ °C hamsters sometimes stirred in response to the opening of the chamber door (essential for animal monitoring). Thus, the times of entry into and arousal from torpor were analyzed only for hamsters held at $T_a = 5$ °C. Additionally, for deep bout duration, each hamster's maximum bout length was analyzed to reduce bias resulting from possible disturbance-induced arousal. Each hamster's longest deep torpor bout was also assessed to calculate its rate of entry into and arousal from deep torpor. Overall rates were calculated using normothermic T_b and minimum T_b as anchoring points. Rates of entry and arousal were also calculated over several T_b ranges, after Kauffman et al. (2001) as described in Table 2.

Body mass and food intake

Body mass was recorded when hamsters were transferred to short days, as well as when they were placed in and removed from the cold chamber. Due to an oversight, four hamsters at $T_a = 5$ °C were not weighed upon removal from the cold chamber.

Food intake was monitored as an index of energy consumption in a subset of hamsters ($n = 7$) at $T_a = 5$ °C, and in all hamsters ($n = 9$) at $T_a = 13$ °C. Cages were provisioned with approximately 250 g of food, and pellets remaining in the food hopper were weighed 1 week later. Pellets hoarded on cage bottoms were collected and included in food mass measurements. Fresh food was provided after each measurement. Because chow pellets absorb moisture at low T_as, 250 g of food was placed in an empty cage each week in each cold chamber, and re-weighed 1 week later to correct for moisture-induced inflation in food weight.

Treatment groups were balanced with respect to body mass at the time of placement in cold chambers, but to avoid disturbance of torpor (particularly at $T_a = 13$ °C), body mass was not measured weekly. Food intake values are therefore presented as gram food consumed/hour spent normothermic, rather than as mass-specific values. We used telemetric data to calculate the amount of time each hamster spent normothermic over the same weekly intervals during which food intake was measured. Food intake was compared between T_as during an initial week of normothermia, and 1, 3, and 5 weeks after the onset of deep torpor bouts.

Statistical analyses

All statistics were performed using JMP 7.0 (SAS Institute Inc., Cary, NC, USA). Except where noted, pairs of means were compared using unpaired *t* tests. More than two means were compared using one-way ANOVAs; significant results were followed with post hoc Tukey–Kramer HSD tests. Relationships between variables were analyzed by linear regression analyses. Differences were considered significant if $P < 0.05$. All data are presented as mean ± SEM.

Results

Normothermia and shallow torpor bouts

Normothermic T_b during the first 72 h of cold exposure at T_as of 5 and 13 °C was 36.9 ± 0.1 °C (groups combined),

and did not differ between the sexes or between T_as (Fig. 1).

At both T_as, most hamsters initiated test bouts within 3 weeks of entry into the cold. Females initiated test bouts sooner than males (7 ± 2 vs. 18 ± 5 days at 5 °C and 16 ± 1 vs. 19 ± 6 days at 13 °C), but these differences were not significant. There was no significant relationship between duration of 10L exposure prior to cold and the timing of initiation of test bouts for either sex at either T_a.

Mean test bout durations, T_b minima, IBIs and ranges for each of these parameters at both T_as are summarized in Table 1. There were no significant differences between sexes or T_as.

Most hamsters generated shallow bouts for fewer than 2 weeks prior to the first deep torpor bout, although three individuals (one 5 °C male and two 13 °C females) manifested shallow bouts for more than 3 weeks. Even after the onset of deep torpor bouts, 9 of 12 hamsters of both sexes (75 %) at $T_a = 5$ °C and 7 of 9 hamsters (78 %) at $T_a = 13$ °C continued to generate shallow, short bouts (<11 h in $T_a = 5$ °C and <20 h in $T_a = 13$ °C) interspersed among deep, multi-day torpor bouts (Fig. 2).

Deep torpor

Deep torpor bouts exceeded 24 h at both T_as. Maximum bout length at $T_a = 5$ °C was significantly longer for females than males; a comparable difference was not evident at $T_a = 13$ °C (one-way ANOVA and post hoc Tukey–Kramer HSD tests; $P < 0.05$; Fig. 3). Hamsters of both sexes at $T_a = 5$ °C had significantly longer bouts than their counterparts at $T_a = 13$ °C (one-way ANOVA and post hoc Tukey–Kramer HSD tests; $P < 0.05$; Fig. 3).

T_b fluctuated by ≤0.5 °C during deep torpor, likely reflecting minor fluctuations in T_a. At $T_a = 5$ °C, minimum T_b during deep torpor bouts was significantly higher for females than for males (unpaired t test; $P < 0.01$; Fig. 1). Mean minimum T_bs were 2.8 ± 0.3 °C (females) and 0.0 ± 0.6 °C (males) above minimum T_as, which ranged from 4.4 to 5.5 °C. At $T_a = 13$ °C, minimum T_b did not differ significantly between sexes (Fig. 1). Under these conditions, mean minimum T_bs were 0.9 ± 0.2 °C (females) and 0.6 ± 0.1 °C (males) above minimum T_as, which ranged from 11.4 °C to 12.0 °C. The $T_b - T_a$ gradient maintained by females at $T_a = 5$ °C was significantly higher than that maintained by all other sex-T_a groups (one-way ANOVA and post hoc Tukey–Kramer HSD tests; $P < 0.05$).

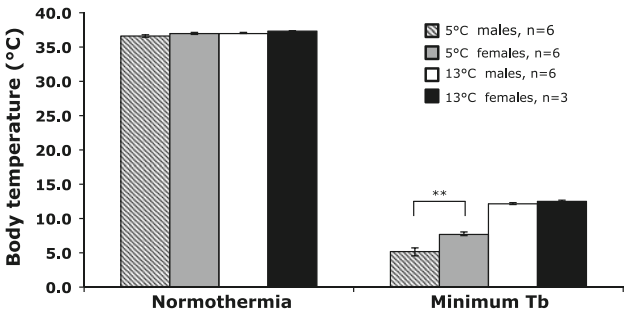

Fig. 1 Mean T_b during the first 72 h of cold exposure (normothermia) and mean minimum T_b during deep torpor (minimum T_b). *Error bars* represent SEM. $^{**}P < 0.01$

Table 1 Test bout duration, minimum T_b, and interbout interval (IBI) at $T_a = 5$ and 13 °C

	5 °C ($n = 12$)	13 °C ($n = 9$)
Bout duration (h)	4.0 ± 0.5 (range 1.3 ± 0.3–7.9 ± 0.8)	5.4 ± 0.7 (range 1.9 ± 0.7–11.5 ± 1.1)
Minimum T_b (°C)	27.4 ± 0.8 (range 21.3 ± 1.5–32.2 ± 0.6)	26.5 ± 1.5 (range 19.1 ± 1.7–31.3 ± 1.3)
IBI (h)	32.9 ± 4.8 (range 15.6 ± 5.0–53.0 ± 5.3)	25.2 ± 4.1 (range 7.4 ± 3.1–55.0 ± 7.7)

Mean IBIs after deep bouts commenced were 22.8 ± 1.9 and 25.0 ± 2.2 h long at $T_a = 5$ and 13 °C, respectively. Mean, minimum, and maximum IBI did not differ between sexes or T_as.

Timing of torpor

A total of 231 torpor bouts were analyzed at $T_a = 5$ °C (Fig. 4). Entry into torpor occurred significantly more often (89 %) during the scotophase (subjective night) than during the photophase (subjective day) (Chi-square goodness of fit test; $P < 0.01$). Timing of arousal was more variable, with 59 % of arousals occurring during the scotophase ($P > 0.05$).

Hamsters at $T_a = 5$ °C showed significantly steeper overall rates of T_b decline, and cooled more rapidly from T_bs of 25–13 °C than hamsters housed at $T_a = 13$ °C (Table 2, one-way ANOVA and post hoc Tukey–Kramer HSD tests, $P < 0.05$). Cooling rates from normothermic T_b to 25 °C did not differ as a function of T_a. There were no sex differences in overall rates of entry into torpor or in rates of cooling from normothermic T_b to 25 °C. From T_bs of 25–13 and 13 °C to minimum T_b, however, males at $T_a = 5$ °C cooled significantly more quickly than females ($P < 0.05$); this difference was not apparent at $T_a = 13$ °C (one-way ANOVA and post hoc Tukey–Kramer HSD tests).

Arousal was achieved more rapidly by hamsters at $T_a = 13$ °C than by those at $T_a = 5$ °C (one-way ANOVA and post hoc Tukey–Kramer HSD tests; $P < 0.05$). From T_bs of 13–25 °C, however, this difference in arousal rate was significant only for males, and there was no difference between T_as from 25 °C to normothermic T_b. Neither

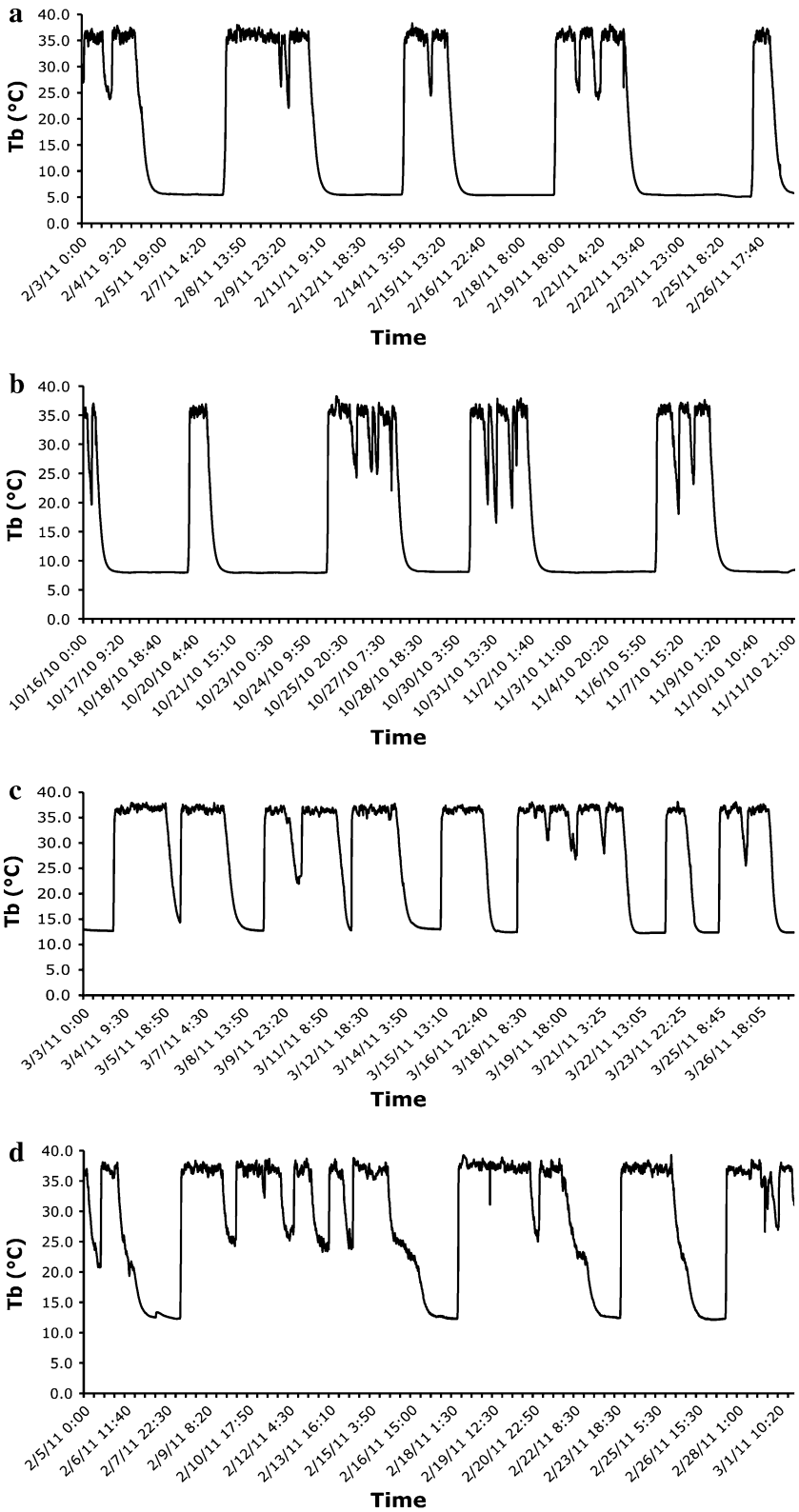

Fig. 2 T_b for a male at 5 °C (**a**), a female at 5 °C (**b**), a male at 13 °C (**c**), and female at 13 °C (**d**) over the course of ~25 consecutive days. For hamsters at both T_as, short, shallow torpor bouts were interspersed among deep, multi-day bouts

overall rates of arousal, nor rewarming from 25 °C to normothermic T_b differed between the sexes, but males at $T_a = 5$ °C rewarmed more rapidly than females from minimum T_b to 13 °C and from 13–25 °C (one-way ANOVA and post hoc Tukey–Kramer HSD tests; $P < 0.05$).

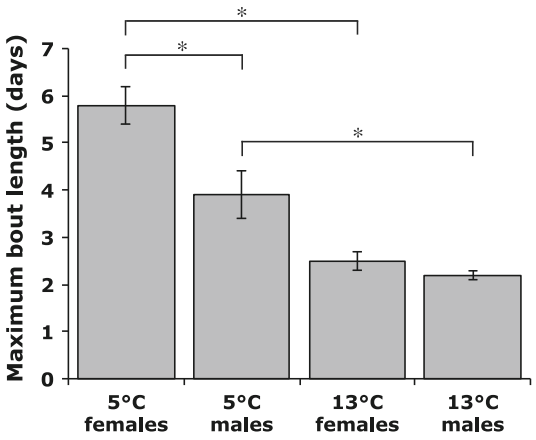

Fig. 3 Maximum bout lengths were longer at $T_a = 5$ °C than at $T_a = 13$ °C, and at $T_a = 5$ °C females had longer maximum bout lengths than males. *$P < 0.05$

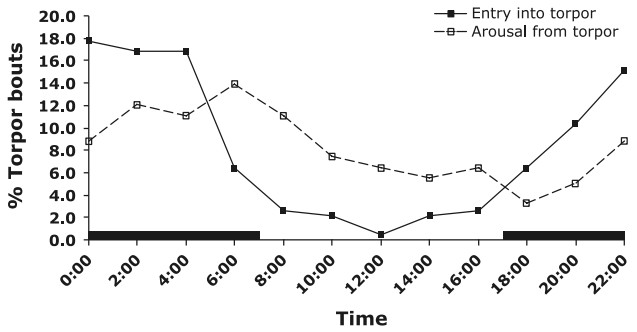

Fig. 4 Times of entry into (*solid line*) and arousals from (*dashed line*) 231 torpor bouts at $T_a = 5$ °C. Entry into torpor occurred predominantly during the scotophase (1700–0700 h; *black horizontal bar*). Timing of arousals was variable

Body mass and food intake

Initial body mass at 16L, 22 ± 2 °C was 138.0 ± 5.8 g for females and 153.1 ± 4.9 g for males ($P > 0.05$). Male body mass decreased (−2.9 ± 1.1 g) during 6–11 weeks of housing in 10L, 22 ± 2 °C (paired t test; $P < 0.05$), but

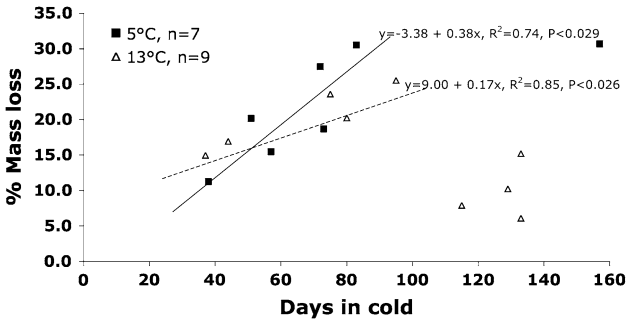

Fig. 5 Body mass decline as a function of time in the cold. At $T_a = 5$ °C, mass loss leveled off at ~30 % for hamsters housed in the cold for >80 days. At $T_a = 13$ °C, mass loss peaked at ~25 % for hamsters housed in the cold for 75–95 days, but was lower for individuals that spent more or less time in the cold. Regression lines for time <100 days in the cold are shown for $T_a = 5$ °C (*solid line*) and $T_a = 13$ °C (*broken line*)

mass loss was not significantly related to time spent in 10L prior to placement in cold chambers. Female body mass did not decrease during housing in 10L, 22 ± 2 °C. At the time of transfer to cold chambers, body mass did not differ significantly between the sexes.

Hamsters were in the 5 °C chamber for a shorter time (73 ± 10 days) than those housed at 13 °C, (93 ± 12 days), reflecting differences in transmitter battery lifespan. All but one hamster ($T_a = 5$ °C) lost weight in the cold; this individual was excluded from mass loss analyses. There were no sex differences in percent mass loss. At $T_a = 5$ °C, mass loss increased with time spent in the cold, reaching a peak of ~30 % for individuals in the cold >80 days. At $T_a = 13$ °C, maximum mass loss was ~25 % in individuals in the cold for 75–95 days, and was lower for those that spent either more or less time in the cold (Fig. 5). For hamsters exposed to cold for <100 days, percent mass loss was significantly predicted by the number of days spent in the cold at both $T_a = 5$ °C [% mass loss = −3.38 + 0.38 (days in cold), $R^2 = 0.74$, $P < 0.029$] and $T_a = 13$ °C [% mass loss = 9.00 + 0.17 (days in cold), $R^2 = 0.85$, $P < 0.026$)]. Neither the slopes

Table 2 Rates of entry into and arousal from torpor (°C/h) at $T_a = 5$ and 13 °C

	5 °C males ($n = 6$)	5 °C females ($n = 6$)	13 °C males ($n = 6$)	13 °C females ($n = 3$)
Overall rate of entry	1.46 ± 0.09[a]	1.36 ± 0.04[a]	1.17 ± 0.03[b]	1.03 ± 0.11[b]
Normothermia–25 °C	4.21 ± 0.34[a]	3.58 ± 0.25[a,b]	3.20 ± 0.35[a,b]	2.22 ± 0.44[b]
25–13 °C or T_{bmin}	3.55 ± 0.21[a]	2.75 ± 0.12[b]	0.80 ± 0.03[c]	0.73 ± 0.05[c]
13 °C–T_{bmin}	0.54 ± 0.04[a]	0.42 ± 0.02[b]	N/A	N/A
Overall rate of arousal	12.84 ± 0.43[a]	11.35 ± 0.43[a]	17.79 ± 1.09[b]	16.12 ± 0.52[b]
T_{bmin}–13 °C	5.85 ± 0.45[a]	3.87 ± 0.20[b]	N/A	N/A
13 °C or T_{bmin}–25 °C	30.90 ± 2.48[a]	22.67 ± 1.39[b]	16.14 ± 0.72[c]	15.62 ± 1.15[b,c]
25 °C–normothermia	19.66 ± 1.89[a]	19.74 ± 1.40[a]	21.49 ± 3.00[a]	16.72 ± 0.51[a]

[a,b,c] Values in the same row that do not share a common letter differ significantly ($P < 0.05$)

nor intercepts of these regression lines differed significantly between the two T_as (ANCOVA; $P > 0.05$).

Normothermic food intake could not be calculated for one individual that entered torpor within a week of placement in the cold chamber. In addition, some hamsters' transmitters failed before they had experienced 3 or 5 weeks of deep torpor, and these individuals were excluded from food intake analyses for those time points; numbers of hamsters included at each time point are indicated in Fig. 6. Nonetheless, at each time point groups were balanced with respect to initial body mass. Food intake did not differ significantly between T_as at any time point; however, we detected trends (unpaired t tests; $P < 0.06$) toward higher consumption at $T_a = 5$ °C during normothermia, and toward higher consumption at $T_a = 13$ °C during the 5th week of deep torpor (Fig. 6).

Discussion

Despite the extensive use of Turkish hamsters in hibernation research (Hall and Goldman 1980; Lyman et al. 1981; Hall et al. 1982; Hall and Goldman 1982; Darrow et al. 1986; Goldman et al. 1986; Goldman and Darrow 1987; Bartness et al. 1991; Yigit et al. 2008), the present study is the first to describe in detail the basic torpor characteristics of this species. In contrast to studies that found variable rates (~20–30 %) of non-responsiveness to short-day cold challenges (e.g., Hall and Goldman 1980; Goldman and Darrow 1987; Bartness et al. 1991), all our hamsters hibernated readily. After brief intervals of short (generally <12 h) shallow ($T_b > 20$ °C) test bouts, hamsters generated multi-day deep torpor bouts. There were no differences in shallow torpor bout duration, depth, or frequency between T_as, but deep bouts were longer at $T_a = 5$ °C (4–6 days) than at $T_a = 13$ °C (2-3 days). Previous studies of Turkish hamster hibernation that maintained T_a as low as 3 °C (Bartness et al. 1991) and as high as 10 °C (e.g., Hall and Goldman 1980, 1982; Hall et al. 1982) reported a range

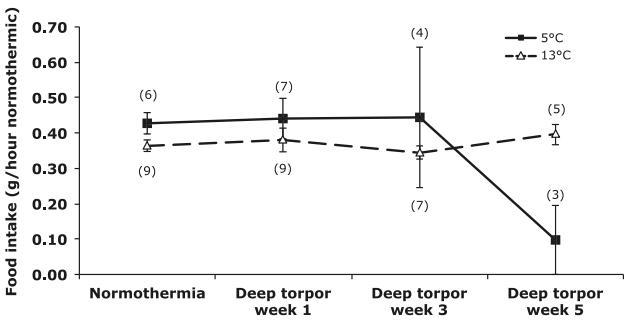

Fig. 6 Food intake did not differ between the two T_as during normothermia or at week 1, 3, or 5 after the onset of deep torpor. The number of individuals at each time point is in *parenthesis*

of estimates for bout duration. Consistent with previous work on other hibernating rodents, which shows an inverse relationship between torpor bout duration and torpor T_b provided that torpid animals are thermoconforming (e.g., Twente and Twente 1965; Geiser and Kenagy 1988; Buck and Barnes 2000), torpor bout duration in Turkish hamsters was inversely related to T_b during torpor, except in the case of sex differences (discussed below).

We found that whereas females at $T_a = 5$ °C maintained T_b nearly 3 °C above T_a, males maintained T_b within only 1 °C of T_a. This difference was not apparent at $T_a = 13$ °C, suggesting that females have a higher critical minimum temperature than males. Despite maintaining a higher minimum T_b, females at $T_a = 5$ °C generated longer bouts than males. This difference is partially attributable to the fact that females took longer to reach T_b nadirs and longer to rewarm to normothermia, but is ultimately difficult to reconcile in light of numerous studies—including this one—indicating an inverse relationship between bout length and depth (e.g., Twente and Twente 1965; Geiser and Kenagy 1988; Buck and Barnes 2000). To our knowledge, this study is the first to report a sex difference in critical minimum temperature; the potential energetic and fitness consequences of this difference merit further investigation.

Hamsters lost up to 30 % of their initial body mass during the first 3–4 months in the cold, as reported previously (e.g., Hall and Goldman 1982; Hall et al. 1982; Goldman and Darrow 1987). Hamsters residing in the 13 °C cold chamber beyond this point had lower % mass loss; recovery of body mass was likely coincident with gonadal recrudescence (Hall and Goldman 1982; Hall et al. 1982).

Turkish hamsters do not fatten in preparation for hibernation, unlike most other deep hibernators (Lyman and O'Brien 1977; French 1988), but rather hoard food and continue to eat during periodic arousals (Vander Wall 1990). Neither body mass loss during the first 100 days in the cold, nor normothermic food intake during the first 5 weeks of deep torpor differed between T_as. The energetic costs may be similar at both T_as, resulting in similar patterns of food intake and mass loss. Hamsters at $T_a = 5$ °C generated significantly longer torpor bouts, but interbout intervals were similar at both T_as, and consequently, arousals from torpor were more frequent at $T_a = 13$ °C. If the higher costs of normothermia and arousal at $T_a = 5$ °C were offset by fewer total arousals—which account for the majority of a hibernator's winter energy expenditure (Wang 1978; Körtner and Geiser 2000), then the overall cost of living at $T_a = 13$ °C may be similar to that at $T_a = 5$ °C. A second possibility is that digestive conversion efficiency increased with the depth and duration of torpor, so that hamsters at $T_a = 5$ °C offset the higher costs of normothermia and arousal by extracting more calories

from the same amount of food, as has been reported for the chipmunk *Tamias striatus*, another food-hoarding species (Humphries et al. 2003). A third possibility is that limitations of sample size prevented detection of differences in food intake between T_as; perhaps consistent with this idea, we detected only a trend ($P < 0.06$) toward higher food intake at $T_a = 5$ °C during an initial period of normothermia. Additional work using respirometry would likely help to discriminate between these hypotheses.

Entries into torpor occurred almost exclusively during the scotophase, as also occurs in golden mantled ground squirrels (Ruby et al. 2002) and Syrian hamsters (Oklejewicz et al. 2001). It is notable that both nocturnally and diurnally active rodent hibernators initiate torpor bouts during the dark phase. Timing of arousals from torpor was variable, with no strong tendency to occur either in the photophase or scotophase. In species that employ daily torpor, timing of arousals appears to be under circadian control—often coupled to either the onset of the species' active phase or the warmest part of the day (Körtner and Geiser 2000)—though timing of arousals is variable in Siberian hamsters (Ruby 2003). Less work has been done on the timing of arousals in hibernators; golden-mantled ground squirrels arouse preferentially during the photophase (Ruby et al. 2002), but Syrian hamsters—closely related to Turkish hamsters—lack circadian organization in the timing of arousals (Oklejewicz et al. 2001).

Like other heterothermic species, Turkish hamsters cool most rapidly during their initial descent into torpor, and cooling slows as T_b approaches its nadir (e.g., Wilz and Heldmaier 2000; Kauffman et al. 2001; Lee et al. 2009). It is difficult to directly compare cooling rates between species, given that differences in T_a, body mass, nest construction, and sociality all potentially affect cooling; nonetheless, both Siberian hamsters (*Phodopus sungorus*) and edible dormice (*Glis glis*) cool at least twice as quickly as Turkish hamsters when housed at 4–5 °C (Kauffman et al. 2001; Wilz and Heldmaier 2000). Alaska marmots (*Marmota broweri*)—much larger than Turkish hamsters and exposed to lower T_a—cool more quickly upon initial descent into torpor, but subsequently cool more slowly than Turkish hamsters as they approach minimum torpor T_b (Lee et al. 2009). Among hamsters at $T_a = 5$ °C, rewarming rates were low at the beginning of arousals and accelerated at T_bs intermediate between minimum T_b and normothermia, as described in other mammals (Hammel 1986, Geiser and Baudinette 1990). Overall rates of arousal in Turkish hamsters are broadly comparable to rates reported for other rodents (Geiser and Baudinette 1990).

A final point worthy of note is that, in most hamsters, shallow torpor bouts were interspersed between deep bouts throughout the hibernation season, in contrast to shallow torpor (so-called test bouts) that many species, including Turkish hamsters, generate at the beginning and end of the hibernation season (Strumwasser 1959; Geiser 2004). Turkish hamsters may thus be capable of both hibernation and daily torpor. Shallow bouts occurred at all times of day and at both T_as. We cannot exclude the possibility that at $T_a = 13$ °C some of these events may reflect premature arousal due to disturbance, but this was not a concern at $T_a = 5$ °C, because hibernators were not disturbed by human presence in the cold chamber. Field observations would be needed to rule out the possibility that these shallow bouts are an artifact of captivity. In addition, studies integrating respirometry data would help determine whether metabolic rate during these short bouts more closely matches the marked reduction in metabolism during bouts of hibernation, or whether the metabolic reduction is shallower, as is the case for most daily heterotherms (Geiser and Ruf 1995; Geiser 2004, but see Wilz and Heldmaier 2000; Geiser and Mzilikazi 2011). Few species are known to utilize both hibernation and daily torpor (e.g., Bartholomew and Hudson 1960; Wilz and Heldmaier 2000; Toussaint et al. 2010), suggesting that Turkish hamsters may be particularly valuable for studying potential physiological differences between these two patterns of heterothermy and the possibility that hibernation evolved from daily torpor.

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