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RESEARCH ARTICLE

Individual differences in torpor expression in adult mice are related to relative birth mass

Goro A. Kato[1,2], Shinsuke H. Sakamoto[3], Takeshi Eto[4], Yoshinobu Okubo[5], Akio Shinohara[1], Tetsuo Morita[3] and Chihiro Koshimoto[1,*]

ABSTRACT

Daily torpor is a physiological adaptation in small mammals and birds, characterised by drastic reductions in metabolism and body temperature. Energy-constraining conditions, such as cold and starvation, are known to cause the expression of daily torpor. However, the reason for high degrees of inter- and intra-individual variation in torpor expression (TE) in similar situations is not clear. As littermates of altricial animals are exposed to an uneven allocation of maternal resources from conception to weaning, we tested whether early nutritional experiences have long-term effects on TE in adults. We used full-sibling littermates of laboratory mice that as adults were starved overnight to induce torpor. We measured body mass from birth until adulthood as an indicator of nutritional status, and calculated the relative body mass (RBM) as an indicator of the difference in nutritional status within a litter. After maturation, we subjected mice to five repeated torpor induction trials involving 24 h of fasting and 5 days of recovery. Half of the female mice displayed great individual variation in TE whereas male mice rarely exhibited daily torpor. In females, RBM at birth influenced TE, irrespective of body mass in adulthood; thus, female mice born with low RBMs displayed high TE in adulthood. In conclusion, we provide evidence that TE in mice differs among littermates, and that this variation is linked closely to heterogeneous nutritional experiences during the fetal period.

KEY WORDS: Daily torpor, Developmental effect, Sibling competition, Phenotypic plasticity, Body mass, *Mus musculus*

INTRODUCTION

To overcome various adverse environmental changes, animals must often react both physiologically and behaviourally. Usually, more than one reaction is possible; therefore, animals ideally respond in such a way as to optimise their fitness via processes such as the optimal allocation of time and energy expenditure (MacArthur and Pianka, 1966). Historically, researchers have had difficulty in determining the effects of 'past experience' on such optimisation. The effects caused by the differences of fetal growth on subsequent traits have, however, been demonstrated (Ryan and Vandenbergh, 2002; Nagao et al., 2004). In addition, recent studies have also demonstrated that some adaptive phenotypic traits are clearly affected by relatively recent experiences (Lima and Dill, 1990; Agrawal, 2001; Milligan et al., 2001; Reznick and Ghalambor, 2001; see also Eto et al., 2015). Therefore, past experience may alter the expression of survival behaviours.

Daily torpor is a physiological adaptation in mammals and birds to cope with energy constraints, such as starvation and cold. Daily torpor is characterised by controlled reductions in metabolic rate and body temperature (T_b), usually during the rest phase of the circadian rhythm (Heldmaier et al., 2004). Daily torpor lasts for <24 h and is interrupted by daily foraging and feeding (Geiser, 2004). Torpor is believed to have originated plesiomorphically with adaptive adjustments to the environmental conditions and ecology of each species (Ruf and Geiser, 2015). Additionally, because the surface area-to-volume ratio of animals increases with decreasing body size, small animals have greater energy requirements than do larger species due to their rate of relative heat loss (Geiser, 2004). Therefore, the ability to engage in daily torpor is crucial and plays an important role in overcoming starvation in small animals by reducing energy expenditure (Ruf and Geiser, 2015).

Torpor expression (TE) in small mammals is adjusted (or triggered) by several exogenous and/or endogenous factors that increase or decrease energy expenditure (see review in Ruf and Geiser, 2015), such as ambient temperature (Swoap, 2008; McKechnie and Mzilikazi, 2011), food availability and foraging costs (Ruf et al., 1991; Schubert et al., 2010), huddling (Eto et al., 2014) and reproductive status (Ruby et al., 1993; Holloway and Geiser, 1996). However, among individuals of the same species, TE is highly variable in both how and how often it is expressed (Canale and Henry, 2011; McKechnie and Mzilikazi, 2011), even if these individuals face similar energy constraints (Lynch et al., 1978; Ruf et al., 1991; Dikic et al., 2008). Furthermore, Brigham et al. (2000) reported that free-ranging Australian owlet-nightjars (*Aegotheles cristatus*) displayed variation in TE among individuals, even though their TE is highly seasonal and only occurs on the coldest days. These previous studies suggest that TE is a plastic physiological characteristic that permits adaptation to stochastic ecological situations.

The house mouse (*Mus musculus* Linnaeus 1758) is a widely used model organism in biology that exhibits daily torpor in response to overnight fasting or food restriction. The methods used to induce torpor in mice are well documented (e.g. Hudson and Scott, 1979; Webb et al., 1982; Rikke et al., 2003). Notably, laboratory mice are usually housed under strictly controlled conditions with the provision of a nutritionally adequate diet and water *ad libitum* until torpor induction. However, TE varies among individuals not only in wild-caught mice (Tomlinson et al., 2007) but also in laboratory strains (Dikic et al., 2008;

[1]Division of Bio-resources, Department of Biotechnology, Frontier Science Research Center, University of Miyazaki, Kihara 5200, Kiyotake, Miyazaki, Miyazaki 889-1692, Japan. [2]Center of Biomedical Research, Research Center for Human Disease Modeling, Graduate School of Medical Sciences, University of Kyushu, Maidashi 3-1-1, Higashi-ku, Fukuoka, Fukuoka 812-8582, Japan. [3]Department of Animal and Grassland Sciences, Faculty of Agriculture, Kibana Campus, University of Miyazaki, Miyazaki 889-2192, Japan. [4]Center for Toki and Ecological Restoration, Niigata University, Niigata 952-0103, Japan. [5]Japan Wildlife Research Center, 3-3-7 Kotobashi, Sumida-ku, Tokyo 130-8606, Japan.

*Author for correspondence (ckos@med.miyazaki-u.ac.jp)

C.K., 0000-0001-9949-508X

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Schubert et al., 2010). Furthermore, Dikic et al. (2008) demonstrated that SWR/J and AKR/J strains of inbred mice had intra-strain variation in torpor; two individual AKR/J mice entered torpor reliably and two other individuals never showed marked hypometabolism or T_b reduction during a 22–24 h combined cold and food deprivation challenge. Based on this report, individual variation in torpor must be moderated by non-genetic factors, given the negligible genetic differences among inbred mice.

In general, mammalian pups share maternal resources, such as milk, nest materials and maternal care with their littermates until they become independent, resulting in competition among littermates (Bautista et al., 2005; Hudson and Trillmich, 2008; Rödel et al., 2008). These competitions can continue from the fetal period and influence one another's physical and physiological development (vom Saal and Bronson, 1980; vom Saal, 1989). Similarly, individual laboratory mice face competition with siblings for food resources prior to weaning whereas, after weaning, all individuals are provided with sufficient food and experience similar environmental conditions. Therefore, we hypothesised that energy-limiting conditions caused by sibling competition prior to weaning may affect TE in adulthood. In this study, we tested the relationship between relative body mass (RBM) among littermates (as an index of differences in nutrient acquisition among littermates before weaning) and TE in adulthood using laboratory mice kept under strictly controlled environmental conditions.

MATERIALS AND METHODS

Animals

The ICR laboratory mouse strain has been used for torpor research (e.g. Hudson and Scott, 1979; Schubert et al., 2010). Females of this strain can raise more pups than their number of teats (10) with a low risk of neglect (12 pups on average; e.g. Eisen, 1978). This trait enhances competition for maternal resources among littermates, and helps us to evaluate differences in growth state among littermates at a fine statistical scale. Therefore, we chose this strain to test our hypothesis.

Ten pairs of Slc:ICR mice were purchased from Japan SLC Inc. (Shizuoka, Japan; this strain was introduced from Charles River Laboratories, Inc., Wilmington, MA, USA, in 1965). Initially, four pairs were mated followed by the remaining six pairs. Each pair of mice was kept within a single cage for two days and separated and held individually when we found vaginal plugs or the presence of spermatozoa in female virginal smear. We used the first-generation offspring for this study. Litter sizes (N) and sex ratios (females:males) in the first mated group were 6 (4:2), 9 (5:4), 11 (7:4) and 12 (5:7); those in the second mated group were 8 (6:2), 12 (7:5), 13 (3:10), 14 (4:10), 14 (7:7) and 15 (12:3). All mice were housed in plastic cages (225×338×140 mm, CLEA Japan, Inc., Tokyo, Japan) with non-autoclaved wood shavings (Soft chip, Japan SLC Inc.) as bedding, and were allowed free access to food (Labo MR Stock, Nosan Corporation, Kanagawa, Japan) and tap water. Room temperature was 23±1°C, with a 12 h:12 h (light:dark) photoperiodic cycle (lights on at 08:00 h). All experimental procedures were reviewed and approved by the Animal Experimentation Committee of the University of Miyazaki (Permission No. 2005–053; 2013-524).

Experimental procedures

Experimental procedures are summarised by the following three steps: (1) continuous measurement of individual body mass from birth to adulthood; (2) implantation of a temperature logger into the abdominal cavity on postnatal day (PND) 63 under anaesthesia; and (3) five repeated torpor induction trials.

After the pups were delivered, we first conducted a series of individual identification processes on all pups: pasted-on tattoo stickers (Model No. 51113; A-ONE Co., Ltd., Tokyo, Japan) on PNDs 0–6, animal markers (FG2200K, Muromachi Kikai Co., Ltd., Tokyo, Japan) on the limbs and back (PNDs 7–17), and ear punching (PNDs 18–20). On PND 21, mice were then weaned and placed individually in plastic cages. Body mass was measured to the nearest 0.01 g at least once per week until the first torpor induction trial (PND 94).

On PND 63 (sexual maturity; Vandenbergh et al., 1972), a temperature logger (iButtons, DS1922L, Maxim Integrated, San Jose, CA, USA) was implanted into the abdominal cavity of each mouse under anaesthesia (i.p.; medetomidine hydrochloride, 0.3 mg kg^{-1}; midazolam, 4 mg kg^{-1}; butorphanol tartrate, 5 mg kg^{-1}) to determine the expression of daily torpor. These loggers were programmed to record temperature every 12 min at 16-bit resolution (0.0625°C), and thus could measure core T_b for 34 consecutive days. In addition, these loggers were coated with thin layers of a paraffin–Evaflex mixture (EV220, Mitsui Du Pont–Mitsui Polychemical Co., Ltd., Tokyo, Japan) following Masaki et al. (2005) to avoid damage from bodily fluids. The average logger mass was 3.39±0.16 g. Most of these loggers were within the recommended mass for implanted devices into small mammals (<10% of body mass, Rojas et al., 2010). Although loggers implanted in seven mice were slightly heavier (range: 10.1–10.6% of body mass), they did not disrupt feeding, activity or any other movement in the mice in which they were implanted (G.A.K., unpublished observations). After implantation, we observed post-operative recovery for at least 3 weeks. This period was sufficient for mice to return to normal (i.e. preoperative) body mass.

After that, we subjected mice to 24 h of fasting (onset at 18:00 h) to induce daily torpor, followed by 5 days of *ad libitum* feeding. These treatments were repeated five times per individual, and TE was estimated using T_b data. Pre-fasting body mass was measured to the nearest 0.01 g. The implanted logger mass was subtracted from body mass. Our protocols were based on those described by Dikic et al. (2008).

Data handling and statistical analysis

In the first mated group, two male pups died immediately after birth and one male pup died after weaning. In addition, four temperature loggers failed to function due to system errors. In the second mated group, four pups (two females and two males) died immediately after birth. Male pups in the second group were not used in subsequent torpor induction trials because TE was rarely observed in male mice in the first group (see the Results section). Therefore, the numbers of mice used in our analysis were 114 (females:males=60:54) on PND 0, 107 (58:49) on PNDs 12 and 21, and 68 (55:13) on PND 94 and for torpor induction trials.

All statistical analyses were performed using the statistical package R (version 3.4.0; R Developmental Core Team, 2017). P-values <0.05 were considered to be statistically significant, and values are reported as means±standard deviations (s.d.).

Body mass data

We analysed body mass data using two methods: actual body mass (ABM) and RBM. RBM was defined to estimate the extent of sibling competition among littermates, because the ABM of each individual was influenced by maternal resources and litter size (Table S1). RBM was calculated within each litter on PNDs 0

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(birth), 12 (eyes opening) and 21 (weaning), according to the formula:

$$\text{RBM} = \text{individual ABM} / \text{mean litter ABM}, \quad (1),$$

where individual ABM and mean litter ABM are measured in g. Body mass data on PND 94 were analysed using ABM alone because pups were already weaned by this time. ABM before fasting during each torpor induction trial was noted as 'BM_{trial}' and defined as an indicator of physical condition just before fasting.

To reveal the long-term effects of differences in birth mass on postnatal development, we estimated the correlations among ABM data from PND 0 to PND 94. The relationships between ABM and litter size were estimated similarly. All correlational tests were performed using Pearson's Correlation analysis. Sex differences in ABM data on PNDs 0, 12, 21 and 94 were identified using analysis of variance (ANOVA). We examined correlations among ABM, RBM and BM_{trial} data to consider the presence of multicollinearity for subsequent analyses.

T_b data and torpor parameters

The mean resting T_b of our mice was maintained at 35.33±0.45°C [N=30, control period (4 days before the first trial)]. Thus, the expression of daily torpor (i.e. TE) was defined as T_b<31°C (Hudson and Scott, 1979). We coded TE as a dummy variable of 'presence (1)' for a torpor trial and 'absence (0)' for a non-torpor trial.

First, we examined whether TE differed by sex in the first mated group using a generalised linear model (GLM). Next, we ran a generalised linear mixed model (GLMM) with the 'glmer' function in the lme4 package (ver. 0.999999-0) (http://CRAN.R-project.org/package=lme4) to examine the factors affecting TE. This analysis was conducted on the basis of sex and used the combined data of both mating groups. To avoid the problem of multicollinearity, highly correlated variables were eliminated or selected from the explanatory variables for the GLMM analysis. This decision was based on the correlation value (r) or variance inflation factor (VIF); in the case of r>0.60 or VIF>10, we chose between the two variables relevant for our purposes. ABMs until PND 21 were strongly correlated with each other (r>0.60, Table S2). RBM on PND 12 also strongly correlated with RBMs on PNDs 0 and 21, while correlation between RBMs on PNDs 0 and 21 was weak (Table S2). There were high VIF values between ABM and RBM on PNDs 0, 12 and 21. Here, we focused on the effects of maternal resources allocation during fetal and lactation periods on TE, thus ABMs and RBMs on PNDs 0 and 21 were more fascinating than those on PND 12. Therefore, we compared three models including: (a) ABM on PND 94 and RBMs on PNDs 0 and 21; (b) ABM on PNDs 0 and 94 and RBM on PND 21; or (c) ABM on PNDs 21 and 94 and RBM on PND 0 as explanatory variables. TE seemed to be influenced by differences between mothers and trials (see Results); thus, 'mother' and 'trial' were considered as random effects. Model selection was conducted using a backward stepwise procedure in which the most parsimonious model corresponded to the model with the lowest Akaike Information Criterion (AIC) value (Anderson et al., 2000).

Furthermore, to determine when and how the physical state before fasting affected TE, we conducted a similar GLMM analysis using BM_{trial} as an explanatory variable and considered 'mother' and 'trial' to be random effects.

For torpor parameters, the time of TE onset, minimum torpor T_b and torpor bout duration (the period of time during which T_b remained below 31°C) were calculated. Linear regression analysis was performed to investigate the relationship between minimum torpor T_b and torpor bout duration.

RESULTS

ABM and RBM

The ABMs of juvenile female mice on PNDs 0, 12, 21 and 94 were 1.87±0.18 g, 7.86±1.46 g, 14.25±2.59 g and 39.11±4.04 g, respectively. In males, ABMs at these time points were 1.91±0.21 g, 7.78±1.08 g, 14.11±2.20 g and 44.90±4.97 g, respectively. We observed no sex difference in ABM, except on PND 94 (ANOVA: PND 0, P=0.25; PND 12, P=0.75; PND 21, P=0.76; PND 94, P<0.01). Similarly, litter size was correlated negatively with ABM when ABM on PND 94 was excluded (Table S1). Interestingly, the correlation between ABM during growth and ABM on PND 0 weakened gradually with time in female mice but not in males (Fig. 1). RBMs were correlated significantly with each other (Tables S2 and S3). These results suggest that mice developed constantly in litters until weaning, irrespective of sex. BM_{trial} seemed to increase over the trials (data not shown); however, this increase was not significant (Tukey's honest significant difference test, P>0.05).

Patterns of torpor

Twenty-six (of 55) female mice exhibited torpor. We observed torpor a total of 74 times in the five repeated torpor induction trials. In contrast, only one male mouse exhibited torpor (Figs S1 and S2). Therefore, subsequent analyses were performed using female data alone.

Torpor in female mice showed high variation among litters and littermates and among and within individuals (Fig. S1). These could be categorised as single (Fig. 2A) and multiple torpor bouts (Fig. 2B). In addition, we observed torpor under *ad libitum* feeding conditions (Fig. 2C; see also Fig. S1). Trial 2 involved the most TE (19 mice); 8–10 mice exhibited torpor in the other trials. One individual (MD06) exhibited torpor in all trials (Fig. 2, Fig. S1).

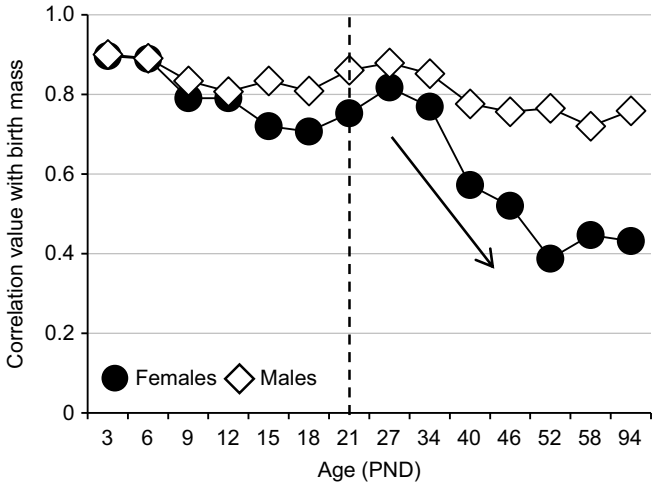

Fig. 1. Correlations between actual body mass (ABM) on postnatal day (PND) 0 and subsequent ABMs. To examine the long-term effects of birth mass on subsequent development, we estimated the relationships between ABM on PND 0 and subsequent ABMs. The *y*-axis indicates the correlation value (Pearson's correlation analysis) between ABM on PND 0 and the subsequent ABMs and the *x*-axis indicates the age in PND. The broken line indicates the day of weaning (PND 21). The correlation weakened after weaning in female mice but did not in male mice. N=35 (21 females, 14 males).

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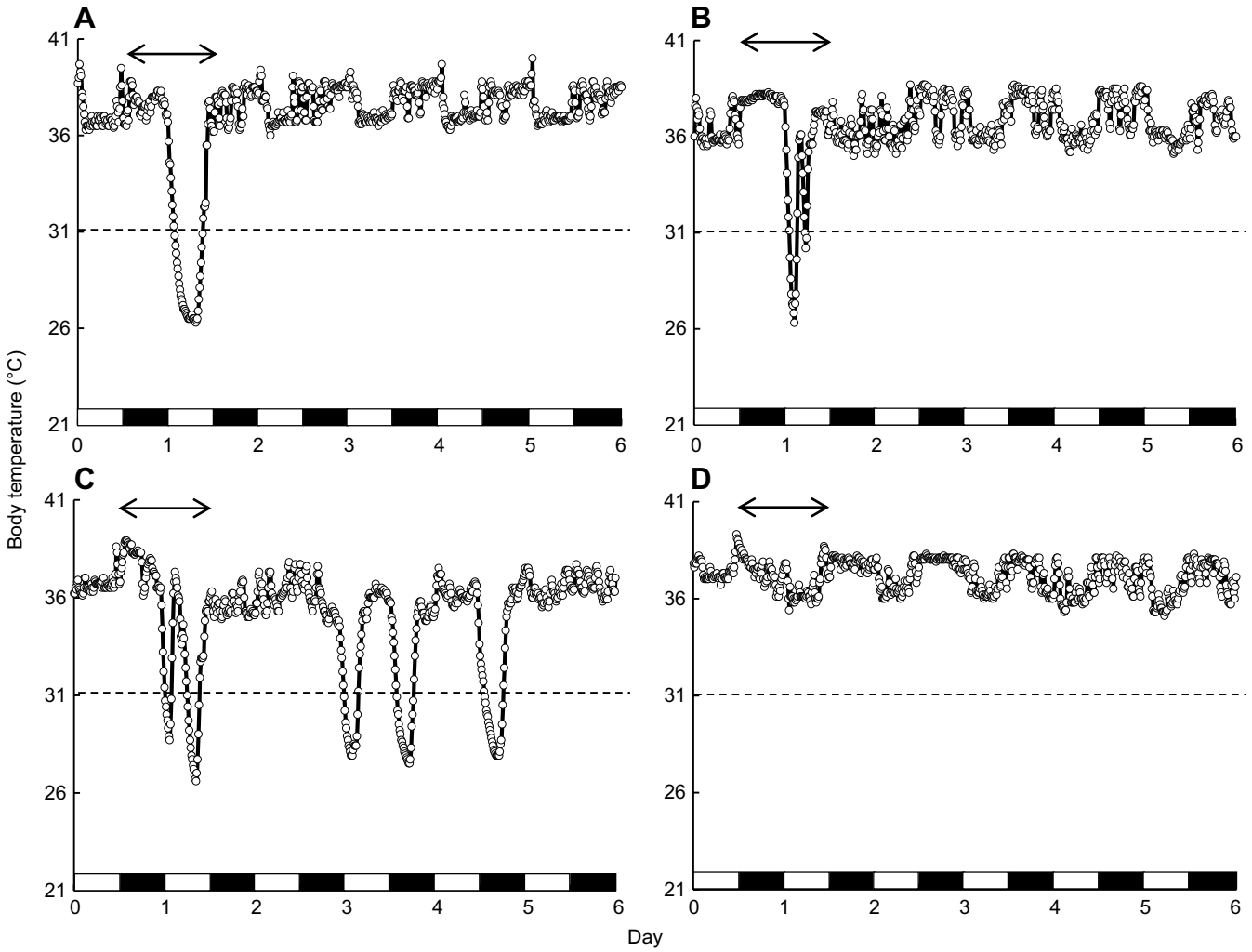

Fig. 2. Representative body temperature (T_b) patterns during torpor induction trials in individual female mice. The patterns of torpor bouts were categorised as single (A), multiple (B and C) and no torpor expression (D). Arrows indicate the fasting period. Black bars represent the scotophase and white bars represent the photophase. The broken bar indicates T_b=31°C, which delineates TE (Hudson and Scott, 1979).

Onset time of torpor was erratic but was mostly observed at the end of the scotophase or near the beginning of the photophase (range 01:54 h–14:44 h, the mode time by hour is 11:10 h). Mean minimum torpor T_b was 28.9±1.7°C (range 23.5–30.9°C), and mean torpor bout duration was 113±98 min (range 5–472 min). A strong negative correlation was observed between minimum torpor T_b and torpor bout duration (P<0.01, r=0.83).

GLMM analyses for TE

Final models in analyses (a) and (c), which include RBM on PND 0 and ABM on PND 94, were the same, i.e. TE occurred more frequently among individuals whose RBM on PND 0 was low (family=binomial, estimate=−10.43, s.e.=3.11, z=−3.36, P<0.001; Fig. 3) whereas ABM on PND 94 had no influence on TE (estimate=−0.09, s.e.=0.06, z=−1.56, P=0.118). In analysis (b), TE occurred more frequently among individuals whose RBM on PND 21 was low (family=binomial, estimate=−5.80, s.e.=1.95, z=−2.97, P=0.003) whereas ABM on PND 94 had no influence on TE (ABM on PND 94: estimate=−0.09, s.e.=0.06, z=−1.45, P=0.147). We determined that the final model in analyses (a) and (c) could be the most parsimonious model for our analysis because it showed the lowest AIC value (254.2) than the other models, including the final model in analysis (b). Although BM on PND 0 seemed to influence TE (family=binomial, estimate=−3.361, s.e.=1.472, z=−2.283, P=0.02; Fig. S3), the models including this variable were not selected as the fitted model. As a result, the individual difference in TE in adulthood could be explained by RBMs rather than ABMs.

In another GLMM analysis, BM_{trial} also influenced TE (family=binomial, estimate=−0.11, s.e.=0.05, z=−2.02, P=0.043, AIC=261.7). Therefore, female mice used torpor when ABM just before fasting was low (Fig. 4).

DISCUSSION

Almost half (26/55) of the female mice exhibited daily torpor throughout the five torpor induction trials, and their TE varied markedly among litters, littermates and individuals (Fig. S1). In accordance with the GLMM analysis, the RBM on the PND 0 was correlated with TE in adulthood (Fig. 3), suggesting that female mice with low birth masses relative to their littermates do increase the use of daily torpor during adult life. ABM and RBM before weaning were correlated significantly (Table S2), as mouse pups grow constantly within a litter. This finding suggests that differences in ABM among littermates reflect growth during fetal

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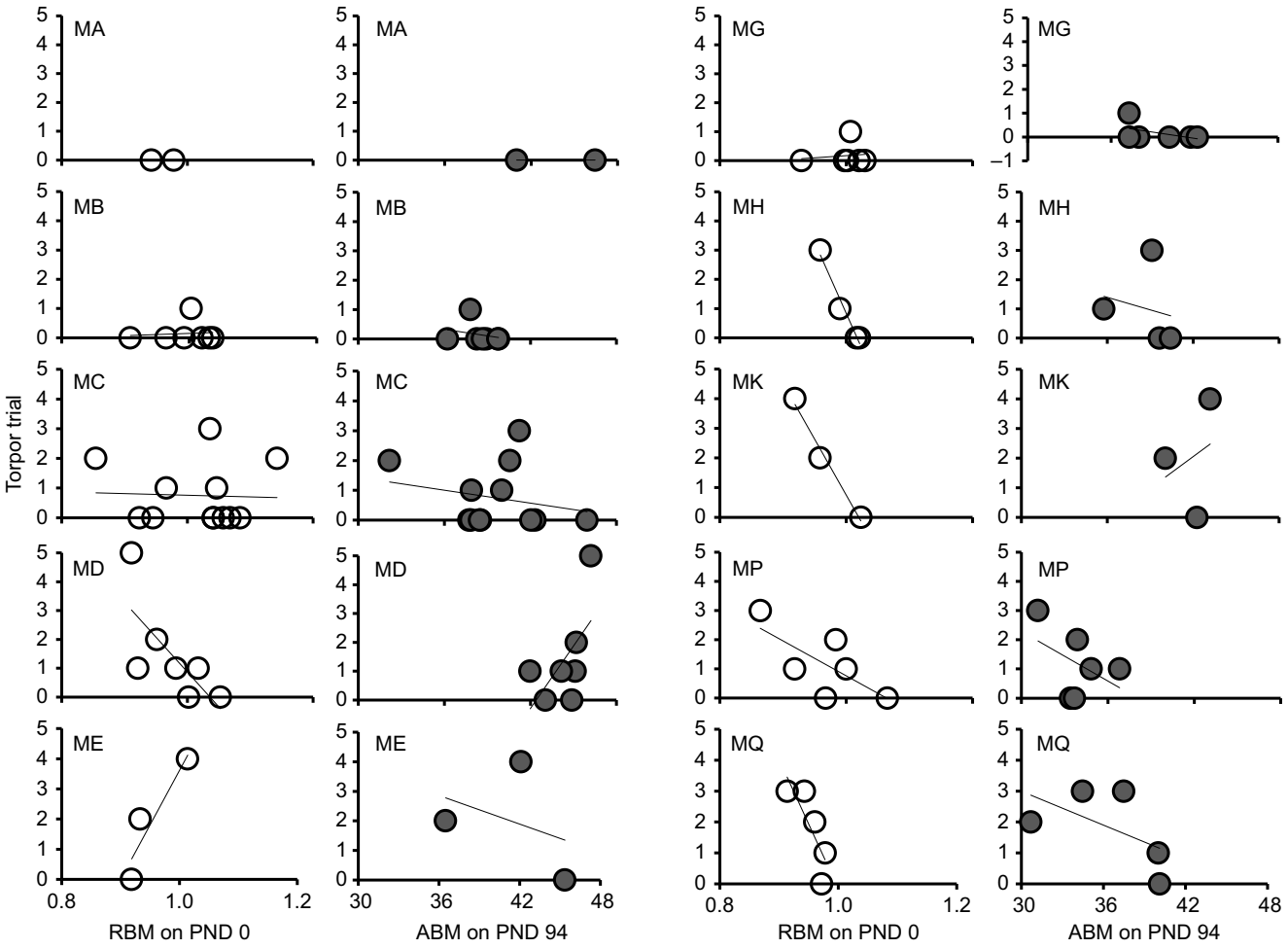

Fig. 3. Relationships between torpor expression (TE) and relative body mass (RBM) on postnatal day (PND) 0 or actual body mass (ABM) on PND 94 in female mice. Mice were subjected to five repeated torpor induction trials. The *y*-axes indicate the number of trials during which daily torpor was exhibited and the *x*-axes represent RBM on PND 0 or ABM on PND 94. MA–MQ indicate litter identification (=mother identification). Approximate trend lines are indicated in the plots. TE in female mice showed high variations between litters, littermates and individuals. The result of the generalised linear mixed model analysis demonstrated that female mice born with low RBMs showed high expression of daily torpor. In addition, BM on PND 94 seemed to be of little relevance to TE.

development. Therefore, we suggest that the capability for TE can differ among littermates, and further demonstrates that individual variation in TE is influenced by fetal development and ensuing sibling interactions. Riek and Geiser (2012) observed intraspecific plasticity in torpor usage and morphological traits in fat-tailed dunnarts (*Sminthopsis crassicaudata*) and noted that the differences in torpor usage were affected by the ambient temperature during the rearing period (from birth until adulthood). They suggested that such short-term phenotypic responses without long-term selection are likely to be important for the ability to cope with different climates over a wide distribution range. This study seems to corroborate our results.

Mammalian pups are forced to share or compete for maternal resources because resources are usually limited (Milligan et al., 2001; Hudson and Trillmich, 2008; Rödel et al., 2008, 2010; Branchi, 2009; Hudson et al., 2011; van Hasselt et al., 2012). Therefore, mammalian fetuses can influence one another's development (vom Saal and Bronson, 1980; vom Saal, 1989). In the present study, we observed that female mice with low birth masses relative to their littermates exhibited daily torpor more frequently. This result suggests that poor fetal development promotes the expression of daily torpor in adult life. As RBM on PND 0 was positively correlated with RBM at later time points, it appears that not only RBM on PND 0, but also the cumulative effects before weaning, influence individual variation in TE. We hypothesise that low acquisition of food (energy, nutrients) during early periods of life will result in stronger sensitivity to food scarcity, likely resulting in high individual variability in TE in mice. Such variation among individuals and littermates in the potential for TE may influence fitness during starvation.

Recently, intrauterine position has been reported to have multiple effects on the subsequent traits of several species of laboratory rodents (Ryan and Vandenbergh, 2002; Nagao et al., 2004). These studies have suggested that interactions associated with gonadal hormones (oestrogen, testosterone and others) are somehow linked to fetal characteristics. For example, mice located between two males in the uterus (2M mice) are exposed to relatively high levels of testosterone compared with mice located between two females (0M mice); subsequently, 2M mice display more sensitive reactions to testosterone throughout their lives (Ryan and Vandenbergh, 2002). Furthermore, 2M mice of either sex are heavier than are 0M mice, which may be caused by differences in metabolism, differential responses to stress or differing levels of aggression among individuals in a population (Palanza et al., 2001;

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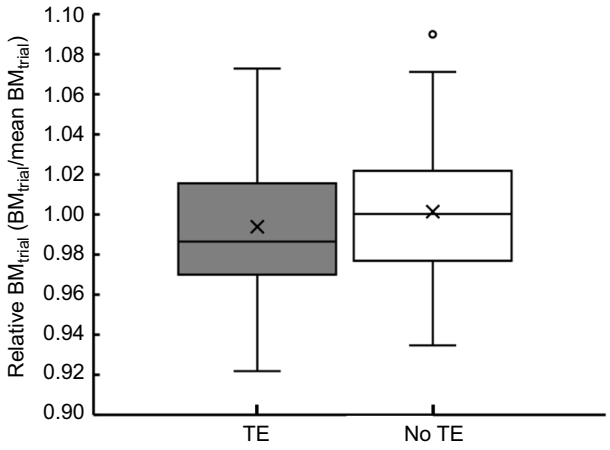

Fig. 4. Relationship between pre-fasting trial body mass (BM_{trial}) and torpor expression (TE) in female mice. We measured BM_{trial} as an indicator of the physical state just before fasting. This figure shows relative BM_{trial} values (BM_{trial}/mean BM_{trial}) because BM_{trial} was influenced by litter size. This conversion enabled us to better understand the relationship between TE and BM_{trial}. A generalised linear mixed model analysis showed that female mice exhibited torpor when BM_{trial} was low (P=0.04). The vertical line indicates the relative values of BM_{trial}. The mean value is indicated with an X. The data are expressed as means±standard deviations (s.d.). The circle indicates an outlier. Two hundred and seventy-five data points from 55 female mice (five torpor induction trials).

Ryan and Vandenbergh, 2002). Although we could not distinguish the intrauterine positions of mice in our study, these previous studies suggest that such prenatal sibling interactions may also affect TE. Interestingly, endogenous hormones, such as testosterone and leptin, are well documented to modify torpor patterns in rodents [*Mesocricetus brandti* (hibernator): Hall and Goldman, 1980; *Phodopus sungorus*: Ruby et al., 1993; *Saccostomus campestris*: Mzilikazi and Lovegrove, 2002; Swoap, 2008]. These characteristics might be also important for understanding why specific litters, such as MA, MB and MG (see Fig. 3 for results in each litter), have exhibited less daily torpors. Our future research will therefore aim to examine whether intrauterine position and resulting variation in exposure to various hormones can affect TE in adulthood and consider how such effects compare with those of RBM.

A final finding of the present study is that daily torpor in female mice was induced when actual body mass before fasting (BM_{trial}) was low (Fig. 4). The frequency of daily torpor increases when food is limited and body mass is low (Christian and Geiser, 2007). Smaller individuals have greater energy requirements per unit body mass than do larger individuals due to their relative heat loss (Geiser, 2004). Hence, both low energy reserves and fasting appear to trigger TE. The relationship between torpor and body condition has been examined using comparative analyses among individuals (e.g. Lynch et al., 1978; Ruf et al., 1991). In the present study, female mice displayed high intra-individual variation in BM_{trial} and recover from the tissue/energy/nutrient loss caused by fasting, as well as in TE, during the repeated trials, even with the same fasting procedure. These results may indicate that the potential for TE in each individual might be masked by intra-individual variation in body condition at the onset of the fasting period. We assumed that this intra-individual variation is likely to be associated with food consumption before fasting and/or foraging effort during the fasting period. Ruf et al. (1991) reported that Djungarian hamsters (*Phodopus sungorus*) use torpor as an intrinsic component of energy balance control; torpor in these hamsters is functionally linked to individual physiological adjustments of food consumption and foraging activities. In addition, Schubert et al. (2010) demonstrated that high foraging costs (poor foraging conditions) result in TE in laboratory mice. Moreover, our previous study (Eto et al., 2015) showed that the magnitude of food overabundance is clearly associated with reduced expression of daily torpor in the large Japanese field mouse (*Apodemus speciosus*). Together, these studies suggest that small rodents can recognise the cost of energy acquisition as well as endogenous energy condition, and they subsequently use this information to determine whether to undergo torpor. We did not examine the parameters for estimating the cost of food acquisition and activity in the present study; this remains an avenue for future study.

We also observed several types of daily torpor. Torpor was observed in 26 of 55 female mice, some of which exhibited a single bout of torpor (Fig. 2A), while others experienced multiple bouts of torpor in a single day (Fig. 2B) or over several days (Fig. 2C). These torpor bouts were observed in different mice (see Fig. S1). Multiple bouts of torpor such as those illustrated in Fig. 2B have been previously reported in some heterothermic animals (*Peromyscus leucopus*: Hill, 1975; *Sminthopsis macroura*: Geiser and Drury, 2003; *Vespadelus pumilus*: Turbill et al., 2003). Interestingly, three female mice exhibited torpor during 2–4 days after fasting (i.e. under *ad libitum* feeding conditions) (Fig. 2C, Fig. S1). We assume that the spontaneous torpor in these cases might have been induced by a continued effect from the fasting on previous days; however, our data do not permit a final conclusion.

With regard to daily torpor in laboratory mice under *ad libitum* feeding conditions, an interesting issue related to leptin metabolism arises. The *ob/ob* mouse strain (Swoap, 2008), which lacks the obese (*ob*) gene related to leptin production (Ingalls et al., 1950), exhibits spontaneous daily torpor. Leptin, which regulates appetite, is secreted primarily in fat cells and thus plasma leptin concentration is typically correlated with body fat content (Maffei et al., 1995). Additionally, fasting can disrupt hormone balances, particularly those hormones related to the regulation of appetite, such as ghrelin and leptin. Several studies have hypothesised that the secretion of such peptides mediated via neuropeptide Y modulates the utilisation and patterns of torpor in mice (Gavrilova et al., 1999; Gluck et al., 2006; Swoap, 2008). If the TE threshold of circulating concentrations of such peptides is influenced by early developmental stages, this threshold may also be an important endocrinological mechanism for regulating daily torpor in adult life.

Male mice in the first mated group rarely exhibited daily torpor (Fig. S2). Some strains of male mice reportedly enter torpor frequently (Sunagawa and Takahashi, 2016) whereas it is uncommon or unknown in other strains (Swoap and Gutilla, 2009). Among other rodents, TE may be inhibited in male hamsters, mainly owing to their high concentrations of endogenous testosterone (Ruby et al., 1993). Therefore, we hypothesise that factors responsible for individual variation in TE may differ between male and female mice.

In conclusion, we demonstrated that laboratory mice bred in a controlled environment displayed a high degree of individual variation in torpor use, even among littermates. Furthermore, we show that differences in fetal development, as reflected by birth mass, have long-term effects on torpor use in adult life. This inter-litter variation in adaptive phenotype may relate to relative fitness. Although various additional studies are necessary to further support specific aspects of our hypothesis, our results provide new insight into mechanisms of TE in small mammals.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: G.A.K., T.E., Y.O., T.M., C.K.; Methodology: G.A.K., T.M.; Validation: G.A.K., S.H.S.; Formal analysis: G.A.K., T.E.; Investigation: G.A.K., S.H.S.; Resources: C.K.; Data curation: G.A.K., T.E., Y.O.; Writing - original draft: G.A.K.; Writing - review & editing: T.E., A.S., T.M., C.K.; Supervision: S.H.S., A.S., T.M., C.K.; Project administration: C.K.; Funding acquisition: C.K.

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Data availability
All data used in the manuscript as well as the R code are available from the Dryad Digital Repository (Kato et al., 2018): https://doi.org/10.5061/dryad.h49g4.

Supplementary information
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