Electronic supplementary material

Measurements of torpor use and hibernating patterns

Nest temperatures were recorded every minute. Torpor onset and termination were determined from the visual inspection of graphs. Torpor entry was defined as a slow and continuous decrease of the nest temperature down to T_a while the animal was present in its nest, until termination of torpor, when the nest temperature returned to the highest value after a steep and continuous rise (Fig. S2). For the period of highest summer mass gain, we determined torpor frequency (the number of torpor bouts displayed by an individual per week) and the total torpor duration (the total time an individual spent in torpor per week). For the winter hibernation period, we determined, by means of the nest temperature measurements, the time each animal spent euthermic between the induction of hibernation (when food and water was removed) and the onset of hibernation (i.e. the first torpor bout longer than 24h), the number of arousals from prolonged torpor, the mean torpor duration and the total and the mean IBE durations. The total euthermic time (i.e. the sum of the euthermic time before the hibernation onset and the total IBE duration) was also computed. These parameters were only calculated for the period when juveniles were hibernating alone. Once per day (8.30am - 12.30am), all animals were quietly scanned for subcutaneous T_b recording by temperature-sensitive PIT tags. Torpor was defined as a decrease of subcutaneous T_b below 32°C. PIT Tag measurements confirmed results of the use of torpor, i.e., torpor frequency, assessed by nest temperature recordings.

Determination of body composition, total energy expenditure and water turnover

We used the multi-point doubly-labelled water (DLW) methodology during a 6-day period [1] to determine body composition, total energy expenditure (TEE) and water turnover (rH_2O) just prior to hibernation (on week 5). For each individual, a baseline urine sample was taken by gently stimulating urination. A premixed 5 g/kg dose of DLW, thinned with 3% NaCl to

physiological osmolarity, was injected intra-peritoneously into each animal. Isotopic equilibration in body water was determined from a blood sample collected at 1-h post-dose from a quick sampling of the saphenous vein. Approximately 300 µl of blood, representing less than 0.5% of the animal body mass, were taken from each individual (~80 g). The blood was collected in micro-capillary tubes, which were immediately flame-sealed. Juvenile dormice were then released into their own cages, and urine samples were taken 24, 48, 72 and 144 h after the equilibration time in cryogenically stable tubes. Blood capillaries were stored at 4°C and urine samples were kept frozen at -20°C until subsequent analyses by isotope ratio mass spectrometry, at the Department of Ecology, Physiology and Ethology (IPHC, CNRS-UdS, Strasbourg, France).

We used the Soxhlet method to assess post-hibernation body fat levels [2]. Dead bodies were weighed, minced and rubbed in sea sand. Afterwards, they were dried in a heat cabinet at 103°C for 48 h and then cooled in an exsiccator. Fat was extracted by repeatedly washing with petroleum ether, under reflux in a glass flask for 6 hours. Fat content was then calculated as percentage of body mass.

Determination of relative telomere lengths (RTL)

Measurements of RTL were performed at the Department of Ecology, Physiology and Ethology (IPHC, CNRS-UdS, Strasbourg, France). We used a quantitative PCR method [3, 4] to estimate the quantity of telomere repeated sequences relative to a reference gene that was predetermined to be non-variable in copy number (non-VCN) among the sample genomes. Indeed, the reference gene (Interphotoreceptor retinoid-binding protein, IRBP, NCBI Accession no. AB253957 and AB253958) for the relative measure was determined to be single copy gene and non-variable in copy number as per Borst *et al.* [5] and Sato *et al.* [6] and already used as a control gene in other mammals for telomere length measurement [7]. The ratio of telomere-to-non-VCN gene (T/S) was further standardised among runs (plates)

using the telomere-to-non-VCN gene ratio obtained from a control sample included in each qPCR run (see equation below). Primers for the non-VCN gene (5'-CACTCACCAACCTCACACAAGA -3', and 5'- CCACATTGCCCTCCAGAAC -3') amplified a 81 bp portion of the IRBP gene. Forward and reverse telomeric primers were 5'-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3' and 5'- GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT-3', respectively [3].

Samples were diluted to a standard DNA concentration of 1 ng/μL. Reactions were done in a final volume of 10 μL and contained 5 μL of BRYT Green® dye (GoTaq®qPCR Master Mix, Promega, France) and 200 nM of each primer for telomere or 1 μM of each primer for non-VCN gene reactions. Cycling conditions included an initial hold at 95°C for 2 min followed for telomere amplification by 30 cycles of 95°C for 45 sec, 56°C for 45 sec, and 72°C for 1 min with fluorescence readings recorded at this step. For the non-VCN gene amplification we programmed 45 cycles of 95°C for 45 sec, 50°C for 45 sec, and 72°C for 1 min with fluorescence readings recorded at this step. A final melt step was included on each run for the telomere and non-VCN gene reactions with the temperature ramping from 50°C to 95°C in 1°C increments. Cycling was performed on a Realplex 2 real-time PCR instrument (Eppendorf, Germany). In each run, we included 2 replicates of 8 DNA samples taken from an even number of individuals from each treatment group, 2 replicates of a no-template control. Each plate (telomere and non-VCN gene) included serial dilutions (5 ng, 2.5 ng, 1.25 ng, 0.625 ng, 0.3125 ng, 0.15625 ng, and 0.078125 ng) of DNA of the same sample ID which were run in duplicate. These serial dilutions were used to generate a reference curve on each plate, in order to control for the amplifying efficiency of the qPCR (both telomere and non-VCN gene). Mean PCR efficiency calculated from the reference curves of the qPCR runs were comprised between 0.99 and 1.01 for the telomere and between 0.99 and 1.00 for non-VCN gene reactions. R^2 calculated from the reference curves of the qPCR runs were comprised between 0.94 and 0.99 for telomere and between 0.98 and 0.99 for non-VCN gene

reaction. To take into account the slight variation of efficiencies between telomere and control gene amplifications, we calculate RTL using the method suggested by Pfaffl *et al.* [8]. Using the mean PCR efficiency per amplicon (E) and the cycle threshold (Cq) value per sample, we calculated the RTL using the formula:

RTLS = (ECT^CqCT / EST^CqST) / (ECN^CqCN / ESN^CqSN),

where: RTLS = RTL for a given sample; ECT = mean efficiency of the control telomere reactions; CqCT = mean cycle threshold of the control telomere replicates; EST = mean efficiency of all sample telomere reactions; CqST = mean cycle threshold of within-sample telomere replicates; ECN = mean efficiency of the control non-VCN gene reactions; CqCN = mean cycle threshold of the control non-VCN gene replicates; ESN = average efficiency of all sample non-VCN gene reactions; and CqSN = average cycle threshold of within-sample non-VCN gene replicates.

Intra-plate mean coefficients of variation for T/S were 9.0% (n=120) and inter-plate coefficients of variation based on repeated samples were 13.0% (T/S again, n=8).

Statistical analyses

For the period of highest summer mass gain, analyses of variance (R-package 'car' [9]) were used to assess effects of group and time (week as a factor) on body mass gain and food intake, and effects of group, time (week as a factor) and food intake on torpor frequency and total torpor duration. Because torpor use and food intake were both correlated with group, we tested with a separate analysis of variance their effects on body mass gain across both feeding groups. Linear mixed effects models were employed to test effects of group and time (week as a factor) on RTL, with torpor frequency and body mass gain included as covariates in the model. Initial inspection of the data gave no evidence for a sex effect on any of the response variables. Therefore, to avoid over-fitting, sex was not included as a predictor in the analyses. To adjust for repeated measurements, individual intercepts were included as random factors in

these mixed models. For torpor frequency and total torpor duration, unpaired t-tests, with the Benjamini-Hochberg correction for multiple comparisons, were used to further assess group differences at a given time-point (week). Unpaired *t*-tests were used to compare body masses at the beginning of our experiment and to compare fat masses and fat-free masses at week 5 of the pre-hibernation period. Because some individuals entered prolonged (> 24h) torpor bouts, torpor frequency and total torpor duration for the late pre-hibernation period (weeks 5 and 6) were excluded from the analyses.

For the first month of the winter hibernation period (juveniles housed single in nests), linear mixed-effects models were again employed to assess group and sex differences in the time spent euthermic before the onset of hibernation, mean torpor duration, number of torpor arousals, total and mean IBE durations, and total euthermic time. The pre-hibernation body mass was used as a covariate in these analyses. Differences between time (pre and post-hibernation), groups and sexes in body mass, fat mass and fat-free mass, as well as in percentage losses were tested using linear mixed-effects models, with the pre-hibernation body mass as a covariate. Group and sex differences in post-hibernation body mass, fat mass and fat-free mass were tested using linear mixed-effects models. We also assessed differences in RTL between pre and post-hibernation by using linear mixed-effects models, with groups and sexes as covariates.

To account for measurement errors in both the dependent and predictor variables, we applied ranged major axis (RMA) models (R package 'lmodel2' [10, 11]) for regression analyses between torpor frequency and, TEE and rH_2O during the late pre-hibernation period, and between total torpid and IBE durations and the percentage change (relative to the initial length in telomeres) in post-hibernation RTL during winter hibernation.

References

1. Blanc S., Géloën A., Pachiaudi C., Gharib C., Normand S. 2000 Validation of the doubly labeled water method in rats during isolation and simulated weightlessness. *Am J Physiol Reg Int Comp Physiol* **279**(6), R1964-R1979.

2. Soxhlet F.V. 1879 Die gewichtsanalytische Bestimmung des Milchfettes. *Polytechn J* **232**, 461-465.

3. Callicott R.J., Womack J.E. 2006 Real-time PCR Assay for Measurement of Mouse Telomeres. *Comp Med* **56**(1), 17-22. http://www.ingentaconnect.com/content/aalas/cm/2006/00000056/00000001/art00003.

4. Cawthon R.M. 2002 Telomere measurement by quantitative PCR. *Nucleic Acids Res* **30**(10), E47. (doi:10.1093/nar/30.10.e47).

5. Borst D.E., Redmond T.M., Elser J.E., Gonda M.A., Wiggert B., Chader G.J., Nickerson J.M. 1989 Interphotoreceptor Retinoid-binding Protein. Gene characterization, protein repeat structure, and its evolution. *J Biol Chem* **264**(2), 1115-1123. http://www.jbc.org/content/264/2/1115.full.pdf.

6. Sato J.J., Hosoda T., Wolsan M., Tsuchiya K., Yamamoto M., Suzuki H. 2003 Phylogenetic Relationships and Divergence Times among Mustelids (Mammalia: Carnivora) Based on Nucleotide Sequences of the Nuclear Interphotoreceptor Retinoid Binding Protein and Mitochondrial Cytochrome *b* Genes. *Zoolog Sci* **20**(2), 243-264. (doi:10.2108/zsj.20.243).

7. Izzo C., Hamer D.J., Bertozzi T., Donnellan S.C., Gillanders B.M. 2011 Telomere length and age in pinnipeds: The endangered Australian sea lion as a case study. *Mar Mammal Sci* **27**(4), 841-851. (doi:10.1111/j.1748-7692.2010.00450.x).

8. Pfaffl M.W. 2001 A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* **29**(9), e45. (doi:10.1093/nar/29.9.e45).

9. Fox J., Weisberg S. 2011 *An R Companion to Applied Regression*. 2nd ed, Sage; xxii, 449 p.

10. Legendre P., Legendre L. 1998 *Numerical Ecology: second english edition* 870 p.

11. Sokal R.R., Rohlf F.J. 1995 *Biometry. The Principles and Practice of Statistics in Biological Research*. 3rd ed. New York, W.H. Freeman; 887 p.