Daily torpor in mice: high foraging costs trigger energy-saving hypothermia

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Many animal species employ natural hypothermia in seasonal (hibernation) and daily (torpor) strategies to cope with energetic challenges. This energy-saving hypothermia is a typical response to fluctuations in food availability, but the relationship between environmental quality, foraging behaviour and torpor responses is poorly understood. We studied body temperature responses of outbred ICR (CD-1) mice exposed to different food reward schedules, simulating variation in habitat quality. Our main comparison was between female mice exposed to low foraging-cost environments and high-cost environments. As controls, we pair-fed a group of inactive animals (no-cost treatment) the same amount of pellets and high foraging-cost animals. Mice faced with high foraging costs were more likely to employ torpor than mice exposed to low foraging costs, or no-cost controls (100% versus 40% and 33% of animals, respectively). While resting-phase temperature showed a non-significant decrease in high-cost animals, torpor was not associated with depressions in active-phase body temperature. These results demonstrate (i) that mice show daily torpor in response to poor foraging conditions; (ii) that torpor incidence is not attributable to food restriction alone; and (iii) that high levels of nocturnal activity do not preclude the use of daily torpor as an energy-saving strategy. The finding that daily torpor is not restricted to conditions of severe starvation puts torpor in mice in a more fundamental ecological context.

Keywords: hypothermia; environmental quality; foraging costs; energy balance

2. MATERIAL AND METHODS

(a) Animals and housing

Virgin female mice (Mus musculus domesticus, Hsd:ICR (CD-1) outbred) were housed at 21 ± 1 °C on an L:D cycle of 12:12 (lights on 0400 GMT +1). At four months of age, we implanted temperature transmitters (Series 3000 XM-FH, Mini Mitter, USA) i.p. under 1.5 per cent isoflurane anaesthesia. Animals were postsurgically injected s.c. with 0.5 mg kg⁻¹ TEMgesic for pain relief and allowed to recover for one week. Body mass (1–2 h before lights out), food intake and wheel-running activity (where applicable) were measured daily.

(b) Experimental manipulation

Detailed methods of the foraging cost manipulation are described by Schubert et al. (2008). Briefly, mice were trained to run in wheels for 45 mg food pellets (TestDiet 5TUM, PJAi, Sandown Chemicals, Hampton, UK) given by a dispenser (Med Associates ENV-203, Sandown Scientific, Hampton, UK) linked to a steering computer (Series 3 Programmable Controller, General Electric). Foraging costs were calculated individually for each animal by collecting baseline data. Low foraging costs were set to the ratio of spontaneous activity to ad libitum food intake, thus representing a balanced energy budget in a priori. High foraging costs started at the baseline level, then increased by 10 per cent every 2 days until 200 per cent of baseline (20 days), and thereafter remained stable. On average, females in the low foraging-cost group needed to run 114 revolutions (50 m) for one 45 mg pellet of food, while mice in the high foraging-cost group needed to run 278 revolutions (122 m) per pellet. As food restriction controls, a no-cost group without running wheels was used. Each of these was pair-fed (once daily, at lights-out) a ration of pellets that matched the previous day’s intake of an individual female from the high foraging-cost group.

We trained 24 mice for the experiment but omitted some animals from analysis due to technical problems (e.g. malfunctioning food dispensers or transmitter batteries). Final sample sizes per group were 5 (low cost), 4 (high cost) and 6 (no cost). We obtained temperature recordings during the baseline period and from experimental day 0 through 20 (depending on transmitter battery life). In addition, we measured euthermic (non-torpor) resting metabolic rate (RMR, kJ m⁻³) once during each experimental period at an ambient temperature of 21°C, placing mice in an open-flow respirometry system for attention for its potential therapeutic applications (Carey et al. 2003), which include neuroprotection in cardiac-arrest patients, preservation of organs for transplantation, and the treatment of neurodegenerative diseases like Alzheimer’s (Malatesta et al. 2007).

Mice are a model for mammalian physiology, but little is known about the biology of natural murine torpor. Although they do not hibernate, house mice (Mus musculus domesticus) exhibit hypothermic responses to overnight fasting (Hudson & Scott 1979), low ambient temperatures (Tomlinson et al. 2007) and pharmacological agents (i.e. adenosine monophosphate (Swoap et al. 2006), H₂S (Blackstone et al. 2005) and 2-deoxy-D-glucose (Freinkel et al. 1972)). Whether these responses are functionally similar to natural torpor has remained unclear (Swoap et al. 2006), in part because manipulations such as fasting entail a non-trivial risk of death (Hudson & Scott 1979).

For small animals exhibiting daily torpor, food consumed during foraging is the most important source of energy (Geiser 2004). To understand the natural context of murine torpor, we studied body temperature regulation in mice exposed to poor environments. We elicited torpor in mice by exposing them to increased foraging costs per food reward (hereafter simply ‘foraging costs’) which simulated increased travel distances between food patches. High and low foraging costs were imposed by exercise-driven reward schedules. To control for the effect of food restriction, an additional group of non-foraging mice received the same amount food as high foraging-cost animals.
showed torpor (binomial GLZ: $\chi^2_{1,15} = 6.36; p = 0.04$; contrasts high–low, high–control, low–control $p < 0.05$). Treatment neither predicted the total number of torpor bouts or minimum torpor temperature, nor did it affect average temperatures in the active or inactive circadian phase (all $p > 0.10$) nor energy intake (kJ; $y = -0.01x + 4.57, R^2 = 0.003, F_{1,6} = 0.02, p > 0.10$) predicted torpor bout duration (h) when averaging data per individual for the subset of animals showing torpor. Assuming that metabolic depression precedes torpor, we estimated metabolic rate in torpor (MR2) from the equation: $MR2 = (MR1 \times (T_b - 2 - T_a)) / (T_b - 1 - T_a)$ (Heldmaier & Ruf 1992); MR1 was euthermic RMR, $T_{b,1}$ was individual euthermic resting-phase temperature (mean $\pm$ s.d. = 35.9 $\pm$ 0.91°C), $T_{b,2}$ was average low torpor temperatures and $T_a$ was ambient temperature (21°C). The change in metabolic rate over time represented energy savings which ranged from 1–8 kJ d$^{-1}$ (figure 2).

### 3. RESULTS

#### (a) General responses

Our experimental manipulation had dramatic effects on both body mass and energy intake (see Schubert et al. 2008). Briefly, while high- and low-cost females ran about the same amount (approx. 9–11 km d$^{-1}$), high-cost females (and their no-cost controls) consumed approximately 25 per cent less energy per day. This directly affected the body mass of high-cost females, which declined steadily over the course of the study with a slope of approximately 0.12 g d$^{-1}$. Estimates of net energy balance from food intake and daily energy expenditure (doubly labelled water method) suggested that high-cost females were in a zero or negative balance, while the other groups remained in a positive balance. All females faced with high foraging costs ceased oestrous cyclicity (Schubert et al. 2008), and of the 10 females who eventually showed torpor, eight had ceased oestrous cyclicity before their first torpor day.

#### (b) Torpor frequency and duration

All mice maintained normal circadian body temperature rhythms in the baseline period. In the experimental period, many animals exhibited daily torpor bouts (figure 1). Although torpor was observed in some mice from each experimental group (table 1), treatment significantly predicted whether an animal ever showed torpor (binomial GLZ: $\chi^2_{1,15} = 6.36; p = 0.04$; contrasts high–low, high–control, low–control $p < 0.05$). On average, torpor bouts lasted longer in high and low foraging-cost groups than the no-cost control group (table 1; linear GLZ: $F_{2,5} = 6.23, p = 0.04$, contrasts of high–control and low–control $p < 0.05$). Treatment neither predicted the total number of torpor bouts or minimum torpor temperature, nor did it affect average temperatures in the active or inactive circadian phase (all $p$-values $> 0.1$).

#### (c) Correlates of torpor bouts

Neither body mass (g; $y = -0.54x + 19.97, R^2 = 0.29, F_{1,6} = 2.452, p > 0.10$) nor energy intake (kJ; $y = -0.01x + 4.57, R^2 = 0.003, F_{1,6} = 0.02, p > 0.10$) predicted torpor bout duration (h) when averaging data per individual for the subset of animals showing torpor. Assuming that metabolic depression precedes torpor, we estimated metabolic rate in torpor (MR2) from the equation: $MR2 = (MR1 \times (T_b - 2 - T_a)) / (T_b - 1 - T_a)$ (Heldmaier & Ruf 1992); MR1 was euthermic RMR, $T_{b,1}$ was individual euthermic resting-phase temperature (mean $\pm$ s.d. = 35.9 $\pm$ 0.91°C), $T_{b,2}$ was average low torpor temperatures and $T_a$ was ambient temperature (21°C). The change in metabolic rate over time represented energy savings which ranged from 1–8 kJ d$^{-1}$ (figure 2).
Table 1. Characteristics of daily torpor in female ICR (CD-1) laboratory mice exposed to varying foraging conditions. Summary data per group are given as means and s.d. Zero or n/a indicates that an animal did not show torpor.

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<th>days scored (no.)</th>
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<th>average active phase body temperature (ºC)</th>
<th>average resting phase temperature (ºC)</th>
<th>total torpor bouts (no.)</th>
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*Average values are presented in parentheses.*

4. DISCUSSION

Female mice exposed to high foraging costs, and to some extent any foraging costs, showed body temperature reductions well below the normal temperature of the inactive circadian phase. The depth and duration of these temperature depressions are consistent with the idea that mice show daily torpor as a response to poor environmental conditions. This is new evidence that torpor in mice is not restricted to conditions of starvation and inactivity, but also occurs in response to high foraging costs in poor quality habitat. As an ecologically relevant strategy to maintain energy balance, natural torpor in mice is likely to be accompanied by a suite of physiological adaptations such as those shown by obligate hibernators (Carey et al. 2003). Our results, therefore, provide an important context for comparing torpor physiology between species and for improving our functional understanding of hypometabolic states.

Although others have reported natural torpor in mice (Hudson & Scott 1979; Tomlinson et al. 2007), ours is the first to show that mice use torpor when food becomes more difficult to obtain. In a sense, mice in our experiment were fed ad libitum, since food was available to them on demand. Rather than increasing activity and expenditure, these animals reduced their total energy budget (Schubert et al. 2008). This somewhat counterintuitive response can be explained by reductions in metabolic rate to balance the reduced energy intake. Although the occurrence of torpor to maintain energy balance in poor foraging conditions has been suggested earlier (Perrigo & Bronson 1983; Vaanholt et al. 2007), ours is the first study to document this phenomenon.

Experimental animals did not appear to decrease their active phase temperature. In this respect, body temperature regulation in mice differs drastically from responses of food-deprived rats, which seem incapable of torpor and show gradual declines in both resting and active-phase body temperature when food restricted (Severinsen & Munch 1999).

Although we chose a strict criterion (<30 ºC) to classify torpor, mice are likely to reduce temperature to varying extents on a daily basis. Future studies profiling temperature rhythms in detail will allow us to relate energy balance to torpor behaviour, but our data have already shown that reduced food intake is only one of the several factors contributing to trigger torpor in mice.

These findings cast murine torpor in a new light. We have little understanding of whether pharmacological interventions to lower metabolism also trigger natural physiological adjustments; it is therefore imperative to validate them against natural torpor. Our experimental approach is promising for studies of tissue- and cell-level processes occurring in torpid animals. Work to understand neuroprotection—one of the most exciting translational aspects of torpor physiology (Drew et al. 2001)—has already moved beyond species following a pattern of obligate hibernation to those that hibernate facultatively (Härtig et al. 2007). Studies of the physiology of foraging-induced torpor in mice will be an exciting next step in understanding the biology of hypometabolic states.

All procedures concerning animal care and treatment were in accordance with the regulations of the Ethical Committee for Use of Experimental Animals (DEC licence 4321B).

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