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Feasting, fasting and freezing: energetic effects of meal size and temperature on torpor expression by little brown bats *Myotis lucifugus*

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SUMMARY

Torpor is an adaptation for energy conservation employed by many species of small-bodied endotherms. However, surprisingly little is known regarding proximate factors influencing day-to-day variation in torpor expression in the wild. We used open-flow respirometry to quantify torpor expression in nine little brown bats (*Myotis lucifugus*, LeConte 1831) at two ambient temperatures (7°C and 17°C) following either sham feeding or consumption of a high-protein meal (50% or 100% of the mass required to reach satiation for each individual). Food consumption significantly increased the time spent normothermic before torpor entry but did not affect either the rate of body cooling or torpid metabolic rate. Bats did not fully exploit potential energy savings by maximising their use of torpor. Instead they varied torpor expression such that total energy expenditure over the course of each 22-h trial was balanced against gross energy intake immediately before the trial, independent of ambient temperature. This was accomplished by adjusting the timing of entry into torpor (thus altering the time spent torpid), rather than by modulating torpid metabolic rate. However, pre-trial body mass was also a significant predictor of torpor expression, which suggests that energy reserves combine with recent foraging success to influence individuals' decisions about depth and duration of their torpor bouts. We also present evidence that little brown bats use the heat generated through digestion (i.e. the heat increment of feeding) to substitute for active thermogenesis at sub-thermoneutral temperatures, thereby reducing the energetic costs of thermoregulation prior to torpor entry.

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Key words: energetics, energy budget, heat increment of feeding, *Myotis lucifugus*, torpor.

INTRODUCTION

Small endotherms possess a relatively low insulatory capacity and a large surface area to volume ratio, which requires a steep increase in heat production to maintain a high body temperature (T_b) as ambient temperature (T_a) drops below the thermoneutral zone. This heightened metabolic cost, coupled with a limited energy storage capacity, can make it challenging for small birds and mammals to endure periodic energetic shortfalls (e.g. during inclement weather) (Geiser, 2004). To compensate, many small endothermic species are able to enter a reversible state of metabolic depression known as torpor. During torpor, T_b is regulated at a reduced set-point, often many degrees below normal, dramatically reducing heat loss and, therefore, daily energy requirements (Geiser, 2004). In fact, the metabolic rate (MR) of thermoconforming heterotherms during torpor (TMR, torpid metabolic rate) may be reduced by up to 90% compared with normothermic resting metabolic rates (RMR), significantly prolonging survival in energetically unfavourable conditions (Geiser, 2004; Willis et al., 2005; McKechnie et al., 2007).

A range of factors influence torpor expression in both the laboratory and field. Sugar gliders and nectar-feeding bats, for example, tend to rely on torpor only during 'energetic emergencies' when fat reserves are low (Christian and Geiser, 2007; Kelm and von Helversen, 2007). By contrast, many species of temperate-zone bats routinely enter torpor during periods of inactivity, apparently whenever environmental conditions and/or the status of energy reserves make it favourable to do so (Hamilton and Barclay, 1994; Willis et al., 2006). Experimental evidence from a range of species indicates that torpor expression is strongly influenced both by fluctuations in energy availability and expenditure, because torpor expression tends to increase when food availability, body fat stores and/or T_a decrease (Brown and Bartholomew, 1969; Song et al., 1998; Lovegrove et al., 2001; Boyles et al., 2007; Wojciechowski et al., 2007). These patterns appear generally applicable although new field evidence suggests that the balance between costs and benefits of torpor may lead to a range of behavioural and metabolic responses. At least for some species during the active season, individuals with large energy reserves may rely heavily on torpor because it allows them to reduce foraging time and potential exposure to predation (Beiber and Ruf, 2009; Stawski and Geiser, 2010). Nonetheless, unexplained intraspecific variation in torpor expression exists among individuals of similar body condition exposed to apparently identical environmental conditions (Hickey and Fenton, 1996; McKechnie et al., 2007). Thus, the interplay of factors that influence individuals in their decision to enter torpor on a day-to-day basis (and decisions about the depth and duration of these torpor bouts) remains unclear.

Despite the apparent benefits of torpor, adopting this physiological state may have indirect costs. For example, use of torpor may increase risk of predation (French, 1988) (but see Wilkinson and South, 1992; Bieber and Ruf, 2009), decrease digestive efficiency (Speakman and Rowland, 1999), reduce immunocompetence (Prendergast et al., 2002; Luis and Hudson, 2006) or slow growth and delay reproduction (Hamilton and Barclay, 1994; Lausen and Barclay, 2006). The recent emergence of white-nose syndrome in hibernating bats of the northeastern United States of America

suggests that deep, long-term torpor may also increase the susceptibility of bats to fungal infection (Blehert et al., 2009; Boyles and Willis, 2010; Gargas et al., 2009). Consequently, it may not be ideal for endotherms to maximise their use of torpor (i.e. remain torpid for as long as possible, and at as low a TMR as possible) but instead to optimise torpor expression by balancing its energetic benefits against its physiological and ecological costs. The best evidence for this comes from food-storing eastern chipmunks (*Tamias striatus*), which decrease their use of torpor when supplied with larger food hoards during the winter (Humphries et al., 2003), and hibernating bats, which may select relatively warm microclimates during hibernation, despite increased energy costs during torpor (Boyles et al., 2007; Wojciechowski et al., 2007). Reproductive bats also regulate their expression of torpor during summer to minimise the costs of delayed foetal growth and development (Hamilton and Barclay, 1994; Dietz and Kalko, 2006). However, to date the optimisation of torpor expression by heterotherms during the active season is not well understood.

Recent feeding is a proximate factor that can vary substantially among individuals on a daily basis, and could help explain individual variation in torpor expression. Recent foraging success may be especially important for animals that must budget their energy on short time-scales (i.e. on a day-to-day basis), as energy expenditure must be closely balanced by energy intake. If ingested food is catabolised as (or soon after) it is obtained, an individual that has recently consumed a meal may be less inclined to enter torpor than one that has not fed recently. Conversely, if individuals are balancing their energy budgets on a longer, more flexible timescale, a weaker relationship between recent feeding and torpor expression might be expected.

In addition to providing energy to offset thermoregulatory costs, recent feeding may also influence torpor expression *via* the obligatory increase in metabolic heat production or heat increment of feeding (HIF), which accompanies digestion. The HIF represents the sum of all heat liberating actions required to process a meal (e.g. mastication, motility, secretion, absorption, transformation and excretion), with the magnitude of this effect typically proportional to meal size (McCue, 2006; Secor, 2009). The scope of the HIF is also strongly dependent upon the composition of the ingested diet, with high protein diets producing larger postprandial metabolic responses (McCue, 2006; Secor, 2009). Interestingly, the HIF has been shown to substitute for active thermogenesis below thermoneutrality in a number of endothermic species (Chappell et al., 1997; Hindle et al., 2003; Bech and Praesteng, 2004; Enstipp et al., 2008) (but see Campbell et al., 2000), thus reducing thermoregulatory costs associated with maintaining a high T_b at low T_a. If heterothermic endotherms can draw upon the HIF for thermoregulation, it may allow them to remain normothermic longer or reduce the depth and/or duration of torpor following feeding and mitigate the potential ecological (e.g. risk of predation) and physiological costs associated with use of torpor. However, to our knowledge the potential for HIF to influence these factors has not been investigated for any heterothermic endotherm.

Little brown bats (*Myotis lucifugus*, LeConte 1831) are small (7–9 g), cavity roosting bats distributed widely across North America (Fenton and Barclay, 1980; Nagorsen and Brigham, 1993). They can enter torpor throughout the year, with bout durations typically lasting 2–3 weeks from October to May (Thomas et al., 1990; Boyles and Brack, 2009) and 1–24 h during the active season from June to September (Kurta and Kunz, 1988; Nagorsen and Brigham, 1993) (T. D. Parkinson, K. J. O. Norquay and C.K.R.W., unpublished data). This species is an opportunistic forager that consumes a wide range of insects, e.g. dipterans, coleopterans and lepidopterans (Nagorsen and Brigham, 1993). Their foraging success and time spent foraging can vary widely between nights (Anthony et al., 1981) but on a typical night of foraging, a little brown bat may eat 4–8 g (Nagorsen and Brigham, 1993; Boyles and Willis, 2010), and may fill and then empty the gut several times (Ormsbee et al., 2007). The little brown bat is thus an ideal model organism to examine the interplay of recent feeding and T_a on torpor expression.

Our overall objective was to assess the effect of recent feeding and T_a on the short-term energy budgets of seasonally acclimatised little brown bats. We tested the hypothesis that little brown bats optimise torpor expression such that energy expenditure closely matches the energy available from recent feeding (i.e. they balance their energy budgets on a relatively short timescale). This hypothesis predicts that: (1) recent feeding will delay the onset of torpor and reduce the rate of entry into torpor at both high (17°C) and low (7°C) T_as; (2) recent feeding will reduce the depth and duration of torpor at both high and low T_as; (3) little brown bats will balance their thermoregulatory costs against the energetic value of their most recent meal, i.e. their postprandial energy expenditure will be closely correlated with the energy content of the ingested meal; and (4) there will be relatively little effect of T_a on overall daily energy expenditure. We also tested whether little brown bats substitute postprandial HIF for active thermogenesis at sub-thermoneutral T_as.

MATERIALS AND METHODS

Study subjects

All methods were approved by the University of Winnipeg Senate Animal Care Committee. Twelve hibernating adult-male little brown bats were collected from 'Dale's cave' (Walter Cook Caves Park Reserve, ~100 km north of Grand Rapids, MB, Canada) on 26 May 2008 under Manitoba Conservation Wildlife Scientific Permit #WB06122. Seven of the bats had been banded previously; unmarked individuals were outfitted with a numbered, lipped, aluminium forearm band (Porzana, Ltd, East Sussex, UK). Bats were then transported to a field laboratory where they were maintained in stainless-steel bat cages (25 cm × 20 cm × 20 cm; six bats per cage). Captive bats were maintained on a diet of mealworms (*Tenebrio molitor* L.) powdered with a mineral supplement (2:0 Calcium/No Phosphorus, T-Rex Products Inc., Chula Vista CA, USA) and crickets (*Acheta domesticus* L.). Bats quickly learned to feed and drink directly from dishes in their cages, and were subsequently provisioned once each evening between 22:00 h and 00:00 h to mimic their nocturnal foraging habits. Water dishes were again replenished each morning between 09:00 h and 10:00 h, after the cages had been cleaned and the food dishes removed. Cages were kept in an isolated, quiet room with a shaded window to provide a dim but natural photoperiod. T_a was held at ~22°C using a thermostat-controlled space heater, and a high relative humidity (≥95%) was maintained using a portable humidifier. For ~one week following the completion of experiments (see below), bats were given 30 min each evening to fly in preparation for release at their point of capture.

Experimental protocol

The experiment was designed so that each bat underwent six separate respirometry trials at two T_as (7°C and 17°C) and following three different feeding treatments (see below). These treatment temperatures were selected to mimic the range of nocturnal temperatures typically encountered by free-ranging bats in late spring/early summer (C.K.R.W., unpublished data). Several days prior to initiating metabolic experiments, each bat was offered as

many crickets as it would voluntarily consume during a 10-minute period and the total ingested mass recorded. This mass was subsequently fed to each animal for its '100%-satiation' trial and halved for the '50%-satiation' trials (see below). Although bats were maintained on both crickets and mealworms during their stay in captivity, they were fed only crickets immediately prior to experimental trials because of their relatively high protein content [64.9% crude protein on an ash-free dry-mass basis (Bernard and Allen, 1997)], which is known to maximise the HIF response (Campbell et al., 2000; Secor, 2009).

The nine bats employed in the experiment were randomly divided into three groups and each group was run together every third day. As only one T_a could be run daily, we could only partially randomise the order in which each bat was exposed to each treatment. The schedule of 17°C and 7°C treatments was thus randomly assigned for each group, with bats randomly assigned a feeding treatment within each set of 17°C and 7°C trials. Approximately 24 h before the start of their metabolic trials, bats were hand-fed mealworms until satiated and then housed together overnight in a cloth bat bag (Kunz, 1988). The following morning, these bats were given water using an eyedropper then returned to their cage between 09:00 h and 10:00 h, after food dishes for the other bats had been removed. All individuals had free access to water for the remainder of the day. Thus, all bats were fasted for a full day prior to their experimental feeding treatments and metabolic trials.

Immediately before each trial, bats were weighed, provided water and then either sham fed or hand fed to 50% or 100% satiation. To ensure that each bat experienced similar treatment of handling prior to their trials, they were given 10 minutes in which to consume their meal. Sham-fed individuals were simply held and provided water *via* an eyedropper during this period. If bats were unwilling to eat their entire meal, the exact mass of crickets consumed in the 10-min pre-trial period was recorded. At the conclusion of each metabolic trial, bats were removed from the metabolic chambers, weighed and isolated in cloth bat bags. If bats were still torpid after ~20 min, they were warmed exogenously by hand. Bats were then provided food and water to ensure they received a substantial meal following their trials, and returned to their holding cages.

Respirometry trials

We used positive pressure, open-flow respirometry to measure the rates of oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) during the 22-h experimental trials. Bats were exposed to an early summer photoperiod (14 h:10 h L:D) during trials *via* a shielded, compact fluorescent light, with trials initiated at midnight to match the bats' natural summer foraging and roosting patterns as closely as possible (Ormsbee et al., 2007). Three airtight 100 ml metabolic chambers constructed from transparent acrylic tubing (diameter=4.5 cm; height=6.5 cm; RC-5-10, Sable Systems International, Las Vegas, NV, USA) were each outfitted with a metal perch and hung vertically inside a temperature-regulated cabinet. Chamber inlet and outlet flow was restricted to ports located at either end of each chamber to ensure ample mixing of air and to minimise washout effects (Lighton, 2008; Willis and Cooper, 2009). T_a within the cabinet and each of the animal chambers was continually monitored using thermocouples.

Room air was scrubbed of H_2O using Drierite (Anhydrous Calcium Sulfate; W. A. Hammond Drierite Co. Ltd, Xenia, OH, USA), then pumped into the respirometry set-up using a diaphragm pump (SG O 115-120 V/60Hz, SCHEGO, Offenbach, Germany). Here it was divided into four streams, one for each of the three metabolic chambers plus a baseline stream. The flow rate of air through each metabolic chamber was maintained between 100 ml min^{-1} and 300 ml min^{-1} using mass flow controllers (GFC17, Aalborg Instruments Inc., Orangeburg NY, USA). Flow rates were adjusted to maintain O_2 levels above 20.2% and CO_2 levels below 0.43% within the chambers at all times (Withers, 2001; Willis and Cooper, 2009), and to preserve a detectable differential between the baseline and excurrent O_2 and CO_2 concentrations while the bats were torpid. Air flowed continuously through the metabolic chambers and baseline tubing, was scrubbed of H_2O, then entered a computer-controlled multiplexer (Intelligent Multiplexer V3, FoxBox, Sable Systems International) that sequentially switched between the four airstreams every 90 s, which we determined, prior to the experiment, was long enough to account for the washout characteristics of the metabolic chamber and associated tubing (Willis and Cooper, 2009). Consequently, only a single stream of dry air [50–80 ml min^{-1}; adjusted to maintain a rate that was at most half of that through the metabolic chamber (Lighton, 2008)] was serially routed through precision CO_2 and O_2 gas analysers (FoxBox, Sable Systems International) at a time. Incurrent flow rates, chamber and cabinet T_as, and outlet O_2 and CO_2 concentrations were recorded using ExpeData once per second (v. 1.0.24, Sable Systems International) and stored on a laptop.

Data analysis

We used the automated drift correction function in ExpeData (based on a fourth order polynomial derived from our baseline measurements of outside air) to correct for analyser drift in our O_2 and CO_2 signals and then calculated $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ (ml min^{-1}) for each individual following eqns 10.6 and 10.7 of Lighton (Lighton, 2008) using ExpeData. To account for the washout characteristics of the metabolic system, we used the last 30 s of each 90-s sampling interval as a proxy for the mean $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ of each individual during the successive 6-min recording periods. Although open-flow respirometry allows for the conversion of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ into units of heat production (kJ h^{-1}), these formulae typically assume that animals are fasted and that protein catabolism is negligible (Campbell et al., 2000). As both of these assumptions were clearly violated for fed individuals, we employed different equations depending on the digestive/metabolic state of each individual. Fed individuals exhibited obvious postprandial increases in RER that lasted 130±55 min (mean ± s.d.) above fasted values of (~0.7). Consequently we used eqn 3 of Campbell et al. (Campbell et al., 2000) for the first 130 min following feeding, or until the onset of torpor, with the assumption that the proximate composition of crickets is 67.8% protein, 24.0% carbohydrate and 8.1% lipids (Barker et al., 1998). From this point forward we employed the equation: $MR=[16+5.164\times(RER)]\times \dot{V}_{O_2}$, which assumes animals are fasted and that protein catabolism is negligible (Lighton, 2008). This equation was adopted for the entire duration of trials for fasted individuals. During periods of torpor we assumed a RER of 0.7 because episodic breathing of bats during deep torpor can prevent accurate determination of RER (Morris et al., 1994). We defined the onset of torpor bouts following Willis (Willis, 2007) based on abrupt, rapid declines of MR from normothermic levels, and the onset of steady-state torpor as the point at which MR stabilised at a reduced level following torpor entry (supplementary material Figs S1 and S2).

In order to test our hypotheses, we used a series of two-factor, repeated-measures analyses of covariance (ANCOVAs) to examine the effects of temperature and meal size on several dependent variables relating to torpor expression (supplementary material Fig. S1). Individual was included as a within-subjects factor to

account for repeated measurements and pre-trial (fasted) body mass was included as a covariate. This analysis allowed us to test for possible interactions between effects of temperature and meal size. If there was no significant interaction, we eliminated the interaction term from the model and repeated the analysis. We also used a series of single-factor, repeated-measures ANCOVAs (with pre-trial body mass included as a covariate) to examine the effect of feeding treatment at both 7°C and 17°C separately, as well as the effect of T_a on each feeding treatment separately. If body mass was not a significant covariate we removed it from the model and repeated the analysis. Tukey's *post-hoc* tests were used to compare means when single-factor ANCOVAs were significant.

All variables were tested for normality using the Shapiro–Wilks test statistic and Bartlett's test was used to check for equality of variances (Zar, 1998). Data were transformed as necessary using either a square root or logarithmic transformation. All analyses were done using either SPSS v. 12.0 (Chicago, IL, USA) or Systat v. 11 (Systat Systems, Inc., Point Richmond, CA, USA). Results are presented as means ± 1 s.d. unless otherwise stated, with $\alpha<0.05$ considered significant.

RESULTS

Eight of the nine bats underwent all six treatments, and 52 of the 54 scheduled metabolic trials were completed. Although all bats appeared healthy and maintained body condition throughout their stay in captivity one individual died unexpectedly the day following its fourth treatment, which prevented us from completing two trials. Mean pre-feeding body mass was 7.59±0.41 g. '100%-satiation' meals averaged 1.32±0.17 g (17.3% of body mass), while '50%-satiation' meals averaged 0.75±0.11 g (9.8% of body mass). Based on the water and energy content of crickets [73.2% water, 22.36 kJ g^{-1} dry mass (Bernard and Allen, 1997)], bats that were fed to satiation ingested 7.88±1.04 kJ, while those fed to half-satiation consumed a mean of 4.48±0.67 kJ.

All bats spent the majority of their trials in deep, steady-state torpor regardless of feeding or temperature treatment and once individuals entered torpor, most remained torpid for the remainder of the trial (supplementary material Fig. S2). Indeed, only 18 spontaneous arousals were recorded, with eight of these expressed by a single individual during five of its trials. Two individuals did not spontaneously arouse during any of their trials. The majority of spontaneous arousals (83%) occurred at 17°C and were followed almost immediately by re-entry into torpor.

The only significant interaction detected was between temperature and meal size for mean MR prior to torpor onset (P=0.015). No other significant interactions between temperature and meal size for any other dependent variables were detected (P>0.05); thus, results presented below for all two-factor ANOVAs represent models with interaction terms removed.

Characteristics of torpor expression

The time each bat spent normothermic between the end of feeding and the start of torpor entry varied widely, ranging from 7 min (17°C, '50%-satiation' meal) to 5 h, 23 min (17°C, '100%-satiation' meal). There was no effect of temperature (P=0.07) but feeding treatment had a significant effect on the time spent normothermic before torpor entry (supplementary material Table S1; Fig. 1; P=0.001). Pre-meal body mass also affected time to torpor entry (P=0.005), with larger individuals tending to enter torpor later than their smaller cohorts, an effect that was driven by the strong effect of body mass on time to torpor entry during the cold treatment (supplementary material Table S1; Fig. 2; see below). Controlling for the other independent

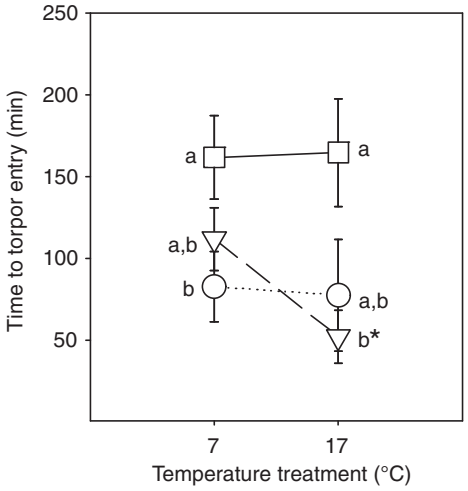

Fig. 1. Mean values (±1 s.e.m.) for the time to torpor entry for nine male little brown bats (*Myotis lucifugus*) held at ambient temperatures (T_as) of 7°C and 17°C following feeding to 50% (triangles) or 100% satiation (squares) on a meal of crickets (*Acheta domesticus*) or following sham feeding (circles). Symbols sharing the same letter at a given temperature are not significantly different (P=0.05). Asterisk denotes significant temperature effects within a feeding treatment.

variables, there was also a significant difference among individuals (supplementary material Table S1; P=0.001), which means that individuals exhibited repeatable differences in their patterns of torpor expression. The duration of the cooling period was significantly influenced by temperature treatment (supplementary material Table S1; Fig. 3; P=0.002), with longer cooling periods at 17°C than at 7°C (Fig. 3). T_a also had a significant effect on the maximum rate of torpor entry (P=0.002) but body mass, individual and feeding treatment did not (supplementary material Table S1). At 7°C, the mean maximum rate of decline in MR was 0.16±0.02 ml O_2 min^{-1} s^{-1}. This increased to 0.10±0.01 ml O_2 min^{-1} s^{-1} at 7°C.

When we divided the dataset and analysed results for the two temperature treatments independently, pre-feeding body mass did not influence any of the dependent variables at 17°C (P>0.05) but at 7°C it did have a significant effect on time to torpor entry (Fig. 1; P=0.004), maximum rate of torpor entry (P=0.01), total energy expended before torpor (P=0.009) and the total energy expenditure over the 22-h trial (P=0.001) (supplementary material Table S2). Time to torpor entry was significantly influenced by feeding treatment at both 7°C (P=0.046) and 17°C (supplementary material Table S2; P=0.03) with bats fed to '100% satiation' spending twice as long (~160 min *vs* 80 min) normothermic compared with fasted individuals (Fig. 1; see also Fig. S2 in supplementary material). Based on *post-hoc* analyses, this effect was due to a significant difference between sham-fed bats and bats fed to 100% satiation at 7°C (Fig. 1; P=0.04), and by a significant difference between bats fed to 50% and 100% satiation at 17°C (Fig. 1; P=0.04).

When the dataset was divided instead by feeding treatment to determine the effect of temperature on each feeding treatment, body mass was not a significant covariate for any of the dependent variables following any feeding treatment (P>0.05). Temperature had a significant effect on time to torpor entry only for bats fed to 50% satiation (Fig. 1; P=0.025). T_a also had a significant effect on the duration of the cooling period for bats fed to 50% satiation (Fig. 3; P=0.003). Temperature affected the maximum rate of entry only

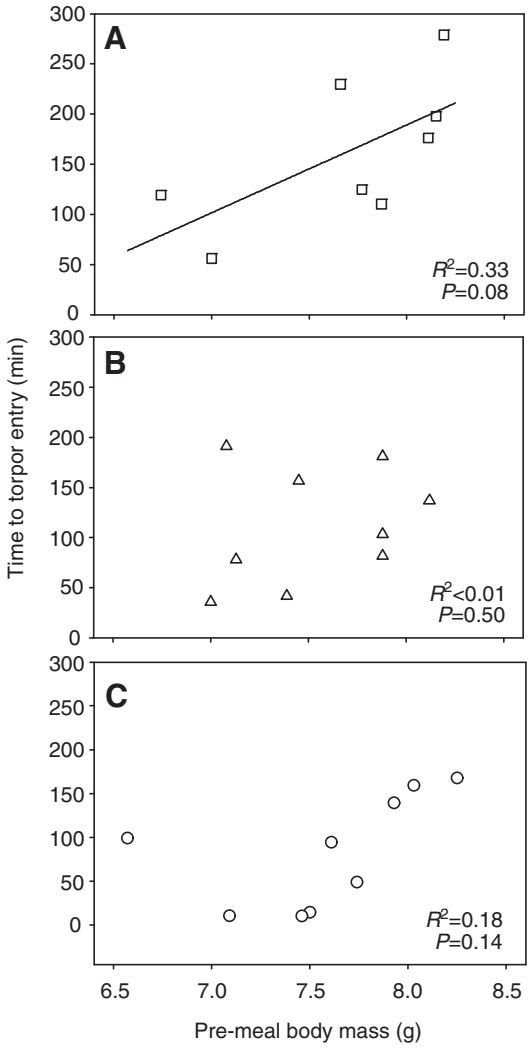

Fig. 2. Relationship between time to torpor entry and pre-meal body mass for nine male little brown bats (*Myotis lucifugus*) at 7°C. Individuals fed to 100% satiation are indicated by squares (A), those fed to 50% satiation by triangles (B) and those that were sham fed are represented by circles (C). The data are divided by feeding treatment into separate panels for clarity. The positive relationship between body mass and time to torpor entry was significant when the effects of feeding and temperature treatments were controlled for using ANCOVA (see text for details).

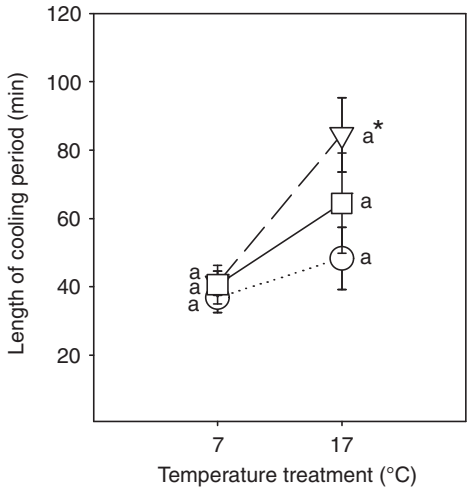

Fig. 3. Mean values (±1 s.e.m.) for the duration of cooling period for nine male little brown bats (*Myotis lucifugus*) held at ambient temperatures (T_as) of 7°C and 17°C following feeding to 50% (triangles) or 100% satiation (squares) on a meal of crickets (*Acheta domesticus*) or following sham feeding (circles). Symbols sharing the same letter at a given temperature are not significantly different (P=0.05). Asterisk denotes significant temperature effects within a feeding treatment.

for bats fed to 100% satiation (supplementary material Table S3; P=0.01).

Measurements of energy expenditure

Temperature and feeding treatment had a significant influence on both mean pre-torpor $\dot{V}_{O_2}$ and mean pre-torpor MR (Fig. 4; supplementary material Table S1). Total energy expenditure (kJ) before torpor entry (supplementary material Table S1) was significantly influenced by feeding treatment (P<0.001), individual (P<0.001), pre-meal body mass (P=0.003) and temperature (P=0.001), with bats expending more energy at 7°C (3.03±2.12 kJ) than 17°C (1.69±1.61 kJ; Fig. 5). Temperature was the only independent variable that influenced mean TMR (supplementary material Table S1; P<0.001), with TMR averaging 9.42±0.39 J h^{-1} (0.48±0.02 ml O_2 h^{-1}) at 7°C and 20.29±0.98 J h^{-1} (1.06±0.05 ml O_2 h^{-1}) at 17°C. Total energy expenditure by bats over the entire trial was significantly influenced by feeding treatment (P=0.001), pre-meal body mass (P<0.001) and individual (P=0.001) but temperature had no significant effect (supplementary material Table S1; P=0.64). Larger meals and larger pre-meal body masses were both positively correlated with total energy expenditure. The change in body mass over the course of each metabolic trial was significantly affected by temperature (P=0.001), feeding treatment (P=0.001), pre-meal body mass (P=0.001) and individual (P=0.002) (supplementary material Table S1). Generally, higher pre-meal body masses and lower temperatures all led to smaller reductions in body mass.

When we examined the effect of feeding treatment on pre-torpor $\dot{V}_{O_2}$ at each temperature separately, there was no effect of feeding treatment at 7°C but we did detect an effect at 17°C (supplementary material Table S2; P=0.002). Based on our *post-hoc* analyses, this was driven by a significantly lower $\dot{V}_{O_2}$ for sham-fed individuals than for those fed to either 50% (P=0.002) or 100% satiation (Fig. 4A; P=0.02). Notably, when $\dot{V}_{O_2}$ data were converted into units of heat production (taking differences in substrate catabolisation between fed and fasted bats into consideration; see Materials and methods), a different pattern emerged (Fig. 4B).

Total energy expenditure (kJ) before torpor entry was significantly influenced by meal size at 17°C but not 7°C (Fig. 5). Total pre-torpor energy expenditure of bats fed to 100% satiation at 17°C was significantly greater than that of sham-fed (P=0.02) and 50%-satiation individuals (P=0.04). Total energy expenditure over the entire trial was significantly affected by feeding treatment (supplementary material Table S2), with the total energy expenditure by sham-fed (2.54±0.52 kJ) and fully satiated bats (5.19±0.88 kJ) being significantly different at 7°C (P=0.02) but not 17°C (Fig. 6). Feeding treatment had a significant effect on the change in body mass at 7°C (P=0.03) but not 17°C (supplementary material Table S2).

When the data were divided to determine the effect of temperature on each feeding treatment separately, we found temperature had a significant effect on mean pre-torpor MR of sham-fed (P=0.007) and fully satiated individuals (P=0.001) (Fig. 4B, supplementary material,

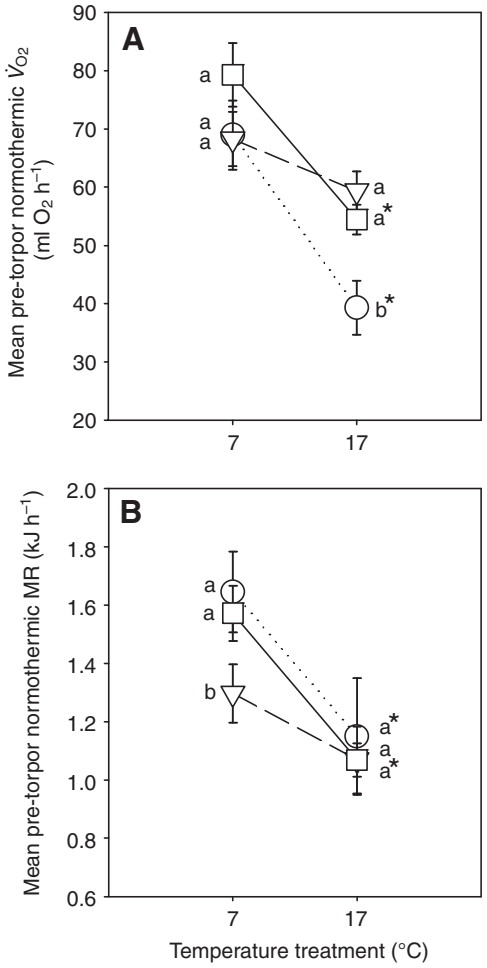

Fig. 4. Mean values (±1 s.e.m.) for (A) the mean pre-torpor (normothermic) rate of oxygen consumption (ml O_2 h^{-1}) and for (B) the average pre-torpor (normothermic) metabolic rate before torpor entry (kJ h^{-1}) of nine male little brown bats (*Myotis lucifugus*) held at ambient temperatures (T_as) of 7°C and 17°C after being fed to 50% (triangles) or 100% satiation (squares) on a meal of crickets (*Acheta domesticus*) or following sham feeding (circles). Symbols sharing the same letter at a given temperature are not significantly different (P=0.05). Asterisks denote significant temperature effects within a feeding treatment.

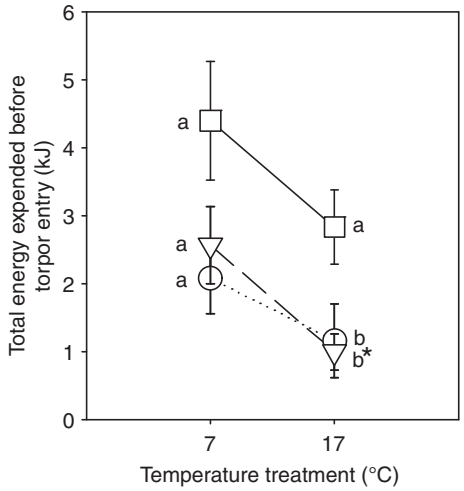

Fig. 5. Mean values (±1 s.e.m.) for the total energy expended (kJ) before torpor entry at ambient temperatures (T_as) of 7°C and 17°C following feeding to 50% (triangles) or 100% satiation (squares) on a meal of crickets (*Acheta domesticus*) or following sham feeding (circles). Symbols sharing the same letter at a given temperature are not significantly different (P=0.05). Asterisk denotes significant temperature effects within a feeding treatment.

Table S3). Temperature also had a significant effect on total energy expenditure before torpor entry for bats fed to 50% satiation (P=0.02) but not for the other feeding treatments (supplementary material, Table S3). There was also a significant effect of temperature on mean TMR for sham-fed bats (P<0.001) and bats fed to 100% satiation (P<0.001) (supplementary material, Table S3).

Temperature had no significant influence on the total energy expenditure over the entire trial for any of the feeding treatments (Fig. 6; supplementary material Table S3). There was a significant effect of temperature on reduction in body mass for sham-fed bats (P=0.01) and bats fed to 50% satiation (P=0.02) but not bats fed to 100% satiation (supplementary material Table S3).

RER and RMR

Qualitatively, the RER values of bats fed to full satiation were consistently higher than those of fasted bats, while the RERs of half-satiated bats were intermediate (supplementary material Fig. S3). RER values of satiated individuals also took longer to return to fasted values (~0.7, indicating a predominantly fat-based metabolism) compared with individuals fed to half satiation. Similarly, the RER of bats consuming the larger meal took longer to return to fasted levels at 17°C than at 7°C.

DISCUSSION

Our findings indicate that little brown bats are able to balance their energy budgets on a relatively short timescale during late spring, and that this balance is primarily achieved by adjusting daily energy expenditure, through the use of torpor, against the energy intake of their most recent meal. Given their small size (7.6 g), ingested food probably represents a particularly important energy reservoir to fuel metabolism during torpor and/or the subsequent normothermic phase. However, because the efficiency of food assimilation by bats declines with temperature (Speakman and Rowland, 1999), it would presumably be most advantageous to postpone torpor until digestion is complete (or *vice versa*). Not surprisingly then, energy intake was positively correlated with time to torpor entry by little brown bats at both 7°C and 17°C, with fully satiated individuals spending significantly more time normothermic following feeding than the other two treatment groups (Fig. 1; see also Fig. S2 in supplementary material). Animals that consumed larger meals also exhibited qualitatively higher RERs and a more protracted postprandial RER response compared with those that ate smaller meals or no meal at all (supplementary material Fig. S3). The mean time for RER values of fed bats to shift to fasted levels (i.e. presumably the time at which metabolism shifted from the recent high protein meal to stored fat) was also qualitatively similar to the mean time to torpor entry for each treatment. Taken together, these findings suggest that little brown bats tend to complete digestion prior to torpor entry. However, there was still substantial variation in time to torpor entry within treatments (e.g. 32 min to 5 h, 15 min for fully satiated bats, 7 min to 2 h, 3 min for bats fed to 50% satiation and 8 min to 5 h, 3 min for sham-fed individuals, all at 17°C).

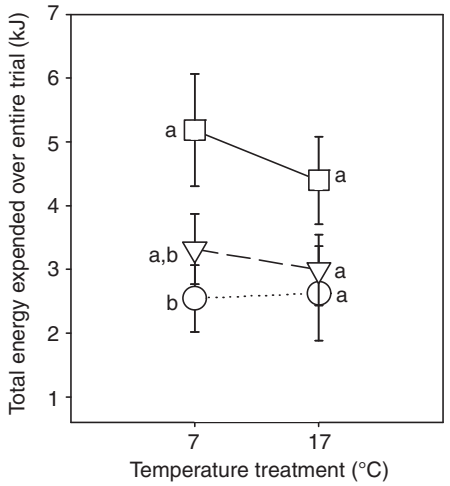

Fig. 6. Mean values (±1 s.e.m.) for the total energy expended (kJ) over the entire 22-h metabolic trial at ambient temperatures (T_as) of 7°C and 17°C following feeding to 50% (triangles) or 100% satiation (squares) on a meal of crickets (*Acheta domesticus*) or following sham feeding (circles). Symbols sharing the same letter at a given temperature are not significantly different (P=0.05). No significant temperature effects were detected.

Some of this variation in torpor expression was explained by differences in body mass, which most probably reflect differences in body condition as pre-meal body mass was positively correlated with time to torpor entry at 7°C (Fig. 2). Given that even the smallest bats in our study had sufficient energy stores to fuel at least one active arousal from torpor, it appears to be more economical for small individuals to delay digestion and enter torpor quickly, especially when exposed to cold temperatures where they would realise large, immediate reductions in energy expenditure. These bats could then delay rewarming from torpor (and digestion) until T_as are warmer [e.g. due to solar heating of the roost site (Geiser and Drury, 2003)], which would reduce overall energy costs and allow small individuals to partition a larger fraction of the meal into fat stores. Accordingly, the mass of accessible fat reserves may supersede recent feeding in the decision of little brown bats to enter torpor. This indicates that bats are flexible in terms of pre-torpor digestion and that recent feeding does not necessarily prevent them from quickly entering torpor.

Gould's long eared bats (*Nyctophilus gouldi*) exhibited substantial increases in both $\dot{V}_{O_2}$ and T_b for up to 9 h following ingestion of a meal, which Morris et al. primarily ascribed to the obligatory HIF response (Morris et al., 1994). Thus, our finding that meal size had no effect on the duration of the torpor entry period (Fig. 3) or on the maximum rate of torpor entry (supplementary material Table S2) is noteworthy. As cooling rate is directly proportional to thermal conductance, which, in turn, is proportional to the differential between T_b and T_a (Speakman and Thomas, 2003), it is not surprising that both variables were significantly influenced by T_a. This finding supports the idea that digestion is either completed or suspended prior to the onset of torpor to allow for rapid cooling. However, there was a noticeably larger variation in the total duration of the cooling period at 17°C than at 7°C (Fig. 3), implying that bats at the higher T_a may have been able to influence (slow) their rate of cooling during torpor onset. This would decrease the time spent in steady-state torpor, and is consistent with our prediction that bats will not necessarily minimise energy expenditure by maximising the time spent torpid. Instead, they may be able to adjust their rate of torpor entry so that they still gain energetic benefits of torpor but reduce the length of time they are exposed to its indirect costs.

Depth of torpor was not affected by recent feeding as T_a was the only factor to influence TMR (supplementary material Table S2). This suggests that energetically expensive processes associated with assimilation (i.e. HIF) are negligible during the torpor bout. We were unable to assess the effect of recent feeding on the duration of subsequent torpor bouts, as spontaneous arousals were observed in less than 1/3 of the trials, with only one individual remaining normothermic following arousal. However, the observation that most (15 out of 18) arousals occurred at 17°C supports the contention that the animals were optimising torpor expression, because the energetic benefits of entering torpor and arousal costs are both smaller at this T_a.

T_a significantly influenced the mean MR of normothermic and torpid little brown bats but had little effect on total energy expenditure over their 22-h trials (Fig. 6). The estimated field MR of free-ranging little brown bats during summer was 29.9 kJ day^{-1} (Nagy et al., 1999), while that measured for solitary, captive bats was 17.4 kJ day^{-1} (O'Farrell et al., 1971). Thus, assuming a digestible energy efficiency of 91.2% (O'Farrell et al., 1971) and urinary energy losses of 5% (Gusztak et al., 2005), bats in our study fed to 50% and 100% satiation obtained 12.9–22.2% (0.862×4.49 kJ= 3.87 kJ) and 22.8–39.2% (6.82 kJ), respectively, of their estimated daily energy requirements. Despite this relatively small proportion, they were still able to closely match their total energy expenditure with their energy intake under both the 'cold' and 'warm' ambient conditions. Again, this seems to highlight that recent feeding combines with available fat reserves to influence torpor expression and, therefore, daily energy expenditure. Many species have been shown to balance activity levels and torpor use under different food and temperature regimes to maintain a constant body mass (Brown and Bartholomew, 1969; Hiebert, 1991; Bozinovic et al., 2007; Gutman et al., 2006). The maintenance of sufficient fat stores may act as insurance for situations where unfavourable ambient conditions (e.g. low temperatures) persist for extended periods.

Based on mean, pre-torpid $\dot{V}_{O_2}$ values alone, a significant HIF response (i.e. elevation in MR) appeared to occur following consumption of both the 50%- and 100%-satiation meals (particularly at 17°C; Fig. 4A), suggesting little brown bats are unable to substitute the HIF for active thermogenesis. However, because the catabolisation of fats, lipids and carbohydrates release varying amounts of heat per unit of O_2 consumed, $\dot{V}_{O_2}$ values tend to overestimate true values of heat production (Campbell et al., 2000). Indeed when we calculated the energetic equivalence of $\dot{V}_{O_2}$, taking meal composition into account, meal size had no influence on the mean normothermic pre-torpor rate of energy expenditure at 17°C (Fig. 4B). This highlights that $\dot{V}_{O_2}$ values alone are not reliable for examining the magnitude of HIF responses or determining whether HIF substitutes for active thermogenesis, especially following consumption of protein-rich meals (Campbell et al., 2000). The same pattern was apparent when we compared resting MR values (to account for possible differences in activity) among the three feeding treatments (data not shown). This lack of association between nutritional state and rate of metabolism strongly suggests that little brown bats can substitute HIF for active thermogenesis. Because the HIF response is obligatory, this heat source could markedly reduce thermoregulatory costs associated with maintaining normothermia (Campbell et al., 2000; Hindle et al., 2003), thus reducing reliance on stored/assimilated energy stores for this purpose.

Conclusions

Our results demonstrate that recent feeding delays torpor entry in little brown bats, and that this response is, for the most part, independent of temperature. This presumably reflects an optimisation of torpor expression, which allows bats to obtain necessary energy savings while minimising exposure to potential physiological or ecological costs of heterothermy. However, our findings indicate that recent feeding does not explain all of the variation between individuals in their physiological or behavioural decision to enter torpor. Within feeding treatments, large bats tended to remain normothermic for longer, probably until digestion was complete while smaller individuals entered torpor immediately, presumably prior to digestion of their meals. Thus, even though they could have conserved more energy by entering torpor immediately, large bats prolonged the time they spent normothermic prior to torpor entry perhaps because they had larger available energy reserves. Taken together, our findings are consistent with Humphries et al.'s (Humphries et al., 2003) optimisation hypothesis and suggest that, while short-term variation in daily energy intake (i.e. foraging success) can strongly influence torpor expression, bats also have considerable flexibility in modulating their daily energy expenditure because of their exceptional ability to exploit torpor. This study also provides evidence that little brown bats exploit the obligatory HIF response to substitute for active thermogenesis during normothermia below thermoneutrality. This may help them offset thermoregulatory costs of remaining normothermic at low T_a while defraying costs associated with torpor such as increased risk of predation, sleep deprivation or build-up of nitrogenous wastes.

LIST OF ABBREVIATIONS

HIF	heat increment of feeding
MR	metabolic rate
RER	respiratory exchange ratio
RMR	resting metabolic rate
T_a	ambient temperature
T_b	body temperature
TMR	torpid metabolic rate
$\dot{V}_{CO_2}$	rate of carbon dioxide production
$\dot{V}_{O_2}$	rate of oxygen consumption

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