© 2017. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2017) 220, 2380-2386 doi:10.1242/jeb.160150

RESEARCH ARTICLE

Specialist–generalist model of body temperature regulation can be applied at the intraspecific level

Anna S. Przybylska[1,*], Jan S. Boratyński[1,2], Michał S. Wojciechowski[3] and Małgorzata Jefimow[1]

ABSTRACT

According to theoretical predictions, endothermic homeotherms can be classified as either thermal specialists or thermal generalists. In high cost environments, thermal specialists are supposed to be more prone to using facultative heterothermy than generalists. We tested this hypothesis at the intraspecific level using male laboratory mice (C57BL/cmdb) fasted under different thermal conditions (20 and 10°C) and for different time periods (12–48 h). We predicted that variability of body temperature (T_b) and time spent with T_b below normothermy would increase with the increase of environmental demands (duration of fasting and cold). To verify the above prediction, we measured T_b and energy expenditure of fasted mice. We did not record torpor bouts but we found that variations in T_b and time spent in hypothermia increased with environmental demands. In response to fasting, mice also decreased their energy expenditure. Moreover, animals that showed more precise thermoregulation when fed had more variable T_b when fasted. We postulate that the prediction of the thermoregulatory generalist–specialist trade-off can be applied at the intraspecific level, offering a valid tool for identifying mechanistic explanations of the differences in animal responses to variations in energy supply.

KEY WORDS: Body temperature, Heterothermy, Fasting, Specialist–generalist trade-off

INTRODUCTION

Endothermic thermoregulation requires large amounts of energy to maintain body temperature (T_b) at a high and relatively constant level, which is particularly evident beyond the thermoneutral zone (TNZ) (Scholander et al., 1950). In active endothermic animals, the only way to balance high energy expenditure resulting from the need to maintain T_b is to increase energy intake (Humphries et al., 2003; McNab, 1974; Scholander et al., 1950). However, mammalian T_b varies continuously on a daily and seasonal basis (Geiser and Ruf, 1995; Refinetti and Menaker, 1992), and the degree of its variation differs among taxa (Boyles et al., 2013). According to theoretical predictions (Angilletta et al., 2006, 2010), thermoregulatory strategies of endothermic homeotherms range from specialists, which regulate their T_b precisely, to generalists, which tolerate greater variations of T_b. It was also proposed that in high cost environments, specialists spend more energy to maintain performance than generalists, which perform well in a wide spectrum of environments. Based on the above, Boyles and Warne (2013) further predicted that in the face of energy limitation, specialists should be more prone to using facultative heterothermy than generalists. Food limitation and cold increase the costs of maintaining high T_b and increase the frequency and lengthen duration of torpor bouts (Bozinovic et al., 2007; for rodent examples, see Ruf et al., 1993; Tomlinson et al., 2007). Hence, thermal generalists, or specialists that use facultative heterothermy, should be favored in environments with low or unpredictable energy supply (Lovegrove, 2000). Endotherms deal with energy supply deficit either by increasing activity and searching for food, or by decreasing it, and reducing energy expenditure, i.e. by entering torpor (Gutman et al., 2007). However, increasing activity may lead to resource exhaustion (Russell et al., 1987), and it increases the risk of predation (Lima and Dill, 1990); nevertheless, it brings about an opportunity to find food (Overton and Williams, 2004; Sakurada et al., 2000). Torpor, in turn, is a state of regulated decrease of T_b and metabolic rate (MR) (Heldmaier and Ruf, 1992; Ruf and Geiser, 2015; Snyder and Nestler, 1990), which brings about benefits when food is unavailable or when costs of foraging are too high (Hudson and Scott, 1979; Ruf and Heldmaier, 2000; Schubert et al., 2010; but see Humphries et al., 2003 and Wojciechowski et al., 2011 for a discussion of increased predation risk associated with torpor). In recent decades, several studies have focused on torpor use as a response to energy deficit (Bae et al., 2003; Gutman et al., 2006; Nespolo et al., 2010; Schubert et al., 2010, 2008). Wild house mice (*Mus musculus*), as well as different laboratory strains, enter daily torpor in the face of low food availability or low ambient temperature (T_a), or both (Morton, 1978; Tomlinson et al., 2007; Webb et al., 1982; Williams et al., 2002), indicating that facultative heterothermy is a response to increased energy demands (Dikic et al., 2008; Gordon, 2012; Hudson and Scott, 1979; Schubert et al., 2010).

To test the hypothesis that mice adjust their thermoregulation in response to environmental demands, we fasted C57BL6/cmdb mice for between 12 and 48 h at different T_a, and measured their T_b continuously. We predicted that variability of T_b and time spent with T_b below normothermy would increase with the increase of environmental demands (i.e. T_a and duration of fasting). As T_b of food-deprived mice showed large variations in response to food deprivation that could not be classified as torpor (see Results), the second aim of this work was to quantify the energetic consequences of such a pattern of heterothermic thermoregulation. To do so, we measured the MR of mice during 48 h food deprivation at constant, moderate T_a. Using data of T_b variability in fed and fasted mice, we tested the hypothesis that facultative heterothermy is more common in thermal specialists than in generalists (Boyles and Warne, 2013); specifically, we examined the prediction that at the intraspecific level, precise regulation of T_b during *ad libitum* food availability would be negatively correlated with greater variability of T_b during food deprivation.

[1]Department of Animal Physiology, Nicolaus Copernicus University, 87-100 Toruń, Poland. [2]Museum and Institute of Zoology, Polish Academy of Sciences, 00-679 Warsaw, Poland. [3]Department of Vertebrate Zoology, Nicolaus Copernicus University, 87-100 Toruń, Poland.

*Author for correspondence (annaprzybylska2808@gmail.com)

A.S.P., 0000-0002-3646-3686

Received 28 March 2017; Accepted 12 April 2017

Journal of Experimental Biology

Journal of Experimental Biology (2017) 220, 2380-2386 doi:10.1242/jeb.160150

List of symbols and abbreviations

EE	energy expenditure
HI_{fast}	heterothermy index during fasting
HI_{fed}	heterothermy index during *ad libitum* food availability
m_b	body mass
MR	metabolic rate
T_a	ambient temperature
T_b	body temperature
$T_{b,hypo}$	hypothermic body temperature
$T_{b,min}$	minimum body temperature

MATERIALS AND METHODS

Animals

All experimental procedures were approved by the Local Committee for Ethics in Animal Research in Bydgoszcz, Poland (decision no. 6/2014 from 16/01/2014, and no. 32/2014 from 27/11/2014). We used two groups of male mice of the C57BL6/cmdb strain originating from the colony maintained at the Centre for Experimental Medicine of the Medical University of Białystok, Poland. In the first series of experiments we used eight individuals, and in the second series we used 12 individuals. Two-month-old mice were transported to the Nicolaus Copernicus University in Toruń and were acclimated for 2 months before starting the experiments. They were kept individually in standard rodent cages (model 1264, Tecniplast, Buguggiate, Italy) in a walk-in climate chamber at T_a=20±2°C and under a 12 h:12 h light:dark cycle, with food (Labofeed B, Kcynia, Poland) and water available *ad libitum*.

Experimental protocol

A week before each experiment (experiments 1 and 2; see below), mice were implanted intraperitoneally with miniaturized temperature-sensitive data loggers (modified iButton, model DS1922L, Dallas Semiconductor, Dallas, TX, USA), under ketamine (40 mg kg^{-1}; Ketamine 10%, Biowet, Puławy, Poland) and xylazine (8 mg kg^{-1}; Sedazin 2%, Biowet, Puławy, Poland) anesthesia. Before implantation, loggers were covered with paraffin wax (final mass: 1.5–2.2 g) and calibrated in a water bath against a high-precision thermometer. After implantation, mice were allowed to recover for 3 days and during that time they were supplemented with antibiotic in drinking water (Enflocyne 5%, Biowet, Puławy, Poland). During experiment 1, T_b was logged in 12-min intervals with resolution of 0.5°C, whereas during metabolic measurements (experiment 2), T_b was logged every 5 min with a resolution of 0.065°C.

Experiment 1: response of male mice to increasing environmental demands

In the first series of experiments we aimed to answer the question of whether the thermoregulatory response to food deprivation depends on environmental demands, namely, fasting and cold. Each mouse was deprived of food six times: for 12, 24, 36 and 48 h at 20°C, and then for 12 and 24 h at 10°C. During experiments at 20°C, mice were randomly divided into two groups of four mice each, which differed in the order of food deprivation trails, except for the 48 h fast, which was set as the last fast in both groups. This was done to minimize confounding effects of repeated food deprivation events on the animal's response to fasting (McCue et al., 2017a). Because the order of trials did not affect T_b [linear mixed-effects (LME) models with order of trials as a fixed factor, body mass (m_b) as a covariate and animal ID as a random factor; diurnal T_b: LME: $F_{1,6}$=0.812, P=0.53, nocturnal T_b: LME: $F_{1,12}$=1.11, P=0.32], the results from both groups were analyzed together. Thereafter, mice were transferred to 10°C, and after 3 days of habituation were deprived of food for 24 and 12 h. Because there is no information about mice being fasted in the cold for more than 24 h, we did not extend food deprivation for safety reasons. Before each trial, food was withdrawn ∼0.5 h before lights off, and cages, especially bedding material, were carefully checked for uneaten food. Water was available *ad libitum* during the entire experiment. Mice were weighed before and after each bout of food deprivation to the nearest 0.1 g (SPU402, OHAUS, Parsippany, NJ, USA); mass of the logger was always subtracted from a mouse's m_b. After each food deprivation bout, mice were offered food and water *ad libitum* and were allowed to recover for between 3 and 6 days depending on fast duration, i.e. until their m_b returned to the pre-fast level.

Data analysis

Out of eight animals used in the first series of experiments, we obtained T_b data for four mice, which were fasted at both T_a. Additionally, we collected data for a further two individuals fasted at 20°C, and for two others fasted at 10°C. In total, we analyzed T_b recordings from six individuals measured at each T_a. Following Boyles et al. (2011b), we used the heterothermy index (HI), which is a statistical description of T_b variations that can be used for all thermoregulating animals:

$$\mathrm{HI} = \sqrt{\frac{\sum (T_{b,\mathrm{mod}} - T_{bi})}{n-1}}, \quad (1)$$

where $T_{b,mod}$ is modal T_b (here, the most frequent T_b recorded when food was available *ad libitum*), T_{bi} is T_b recorded at time i, and n is the number of samples. HI during food deprivation (HI_{fast}) was calculated for each trial separately. The HIs of mice fed *ad libitum* (HI_{fed}) at both T_a were calculated from data obtained during 3-day recordings before the start of food deprivations. To compare HI_{fed} and HI_{fast}, we used LME models with duration of food deprivation included as a fixed factor; this analysis was done separately for 10 and 20°C. To account for repeated observations from the same individuals, in all LME models animal ID was included as a random factor.

Because the thermal state of fasted mice could not be unambiguously described as torpor (see Results; mice were also responsive to the stimuli), we defined it as hypothermia. According to the 'Glossary of Terms for Thermal Physiology' (IUPS Thermal Commission, 2001), heterothermy is 'a pattern of T_b regulation that exceeds in range that characteristic for homeothermy', while hypothermia is 'the condition of a temperature regulator when core temperature is below its range specified for the normal active state of the species', being either regulated or forced (pathological).

The lower limit for normothermic T_b was determined following Wojciechowski and Pinshow (2009) and was calculated separately for each animal. We assumed that normothermic T_b is normally distributed with a center on diurnal $T_{b,mod}$. Then we fitted the normal distribution curve shaped by the data equal to or higher than the $T_{b,mod}$. Hypothermic T_b ($T_{b,hypo}$) was accepted as T_b lower than diurnal (rest-phase) $T_{b,mod}$ minus two standard deviations. Then we calculated the length of time for which animals were hypothermic during each trial.

Minimum T_b ($T_{b,min}$) was calculated separately for day and night as the mean of three consecutive lowest recordings during food deprivation that were taken 12 min apart. Body mass loss (Δm_b) resulting from food deprivation was calculated as the difference

Journal of Experimental Biology (2017) 220, 2380-2386 doi:10.1242/jeb.160150

between initial m_b before food deprivation and final m_b after food deprivation.

To analyze the effect of increasing environmental demands on HI_{fast}, time with $T_{b,hypo}$ and $T_{b,min}$, and Δm_b of fasted mice, we used the LME model in which initial m_b and $T_{b,mod}$ were included as covariates. Duration of food deprivation and T_a were included as fixed factors and animal ID as a random factor. In all analyses, the interaction between duration of food deprivation and T_a was not significant and thus it was excluded from final analyses.

Experiment 2: metabolic rate and body temperature during 48 h food deprivation

To determine the energetic consequences of T_b variations recorded in experiment 1, the MR of food-deprived mice was measured by indirect calorimetry in an open-flow respirometry system. At the same time, we measured T_b of these mice as described above. Measurements were commenced ∼1 h before the active phase and lasted for ∼48 h. During measurements, mice were exposed to T_a=20°C and had access to water *ad libitum*. Mice were sealed for the duration of the experiment in 0.85 liter chambers constructed of translucent polypropylene containers (HPL 808, Lock&Lock, Hana Cobi, South Korea). To ensure that mice had access to water, ∼150 ml drinking bottle (model ACBT0152, Tecniplast) was mounted in the chamber's lid. Moreover, we put 3 g of sawdust from animal's home cage into each respirometry chamber; this amount of bedding was insufficient to build a nest but may have reduced the stress of a new environment.

We measured respiratory gas exchange of three animals simultaneously, using three parallel respirometry systems. In two systems, rates of O_2 consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$) were measured, and in the third one only $\dot{V}_{O_2}$. Air was pulled from outside the building using an air pump (5HCE-10-M553, Gast Manufacturing, Benton Harbor, MI, USA) and compressed in a balloon, then dried and scrubbed of CO_2 with a PureGas Generator (Puregas, Westminster, CO, USA). The main air stream was split into three chambers and a reference gas stream. The air flow was regulated at ∼500 ml min^{-1} and measured upstream of animal chambers using a mass flow meter (FlowBar-8, Sable Systems International, North Las Vegas, NV, USA). Gases leaving respirometry chambers were selected sequentially with a computer-controlled multiplexer (Intelligent Multiplexer V3, Sable Systems). Then, air from each gas stream was subsampled at ∼100 ml min^{-1} and water vapor pressure of the subsampled air was measured with a water vapor analyzer (RH-300, Sable Systems). Air was then dried in a column of magnesium perchlorate (product number 11636.36, VWR International, Gdańsk, Poland) and subsequently the fractional concentrations of CO_2 (F_{CO_2}) and O_2 (F_{O_2}) were measured using a FoxBox-C integrated CO_2 and O_2 analyzer or with separate CO_2 (CA-10) and O_2 analyzers (FC-10a; both Sable Systems). All electronic elements of the respirometry system were connected to a PC via an analog-to-digital interface (UI2, Sable Systems) and data were acquired using ExpeData software (Sable Systems) at 0.5 Hz. Animal m_b was measured before and after MR measurements to the nearest 0.1 g (Scout Pro 200, OHAUS).

Mean normothermic T_b was calculated from recordings obtained during 3 days before fasting, and mean T_b was calculated separately for the first and second day of food deprivation in a metabolic chamber.

Data analysis

$\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ of animals for which both F_{O_2} and F_{CO_2} were measured were calculated using eqns 10.6 and 10.7 in Lighton (2008). To calculate $\dot{V}_{O_2}$ of animals for which only F_{O_2} was measured, we assumed a respiratory exchange ratio (RER; $\dot{V}_{CO_2}/\dot{V}_{O_2}$) equal to the mean RER calculated for other animals. Then $\dot{V}_{O_2}$ was used to calculate metabolic rate (in W) using RER and oxyjoule equivalent after Lighton et al. (1987) as:

$$\mathrm{MR} = \frac{\dot{V}_{O_2}(16 + 5.164 \cdot \mathrm{RER})}{60}. \quad (2)$$

For each mouse, its total energy expenditure (EE) during a 46 h food deprivation was calculated by integrating the area below the EE curve (we uniformly subtracted 1 h from the beginning each day during which we paused the recording to exchange desiccating columns and span gas analyzers; during that time, mice remained untouched in respirometry chambers; see below). Energy expenditure for the first day of food deprivation (EE1) was calculated from the beginning of dark phase (17:00 h) until 16:00 h the next day. Energy expenditure for the second day (EE2) was calculated in the same way. Total EE was the sum of EE1 and EE2.

To analyze the relationship between duration of food deprivation and T_b, we used an LME model with initial m_b as a covariate, day of food deprivation as a fixed factor and animal ID as a random factor; in the analysis of the effect of food deprivation on EE, we included mean T_b and HI during each particular day as covariates.

Finally, to determine whether individuals that regulated their normothermic T_b more precisely showed greater variations of T_b during fast, we used an LME model with HI_{fast} as a dependent variable, HI_{fed} (here calculated from 2-day recordings before the experiment and used for analysis of the first and second day of food deprivation) and m_b as covariates, day of food deprivation as a fixed factor and animal ID as a random factor. Because m_b was not a significant covariate ($F_{1,12}$=0.00, P=1.00), it was removed from the final model. We performed two analyses: first, for data obtained only during the active phase of the day (i.e. night), as suggested by Boyles and Warne (2013); and second, for data obtained for the whole 24 h, because fasted mice showed large variations in T_b both during active and inactive phases of the day.

All analyses were performed in SPSS Statistics v. 23 (IBM, Armonk, NY, USA). In all LME models, the restricted maximum likelihood method was used to estimate variance components. Assumptions of the linear modeling were checked *post hoc* by inspecting the distribution of residuals obtained from LME (check of histograms and quantile-quantile plots; Grafen and Hails, 2002). Data are presented as estimated marginal means±s.e.m., and statistical significance was accepted at α<0.05.

RESULTS

Experiment 1: response of mice to increasing environmental demands

Overall, when food deprived for 24 h, mice maintained T_b lower by ∼0.5°C than when they were fed *ad libitum* (LME with feeding status and T_a as fixed factors and animal ID as a random factor: $F_{1,20}$=24.42, P<0.001). However, T_b was not maintained at the low level continuously, but after each drop it returned almost immediately to normothermy (Fig. 1). Body temperature was more variable during food deprivation than when mice were fed *ad libitum*, at both 20°C ($F_{4,28}$=13.91, P<0.001) and 10°C ($F_{2,28}$=20.13, P<0.001). HI during food deprivation also increased with the decrease in T_a ($F_{1,28}$=7.68, P=0.01), and during 12 h fast at 20°C it equaled 1.56±0.17°C, whereas at 10°C it was 2.27±0.20°C (Table 1). Lengthening of food deprivation also

Journal of Experimental Biology (2017) 220, 2380-2386 doi:10.1242/jeb.160150

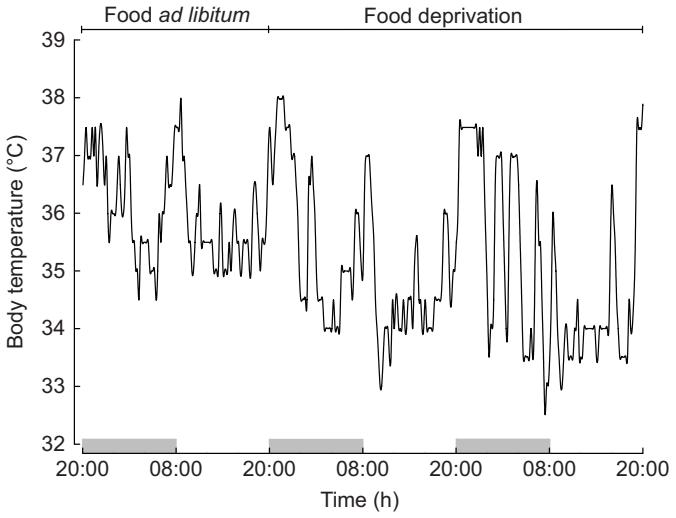

Fig. 1. Time course of body temperature of a representative mouse [ID W1; initial body mass (m_b)=26.8 g, final m_b=22.8 g] during 24 h food *ad libitum* and 48 h of food deprivation. Night is indicated by gray bars.

resulted in higher HI_{fast} ($F_{3,28}$=3.36, P=0.03), and during 24 h food deprivation at 20°C HI_{fast} was 1.63±0.17°C, while during 48 h it increased to 2.34±0.20°C (Table 1). The normothermic T_b of mice was 36.00±0.45°C and the lower limit for normothermy was 34.20±0.64°C at T_a=20°C and 33.24±0.55°C at T_a=10°C. Time spent with $T_{b,hypo}$ increased with the duration of food deprivation ($F_{3,28}$=12.13, P<0.001; Table 1). During the 12 h fast at T_a=20°C, mice spent 34.45±114.39 min with $T_{b,hypo}$, and during the 48 h fast they spent 1072.29±135.43 min with $T_{b,hypo}$ (Table 1). Ambient temperature did not significantly affect time with $T_{b,hypo}$ ($F_{1,28}$=1.31, P=0.26; Table 1). Diurnal $T_{b,min}$ decreased with T_a ($F_{1,28}$=14.31, P=0.01) and with duration of food deprivation ($F_{3,28}$=22.07, P<0.001; Table 1). Nocturnal $T_{b,min}$ did not change with T_a ($F_{1,28}$=2.32, P=0.14) but decreased as food deprivation lengthened ($F_{3,28}$=10.68, P<0.001; Table 1). Mice lost more body mass (Δm_b) as food deprivation was lengthened ($F_{3,28}$=13.25, P<0.001) and T_a was decreased ($F_{1,28}$=23.46, P<0.001; Table 1). Δm_b did not correlate with initial m_b ($F_{1,28}$=0.01, P=0.94) or with $T_{b,mod}$ ($F_{1,28}$=0.07, P=0.79).

Experiment 2: metabolic rate and body temperature during 48 h food deprivation

Food deprivation led to a decrease of MR and T_b (Fig. 2). Mean normothermic T_b before food deprivation was 36.36±0.12°C. There was a significant effect of fasting duration on mean T_b ($F_{2,8}$=130.15, P<0.001). During the first day, mean T_b decreased to 33.95±0.42°C, and during the second day to 31.58±0.99°C. Total EE during 46 h of food deprivation was 66.14±2.88 kJ. Energy expenditure was not significantly different between the two days of food deprivation (day 1: 34.74±155 kJ; day 2: 31.40±1.55 kJ; $F_{1,11}$=1.41, P=0.26). Energy expenditure did not depend on initial m_b ($F_{1,11}$=0.22, P=0.64) or on HI ($F_{1,11}$=2.32, P=0.15). Differences in energy expenditure during food deprivation were explained only by mean T_b ($F_{1,11}$=5.51, P=0.04). The HI of food-deprived mice during their active phase did not correlate with active-phase HI of mice fed *ad libitum* ($F_{1,13}$=2.43, P=0.14; Fig. 3A). However, when calculated for the entire 24 h, HI_{fast} correlated negatively with HI_{fed} ($F_{1,13}$=5.41, P=0.04; Fig. 3B). It was true for both days of food deprivation, and HI_{fast} on the second day of fast was higher than that on the first day ($F_{1,13}$=27.04, P<0.001).

Table 1. Effect of duration of food deprivation and ambient temperature (T_a) on heterothermy index (HI_{fast}), time spent with T_b below normothermy (time $T_{b,hypo}$), diurnal and nocturnal minimum T_b ($T_{b,min}$) and body mass loss (Δm_b)

T_a	20°C				10°C		Duration of food deprivation		T_a	
Duration of food deprivation	12 h	24 h	36 h	48 h	12 h	24 h	$F_{3,28}$	P	$F_{1,28}$	P
HI_{fast} (°C)	1.56±0.17	1.63±0.17	1.82±0.19	2.34±0.20	2.27±0.20	2.35±0.20	3.35	**0.03**	7.68	**0.01**
Time $T_{b,hypo}$ (h)	34.45±114.39	274.23±117.84	500.53±133.02	1072.29±135.43	238.36±135.55	478.13±132.99	12.13	**<0.001**	1.31	0.26
Diurnal $T_{b,min}$ (°C)	[a]	33.32±0.35	34.31±0.36	30.86±0.37	[a]	30.96±0.45	22.07	**<0.001**	14.31	**<0.001**
Nocturnal $T_{b,min}$ (°C)	33.44±0.45	33.39±0.47	30.32±0.53	31.16±0.54	32.37±0.54	32.31±0.53	10.68	**<0.001**	2.33	0.14
Δm_b (g)	1.75±0.31	2.56±0.32	2.98±0.36	4.66±0.36	4.06±0.36	4.87±0.36	13.25	**<0.001**	23.46	**<0.001**

Results are presented as estimated marginal means±s.e.m., obtained from linear mixed-effects models, where initial m_b and $T_{b,mod}$ were used as covariates (initial m_b=27.32 g and $T_{b,mod}$=36.58°C), duration of food deprivation and T_a were included as fixed factors and animal ID as a random factor. Significant effects of duration of food deprivation and T_a are indicated with bold type.
[a]During 12 h trials, mice were always fasted at night.

Journal of Experimental Biology (2017) 220, 2380-2386 doi:10.1242/jeb.160150

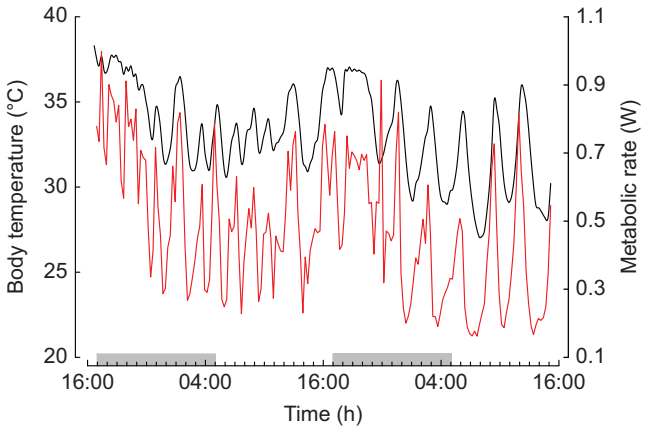

Fig. 2. Time course of body temperature (black line) and metabolic rate (red line) of a representative mouse [ID V3, initial m_b=27.96 g and final m_b=22.01 g] during 48 h of food deprivation. Night is indicated by gray bars.

DISCUSSION

Any T_a lower than thermoneutral is an environmental stress for animals (reviewed in Kingma et al., 2012; Ravussin et al., 2012). We found that laboratory mice (strain C57BL6/cmdb) adjusted their thermoregulation in response to increased environmental demands. Their T_b was more variable and they spent more time below normothermy when they were deprived of food, and this effect was augmented by cold. Our findings give strong support to Angilletta and co-authors' (2010) model of thermal physiology of endotherms, which posits that variations in T_b increase when food is limited and also when operative temperature (here, T_a) decreases. Moreover, we found that mice that showed more precise thermoregulation when fed had more variable T_b when fasted, supporting the prediction of the thermoregulatory generalist–specialist trade-off (Angilletta et al., 2010; Boyles and Warne, 2013) at the intraspecific level.

Thermoregulation depends on several factors such as T_a (Geiser, 2004; Overton and Williams, 2004; Williams et al., 2002), availability of bedding material and social interactions (Gordon, 2004), but also on sex (Geiser and Mzilikazi, 2011; Lovegrove and Raman, 1998). Variations of T_b that were observed in fasted male mice (Figs 1 and 2) could not be defined as torpor. Nevertheless, the use of heterothermy correlated positively with the duration of food deprivation. Such a correlation agrees with thermoregulatory adjustments observed in response to prolonged fasting or food restriction in rats (Wang et al., 2006; Yoda et al., 2000; McCue et al., 2017a), laboratory mice (McCue et al., 2017b), spiny mice (*Acomys russatus*; Gutman et al., 2006) and Chilean mouse-opossums (*Thylamys elegans*; Bozinovic et al., 2007). The lack of prolonged torpor in fasted male mice might be related to sex. In many species, females have been shown to be more prone to using torpor, whereas males often showed only slight variations of T_b (Geiser and Mzilikazi, 2011; Lovegrove and Raman, 1998). Swoap and Gutilla (2009) suggested that it was easier to induce torpor in female than in male C57BL/6 mice. This was supported by the present results; we used male mice and we did not find torpor bouts described for females of the species elsewhere (Dikic et al., 2008; Hudson and Scott, 1979; Schubert et al., 2010; Swoap and Gutilla, 2009). However, contrary to animals that use torpor spontaneously and lower their T_b only during rest phase (Ruf et al., 1993), fasted animals enter torpor regardless of the time of day (Dikic et al., 2008; Swoap and Gutilla, 2009), which is in line with the present results. T_b of mice started to decrease on the first day of the experiment, and both diurnal and nocturnal $T_{b,min}$ decreased as food deprivation prolonged (Figs 1 and 2). Note, however, that Kanizsai and co-authors (2009), who fasted mice at T_a=28°C, found that the mice lowered normothermic T_b only at night, and only during the second day of fasting.

The present results show that T_b decreased with the duration of fasting (Table 1), which led to a decrease in daily energy expenditure. Hence, it seems that although mice did not enter deep torpor, they clearly could benefit from shallow and interrupted hypothermia in the face of increased environmental demands. Thus, even low variability of T_b and a concomitant decrease in MR seems to be an effective way to save energy in the face of a decrease in energy supply. Cold resulted in increased HI and Δm_b, and in lower $T_{b,min}$, yet we did not find a significant effect of T_a on time spent with T_b below normothermy (Table 1). Williams and co-authors (2002) reported that food-restricted C57BL/6J mice reduced their energy expenditure much more at 23°C than at 30°C (TNZ), which could support the idea that variability of T_b increases with decreasing T_a (Gordon, 2009; but see Ravussin et al., 2012). Also, Overton and Williams (2004) showed that response to caloric restriction leads to greater changes in physiology at T_a values below TNZ, and in other studies, the effects of limited energy supply and decreased T_a on heterothermy use were found to be additive (Gordon, 2012; Nespolo et al., 2010; but see Ravussin et al., 2012; Williams et al., 2002).

Fasting or caloric restriction is a challenge, especially for small endothermic animals, and it significantly affects their physiology and behavior (Overton and Williams, 2004; Sakurada et al., 2000; Tucci et al., 2006). During fasting or prolonged food restriction in mice, heart rate, blood pressure, oxygen consumption and pulse are reduced gradually with increasing duration of negative energy balance (Swoap and Gutilla, 2009; Williams et al., 2002). Fasting may also lessen exploratory behavior and result in memory disorders (Tucci

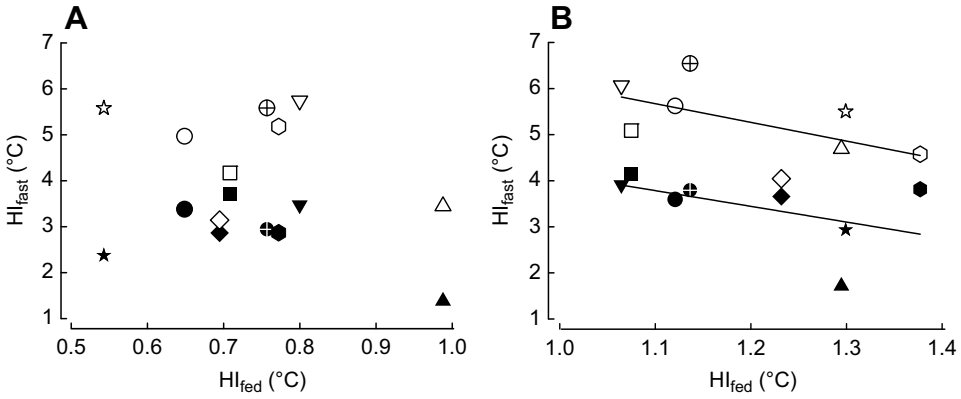

Fig. 3. Correlation between heterothermy indices of fed (HI_{fed}) and fasted (HI_{fast}) mice (n=8) during the first (filled symbols) and second day (open symbols) of fasting in a metabolic chamber at T_a=20°C. (A) During the active phase of day; (B) during whole-day recordings. Each mouse is indicated by different symbols. Note the different scales on the *x*-axes.

et al., 2006). However, it is known that regulating T_b at low levels during fasting provides energy savings (Hudson and Scott, 1979; Schubert et al., 2010), which may enhance survival in the face of adverse conditions (Geiser and Turbill, 2009), and lead to improved fitness of heterothermic animals compared with homeothermic ones (Angilletta et al., 2010). In line with that, we found a significant correlation between EE and mean T_b of fasted mice.

According to models of Gilchrist (1995) and Angilletta et al. (2010), animals can be classified as specialists or generalists, and they should differ in thermoregulatory responses to increasing environmental demands, such as food deprivation or cold (Angilletta et al., 2010; Boyles and Warne, 2013). Specialists should thermoregulate more precisely when energy supply is not limited, but in the face of high energy demands, their T_b should be more variable, leading to lower costs of maintaining homeothermy (Boyles and Warne, 2013). In support of this prediction, male mice that were characterized by lower HI_{fed} showed higher HI during both the first and second days of fasting (Fig. 3B). This negative correlation between variability of T_b under feeding and fasting conditions is the first experimental support for a specialist–generalist trade-off at the intraspecific level (Boyles and Warne, 2013). However, the observed intra-individual variability of thermoregulatory responses to fasting (Fig. 3B) may indicate the proposed specialist–generalist trade-off is a continuum that includes a wide spectrum of thermal sensitivities, similar to what was inferred from interspecific analyses (Boyles et al., 2013). Moreover, intraspecific variability in the use of heterothermy might be important for predicting animal responses to changing conditions on the biogeographic scale (Bozinovic et al., 2011). Heterothermy could mitigate the effects of abiotic conditions on species distribution, enhancing their tolerance to increasing environmental demands (Boyles et al., 2011a). The presence of the proposed specialist–generalist trade-off at the intraspecific level also suggests that heterothermy could be correlated with selection for high mass-independent metabolic rates (Bozinovic et al., 2011; Boyles and Warne, 2013). Our results indicate that hypotheses testing trade-offs in the evolution of thermoregulatory strategies in endotherms can also be tested at the intraspecific level, offering a valid tool to seek mechanistic explanations of the observed differences in animal responses to variations in energy supply and environmental demands.

Acknowledgements
We thank Dr Justin G. Boyles for critical reading and comments on the manuscript. We also thank two anonymous reviewers for their constructive comments.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: A.S.P., J.B., M.S.W., M.J.; Methodology: A.S.P., J.B., M.S.W., M.J.; Formal analysis: A.S.P., M.S.W.; Investigation: A.S.P., M.S.W., M.J.; Writing - original draft: A.S.P., J.B., M.S.W., M.J.; Visualization: A.S.P.; Funding acquisition: J.B., M.J.

Funding
The study was partially funded by the Faculty of Biology and Environmental Protection of Nicolaus Copernicus University funds to M.J. and partially by a grant from the Faculty of Biology and Environmental Protection of Nicolaus Copernicus University awarded to J.S.B. (grant no. 1915-B).

References
Angilletta, M. J., Jr, Bennett, A. F., Guderley, H., Navas, C. A., Seebacher, F. and Wilson, R. S. (2006). Coadaptation: a unifying principle in evolutionary thermal biology. *Physiol. Biochem. Zool.* **79**, 282-294.

Angilletta, M. J., Jr, Cooper, B. S., Schuler, M. S. and Boyles, J. G. (2010). The evolution of thermal physiology in endotherms. *Front. Biosci. (Elite Ed)* **2**, 861-881.

Bae, H. H., Larkin, J. E. and Zucker, I. (2003). Juvenile Siberian hamsters display torpor and modified locomotor activity and body temperature rhythms in response to reduced food availability. *Physiol. Biochem. Zool.* **76**, 858-867.

Boyles, J. G. and Warne, R. W. (2013). A novel framework for predicting the use of facultative heterothermy by endotherms. *J. Theor. Biol.* **336**, 242-245.

Boyles, J. G., Seebacher, F., Smit, B. and McKechnie, A. E. (2011a). Adaptive thermoregulation in endotherms may alter responses to climate change. *Integr. Comp. Biol.* **51**, 676-690.

Boyles, J. G., Smit, B. and McKechnie, A. E. (2011b). A new comparative metric for estimating heterothermy in endotherms. *Physiol. Biochem. Zool.* **84**, 115-123.

Boyles, J. G., Thompson, A. B., McKechnie, A. E., Malan, E., Humphries, M. M. and Careau, V. (2013). A global heterothermic continuum in mammals. *Glob. Ecol. Biogeogr.* **22**, 1029-1039.

Bozinovic, F., Muñoz, J. L. P., Naya, D. E. and Cruz-Neto, A. P. (2007). Adjusting energy expenditures to energy supply: food availability regulates torpor use and organ size in the Chilean mouse-opossum *Thylamys elegans*. *J. Comp. Physiol. B* **177**, 393-400.

Bozinovic, F., Calosi, P. and Spicer, J. I. (2011). Physiological correlates of geographic range in animals. *Annu. Rev. Ecol. Evol. Syst.* **42**, 155-179.

Dikic, D., Heldmaier, G. and Meyer, C. W. (2008). Induced torpor in different strains of laboratory mice. In *Hypometabolism in Animas: Hibernation, Torpor and Crybiology* (ed. B. G. Lovegrove and A. E. McKechnie), pp. 223-230. Pietermaritzburg: University of KwaZulu-Natal.

Geiser, F. (2004). Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annu. Rev. Physiol.* **66**, 239-274.

Geiser, F. and Mzilikazi, N. (2011). Does torpor of elephant shrews differ from that of other heterothermic mammals? *J. Mammal.* **92**, 452-459.

Geiser, F. and Ruf, T. (1995). Hibernation versus daily torpor in mammals and birds: physiological variables and classification of torpor patterns. *Physiol. Zool.* **68**, 935-966.

Geiser, F. and Turbill, C. (2009). Hibernation and daily torpor minimize mammalian extinctions. *Naturwissenschaften* **96**, 1235-1240.

Gilchrist, G. W. (1995). Specialists and generalists in changing environments. 1. Fitness landscapes of thermal sensitivity. *Am. Nat.* **146**, 252-270.

Gordon, C. J. (2004). Effect of cage bedding on temperature regulation and metabolism of group-housed female mice. *Comp. Med.* **54**, 63-68.

Gordon, C. J. (2009). Quantifying the instability of core temperature in rodents. *J. Therm. Biol.* **34**, 213-219.

Gordon, C. J. (2012). Thermal physiology of laboratory mice: defining thermoneutrality. *J. Therm. Biol.* **37**, 654-685.

Grafen, A. and Hails, R. (2002). *Modern Statistics for the Life Sciences*. Oxford: Oxford University Press.

Gutman, R., Choshniak, I. and Kronfeld-Schor, N. (2006). Defending body mass during food restriction in *Acomys russatus*: a desert rodent that does not store food. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **290**, R881-R891.

Gutman, R., Yosha, D., Choshniak, I. and Kronfeld-Schor, N. (2007). Two strategies for coping with food shortage in desert golden spiny mice. *Physiol. Behav.* **90**, 95-102.

Heldmaier, G. and Ruf, T. (1992). Body temperature and metabolic rate during natural hypothermia in endotherms. *J. Comp. Physiol. B* **162**, 696-706.

Hudson, J. W. and Scott, I. M. (1979). Daily torpor in the laboratory mouse, *Mus musculus* var. albino. *Physiol. Zool.* **52**, 205-218.

Humphries, M. M., Thomas, D. W. and Kramer, D. L. (2003). The role of energy availability in mammalian hibernation: a cost-benefit approach. *Physiol. Biochem. Zool.* **76**, 165-179.

IUPS Thermal Commission (2001). Glossary of terms for thermal physiology. *Jpn. J. Physiol.* **51**, 245-280.

Kanizsai, P., Garami, A., Solymár, M., Szolcsányi, J. and Szelényi, Z. (2009). Energetics of fasting heterothermia in TRPV1-KO and wild type mice. *Physiol. Behav.* **96**, 149-154.

Kingma, B., Frijns, A. and van Marken Lichtenbelt, W. (2012). The thermoneutral zone: implications for metabolic studies. *Front. Biosci. (Elite Ed)* **4**, 1975-1985.

Lighton, J. R. (2008). Measuring metabolic rates: a manual for scientists. New York: Oxford University Press, Inc.

Lighton, J. R. B., Bartholomew, G. A. and Feener, D. H. (1987). Energetics of locomotion and load carriage and a model of the energy cost of foraging in the leaf-cutting ant *Atta colombica* Guer. *Physiol. Zool.* **60**, 524-537.

Lima, S. L. and Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool. Rev. Can. Zool.* **68**, 619-640.

Lovegrove, B. G. (2000). Daily heterothermy in mammals: coping with unpredictable environments. In *Life in the Cold: Eleventh International Hibernation Symposium* (ed. G. Heldmaier and M. Klingenspor), pp. 29-40. Berlin: Springer.

Lovegrove, B. G. and Raman, J. (1998). Torpor patterns in the pouched mouse (*Saccostomus campestris*; Rodentia): a model animal for unpredictable environments. *J. Comp. Physiol. B* **168**, 303-312.

McCue, M. D., Albach, A. and Salazar, G. (2017a). Previous repeated exposure to food limitation enables rats to spare lipid stores during prolonged starvation. *Physiol. Biochem. Zool.* **90**, 63-74.

McCue, M. D., Sandoval, J., Beltran, J. and Gerson, A. R. (2017b). Dehydration causes increased reliance on protein oxidation in mice: a test of the protein-for-water hypothesis in a Mammal. *Physiol. Biochem. Zool.* **90**, 1-11.
McNab, B. K. (1974). The energetics of endotherms. *Ohio J. Sci.* **74**, 370-380.
Morton, S. R. (1978). Torpor and nest-sharing in free-living *Sminthopsis crassicaudata* (Marsupialia) and *Mus musculus* (Rodentia). *J. Mammal.* **59**, 569-575.
Nespolo, R. F., Verdugo, C., Cortés, P. A. and Bacigalupe, L. D. (2010). Bioenergetics of torpor in the microbiotherid marsupial, monito del monte (*Dromiciops gliroides*): the role of temperature and food availability. *J. Comp. Physiol. B* **180**, 767-773.
Overton, J. M. and Williams, T. D. (2004). Behavioral and physiologic responses to caloric restriction in mice. *Physiol. Behav.* **81**, 749-754.
Ravussin, Y., LeDuc, C. A., Watanabe, K. and Leibel, R. L. (2012). Effects of ambient temperature on adaptive thermogenesis during maintenance of reduced body weight in mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **303**, R438-R448.
Refinetti, R. and Menaker, M. (1992). The circadian rhythm of body temperature. *Physiol. Behav.* **51**, 613-637.
Ruf, T. and Geiser, F. (2015). Daily torpor and hibernation in birds and mammals. *Biol. Rev. Camb. Philos. Soc.* **90**, 891-926.
Ruf, T. and Heldmaier, G. (2000). Djungarian hamsters – small graminivores with daily torpor. In *Activity Patterns in Small Mammals: An Ecological Approach* (ed. S. Halle and N. C. Stenseth), pp. 217-234. Berlin: Springer.
Ruf, T., Stieglitz, A., Steinlechner, S., Blank, J. L. and Heldmaier, G. (1993). Cold exposure and food restriction facilitate physiological responses to short photoperiod in Djungarian hamsters (*Phodopus sungorus*). *J. Exp. Zool.* **267**, 104-112.
Russell, J. C., Epling, W. F., Pierce, D., Amy, R. M. and Boer, D. P. (1987). Induction of voluntary prolonged running by rats. *J. Appl. Physiol.* **63**, 2549-2553.
Sakurada, S., Shido, O., Sugimoto, N., Hiratsuka, Y., Yoda, T. and Kanosue, K. (2000). Autonomic and behavioural thermoregulation in starved rats. *J. Physiol.* **526**, 417-424.
Scholander, P. F., Hock, R., Walters, V., Johnson, F. and Irving, L. (1950). Heat regulation in some arctic and tropical mammals and birds. *Biol. Bull.* **99**, 237-258.
Schubert, K. A., Vaanholt, L. M., Stavasius, F., Demas, G. E., Daan, S. and Visser, G. H. (2008). Female mice respond differently to costly foraging versus food restriction. *J. Exp. Biol.* **211**, 2214-2223.
Schubert, K. A., Boerema, A. S., Vaanholt, L. M., de Boer, S. F., Strijkstra, A. M. and Daan, S. (2010). Daily torpor in mice: high foraging costs trigger energy-saving hypothermia. *Biol. Lett.* **6**, 132-135.
Snyder, G. K. and Nestler, J. R. (1990). Relationships between body temperature, thermal conductance, Q10 and energy metabolism during daily torpor and hibernation in rodents. *J. Comp. Physiol. B* **159**, 667-675.
Swoap, S. J. and Gutilla, M. J. (2009). Cardiovascular changes during daily torpor in the laboratory mouse. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **297**, R769-R774.
Tomlinson, S., Withers, P. C. and Cooper, C. (2007). Hypothermia versus torpor in response to cold stress in the native Australian mouse *Pseudomys hermannsburgensis* and the introduced house mouse *Mus musculus*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **148**, 645-650.
Tucci, V., Hardy, A. and Nolan, P. M. (2006). A comparison of physiological and behavioural parameters in C57BL/6J mice undergoing food or water restriction regimes. *Behav. Brain Res.* **173**, 22-29.
Wang, T., Hung, C. C. Y. and Randall, D. J. (2006). The comparative physiology of food deprivation: from feast to famine. *Annu. Rev. Physiol.* **68**, 223-251.
Webb, G. P., Jagot, S. A. and Jakobson, M. E. (1982). Fasting-induced torpor in Mus Musculus and its implications in the use of murine models for human obesity studies. *Comp. Biochem. Physiol. A Physiol.* **72**, 211-219.
Williams, T. D., Chambers, J. B., Henderson, R. P., Rashotte, M. E. and Overton, J. M. (2002). Cardiovascular responses to caloric restriction and thermoneutrality in C57BL/6J mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **282**, R1459-R1467.
Wojciechowski, M. S. and Pinshow, B. (2009). Heterothermy in small, migrating passerine birds during stopover: use of hypothermia at rest accelerates fuel accumulation. *J. Exp. Biol.* **212**, 3068-3075.
Wojciechowski, M. S., Jefimow, M. and Pinshow, B. (2011). Heterothermy, and the energetic consequences of huddling in small migrating passerine birds. *Integr. Comp. Biol.* **51**, 409-418.
Yoda, T., Crawshaw, L. I., Yoshida, K., Su, L., Hosono, T., Shido, O., Sakurada, S., Fukuda, Y. and Kanosue, K. (2000). Effects of food deprivation on daily changes in body temperature and behavioral thermoregulation in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **278**, R134-R139.