An Energy-Based Body Temperature Threshold between Torpor and Normothermia for Small Mammals

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ABSTRACT

Field studies of use of torpor by heterothermic endotherms suffer from the lack of a standardized threshold differentiating torpid body temperatures (T_b) from normothermic T_b's. This threshold can be more readily observed if metabolic rate (MR) is measured in the laboratory. I digitized figures from the literature that depicted simultaneous traces of MR and T_b from 32 respirometry runs for 14 mammal species. For each graph, I quantified the T_b measured when MR first began to drop at the onset of torpor ($T_{b\text{-onset}}$). I used a general linear model to quantify the effect of ambient temperature (T_a) and body mass (BM) on $T_{b\text{-onset}}$. For species lighter than 70 g, the model was highly significant and was described by the equation $T_{b\text{-onset}} = (0.055 \pm 0.014)\text{BM} + (0.071 \pm 0.031)T_a + (31.823 \pm 0.740)$. To be conservative, I recommend use of these model parameters minus 1 standard error, which modifies the equation to $T_{b\text{-onset}} - 1\ \text{SE} = (0.041)\text{BM} + (0.040)T_a + 31.083$. This approach provides a standardized threshold for differentiating torpor from normothermia that is based on use of energy, the actual currency of interest for studies of torpor in the wild. Few laboratory studies have presented the time-course data required to quantify $T_{b\text{-onset}}$, so more data are needed to validate this relationship.

Introduction

Heterothermy, or torpor, is fundamental to the lives of many endotherms, and there is continued interest in quantifying its use in the laboratory and field (e.g., Geiser 2004; Solick and Barclay 2006; Willis et al. 2006). Torpid animals save large amounts of energy by lowering their body temperature (T_b) set point and abandoning metabolic heat production until T_b falls to the new, reduced set point. The IUPS Thermal Commission (2003, p. 102; after Bligh and Johnson 1973) defines torpor as "a state of inactivity and reduced responsiveness to stimuli (e.g., during hibernation, hypothermia, or estivation)." However, there is growing appreciation that, in terms of its importance for ecology and evolution, torpor is better defined by its implications for energy savings owing to reduced heat production than by some level of behavioral responsiveness. Many endotherms are still active and capable of performing demanding activities (including flight) at levels of metabolic heat production and T_b low enough to result in large energy savings (between 20° and 30°C; Augee 1969; Austin and Bradley 1969; Bradley and O'Farrell 1969; Hirshfeld and O'Farrell 1976; Willis and Brigham 2003). The phrase "reduced responsiveness" in the definition is particularly subjective and, thus, not useful for quantifying a difference between torpor and homeothermy. Consider that torpid animals respond to disturbance by arousing from torpor, even at the lowest values of T_b (Geiser 2004). The magnitude of these responses can be enormous if changes in T_b or metabolic rate (MR) are appropriately considered responses. These problems with the traditional definition of torpor, and the fact that different categories of heterothermy are recognized, have prompted the use of new terminology to describe energy-saving heterothermy in general (e.g., facultative heterothermic responses; McKechnie and Lovegrove 2002). For simplicity, in this article I define "torpor" as an energy-saving state of heterothermy during which metabolic heat production and T_b are below normal (i.e., normothermic or homeothermic) levels.

Many laboratory studies rely on open-circuit respirometry for indirect measurement of MR. Time courses recorded during these trials reveal obvious and abrupt declines in MR at the onset of torpor. Thus, in laboratory studies, in addition to overall energy savings, it is relatively easy to quantify temporal patterns (bout duration, time of onset) and magnitude (change in MR) of torpor under different conditions because the start and end of each torpor bout are obvious from the MR trace. Confinement in a metabolic chamber is not a natural circumstance for any species, so MR data, while valuable, are limited in terms of what they reveal about use of torpor in the wild (Willis and Cooper, forthcoming). A growing number of studies use temperature-sensitive transmitters or dataloggers to record T_b or skin temperature (T_{sk}) in free-living animals (Körtner and Geiser 2000; Lausen and Barclay 2003; Turbill et al. 2003; Dietz and Kalko 2005; Munro et al.

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2005; Solick and Barclay 2006; Turbill 2006) or under seminatural conditions in laboratory enclosures (Song et al. 1998*a*; Schmid 2000; Cooper and Withers 2004; Willis et al. 2005*a*). As in the laboratory, these studies often aim to quantify temporal patterns and magnitude of torpor bouts because these variables affect energy balance with implications for reproductive fitness. A persistent challenge, however, is defining some T_b or T_{sk} threshold to differentiate torpor from normothermia in the absence of metabolic data. Such a threshold is essential if depth and duration of torpor bouts are to be calculated, but the subjective nature of the traditional definition for torpor (IUPS Thermal Commission 2003) has led to uncertainty about how it should be quantified.

Barclay et al. (2001) identified more than 20 values of T_b and T_{sk} used to define torpor bouts in the literature, as well as other definitions based on criteria such as $T_b - T_a$ differentials or behavior, but few of these were derived with any physiological justification. The most popular value used in studies reviewed by Barclay et al. (2001) was a conservative (though no less arbitrary) 30°C. A conservative threshold is important to avoid overestimating use of torpor, but a potential consequence of being overly exclusive is the failure to detect shallow torpor bouts. Because of Q_{10} effects, torpor is characterized by diminishing energetic savings as T_b falls (Geiser 2004). Thus, shallow bouts are likely to be of great ecological and evolutionary importance because they result in large energy savings while potentially mitigating the selective costs of torpor, such as vulnerability to predation, reduced growth rate, and delayed reproduction.

Barclay et al. (2001) proposed an important step toward a standardized threshold with their concept of active temperature (T_{act}). Active temperature is measured when an individual under study is known to be behaviorally active. For the studies of bats discussed by Barclay et al. (2001), this means recording T_{sk} or T_b immediately before dusk emergence each night of a study and then, to be conservative, defining T_{act} (the threshold temperature) as the lowest dusk emergence T_{sk} or T_b value recorded for an individual during the study (Barclay et al. 2001). The approach could also be applied to dusk emergence of nocturnal, fossorial mammals or morning observations of diurnal birds. While this improves on past practice of arbitrarily selecting a threshold temperature, T_{act} does have potential limitations. Most important, as mentioned above, many heterothermic endotherms can be active while still saving energy because of a reduced T_b set point (Augee 1969; Austin and Bradley 1969; Bradley and O'Farrell 1969; Hirshfeld and O'Farrell 1976; Willis and Brigham 2003; Willis and Cooper, forthcoming). Therefore, there is little energetic justification for linking the threshold temperature with behavior. Furthermore, T_{act} cannot account for variation in $T_b - T_a$, and especially $T_{sk} - T_a$, differentials that can occur even during normothermia, for animals exposed to different T_a's at the time when T_{act} is defined (Withers 1992; Willis and Brigham 2003; Geiser 2004). For example, if T_a happens to be especially low one evening, the T_{act} threshold may be set very low for an animal measured that night, and as a result, ecologically important shallow bouts of torpor would go undetected for that individual. Torpor patterns of individuals for which T_{act} was not determined on the cold evening would not be comparable.

McKechnie et al. (2007) proposed an innovative alternative for defining the torpor threshold based on frequency distributions of T_b or T_{sk} measurements in their field study of freckled nightjars (*Caprimulgus tristigma*). Their method depends on the premise that the distribution of T_b measurements for a heterothermic endotherm should fit a multimodal pattern (i.e., with one or more torpid peaks and one normothermic peak). The lower tail of the normothermic distribution and the upper tail of the torpor distribution will overlap and conceal each other, especially if shallow torpor is used frequently, but McKechnie et al. (2007) addressed this by assuming that normothermic T_{sk} measurements were normally (i.e., symmetrically) distributed about the upper modal value. Based on this assumption, they fitted a bell-shaped curve to T_b data for each nightjar, using the shape of the upper half of the distribution (i.e., values greater than the upper modal peak) to infer the shape of the lower half. Values below the lower 99% confidence limit of the fitted distribution were then considered to reflect use of torpor (McKechnie et al. 2007). This method is clearly more systematic than selecting an arbitrary temperature and is appealing for several reasons. First, the curve can be fitted with 99% confidence based solely on the normothermic modal and maximum T_b's recorded for each individual as long as maximum T_b is assumed to reflect the upper 99% confidence limit for the distribution (Zar 1999). Second, it may also help to control for differences in measurement error between individual animals because data for each individual are fitted to their own curve. However, the central assumption of the method (i.e., that normothermic T_b necessarily fits a normal distribution for a small-bodied heterothermic endotherm) may not always apply because of factors that can influence T_b within the normothermic range. To begin with, as a result of circadian T_b variation, normothermic T_b measurements may often be better described using bimodal rather than unimodal patterns (e.g., Aujard and Vasseur 2001; Warnecke et al. 2007), which may complicate assumptions about the symmetry of distributions. Responses to T_a variation could also play a role. For example, if it tends to be cool during the study, an animal may spend most of that time at a lower normothermic T_b, but measurements obtained on a few hot days, during which T_b is regulated at higher levels, will skew the distribution (Refinetti 1997; Aujard and Vasseur 2001). Measurements of T_{sk} may be especially sensitive to these kinds of effects because of the potential influence of T_a on external temperature-sensitive radiotransmitters (Barclay et al. 1996; Audet and

Thomas 1996; Willis and Brigham 2003; McKechnie et al. 2007). Even differences in foraging success on different days could influence the shape of the distribution because the heat increment of feeding can affect T_b variation within the normothermic range (e.g., Campbell et al. 2000). If a normothermic animal typically thermoregulates about some modal value, a small number of particularly successful (or unsuccessful) foraging bouts during the measurement period could also skew the distribution. Given these potential sources of error, it is important to validate the central assumption of the normal-distribution method.

The eventual goal for field studies of torpor is to quantify the energetic and, by extension, fitness consequences of heterothermy for free-living endotherms. Therefore, a threshold temperature that reflects energy savings due to torpor should have the greatest relevance to energy balance and fitness. To address the issue of defining torpor bouts for field and laboratory studies where it is not possible to measure MR, here I present a new approach for quantifying a threshold temperature. My objective was to quantify the effect of the independent variables BM and T_a on the dependent variable T_b at the immediate onset of torpor bouts (hereafter $T_{b\text{-onset}}$) for mammals. Body size and T_a influence most physiological traits of endotherms, including thermal conductance, the rate of heat exchange with the environment. Small animals have higher rates of heat loss than large animals, and this effect is more pronounced at lower values of T_a (Withers 1992; Geiser 2004). This will influence measurements of T_b at the start of a torpor bout and must be incorporated into a T_b-based threshold for torpor. I predicted that $T_{b\text{-onset}}$ would scale positively with BM and that this effect would be most pronounced at low values of T_a because higher rates of heat loss for small species should result in a more rapid initial decline of T_b immediately after thermoregulation of the normothermic set point is abandoned. I also compared values of threshold temperatures calculated using McKechnie et al.'s (2007) normal-distribution method with values of $T_{b\text{-onset}}$ that I determined for the same species.

Material and Methods

I obtained data on $T_{b\text{-onset}}$ from 18 studies of 14 species of mammals ranging in size from 14.7 to 406.0 g (Table 1). Ambient temperature ranged from 2.5° to 28.4°C at the time of torpor onset during experimental runs (Table 1). Data were obtained from figures depicting simultaneous traces of T_b and oxygen consumption during bouts of normothermy and torpor. I digitized each figure using TechDig, version 2.0 (Jones 1998), and identified the point on each time course when MR first declined abruptly from the resting normothermic level at the start of a torpor bout (Fig. 1). In all studies, the time of torpor entry was clear from a distinct and rapid reduction in MR. I selected $T_{b\text{-onset}}$ for each figure as the T_b recorded at the same time as the initial reduction in MR. In most studies, T_a was reported in the text or figure caption, but in a few, it was depicted as a separate trace in the time course figure. In these latter instances I also used TechDig to identify T_a at the time of torpor entry. In all studies, T_a was stable within 4°C for at least 30 min before and after entry into torpor. I was able to obtain multiple values of $T_{b\text{-onset}}$ over a range of T_a and BM values for nine species (two to four values per species) either because time courses for multiple individuals were presented in one study or because more than one study was published for an individual species.

Although there was clear agreement between T_b and MR traces from all studies in terms of timing of torpor entry (e.g., Fig. 1), there was the potential that washout characteristics of respirometry systems used in the original studies had affected my results. For example, a low flow rate would extend the washout time and could delay detection of the MR decline at torpor onset (Lasiewski et al. 1966; Bartholomew et al. 1981). Fifteen studies provided information on chamber volume and flow rate, which allowed me to estimate the washout time needed to reach 99% equilibrium in the metabolic chamber. I used the equation

$$\text{washout time} = 4.6\left(\frac{V}{\text{FR}}\right), \tag{1}$$

where V equals chamber volume and FR equals flow rate (Lasiewski et al. 1966). To rule out the influence of washout on my findings, I tested for correlations between washout estimates and both independent variables, as well as values of $T_{b\text{-onset}}$.

In addition to potential washout effects, I tested for an effect of mammalian subclass on $T_{b\text{-onset}}$ before analyzing the pooled data set because normothermic T_b of marsupial mammals is known to be lower than that of placentals (Withers 1992). To evaluate whether $T_{b\text{-onset}}$ represents a realistic threshold for torpor, I also compared values of $T_{b\text{-onset}}$ to normothermic T_b values reported by Geiser (2004) and to average normothermic values I determined from T_b time courses in each of the studies I analyzed (see below).

I calculated torpor threshold temperatures using McKechnie et al.'s (2007) normal-distribution method. I used TechDig to sample between five and 12 representative values (mean ± SD = 9.9 ± 2.3 samples) of normothermic T_b from each of the time course figures. Values were sampled at times when animals were clearly normothermic based on their corresponding MR trace. I sampled at equivalent time intervals for each time course (e.g., every 0.5 h), but the sampling interval varied from 0.5 to 2.5 h for different time courses, depending on the duration of normothermic recordings. I selected sampling intervals such that values spanned the entire duration of each time course. I also sampled the maximum T_b recorded during each run. For

Table 1: Data for body mass (BM), ambient temperature (T_a), and body temperature at the time of torpor onset ($T_{b\text{-}onset}$) obtained from the literature, as well as values of $T_{b\text{-}onset} - 1$ SE calculated for each species with equation (4)

Species	BM (g)	T_a (°C)	$T_{b\text{-}onset}$ (°C)	$T_{b\text{-}onset} - 1$ SE (°C)	$T_{b\text{-}norm}$ (°C)	Reference
Eutheria:						
Elephantulus rozeti	49.2	20.0	36.3	33.9	37.3 ± .31	Lovegrove et al. 2001
	49.2	15.0	36.5	33.7	37.2 ± .57	Lovegrove et al. 2001
	49.2	10.0	36.0	33.5	36.8 ± 1.07	Lovegrove et al. 2001
	49.2	5.0	36.9	33.3	37.5 ± .56	Lovegrove et al. 2001
Eptesicus fuscus	14.7	2.5	32.9	31.8	36.5 ± .79	Willis et al. 2005*b*
Glis glis	140.0	12.8	36.4	…	36.0 ± .57	Wilz and Heldmaier 2000
	140.0	5.1	34.9	…	33.7 ± .61	Wilz and Heldmaier 2000
	140.0	15.0	35.3	…	35.3 ± .58	Wilz and Heldmaier 2000
Macroglossus minimus	15.5	15.0	34.2	32.3	35.7 ± .69	Bartels et al.1998
	15.5	20.0	34.3	32.5	36.6 ± .96	Bartels et al.1998
	15.5	24.0	35.0	32.7	37.3 ± .79	Bartels et al.1998
Microcebus murinus	62.0	9.8	36.0	34.0	35.5 ± 1.65	Schmid 1996
	59.0	22.8	34.9	34.4	36.5 ± .96	Schmid 2000
	52.5	16.2	35.4	33.9	36.4 ± 1.21	Schmid 2000
Microcebus myoxinus	37.0	11.8	33.2	33.1	33.9 ± 1.68	Schmid 1996
	29.0	14.7	33.4	32.9	34.1 ± 1.97	Schmid 1996
	30.0	12.9	35.6	32.8	35.6 ± .91	Schmid et al. 2000
Perognathus hispidus	40.1	11.0	34.9	33.2	35.2	Wang and Hudson 1970
Peromyscus eremicus	15.0	19.5	33.2	32.5	37.0 ± 1.58	MacMillen 1965
Phodopus sungorus	25.0	10.0	32.7	32.5	36.2 ± 1.03	Heldmaier and Ruf 1992
Spermophilus lateralis	280.0	4.0	35.1	…	37.0	Steiger 1992
Spermophilus richardsonii	406.0	15.0	35.0	…	37.1	Wang 1978
	406.0	5.0	34.9	…	37.1	Wang 1978
Syconycteris australis	18.0	18.0	35.3	32.5	35.8 ± .85	Coburn and Geiser 1998
	18.0	11.1	33.2	32.3	34.6 ± .60	Geiser et al. 1996*a*
Marsupialia:						
Cercartetus nanus	36.2	19.8	34.5	33.4	35.0 ± 1.23	Song et al. 1998*b*
	36.2	28.4	36.8	33.7	35.8 ± 1.28	Song et al. 1998*b*
	36.7	12.0	33.9	33.1	34.3 ± .55	Westman and Geiser 2004
	36.7	20.0	35.9	33.4	34.5 ± 1.23	Westman and Geiser 2004
Sminthopsis macroura	24.7	19.0	33.5	32.9	35.6 ± 1.57	Geiser et al. 1996*b*
	22.5	18.0	34.0	32.7	34.2 ± 1.73	Song and Geiser 1997
	22.5	28.0	35.9	33.1	35.5 ± 1.22	Song and Geiser 1997

Note. Data for normothermic T_b ($T_{b\text{-}norm}$) are mean ± SD of values sampled from T_b time courses, except for those from *P. hispidus*, *S. lateralis*, and *S. richardsonii*, which are from Geiser (2004).

each set of T_b values, I calculated the average T_b and fitted a normal distribution, assuming that the maximum recorded value represented the upper 99% confidence limit (CL; McKechnie et al. 2007). By definition, a normal distribution is symmetrical about the mean (Zar 1999), so if the upper 99% CL is known (or assumed), the lower limit can be calculated by simple subtraction using the equation

$$\text{lower CL} = \text{mean} - (\text{upper CL} - \text{mean}). \quad (2)$$

I defined this lower limit as the torpor threshold after McKechnie et al. (2007). For each distribution, I tested for skewness and concluded significance if skewness values divided by the standard error of skewness were greater than 2 (Zar 1999). I also compared torpor threshold values calculated using equation (2) with values of $T_{b\text{-}onset}$ that I determined for the same species. I restricted this comparison to species with body mass smaller than 70 g because of my results for $T_{b\text{-}onset}$ (see below).

I used Systat, version 11 (Systat Software, San Jose, CA), for all statistical analyses. I log transformed nonnormal data where necessary and used an α level of 0.05 to assess signif-

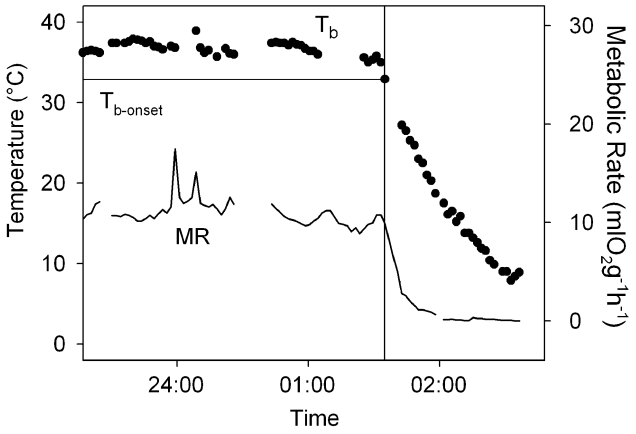

Figure 1. Typical time course plot showing representative traces of T_b and metabolic rate (MR) from which I obtained values of $T_{b\text{-}onset}$. The vertical line corresponds to a clear decline in MR from the resting normothermic level. I defined the T_b measured at this time as $T_{b\text{-}onset}$. Data replotted from Willis et al. (2005*b*) for *Eptesicus fuscus*.

icance. Values reported are the mean ± SD unless stated otherwise.

Results

There was no correlation between washout estimates for respirometry chambers used in the original studies and T_a, BM, or values of $T_{b\text{-}onset}$ (Pearson $r < 0.20$), which suggests that washout did not influence my results. When I controlled for BM and T_a, there was no effect of subclass on $T_{b\text{-}onset}$ (ANCOVA: $F_{28,1} = 0.44$, $P = 0.52$), so I pooled the data from all species in subsequent analyses. A general linear model (GLM) relating the independent variables, T_a and the base-10 logarithm of BM, with the dependent variable, $T_{b\text{-}onset}$, for all mammals was significant ($F_{2,28} = 5.28$, $P = 0.01$, $r^2 = 0.27$). However, there was a clear plateau in the relationship between BM and $T_{b\text{-}onset}$ for values of body mass between 70 and 140 g (Fig. 2*A*). Therefore, I repeated the analysis excluding mammals heavier than 70 g, using nontransformed BM. This left only 11 species (range of body mass = 14.7–62.0 g) but dramatically improved the explanatory power of the model (Fig. 2*B*; $F_{2,23} = 9.9$, $P = 0.001$, $r^2 = 0.46$). Effects of BM ($t = 3.9$, $P < 0.001$) and T_a ($t = 2.26$, $P = 0.03$) both contributed significantly, and the linear model was described by the equation

$$T_{b\text{-}onset} = (0.055 \pm 0.014)\text{BM} + (0.071 \pm 0.031)T_a + (31.823 \pm 0.740), \quad (3)$$

where values represent parameter estimates ± 1 SE (Table 2). Standard error values in this equation reflect the precision of model parameter estimates. A conservative approach, then, which accounts for error associated with the precision of parameter estimation and avoids overestimating use of torpor, would be to use $T_{b\text{-}onset} - 1$ SE to calculate the threshold T_b (Tables 1, 2; Fig. 3). Subtracting SE for each parameter estimate modifies equation (3) to

$$T_{b\text{-}onset} - 1 \text{ SE} = (0.041)\text{BM} + (0.040)T_a + 31.083. \quad (4)$$

Values of $T_{b\text{-}onset}$ used to generate these equations were slightly but significantly lower than values of normothermic T_b reported by Geiser (2004) for the same species (34.8° ± 1.3° vs. 36.0° ± 1.4°C; paired *t*-test: $t = 3.9$, df = 25, $P = 0.001$) as well as average normothermic values obtained from the T_b

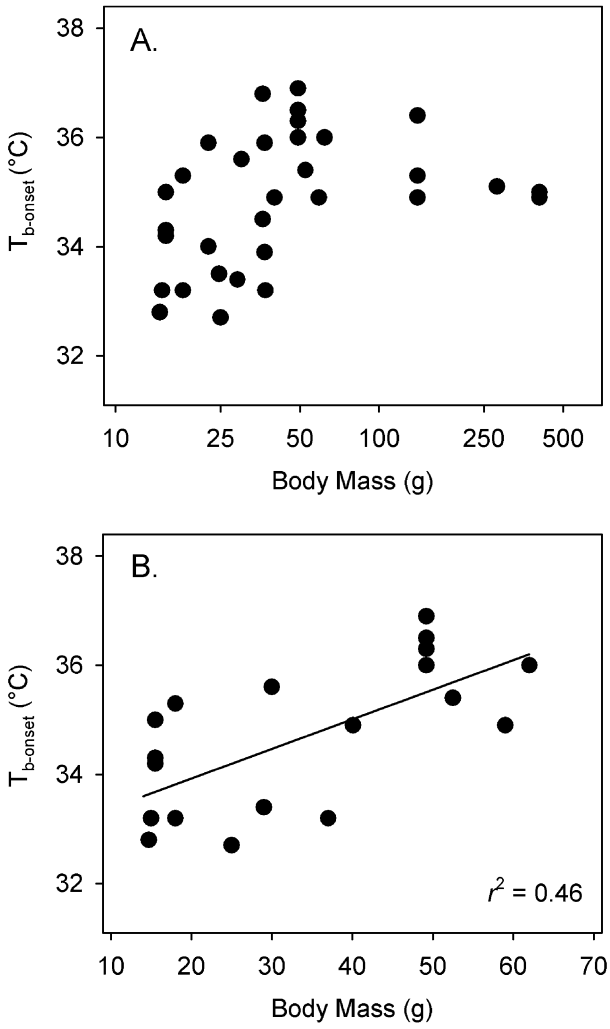

Figure 2. Plots of body mass (BM) versus $T_{b\text{-}onset}$ for all mammals for which data are available (*A*) and for mammals lighter than 70 g (*B*). Note that body mass in *A* is plotted on a log scale. See text (eq. [3]) for general linear model results. The solid line in *B* represents predicted values of $T_{b\text{-}onset}$ for body masses smaller than 70 g, calculated using equation (3) and assuming $T_a = 14$°C, the mean T_a to which animals were exposed in the original studies.

Table 2: Sample values for $T_{b\text{-}onset}$ predicted by equation (3) and $T_{b\text{-}onset} - 1$ SE predicted by equation (4) for mammals of body mass 5–40 g at three different values of T_a

T_a and BM (g)	$T_{b\text{-}onset}$ (°C)	$T_{b\text{-}onset} - 1$ SE (°C)
5.0°C:		
5.0	32.5	31.5
10.0	32.7	31.7
20.0	33.3	32.1
30.0	33.8	32.5
40.0	34.4	32.9
10.0°C:		
5.0	32.8	31.7
10.0	33.1	31.9
20.0	33.6	32.3
30.0	34.2	32.7
40.0	34.7	33.1
20.0°C:		
5.0	33.5	32.1
10.0	33.8	32.3
20.0	34.3	32.7
30.0	34.9	33.1
40.0	35.4	33.5

Note. $T_{b\text{-}onset} - 1$ SE represents a conservative threshold between torpor and normothermia for field studies of small mammals.

traces in the studies I analyzed (35.8° ± 1.1°C; Table 1; paired *t*-test: $t = 4.1$, df = 31, $P < 0.001$). This suggests that $T_{b\text{-}onset}$ represents a realistic threshold between torpor and normothermia.

None of the distributions of normothermic T_b that I obtained from the original studies were significantly skewed. There was a weak but significant linear relationship between values of $T_{b\text{-}onset}$ and torpor threshold values calculated for the same respirometry trials using the normal-distribution method ($F_{1,24} = 7.56$, $P = 0.01$, $r^2 = 0.24$). I used a paired *t*-test to control for differences in T_a and BM between trials and found that values of $T_{b\text{-}onset}$ obtained from the original studies (34.8° ± 1.3°C) were slightly but significantly higher than values calculated by the normal-distribution method (34.1° ± 1.6°C; paired *t*-test: $t = 2.3$, df = 25, $P = 0.03$). However, values of $T_{b\text{-}onset} - 1$ SE calculated based on BM and T_a by equation (4) (33.1° ± 0.6°C) were significantly lower than those calculated using the normal-distribution method (Table 1; paired *t*-test: $t = 3.3$, df = 25, $P = 0.003$).

Discussion

Oxygen consumption, as a proxy for MR, is a far better variable to measure than T_b for determining when endotherms begin to save energy as a result of heterothermy, but for many studies, especially in the field, we are limited to measurement of some less precise correlate of MR. A few studies of small endotherms have employed heart rate telemetry as a metabolic proxy, and heart rate has potential for future studies of torpor in small mammals (Butler et al. 2004; Cooke et al. 2004; Bowlin et al. 2005). However, the vast majority of field and enclosure studies of torpor employ measurements of T_b or T_{sk}. Thus, it is necessary to quantify T_b- or T_{sk}-based boundaries between torpor and normothermia as objectively as possible. Over some timescale, every animal must achieve a balance between energy acquisition and consumption to survive and reproduce, so energy is fundamental to reproductive fitness and is the currency we must address to understand use of torpor in the wild. Values of $T_{b\text{-}onset}$ calculated by the models presented here (eqq. [3], [4]; Table 2) have a stronger physiological justification than thresholds for torpor based on arbitrary values of T_b, $T_b - T_a$ differentials, behavioral activity, or frequency distributions of T_b measurements because they reflect the onset of energy savings that result from abandoning regulation of the normothermic T_b set point at the start of a torpor bout. This said, only a small number of studies have presented the data required to determine $T_{b\text{-}onset}$, so the sample size for my analysis was small. More data are needed to validate the models, but until they are available, $T_{b\text{-}onset}$ may be useful for defining torpor bouts when MR cannot be measured. To be conservative and account for error associated with the precision of model parameter estimates, I recommend using values of $T_{b\text{-}onset} - 1$ SE, calculated by equation (4).

For mammals heavier than a certain threshold (between 62 and 140 g), my findings tentatively justify use of a single value

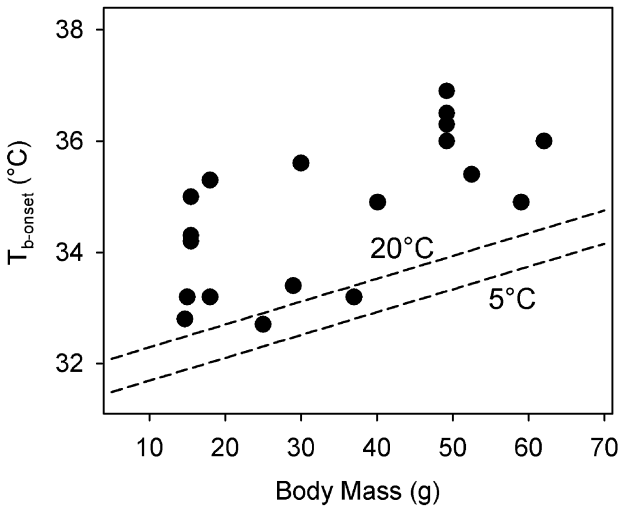

Figure 3. Data from Figure 2*B*, replotted with lines representing $T_{b\text{-}onset} - 1$ SE calculated from equation (4), assuming $T_a = 5$°C (*lower line*) and 20°C (*upper line*).

of T_b to differentiate torpor from normothermia because there was an obvious plateau in $T_{b\text{-onset}}$ above this threshold (Fig. 1*A*). The mean value of $T_{b\text{-onset}}$ for mammals >62 g was 35.3° ± 0.4°C, much higher than the 30°C cutoff used in most studies (Barclay et al. 2001). A plateau in $T_{b\text{-onset}}$ above a threshold BM is not surprising. Large endotherms have high thermal inertia and will cool slowly at the immediate onset of torpor (Geiser 2004). This also explains the strong relationship between $T_{b\text{-onset}}$ and BM for mammals lighter than 62 g. However, normothermic T_b of mammals obviously has an upper limit, so the positive relationship between BM and $T_{b\text{-onset}}$ can extend only so far. There was a large gap in my data set between BM = 62 and 140 g, so more data are needed to determine the BM at which this plateau actually occurs. The gap reflects, in part, the fact that most heterothermic mammals are smaller than this but also that few studies of torpor in mammals >62 g have presented concurrent traces of MR and T_b. In any case, small mammals below this threshold BM are the most likely to be heterothermic (Geiser 2004), so the relationship between $T_{b\text{-onset}}$ and BM should apply to the majority of heterothermic species. For large species, McKechnie et al.'s (2007) normal-distribution approach may work well if it can be confirmed that normothermic T_b fits a normal distribution for free-ranging individuals of the species under study.

A significant proportion (46%) of variation in $T_{b\text{-onset}}$ was explained by BM and T_a, but there was considerable residual variation, which could be influenced by a number of factors, including phylogenetic effects. I was surprised not to detect an effect of subclass on $T_{b\text{-onset}}$. Given the difference in normothermic T_b between marsupial and placental mammals (Withers 1992), taxon-specific effects are likely to influence the true threshold differentiating torpor and normothermia. The fact that I did not detect this difference may reflect the small sample of $T_{b\text{-onset}}$ data available, especially for marsupials. Taxon-specific effects at levels below subclass could also influence normothermic T_b and cooling rates, so, ideally, to control for phylogenetic effects, analyses of $T_{b\text{-onset}}$ should be conducted within species. Future studies should undertake trials recording MR during torpor entry over a range of T_a for conspecifics that vary in BM. By controlling for phylogenetic influence, species-specific equations for $T_{b\text{-onset}}$ are likely to provide more accurate threshold T_b's than those calculated by equation (4).

My results provide tentative support for McKechnie et al.'s (2007) normal-distribution method as another means for defining the torpor threshold. None of the normothermic T_b frequency distributions I analyzed were significantly skewed, so the central assumption of the method was valid for the subset of mammals I studied. I also found a significant correlation between values of $T_{b\text{-onset}}$ and T_b values calculated by the normal-distribution approach, and differences between values predicted by the two methods, while significant, were small (about 1°C). These findings indicate that, again, for this subset of mammals, the two methods provide comparable results. However, support for the normal-distribution approach remains equivocal because I was able to analyze only very small numbers of normothermic T_b data points for each respirometry run. Data sets from free-living animals are certain to be many times larger. Furthermore, most animals included in my analysis were post-absorptive and measured in the laboratory, so, relative to a field setting, there would have been much less variation in factors that could affect the symmetry of T_b distributions. In any case, within-species analyses of effects of T_a and BM on $T_{b\text{-onset}}$ are likely to provide estimates of the torpor threshold that are more relevant to energy balance and reproductive fitness than either equation (4) or the normal-distribution method. These analyses would control for phylogenetic effects while providing threshold T_b's that account for physiological attributes of the animals themselves (i.e., heat loss under different conditions and the onset of energy savings during torpor) rather than statistical properties of measurements.

For a large number of species, the use of implanted devices to measure T_b is impractical if not impossible in the field. For example, many insect-eating bats are small bodied but have very large home ranges. This means that surgically implanted radiotransmitters are not useful because of their small range of signal detection (Barclay et al. 2001; Willis and Cooper, forthcoming). For threatened species or populations, surgical procedures for transmitter implantation may pose too great a risk. In these cases, T_{sk} must be measured as a proxy for T_b and can yield valuable data if its limitations are taken into account (Barclay et al. 1996; Audet and Thomas 1996; Willis and Brigham 2003; Dausmann et al. 2005). Measurements of T_{sk} may be affected by both T_a and BM because of the influence of both variables on thermal conductance. In terms of the torpor threshold, effects of T_a in particular will probably be larger for values of $T_{sk\text{-onset}}$ than for those of $T_{b\text{-onset}}$. External transmitters are subject to ambient heating and cooling, which can result in $T_b - T_{sk}$ differentials of 6°C or more during steady state normothermia, and the magnitude of these differentials is influenced by T_a (Barclay et al. 1996; Audet and Thomas 1996; Willis and Brigham 2003; McKechnie et al. 2007). To my knowledge there are no published time course data depicting concurrent traces of T_{sk} and MR from which values for $T_{sk\text{-onset}}$ could be determined. These data are needed to quantify an energy-relevant T_{sk} threshold for torpor to be used in field studies of small, free-ranging mammals.

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