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Daily torpor is associated with telomere length change over winter in Djungarian hamsters

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Ageing can progress at different rates according to an individual's physiological state. Natural hypothermia, including torpor and hibernation, is a common adaptation of small mammals to survive intermittent or seasonal declines in environmental conditions. In addition to allowing energy savings, hypothermia and torpor have been associated with retarded ageing and increased longevity. We tested the hypothesis that torpor use slows ageing by measuring changes in the relative telomere length (RTL) of Djungarian hamsters, *Phodopus sungorus*, a highly seasonal rodent using spontaneous daily torpor, over 180 days of exposure to a short-day photoperiod and warm (approx. 20°C) or cold (approx. 9°C) air temperatures. Multi-model inference showed that change in RTL within individuals was best explained by positive effects of frequency of torpor use, particularly at low body temperatures, as well as the change in body mass and initial RTL. Telomere dynamics have been linked to future survival and proposed as an index of rates of biological ageing. Our results therefore support the hypothesis that daily torpor is associated with physiological changes that increase somatic maintenance and slow the processes of ageing.

Keywords: ageing; hypothermia; senescence; torpor; telomere

1. INTRODUCTION

When exposed to unfavourable environmental conditions, such as food restriction, many organisms initiate physiological adjustments that appear to slow the processes of ageing [1]. The use of natural hypothermia, including daily torpor and hibernation, is a common response of small mammals to survive intermittent or seasonal periods of energy shortage. In addition to providing energy savings, hypothermia and torpor have been associated with a slowing of ageing-related processes and increased longevity [2–5].

Rate of change in telomere length could provide an indicator of biological ageing [6]. Telomeres are repeated sequences of DNA that cap and protect the ends of eukaryotic chromosomes. Telomeres shorten with each cell division at a rate strongly affected by oxidative stress [7]. In somatic tissue of small mammals, telomere loss is countered by the action of telomerase, a reverse transcriptase. Nevertheless, oxidative stress can disrupt this homeostasis and cause telomeres to shorten over time (e.g. [8]). Telomere dynamics therefore provide an index of cumulative oxidative damage to DNA and possibly also rate of ageing [6]. Critically short telomeres and relatively high rates of telomere shortening have been linked to reduced future survival rates, increased risk of reproductive failure and accelerated ageing in studies of vertebrates, including humans [9].

In the present study, we test the hypothesis that torpor use positively affects the change in telomere length over winter in a highly seasonal small rodent: the Djungarian hamster *Phodopus sungorus*. Exposure to a short-day photoperiod initiates a winter phenotype in this species, which is characterized by a lighter fur colour, the regression of reproductive organs, a reduction in body mass and the use of spontaneous daily torpor bouts (i.e. less than 24 h lasting bouts of voluntary hypothermia and suppressed metabolic rate [10]). We exposed female *P. sungorus* to a short-day photoperiod and either cold or warm temperatures over 180 days and tested for an association between frequency of daily torpor use, which is known to be highly variable among individuals [11], and the change in relative telomere length (RTL).

2. MATERIAL AND METHODS

Twenty-five mature virgin female hamsters (aged: three to eight months) were switched from a long-day (16 L : 8 D) to a short-day photoperiod (8 L : 16 D, lights on: 07.00–15.00 h) and exposed to either a warm (20.0°C ± 0.86°C s.d.) or cold (9.2°C ± 3.7°C) air temperature over 180 days. Food and water were provided ad libitum. We weighed each animal and its consumed pellet food (spilled food was negligible) once per week and returned animals to clean cages. To measure subcutaneous body temperature (T_{subc}), we inserted a micro-transponder (Lifechip, Destron Fearing, Saint Paul, USA) under the dorsal skin of each animal. From day 50, we recorded T_{subc} once per weekday between 08.30 and 09.30 h (83 recorded days) by quietly scanning under each box with a transponder reader (Pocket-reader Ex, Destron Fearing, Saint Paul, USA). This technique provided an index of the daily frequency of shallow (i.e. $T_{subc} < 29$°C) or deep torpor ($T_{subc} < 25$°C). On days 0 and 180, we took a 2 mm punch of ear tissue from each individual. This was stored in 70 per cent ethanol at −20°C until DNA extraction (Mammalian Genomic DNA Miniprep Kit, Sigma-Aldrich, USA). We derived an estimate of RTL for each sample of DNA using a robust and well-validated qPCR method [12–14] (for details, see the electronic supplementary material).

(a) *Data analysis*

To test simple hypotheses, we used *t*-tests and ANOVA tables of linear regression models. To explain variation in the change in RTL among individuals, we used a multi-model inference approach [15] implemented by the package 'MuMIn' [16] in the program R (v. 2.11.1) [17]. We started with a global set of linear models, including all combinations of additive and two-way interaction effects of the parameters: initial RTL at day 0, group (cold or warm), difference in body mass (gram) from day 27 to 171, age (months) and frequency of shallow or deep torpor use (arcsine square-root proportion of measurement days). We then estimated the importance and model-averaged coefficients of parameters using a set of models with the highest support (i.e. ΔAICc < 2) [15] (for detail, see the electronic supplementary material).

3. RESULTS

(a) *Body mass, food consumption and torpor use*

Mean body mass decreased from 27.7 ± 3.2 g (s.d.) when first recorded on day 27 to 24.3 ± 3.2 g on day

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Table 1. Summary of linear models with the highest support (ΔAICc < 2) explaining the change in RTL. Model-averaged estimates of coefficients, unconditional standard errors, and relative importance (sum of AICc weights) were derived for each parameter included the set of well-supported models. Independent model sets included the proportion of days (arcsine square-root transformed) with either (a) shallow ($T_{subc} < 29°C$) or (b) deep ($T_{subc} < 25°C$) daily torpor bouts.

	parameters in models with ΔAICc < 2			estimate coefficients		95% CI		
				estimate	s.e.	lower	upper	relative importance
(a) model sets including a shallow classification of torpor (i.e. $T_{subc} < 29°C$)								
body mass change (g)	—	—		0.03	0.01	0.008	0.061[a]	1.00
shallow torpor (prop. days)	—			0.39	0.15	0.074	0.709[a]	0.65
initial RTL	—			−0.15	0.07	−0.988	−0.040[a]	0.65
group (warm)		—		−0.14	0.07	−0.286	0.012	0.35
r^2	0.52	0.42						
AICc	−16.7	−15.5						
Akaike weight	0.63	0.35						
(b) model sets including a deep classification of torpor (i.e. $T_{subc} < 25°C$)								
body mass change (g)	—	—	—	0.03	0.01	0.008	0.049[a]	1.00
deep torpor (prop. days)	—	—		0.35	0.14	0.052	0.650[a]	0.81
initial RTL	—			−0.37	0.21	−0.801	0.066	0.43
group (warm)			—	·0.14	0.07	·0.286	0.012	0.19
r^2	0.52	0.45	0.42					
AICc	−17.1	−16.8	−15.5					
Akaike weight	0.43	0.38	0.19					

[a]Indicates CI does not include zero.

171, with a greater change in body mass for hamsters in the warm (−1.7 ± 1.7 g) than the cold (−5.0 ± 3.8 g; $t = 2.87$, $p = 0.010$).

Daily torpor was entered most frequently between days 100 and 160 but did not differ significantly between the cold (19 ± 14% recorded days) or warm group (11 ± 10%; $p = 0.22$). As expected, T_{subc} was lower during torpor for hamsters in the cold; hence, deep torpor (i.e. $T_{subc} < 25°C$) occurred more frequently in the cold (15 ± 12%) compared with the warm group (2 ± 3% of days; $t = 3.6$, $p = 0.0029$). Mean T_{subc} was 32.72°C ± 1.7°C in the cold and 33.18°C ± 1.4°C in the warm group ($p = 0.48$).

Food consumption was significantly greater for all hamsters in the cold (4.7 ± 0.6 g d^{-1}) compared with the warm group (3.1 ± 0.4 g d^{-1}; $F_{1,\ 22} = 253.5$, $p < 0.0001$) and varied in a negative linear relationship with frequency of torpor use ($F_{1,22} = 14.9$, $p = 0.0008$; figure 1).

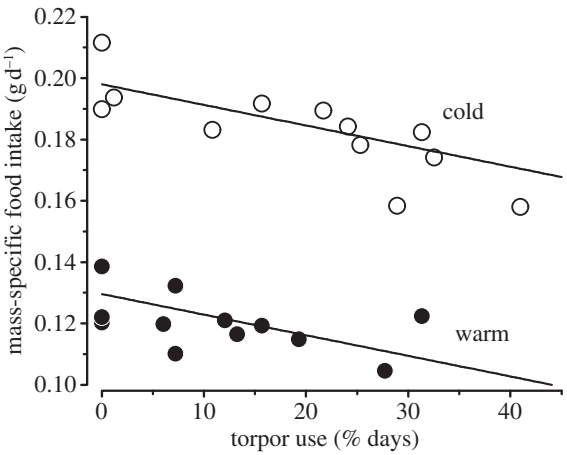

Figure 1. Average mass-specific daily food consumption of individual hamsters as a function of their use of daily torpor over 180 days of exposure to a short-day photoperiod and a warm (approx. 20°C) or cold (approx. 9°C) air temperature.

(b) *Relative telomere length*

Initial RTL did not differ between the groups (RTL on day 0: 1.21 ± 0.14; $t = 0.34$, $p = 0.73$). Over 180 days on a short-day photoperiod, the change in RTL differed significantly between the cold and warm groups ($t = 3.17$, $p = 0.005$). Eleven out of 12 hamsters kept in the cold experienced an increase in their RTL (average change in RTL: 0.25 ± 0.19), whereas RTL decreased or increased approximately equally among hamsters in the warm group (average change: 0.03 ± 0.14).

The best linear model explaining the difference in RTL between day 0 and 180 included effects of the frequency of torpor use, difference in body mass and initial RTL (table 1 and figure 2). The same model structure was favoured when including shallow or deep torpor. Importance of effects was inferred over a set of well-supported models (i.e. ΔAICc value < 2). When using a shallow classification of torpor, unconditional 95% confidence intervals (CIs) around the model-averaged coefficients excluded zero for the parameters: difference in body mass, frequency of torpor use and initial RTL; whereas, when including only deep torpor, 95% CIs excluded zero only for body mass and torpor use. The relative importance of parameters (sum of Akaike weights, range: 0–1) was greatest (equal to 1) for the difference in body mass, and was 0.65 and 0.81 for the frequency of shallow or deep torpor, respectively.

An effect of food intake on the change in RTL was examined using a separate set of well-supported

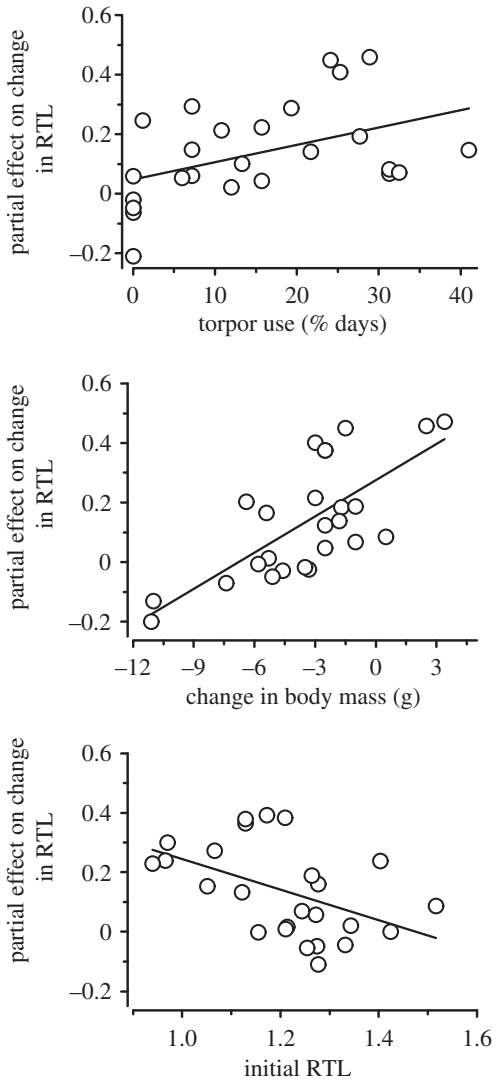

Figure 2. Partial residual plots showing the statistically independent effect of parameters included in the best model ($r^2 = 0.52$) explaining the change in RTL of hamsters over 180 days on a short-day photoperiod.

(ΔAICc < 2) models that included all parameters specified above but replaced frequency of torpor use with mass-specific food intake, which was strongly correlated with torpor (see figure 1). Inferring from this set of models, the difference in body mass and initial RTL were the most important parameters (relative importance: 0.89 and 0.85, respectively) explaining the difference in RTL from day 0 to 180, whereas food intake was rather unimportant (relative importance value of 0.35). Unconditional 95% CIs of model-averaged coefficients for food intake spanned zero.

4. DISCUSSION

We found that hamsters frequently using daily torpor bouts, particularly at low body temperatures, experienced an increase in their RTL over the 180 day period of a short photoperiod. This is not unrealistic because of the activity of telomerase in somatic tissues of small mammals. Telomere dynamics (i.e. the net outcome of loss and renewal) can reflect exposure to chronic oxidative stress [7], predict future survival (e.g. [18]), and indicate variation in rates of biological ageing [6]. Hence, our results support the hypothesis that torpor is associated with a physiological state of increased somatic maintenance and retarded ageing.

An effect of torpor was not associated with a reduction in metabolic energy expenditure: food intake was approximately 50 per cent greater in cold-exposed hamsters, regardless of torpor use, yet neither group nor food intake was important in explaining the change in RTL. This is consistent with studies finding that a cold-induced increase in metabolism has no effect on lifespan in small mammals [19]. The negative effect of a decrease in body mass on RTL could be related to an associated increase in mass-specific basal metabolism [10]. Cell turnover is slowed by torpor use [4], which partly explains why deep torpor especially could minimize the loss of RTL. Oxidative stress affects the rate of telomere loss [7]. Daily torpor by *P. sungorus* is associated with changes in mitochondrial activity [20] that might reduce the production of free radicals. In addition, frequent torpor use could be associated with a state of increased cellular stress resistance, which is characteristic of quiescent states in invertebrate model species [1].

In this experiment, the use of daily torpor was associated with an increase in the length of telomeres in hamsters exposed to a short-day photoperiod. Our results support the hypothesis that, in addition to reducing energy expenditure, the use of torpor might also serve to retard somatic ageing during periods of unfavourable environmental conditions.

All procedures were in accordance with Austrian legislation and approved by the ethics commission of the University of Veterinary Medicine Vienna.

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