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Specialist-generalist model of body temperature regulation can be applied on the intraspecific level

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Summary statement:

Mice with more precise thermoregulation when fed, had more labile body temperature when fasted, supporting the hypothesis that thermal specialists are more prone to use facultative heterothermy than thermal generalists.

Abstract

According to theoretical predictions endothermic homeotherms can be classified as either thermal specialists and thermal generalists. In high cost environments thermal specialists are supposed to be more prone to use facultative heterothermy than generalists. We tested this hypothesis on the intraspecific level using laboratory male 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 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, we found that 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 on the intraspecific level, offering a valid tool to seek for mechanistic explanations of the differences in animal responses to variations in energy supply.

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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 their need of maintaining T_b, is to increase energy intake (Humphries et al., 2003; McNab, 1974; Scholander et al., 1950). However, mammalian T_b varies continuously on 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; Angilletta et al., 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 above, Boyles and Warne (2013) further predicted that in the face of energy limitation, specialists should be more prone to use facultative heterothermy than generalists. Food limitation and cold increase the costs of maintaining high T_b and increase frequency and lengthen duration of torpor bouts (Bozinovic et al., 2007; for rodent examples: 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.a. by entering torpor (Gutman et al., 2007). However, increasing activity may even lead to resource exhaustion (Russell et al., 1987), and it increases 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 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 the discussion of increased predation risk associated with torpor). In recent decades several studies have focused on torpor use as a response to energy supply deficit (Bae et al., 2003; Gutman et al., 2006; Nespolo et al., 2010; Schubert et al., 2010; Schubert et al., 2008). Wild house mice (*Mus*

musculus), as well as different laboratory strains, enter daily torpor in face of high energy demands (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 (low food availability or low T_a, or both) (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 between 12 and 48h at different T_a's, and measured their T_b continuously. We predicted that variability of T_b and time spent with T_b below normothermy 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 energetic consequences of such a pattern of heterothermic thermoregulation. To do so, we measured metabolic rate (MR) of mice during 48h food deprivation at constant, moderate T_a. Using data of T_b variability in fed and fasted mice we tested hypothesis that facultative heterothermy is more common in thermal specialists than in generalists (Boyles and Warne, 2013); specifically, we examined the prediction that on the intraspecific level, precise regulation of T_b during *ad libitum* food availability negatively correlates with greater variability of T_b during food deprivation.

Materials and Methods

Animals

All experimental procedures were approved by the Local Committee for Ethics in Animal Research in Bydgoszcz, Poland (Decisions no. 6/2014 from 16.01.2014, and no. 32/2014 from 27.11.2014). We used 2 groups of male mice of C57BL6/cmdb strain originating from the colony maintained in 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 - twelve individuals. Two-month-old mice were transported to the Nicolaus Copernicus University in Toruń and were acclimated for two months before starting the experiments. They were kept individually in standard rodent cages (model 1264, Tecniplast, Italy) in a walk-in climate chamber at T_a = 20 ± 2°C and under 12L:12D day:night cycle, with food (Labofeed B, Kcynia, Poland) and water available *ad libitum*.

Experimental protocol

A week before each experiment (experiment 1 and 2; see below) mice were implanted intraperitoneally with miniaturized temperature-sensitive data loggers (modified iButton, model DS1922L, Dallas Semiconductors, 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 three 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-minute intervals with resolution of 0.5°C, whereas during metabolic measurements (experiment 2) it was logged every five minutes with 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 whether 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 48h at 20°C, and then for 12h and 24h, at 10°C. During experiments at 20°C mice were randomly divided into 2 groups of 4 mice each, which differed in the order of food deprivation trails, except for the 48h fast which was done as the last one in both groups. This was done to minimize confounding effects of repeated food deprivation events on animal response to fasting (McCue et al., 2017 a). Because the order of trials did not affect T_b (linear mixed-effects (LME) models with order of trials as a fixed factor, 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-day habituation were deprived of food for 24 and 12h. Because there is no information about mice being fasted in the cold for more than 24h, we did not extend food deprivation for the safety reasons. Before each trial, food was withdrawn ~0.5h 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 of 0.1g (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.

Experiment 1: Data analysis

Out of eight animals used in the first series of experiments we obtained T_b data for 4 mice, which were fasted at both T_a's. Additionally, we collected data for further 2 individuals fasted at 20°C, and for 2 others fasted at 10°C. In total, we analyzed T_b recordings from six individuals measured at each T_a. Following Boyles et al. (2011 b) we used heterothermy index (HI) which is a statistical description of T_b variations that can be used for all thermoregulating animals:

$$HI = \sqrt{\frac{\Sigma(T_{b\text{-}mod} - T_{b\text{-}1})^2}{n\text{-}1}},$$

where $T_{b\text{-}mod}$ is modal T_b (here: the most frequent T_b recorded when food was available *ad libitum*), $T_{b\text{-}i}$ 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 HI's of mice fed *ad*

libitum (HI_{fed}) at both T_a's 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.

Since 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 after Wojciechowski and Pinshow (2009) and was calculated separately for each animal. We assumed that normothermic T_b is normally distributed with center on modal diurnal T_b. Then we fitted the normal distribution curve shaped by the data equal or higher than the modal T_b. Hypothermic T_b ($T_{b\text{-}hypo}$) was accepted as T_b lower than modal diurnal (rest-phase) T_b minus two SD's. Then we calculated the time when animals were hypothermic during each trial.

Minimum T_b ($T_{b\text{-}min}$) was calculated separately for day and night as the mean of three consecutive lowest recordings during food deprivation that were taken 12 minutes apart. Body mass loss (Δm_b) resulting from food deprivation was calculated as a difference 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 $T_{b\text{-}hypo}$, $T_{b\text{-}min}$, and Δm_b of fasted mice we used LME model in which initial m_b and $T_{b\text{-}mod}$ were included as covariates. Duration of food deprivation and T_a were included as fixed factors and animal ID as random factor. In all analyses 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 48h food deprivation

To determine energetic consequences of T_b variations recorded in Experiment 1, metabolic rate 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 ~1h before the active phase and lasted for ~48h.

During measurements, mice were exposed to T_a = 20°C and had access to water *ad libitum*. Mice were sealed for the duration of experiment in 0.85L chambers constructed of translucent polypropylene containers (HPL 808, Lock&Lock, Hana Cobi, South Korea). To ensure that mice had access to water, a ~150 ml drinking bottle (model ACBT0152, Tecniplast, Italy) was mounted in chamber's lid. Moreover, we put 3g of sawdust from animal's home cage to each respirometry chamber; this amount of bedding was insufficient to build a nest but may have reduced 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 Inc., 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 3 chambers and to 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 System Int. USA). Gases leaving respirometry chambers were selected sequentially with a computer-controlled multiplexer (Intelligent Multiplexer V3, Sable System Int.). 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 Int.). Air was then dried in a column of magnesium perchlorate (product number 11636.36, VWR International, Gdańsk, Poland) and subsequently fractional concentration of CO_2 (FCO_2) and O_2 (FO_2) were measured using a FoxBox-C integrated CO_2 and O_2 analyzer or with separate CO_2 (CA-10, Sable System Int.) and O_2 analyzers (FC-10a Sable System Int.). All electronic elements of the respirometry system were connected to a PC via analog-to-digital interface (UI2, Sable Systems Int.) and data were acquired using ExpeData software (Sable System Int.) at 0.5 Hz. Animal m_b was measured before and after MR measurements to the nearest 0.1g (Scout Pro 200, O'Haus, USA).

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

Experiment 2: Data analysis

$\dot{V}O_2$ and $\dot{V}CO_2$ of animals for which both FO_2 and FCO_2 were measured were calculated using equations 10.6 and 10.7 after Lighton (2008). To calculate $\dot{V}O_2$ of animals for which only FO_2 was measured, we assumed 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 Watts (W) using RER and oxyjoule equivalent after Lighton et al. (1987) as:

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

For each mouse, its total energy expenditure (EE) during a 46h food deprivation was calculated by integrating the area below the EE curve (we uniformly subtracted 1h 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 (5:00 p.m.) until 4:00 p.m. the next day. Energy expenditure for the second day (EE2) was calculated in the same way. Total energy expenditure (EE) was the sum of EE1 and EE2.

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

Finally, to determine whether individuals which regulated their normothermic T_b more precisely showed greater variations of T_b during fast, we used LME model with HI_{fast} as dependent variable, HI_{fed} (here calculated from 2-day recordings before experiment and used for analysis of 1st and 2nd day of food deprivation) and m_b as covariates, day of food deprivation as a fixed factor and animal ID as 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 did two analyses: first for data obtained only during active phase of day (*i.e.* night) as suggested Boyles and Warne (2013), and second for data obtained for whole 24h, because fasted mice showed large variations in T_b both during active and inactive phase of the day.

All analyses were done in IBM SPSS Statistics v. 23 (IBM_Corp, 2014). 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 were presented as estimated marginal means ± SE, and statistical significance was accepted at $\alpha < 0.05$.

Results

Experiment 1: Response of mice to increasing environmental demands

Overall, when food deprived for 24h, 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 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 the 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 ($F_{(2, 28)} = 20.13$, $p < 0.001$). Heterothermy index during food deprivation also increased with decrease of T_a ($F_{(1, 28)} = 7.68$, $P = 0.01$), and during 12h 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 resulted in higher HI_{fast} ($F_{(3, 28)} = 3.36$, $P = 0.03$), and during 24h food deprivation at 20°C HI_{fast} was 1.63 ± 0.17°C, while during 48h it increased to 2.34 ± 0.20°C (Table 1).

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 $T_{b\text{-}hypo}$ increased with the duration of food deprivation ($F_{(3, 28)} = 12.13$, $P < 0.001$; Table 1). During 12h fast at T_a = 20°C, mice spent 34.45 ± 114.39 min with $T_{b\text{-}hypo}$, and during the 48h they spent 1072.29 ± 135.43 min with $T_{b\text{-}hypo}$ (Table 1). Ambient temperature did not significantly affect time with $T_{b\text{-}hypo}$ ($F_{(1, 28)} = 1.31$, $P = 0.26$; Table 1).

Diurnal $T_{b\text{-}min}$ decreased with T_a ($F_{(1, 28)} = 14.31$, $P = 0.01$) and with lengthening of food deprivation ($F_{(3, 28)} = 22.07$, $P < 0.001$, Table 1). Nocturnal $T_{b\text{-}min}$ 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 $T_{b\text{-}mod}$ ($F_{(1, 28)} = 0.07$, $P = 0.79$).

Experiment 2: Metabolic rate and body temperature during 48h 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 ($F_{(2, 8)} = 130.154$, $P < 0.001$). 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 46h 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 ($F_{(1, 11)} = 5.51$, $P = 0.04$).

Heterothermy index 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 24h, 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 ($F_{(1, 13)} = 27.04$, $P< 0.001$).

Discussion

Any T_a lower than thermoneutral is an environmental stress for animals (review Kingma et al., 2012; Ravussiv 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 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: ambient temperature) decreases. Moreover, we found that mice, which 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) on the intraspecific level.

Thermoregulation depends on several factors like T_a (Geiser, 2004; Overton and Williams, 2004; Williams et al., 2002), availability of bedding material, 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 (Fig. 1 and Fig. 2) could not be defined as torpor. Nevertheless, the use of heterothermy correlated

positively with the duration of food deprivation. Such 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., 2017 a), laboratory mice (McCue et al., 2017 b), spiny mice *Acomys russatus* (Gutman et al., 2006), or 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 were more prone to use 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 females than in males C57BL/6 mice. This was supported by 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 which 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 present results. Mice T_b started to decrease on the first day of experiment, and both, diurnal and nocturnal minimum T_b decreased as food deprivation prolonged (Fig. 1, Fig. 2). Note however, that Kanizsai and co-authors (2009), who fasted mice at T_a = 28°C, found that they lowered normothermic T_b only at night, and only during second day of fasting.

Present results show that T_b decreased with the duration of fasting (Table 1), which lead 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 face of increased environmental demands. Thus, even small variability of T_b and concomitant decrease in MR seems to be an effective way to save energy in face of energy supply deficit. Cold resulted in increased HI, Δm_b, and in lower $T_{b\text{-}min}$, yet we did not find significant effect of T_a on time spent with T_b below normothermy (Tab. 1). Williams and co-authors (2002) reported that, when food-restricted C57BL/6J mice reduced their energy expenditure much more at 23°C than at 30°C (TNZ), what 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's below TNZ, and 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 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 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 et al., 2006). However, it is known that regulating T_b at low level during fasting provides energy savings (Hudson and Scott, 1979; Schubert et al., 2010) which may enhance survival in face of adverse conditions (Geiser and Turbill, 2009), and lead to improved fitness of heterothermic animals compared to homeothermic ones (Angilletta et al., 2010). In line with that, we found significant correlation between EE and mean T_b of fasted mice.

According to models of Gilchrist (1995) and Angiletta et al. (2010), animals can be classified as specialist or generalist, and they should differ in thermoregulatory responses to increasing environmental demands, like food deprivation or cold (Angilletta et al., 2010; Boyles and Warne, 2013). Specialist 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 fast (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., 2011 a). The presence of the proposed specialist-generalist trade-off at the intraspecific level also suggests that heterothermy could be correlated to 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 on 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.

List of abbreviations

T_b – body temperature

T_a – ambient temperature

HI_{fed} – heterothermy index during *ad libitum* food availability

HI_{fast} – heterothermy index during fasting

EE – energy expenditure

m_b – body mass

$T_{b\text{-}hypo}$ – hypothermic body temperature

$T_{b\text{-}min}$ – minimum body temperature

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Author contributions:

All authors planned the work, all contributed to writing the paper, and all gave final approval for publication.

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**, 282-94.

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**, 861-81.

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**, 858-67.

Boyles, J. G., Seebacher, F., Smit, B., McKechnie, A. E. (2011 a). Adaptive thermoregulation in endotherms may alter responses to climate change. *Integr Comp Biol* **51,** 676-90.

Boyles, J. G., Smit, B. and McKechnie, A. E. (2011 b). A new comparative metric for estimating heterothermy in endotherms. *Physiol Biochem Zool* **84**, 115-23.

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

Boyles, J. G. and Warne, R. W. (2013). A novel framework for predicting the use of facultative heterothermy by endotherms. *J Theor Biol* **336**, 242-5.

Bozinovic, F., Munoz, J. L., 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*, eds. B. G. Lovegrove and A. E. McKechnie). Pietermaritzburg: University of KwaZulu-Natal.

Geiser, F. (2004). Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annu Rev Physiol* **66**, 239-74.

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

Geiser, F. and Ruf, T. (1995). Hibernation Versus Daily Torpor in Mammals and Birds - Physiological Variables and Classification of Torpor Patterns. *Physiological Zoology* **68**, 935-966.

Geiser, F. and Turbill, C. (2009). Hibernation and daily torpor minimize mammalian extinctions. *Naturwissenschaften* **96**, 1235-40.

Gilchrist, G. W. (1995). Specialists and Generalists in Changing Environments .1. Fitness Landscapes of Thermal Sensitivity. *American naturalist* **146**, 252-270.

Gordon, C. J. (2004). Effect of cage bedding on temperature regulation and metabolism of group-housed female mice. *Comp Med* **54**, 63-8.

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

Gordon, C. J. (2012). Thermal physiology of laboratory mice: Defining thermoneutrality. *Journal of Thermal Biology* **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**, R881-91.

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. *Physiological Zoology* **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**, 165-79.

IBM_Corp. (2014). IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

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

Kanizsai, P., Garami, A., Solymar, M., Szolcsanyi, J. and Szelenyi, Z. (2009). Energetics of fasting heterothermia in TRPV1-KO and wild type mice. *Physiol Behav* **96**, 149-54.

Kingma, B., Frijns, A. and van Marken Lichtenbelt, W. (2012). The thermoneutral zone: implications for metabolic studies. *Front Biosci (Elite Ed)* **4**, 1975-85.

Lima, S. L. and Dill, L. M. (1990). Behavioral Decisions Made under the Risk of Predation - a Review and Prospectus. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **68**, 619-640.

Lovegrove, B. G. (2000). Daily heterothermy in mammals: Coping with unpredictable environments. *Life in the Cold*, 29-40.

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**, 303-12.

McCue, M.D., Albach, A. and Salazar, G. (2017 a). 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. (2017 b). 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). *Journal of Mammalogy* **59**, 569-575.

Nespolo, R. F., Verdugo, C., Cortes, 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**, 767-73.

Overton, J. M. and Williams, T. D. (2004). Behavioral and physiologic responses to caloric restriction in mice. *Physiol Behav* **81**, 749-54.

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**, R438-48.

Refinetti, R. and Menaker, M. (1992). The circadian rhythm of body temperature. *Physiology & Behavior* **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*, eds. S. Halle and N. C. Stenseth). 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**, 104-12.

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 (1985)* **63**, 2549-53.

Sakurada, S., Shido, O., Sugimoto, N., Hiratsuka, Y., Yoda, T. and Kanosue, K. (2000). Autonomic and behavioural thermoregulation in starved rats. *J Physiol* **526 Pt 2**, 417-24.

Scholander, P. F., Hock, R., Walters, V., Johnson, F. and Irving, L. (1950). Heat Regulation in Some Arctic and Tropical Mammals and Birds. *Biological Bulletin* **99**, 237-258.

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**, 132-5.

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**, 2214-23.

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**, R769-74.

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**, 645-50.

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**, 22-9.

Wang, T., Hung, C. C. and Randall, D. J. (2006). The comparative physiology of food deprivation: from feast to famine. *Annu Rev Physiol* **68**, 223-51.

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. *Comparative Biochemistry and Physiology a-Physiology* **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**, R1459-67.

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.

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**, 3068-75.

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**, R134-9.

Figures

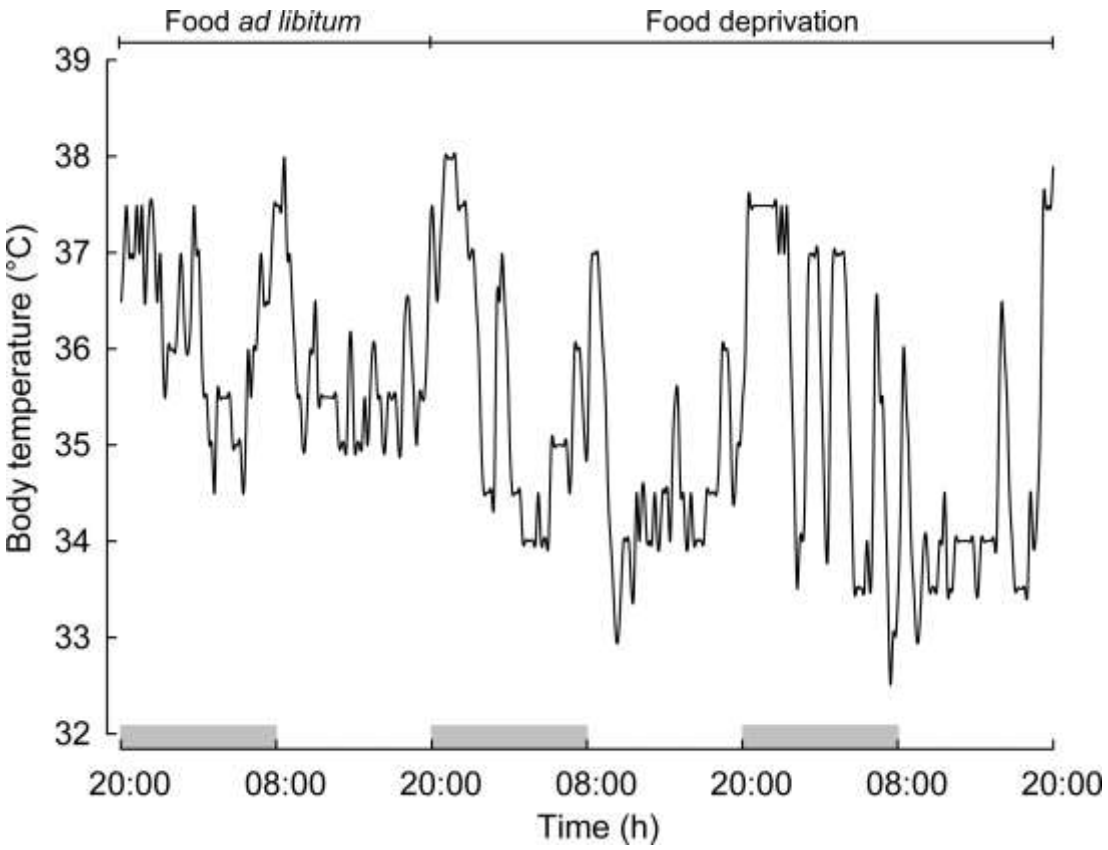

Figure 1. **Time course of body temperature in a representative mouse (ID: W1; initial m_b = 26.8 g, final m_b = 22.8 g) during 24h food *ad libitum* conditions and 48h of food deprivation**. Night is indicated by grey bars.

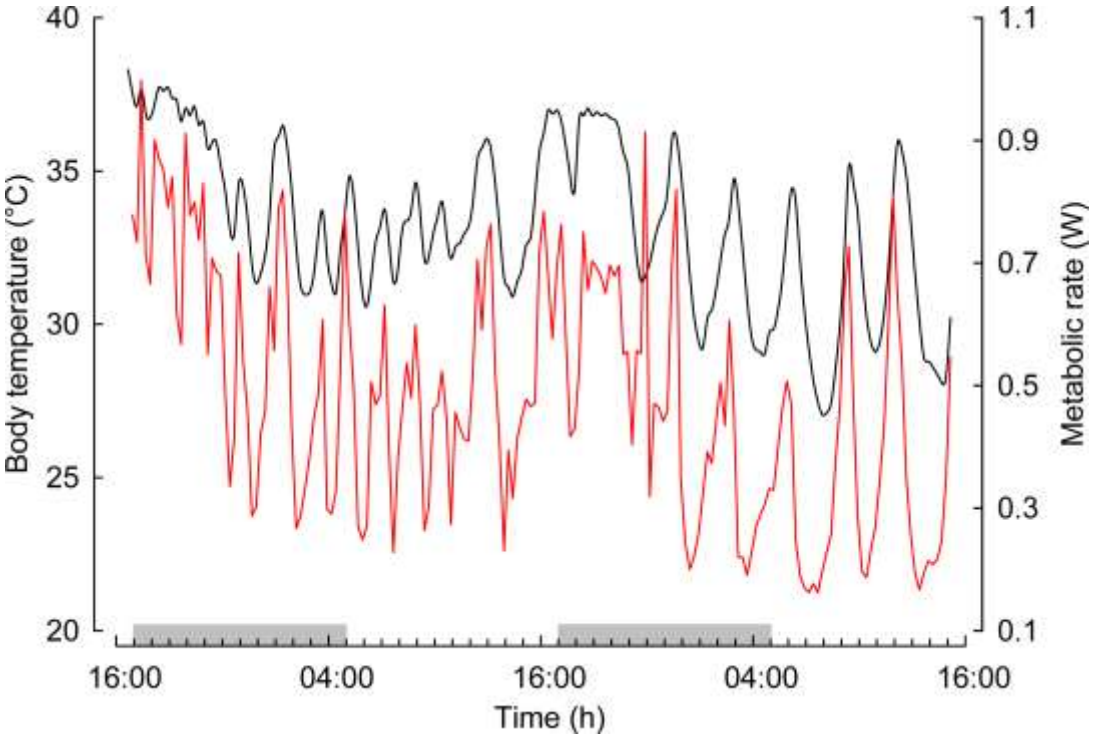

Figure 2: **Time course of body temperature (black line) and metabolic rate (red line) of representative mouse (ID: V3, initial m_b = 27.96 g and final m_b = 22.01 g) during 48h of food deprivation.** Night is indicated by grey bars.

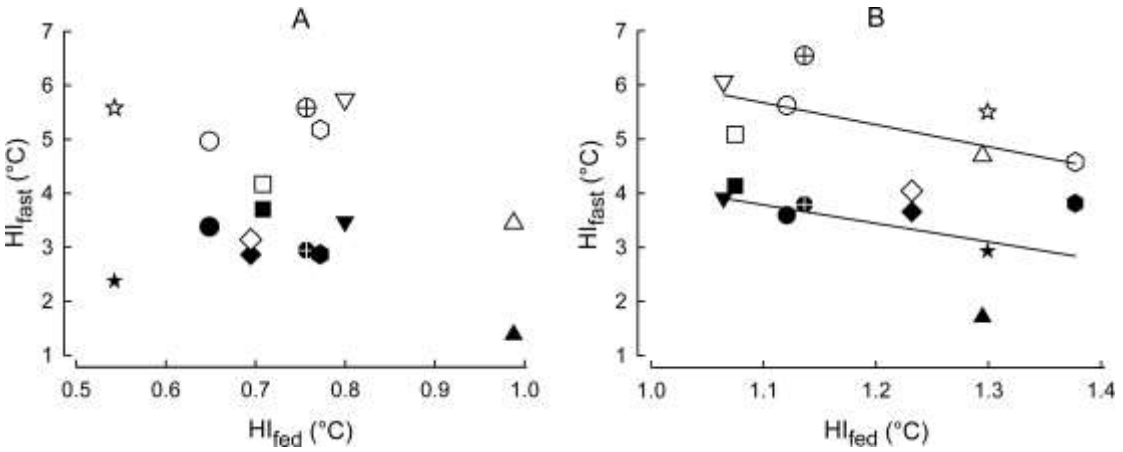

Figure 3. **Correlation between heterothermy indices of fed (HI_{fed}) and fasted (HI_{fast}) mice (n=8) during active phase of day (panel A) and during whole day recordings (panel B), during first (filled symbols) and second day (open symbols) of fasting in metabolic chamber at T_a = 20°C.** Each mouse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

Table 1. **Effect of duration of food deprivation (FD) and ambient temperature (T_a) on heterothermy index (HI), time spent with T_b below normothermy (time $T_{b\text{-}hypo}$), diurnal and nocturnal minimum T_b ($T_{b\text{-}min}$), and body mass loss (Δm_b).** Results were presented as estimated marginal means ± SE, obtained from linear mixed effects models, where initial m_b and $T_{b\text{-}mod}$ were used as covariates, duration of food deprivation and T_a were included as fixed factors and animal ID as random factor. Significant effects of duration of FD and T_a are indicated with bold type.

	20°C				10°C		duration of FD		T_a	
	12	24	36	48	12	24	$F_{(3, 28)}$	*P*	$F_{(1, 28)}$	*P*
HI_{fast} (°C) [b]	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 $T_{b\text{-}hypo}$ (h) [b]	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 $T_{b\text{-}min}$ (°C) [b]	- [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 $T_{b\text{-}min}$ (°C) [b]	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) [b]	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**

[a] Note that during 12h trials mice were always fasted at night

[b] Estimated marginal means were calculated at the following covariates: initial m_b = 27.32g and $T_{b\text{-}mod}$ = 36.58°C