

Passive rewarming from torpor in hibernating bats: minimizing metabolic costs and cardiac demands

Shannon E. Currie, Kodie Noy, and Fritz Geiser

Centre for Behavioral and Physiological Ecology, Zoology, University of New England, Armidale, Australia

Submitted 14 August 2014; accepted in final form 11 November 2014

Currie SE, Noy K, Geiser F. Passive rewarming from torpor in hibernating bats: minimizing metabolic costs and cardiac demands. *Am J Physiol Regul Integr Comp Physiol* 308: R34–R41, 2015. First published November 19, 2014; doi:10.1152/ajpregu.00341.2014.—Endothermic arousal from torpor is an energetically costly process and imposes enormous demands on the cardiovascular system, particularly during early stage arousal from low body temperature (T_b). To minimize these costs many bats and other heterothermic endotherms rewarm passively from torpor using solar radiation or fluctuating ambient temperature (T_a). Because the heart plays a critical role in the arousal process in terms of blood distribution and as a source of heat production, it is desirable to understand how the function of this organ responds to passive rewarming and how this relates to changes in metabolism and T_b . We investigated heart rate (HR) in hibernating long-eared bats (*Nyctophilus gouldi*) and its relationship to oxygen consumption ($\dot{V}O_2$) and subcutaneous temperature (T_{sub}) during exposure to increasing T_a compared with endogenous arousals at constant low T_a . During passive rewarming, HR and $\dot{V}O_2$ remained low over a large T_{sub} range and increased concurrently with increasing T_a (Q_{10} 2.4 and 2.5, respectively). Absolute values were higher than during steady-state torpor but below those measured during torpor entry. During active arousals, mean HR and $\dot{V}O_2$ were substantially higher than during passive rewarming at corresponding T_{sub} . In addition, partial passive rewarming reduced the cost of arousal from torpor by 53% compared with entirely active arousal. Our data show that passive rewarming considerably reduces arousal costs and arousal time; we suggest this may also contribute to minimizing exposure to oxidative stresses as well as demands on the cardiovascular system.

torpor; passive rewarming; heart; metabolism; bat

TORPOR is central to the biology of many small mammals and birds worldwide and involves the coordination of a complex array of costs and benefits that change seasonally and with climate, body condition, and age (26, 31). Torpor is characterized by the controlled and reversible reduction of metabolic processes, body temperature (T_b), ventilation, and cardiac function. While energy requirements of torpid animals are substantially reduced, adequate perfusion of essential organs is still required, and it has long been established that animals capable of entering torpor possess a suite of physiological adaptations to preserve coordinated functioning of the cardiovascular system at low T_b (33). Increased peripheral resistance and reduced venous return, related to increased viscosity of cold blood, and low heart rates (HR) are offset by an increase in stroke volume and alterations of contractile proteins in the myocardium (21). Antiarrhythmic protection ensures that coordinated conduction across all chambers of the heart is maintained throughout torpor (38). These adaptations are also likely

to enable a safe return to euthermia during arousal from torpor, which is extremely demanding and can occur frequently.

Endothermic rewarming from torpor is extremely energetically expensive and can deplete most of the energy needed during the hibernation season (34). During arousal the massive increase in metabolic rate (MR) along with reperfusion of dormant tissues exposes animals to oxidative stress, as increased production of reactive oxygen species (ROS) may outweigh the animal's antioxidant defenses (5). In addition, the cardiovascular system must work to support the increasing oxygen demands of thermogenic organs and surrounding tissues. Rewarming is controlled by the sympathetic-adrenal system, which drives HR and metabolism at maximum rates corresponding to rising T_b (15). In addition, rapid beating of the heart provides a vital source of heat for rewarming (19). Intricate coordination of the circulatory system during this phase results in different rates of oxygen consumption in the body, with significantly greater perfusion of heart, brain, liver, and brown adipose tissue compared with the posterior body, peripheral tissues, and digestive tract (25). The restriction of blood to the anterior portion of the body increases systemic vascular resistance and results in a rapid increase in blood pressure, which reaches maximum levels early in arousal (15). The heart, however, is rate limited by low T_b and forced to work against high blood pressures, reducing its efficiency as a pump and likely introducing mechanical stress. Therefore, it can be argued that early stage arousal from low T_b imposes the greatest demands on the cardiorespiratory system. As such it is important for arousing animals to maintain a balance, reducing stresses on the heart and exposure to ROS while still producing enough energy to rewarm.

The energy demands of rewarming can be offset to some extent by the use of passive rewarming, which often reduces arousal costs by >50%, and can result from heat transfer between nest/roost mates, increases in ambient temperature (T_a), or direct exposure to solar radiation (basking) (8). The benefits of passive rewarming are obvious for daily heterotherms that must rewarm from torpor to forage on a daily basis. In contrast, accounts of passive rewarming in hibernating species are rare, which is largely due to the fact that many hibernators roost or nest in thermally stable microclimates. Bats, however, hibernate in an array of microhabitats from caves to exposed foliage and can spend more than half of their lives in torpor (12). In particular, tree-dwelling bat species generally roost under exfoliating bark and in hollows or rock crevices and may even overwinter in these areas, exposing themselves to large fluctuations in T_a (10, 36).

The cost of endothermic arousals in bats may, in some cases, be proportionately greater than for other species, considering the higher relative surface area for heat loss attributed to wing membranes and the solitary nature of many tree-dwelling

Address for reprint requests and other correspondence: S. E. Currie, Univ. of New England, Armidale NSW 2351, Australia (e-mail: scurrie3@myune.edu.au).

species. During torpor at low T_b , bats maintain average HRs below 40 beats/min and are capable of returning to euthermic T_b ($\sim 35^\circ\text{C}$) and HRs upwards of 600 beats/min in less than 1 h (6, 11, 24). As many of these hibernating bats use torpor year round, often regardless of food availability or weather conditions (31), energy savings can be maximized by the regular use of passive rewarming, particularly in summer when arousals are frequent, daily fluctuations in T_a are large and radiant heat is generally available (2).

Many studies of free-ranging bats have shown that T_b during torpor fluctuates widely with T_a , from $\sim 7^\circ\text{C}$ in northern hemisphere bats (9) up to 20°C in Australian vespertilionids (35). Although for most bats passive rewarming is partial and complete arousal includes an active component, recent studies provide evidence that some desert-dwelling bats rewarm entirely passively; using passive rewarming to increase T_b by more than 20°C (3). Some bats select thermally labile roosts often choosing areas with afternoon sun exposure enabling them to maximize energy savings associated with fluctuating T_a (10, 35). Thermal lability of roost microclimates allow bats to exploit cool morning T_a to minimize T_b and maximize energy savings and warming temperatures/radiant heat later in the day to passively rewarm and reduce costs of normothermic thermoregulation (37, 42). Although passive rewarming has been documented in a number of species, there are currently no published data detailing cardiovascular changes during passive rewarming from torpor or the relationship among HR, MR, and T_b during this phase of arousal.

Because passive rewarming may be important to minimize the energetic costs of endogenous arousal as well as oxidative and mechanical stress on the heart, we aimed to provide the first data on HR and MR during passive arousal from torpor in captive insectivorous long-eared bats (*Nyctophilus gouldi*, ~ 10 g). Bats exposed to fluctuating T_a profiles were compared with individuals maintained at a constant low T_a and required to rewarm actively. *N. gouldi* is a hibernating bat that uses torpor throughout the year in thermally labile roosts and often but not always passively rewarms from torpor in the wild (35). As these hibernators maintain low MR during torpor through metabolic inhibition, we also were interested in the interrelations between HR, MR, and T_b during 1) entry into torpor and 2) passive rewarming compared with 3) steady-state minimum values during torpor. During passive rewarming we predict a synchronized increase in HR and MR with increasing temperature qualitatively similar to that shown during active arousal; however, rates should reflect minimal values at corresponding T_b values in torpor well below rates during active arousal, or follow an intermediate trajectory between steady-state torpor values and those during active rewarming.

METHODS

We used open-flow respirometry, ECGs, and temperature-sensitive passive integrated transponders to measure the relationship between HR, MR (measured as rate of oxygen consumption $\dot{V}O_2$) and subcutaneous temperature (T_{sub}) during passive rewarming from torpor in *N. gouldi*. A total of 15 individuals were used for measurements (mass at capture 10.1 ± 1.0 g). Bats were captured with mist nets at Imbota Nature Reserve and Newholme Field Station near Armidale, New South Wales ($30^\circ 35'S$, $151^\circ 44'E$) and kept in outdoor aviaries at the University of New England. Bats were provided with meal worms and water ad libitum, and this was supplemented by moths and other flying

insects that were attracted into cages by an ultraviolet light. Twice weekly mealworms were dusted with a supplement of Wombaroo Insectivore Rearing Mix. Bats remained within 1 g of their body mass at the time of capture while in captivity.

This study was conducted under a scientific license provided by the NSW Parks and Wildlife Authority (SL100084) and with Animal Ethics approval from the University of New England (AEC12-043).

Transponder implantation. T_{sub} was measured using temperature-sensitive transponders (0.13 g, 14 mm \times 2 mm, IPTT-300 Bio Medic Data Systems Implantable Programmable Temperature Transponder) implanted interscapularly. Transponders were calibrated over a range of 5°C to 40°C to the nearest 0.1°C against a precision reference thermometer in a water bath before use (39).

Before implantation bats were given a minimum of 3 days to acclimate to captivity and ensure they maintained a stable body mass. Bats were anesthetized using general isoflurane-oxygen anesthesia, and the skin was sterilized with 70% alcohol before a small (~ 3 mm) incision was made just below the shoulder blades for transponder insertion. The insertion site was closed with a single suture (chromic gut, Ethicon, Somerville, NJ), and the entire process was complete within 15 min. Bats were given 24 h to recover in a warm room before being returned to outdoor flight cages.

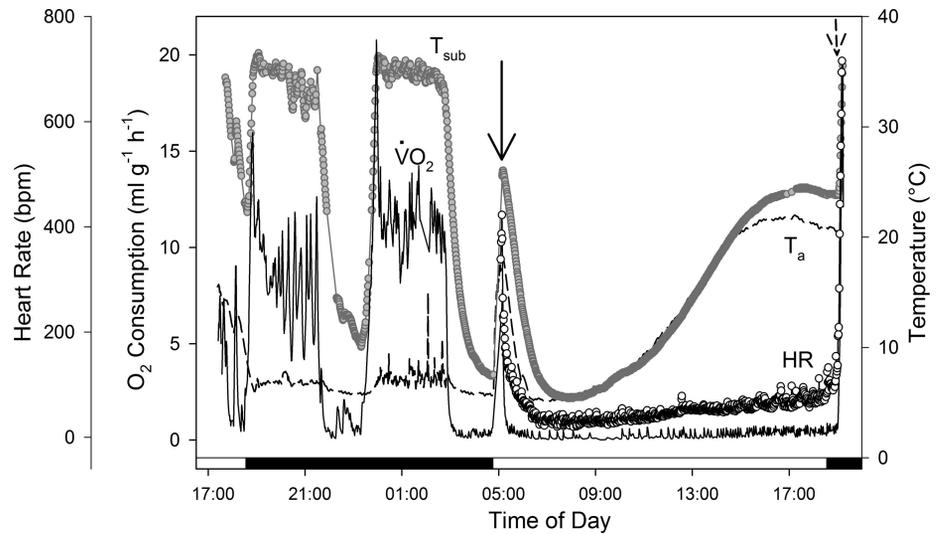
Respirometry and T_a profile. Bats were placed into respirometry chambers in the early evening and were fasted to ensure they were postabsorptive. $\dot{V}O_2$ was monitored overnight and throughout the following day(s) to allow animals to undergo their usual daily thermal cycle. Information regarding open-flow respirometry equipment and techniques are detailed in Currie et al. (6). Respirometry chambers (0.40 or 0.53 liters) were made from modified polycarbonate enclosures with clear lids, lined with a small patch of hessian cloth (burlap) from which the bats could roost. Flow rate (170–200 ml/min) was adjusted based on chamber size to ensure that 99% equilibrium was reached within 11 min. $\dot{V}O_2$ measurements were time adjusted to correspond with measurements of HR, accounting for lag of the system.

Chambers were placed inside a temperature-controlled cabinet with an incandescent light source set to the natural photoperiod at the time of year. Bats were either measured at a single T_a (between 5 and 25°C , $n = 12$), which remained constant throughout the torpor bout, or individuals were exposed to a diurnal T_a increase (from 5 to 23°C , $n = 5$) similar to that experienced in their roosts in the warm season (adapted from Ref. 37). Bats were either exposed to the T_a profile following entry into torpor on the first day of measurement or following up to 4 days of hibernation. One bat remained in torpor when the T_a had reached 23°C and in this case T_a was gradually increased to 30°C before active arousal was induced. Basal metabolic rate (BMR) and basal heart rate (BHR) were measured following arousal from torpor while animals were resting and within the thermoneutral zone previously determined for this species (29 – 34°C ; 7).

ECGs and ventilation. HR was recorded using ECG following the methods of Currie et al. (6). Individuals were placed in respirometry chambers in the evening, and ECG wires (Lead I arrangement) were attached to adhesive electrodes on the bat's forearm just after lights on the following morning. Ventilatory movements were measured using a pulse transducer (MLT1010, ADInstruments) that lay flush with the bat's chest and was sensitive enough to also detect cardiac contractions during apnoeic periods.

Statistical analyses. Average minimum values of $\dot{V}O_2$, HR, and T_{sub} during torpor were taken from times when all variables were lowest for at least 30 min. During torpor all bats exhibited an episodic breathing pattern, which ceased when individuals were actively rewarming; thus active arousal was determined from the start of continuous ventilation. Arousal was assumed to end following a peak (overshoot) in $\dot{V}O_2$ and was measured until $\dot{V}O_2$ fell to $\leq 75\%$ of that maximum. Energy expenditure of arousal (kJ) was calculated from mean $\dot{V}O_2$ (l/h) multiplied by the time taken to arouse (h) and a conversion factor of 20.083 (27). Total torpor bout energy expenditure

Fig. 1. Representative example of heart rate (HR; open circles and line), oxygen consumption ($\dot{V}O_2$; solid line), and subcutaneous temperature (T_{sub} ; grey filled circles) of *Nyctophilus gouldi* exposed to an increasing ambient temperature (T_a); the dark horizontal bar represents scotophase. The animal entered torpor in the early morning before lights on, exhibited a partial arousal associated with ECG lead attachment (indicated by solid arrow), and then proceeded to reenter and remain in torpor until spontaneously arousing at lights off in the evening (indicated by dashed arrow).



was calculated from the peak $\dot{V}O_2$ following the induced partial arousal before entry in the early morning until the peak following active arousal (indicated by arrows in Fig. 1). Total torpor energy expenditure had to be time adjusted for one animal that rewarmed following 4 days of hibernation. This was done by integrating energy expenditure over torpor entry and part of steady-state torpor on the first day added to passive rewarming and arousal on the final day, for an overall torpor bout $\dot{V}O_2$ value. Fat requirements were calculated from energy expenditure assuming that metabolism of 1 mg of fat releases 39.3 J (34).

Average $\dot{V}O_2$ and HR during passive rewarming, active arousal, and entry (following partial arousal $T_{sub} > 17^\circ\text{C}$) were averaged over the same T_{sub} intervals to enable comparison. Values for resting $\dot{V}O_2$, HR, and T_{sub} were taken from the period following arousal. Because of impedance of the ECG associated with bat movement and/or individuals' intolerance of the electrodes, resting values could only be averaged over a 5-min period. Furthermore, after arousal from torpor, bats often moved out of range of the transponder scanner, which was ~ 5 cm, and therefore T_{sub} was occasionally unavailable. The Q_{10} for rates of $\dot{V}O_2$ or HR (R) of thermo-conforming torpid bats and passively rewarming bats was calculated using the following equation: $Q_{10} = (R1/R2)^{10/(T_{sub1} - T_{sub2})}$.

Statistical analyses were performed using *R* v3.1.0 (22). Two sample *t*-tests were used to compare individual's mean $\dot{V}O_2$ and HR following arousal as well as the time taken to reach maximum $\dot{V}O_2$ at different T_a . Repeated measures analysis of variance (ANOVA) was used to compare mean $\dot{V}O_2$ and HR between passive rewarming and corresponding values during active arousal and torpor entry. Analysis of covariance (ANCOVA) was used to compare slopes and intercepts of the curves fitted to passive rewarming and torpor values. Standardized major axis regression was performed to assess the relationships between HR and $\dot{V}O_2$ using the *smatr* package, we accounted for pseudoreplication by adjusting the degrees of freedom as for mixed effect linear modelling that are adjusted for repeated measures (41). Means are reported \pm SD for the number of individuals (n).

RESULTS

Effects of T_a on resting HR and $\dot{V}O_2$. During periods of normothermy metabolism and HR of bats declined linearly in a qualitatively similar pattern with exposure to increasing T_a (Fig. 2). Average resting $\dot{V}O_2$ decreased from 10.37 ± 2.75 $\text{ml}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ at a minimum average T_a of 5.9°C ($n = 7$, $T_{sub} = 34.8 \pm 1.1^\circ\text{C}$) to 4.86 ± 1.26 $\text{ml}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ at an average T_a of 20.2°C ($n = 8$, $T_{sub} = 34.7 \pm 1.1^\circ\text{C}$) (Fig. 2). Correspond-

ingly, resting HR fell inversely with T_a from an average 629 ± 84 beats/min ($n = 7$, $T_{sub} = 34.8^\circ\text{C}$) to 412 ± 95 beats/min ($n = 8$, $T_{sub} = 34.7^\circ\text{C}$). When T_a increased to $30.5 \pm 0.5^\circ\text{C}$, HR fell to basal levels at 227 ± 34 beats/min with corresponding BMR of 1.34 ± 0.16 $\text{ml}\cdot\text{O}_2\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ($n = 4$, mass = 9.9 ± 1.0 g, $T_{sub} = 34.3 \pm 0.6^\circ\text{C}$).

HR and MR during entry into torpor. All bats, at all T_a tested, entered torpor either overnight or in the early morning following lights on (Fig. 1). After a partial arousal associated with attachment of ECG leads in the morning, all bats reentered torpor. As animals reentered torpor, HR and $\dot{V}O_2$ fell exponentially with decreasing T_{sub} (HR $Q_{10} = 2.2$, $\dot{V}O_2$ $Q_{10} = 3.1$) (Fig. 3, A and B), and steady-state minimum values of HR, $\dot{V}O_2$, and T_{sub} during torpor were reached within 2–3 h of partial arousal. Figure 4 shows a representative ECG of one animal at T_a of 15°C at rest (Fig. 4A) and during steady-state

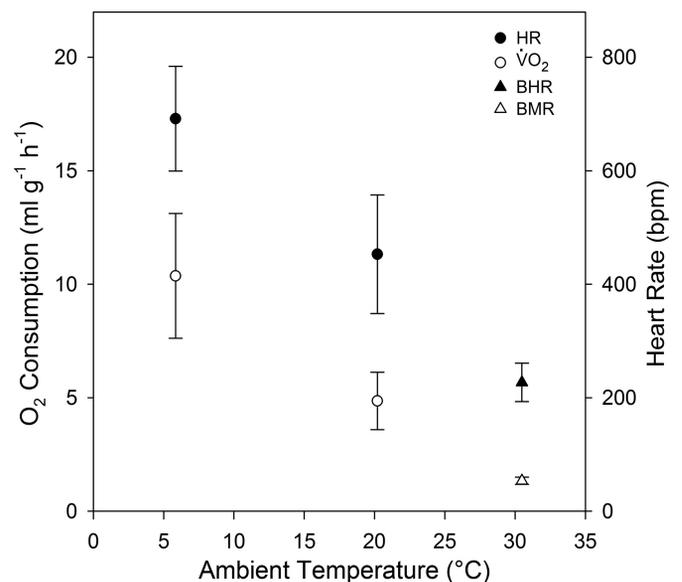


Fig. 2. Relationship for HR (filled circles) and $\dot{V}O_2$ (open circles) against T_a in normothermic *N. gouldi* at rest and within the thermoneutral zone (filled and open triangles). Both HR and $\dot{V}O_2$ increased linearly as temperatures decreased outside of the thermoneutral zone. BHR, basal HR; BMR, basal metabolic rate.

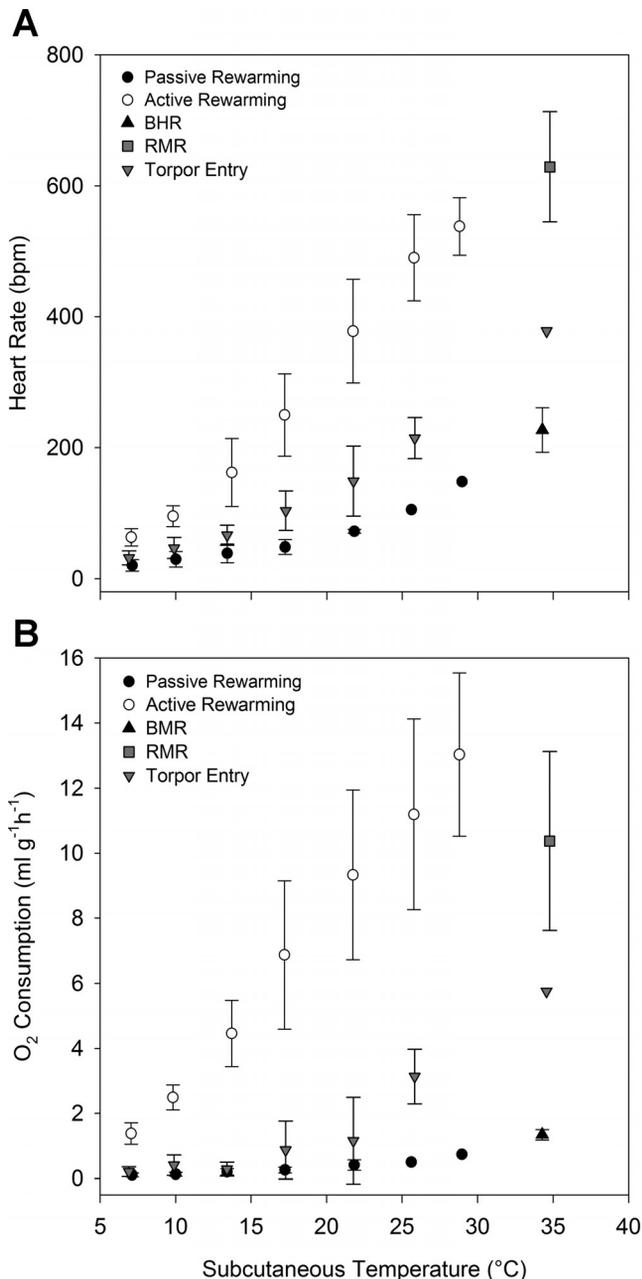


Fig. 3. A: relationship between average HR and T_{sub} of *N. Gouldi* during entry into torpor (grey inverted triangles), passive rewarming from torpor (filled circles), active arousal from torpor at average $T_a = 5.5^\circ\text{C}$ (open circles), resting HR at $T_a = 5.9^\circ\text{C}$ (grey square), and BHR (black triangle). Torpor entry and passive rewarming showed a curvilinear response to changing T_{sub} and were well below HR at the corresponding T_{sub} during active arousal. B: corresponding $\dot{V}O_2$ averages as plotted against T_{sub} (symbols are as for A). $\dot{V}O_2$ showed a similar temperature-dependent response to HR during entry into torpor and passive rewarming, again corresponding values during active arousal were substantially higher. Data points for passive rewarming beyond 20°C and entry beyond 25°C are averages taken from a single animal.

torpor (Fig. 4B). When animals were in torpor at $T_{sub} 16.5 \pm 1.2^\circ\text{C}$ HR fell to 31 ± 11 beats/min with average PR interval 0.11 s, QRS width 0.03 s, and QT time 0.09 s; it was not possible to derive these values from ECGs of normothermic bats. As expected, HR and $\dot{V}O_2$ were significantly lower during cooling than when animals actively rewarmed (ANOVA, $P < 0.05$).

Interestingly, however, at any given T_{sub} during entry into torpor ($T_a = 8.1 \pm 2.7^\circ\text{C}$), both HR and $\dot{V}O_2$ were significantly higher compared with corresponding T_{sub} of animals in steady-state torpor (ANOVA, $P < 0.001$). The slope of the relationship between $\log_{10}\text{HR}$ and T_{sub} differed significantly for animals that were entering torpor compared with animals that passively rewarmed (ANCOVA, $P < 0.01$). In contrast, there was no significant difference between torpor entry and passive rewarming with regard to the slope of $\log_{10}\dot{V}O_2$ against T_{sub} (ANCOVA, $P = 0.08$); however, the intercept for passive rewarming was significantly higher (ANCOVA, $P < 0.05$).

Effect of increasing T_a on HR, $\dot{V}O_2$, and initiation of active arousal. When bats were exposed to an increasing T_a , 3 of 4 individuals aroused from torpor before the T_a profile reached a 23°C plateau; active arousal began at an average T_{sub} of $19.5 \pm 1.2^\circ\text{C}$ after passive rewarming. One individual rewarmed passively with the T_a profile but did not arouse actively until the stimulus of lights off when T_{sub} was 23.9°C and HR had reached 137 beats/min (Fig. 1). Both $\dot{V}O_2$ and HR increased exponentially with increasing T_{sub} during passive rewarming with an average $\dot{V}O_2 Q_{10}$ of 2.5 and HR Q_{10} 2.4 (Fig. 5, A and B). $\dot{V}O_2$ increased ~ 6 -fold from a minimum of 0.06 ± 0.04 $\text{ml}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ($n = 5$, $T_a = 7.0 \pm 0.1^\circ\text{C}$, $T_{sub} = 6.2 \pm 1.0^\circ\text{C}$) at the beginning of T_a increase to 0.35 ± 0.06 $\text{ml}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ($n = 3$, $T_a = 18.8 \pm 1.2^\circ\text{C}$, $T_{sub} = 19.5^\circ\text{C}$) just before endogenous arousal. Across the same temperature range HR only increased approximately threefold from 17 ± 5 beats/min ($n = 5$, $T_{sub} = 6.2^\circ\text{C}$) to 48 ± 18 beats/min ($n = 3$, $T_{sub} = 19.5^\circ\text{C}$). The Q_{10} of highest average $\dot{V}O_2$ during passive rewarming compared with BMR was 3.2, and for highest average passive rewarming HR to BHR it was 2.3 (Fig. 5, A and B).

The curve fitted to average $\dot{V}O_2$ during passive rewarming fell above values for animals that were thermoconforming in steady-state torpor at a similar T_{sub} (Fig. 5B). There was no significant difference between slopes (ANCOVA, $P = 0.26$) but the intercept for passive rewarming $\dot{V}O_2$ was significantly higher than for steady-state torpor (ANCOVA, $P < 0.001$). In contrast, both the slope and intercepts of the curve fitted to HR against T_{sub} for bats in steady-state torpor were not significantly different from passive rewarming (ANCOVA, $P = 0.64$ and $P = 0.27$, respectively) (Fig. 5A).

Comparison of active and passive arousal and effect of T_a . On average, $\dot{V}O_2$ was 20.4-fold higher during active arousal compared with passive rewarming, with a maximum 26-fold difference in $\dot{V}O_2$ at $T_{sub} 17.2 \pm 0.4^\circ\text{C}$ (Fig. 3B). Although still significant, mean HR was only 4.2-fold higher on average in bats that actively aroused compared with passive rewarming, and the maximum difference of 5.3-fold occurred at $T_{sub} 21.8 \pm 0.8^\circ\text{C}$ (Fig. 3A). During active arousal HR and $\dot{V}O_2$ differed in their relationship to increasing T_{sub} with HR increasing in a sigmoidal pattern while $\dot{V}O_2$ increased almost linearly (Fig. 3, A and B). Interestingly, however, both HR and $\dot{V}O_2$ exhibited an overall ninefold increase between $T_{sub} 7.1 \pm 0.5^\circ\text{C}$ and $28.8 \pm 0.3^\circ\text{C}$.

After passive rewarming at the beginning of active arousal, clearly delineated by restoration of continuous breathing, HR increased substantially (>2 -fold) over a 1-min period (2 consecutive readings) from 70 ± 47 to 150 ± 45 beats/min ($n = 4$). In contrast, when bats actively rewarmed from a low T_a (average $5.7 \pm 1.2^\circ\text{C}$), the initial change in HR was negligible, increasing from 40 ± 9 to only 54 ± 23 beats/min ($n = 6$) over

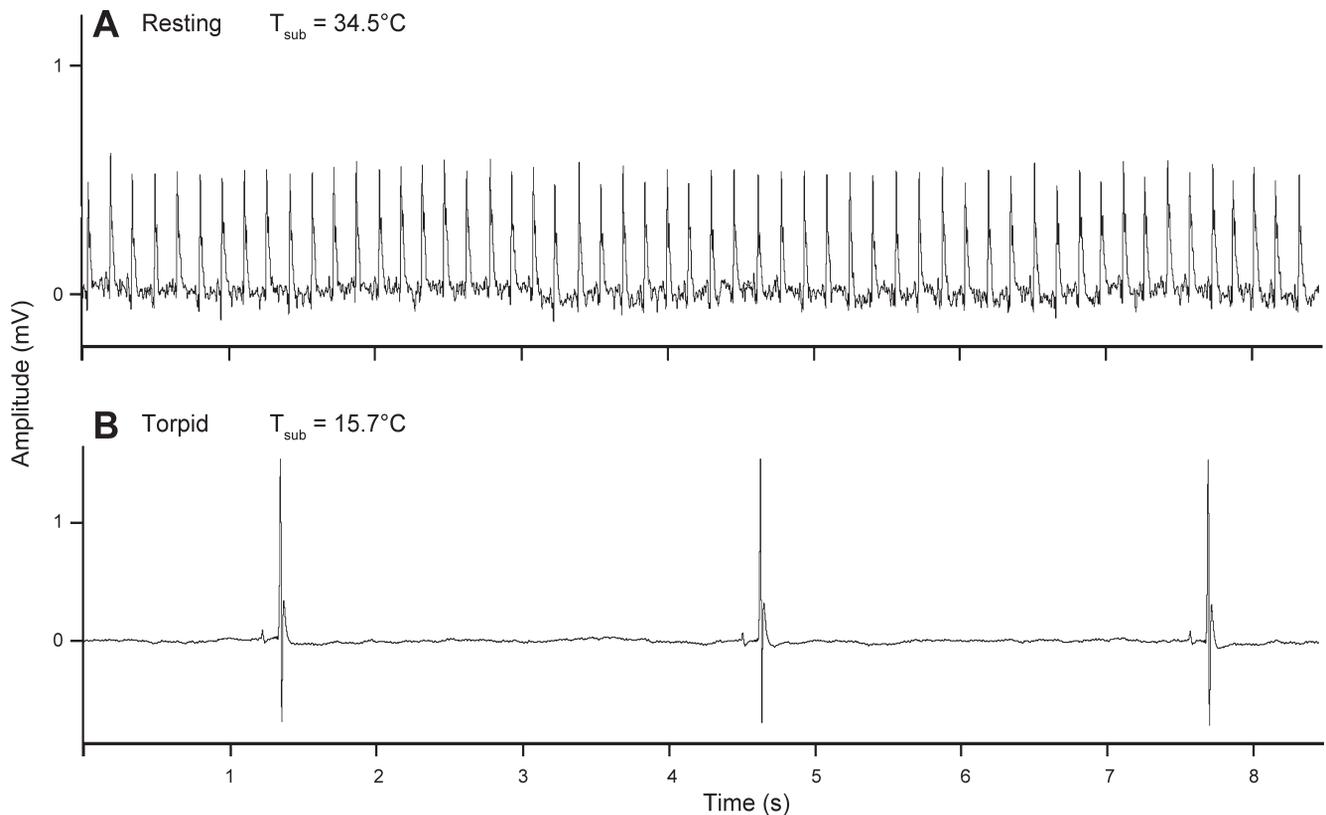


Fig. 4. Representative trace of an ECG for one individual *N. Gouldi* at $T_a = 15^\circ\text{C}$ at rest (A; HR ~ 400 beats/min, $T_{\text{sub}} = 34.5^\circ\text{C}$) and during torpor (B; HR ~ 18 beats/min, $T_{\text{sub}} = 15.7^\circ\text{C}$). Note the y-axis scaling.

1 min. Unlike HR, the initial minute increase in \dot{V}_{O_2} at active arousal was similar for both thermal conditions; increasing from 0.42 ± 0.13 to 0.56 ± 0.32 $\text{ml}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ($n = 4$) after passive rewarming and from 0.35 ± 0.28 to 0.54 ± 0.33 $\text{ml}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ($n = 6$) at constant T_a 5.7°C .

The peak arousal \dot{V}_{O_2} following passive rewarming was 10.96 ± 1.41 $\text{ml}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ($n = 3$) and did not differ significantly from peak \dot{V}_{O_2} following entirely active arousal at T_a $20.6^\circ\text{C} \pm 1.1$ (9.6 ± 0.99 $\text{ml}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$, $n = 6$; two-sample t -test, $P = 0.2122$). There was also no significant difference between maximum arousal HR after passive rewarming (543 ± 45 beats/min, $n = 3$) and the peak HR at constant T_a $20.6^\circ\text{C} \pm 1.1$ (468 ± 95 beats/min, $n = 6$; two-sample t -test, $P = 0.2493$). Not surprisingly, at low T_a (5.7°C) both maximum \dot{V}_{O_2} (14.32 ± 1.96 $\text{ml}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$, $n = 6$) and maximum HR (679 ± 65 beats/min, $n = 6$) were significantly higher following active arousal (two sample t -test, \dot{V}_{O_2} : $P < 0.01$, HR: $P < 0.01$) than at T_a 20.6°C or after passive rewarming.

The time taken to reach maximum HR during active arousal did not differ significantly between animals that rewarmed passively and those kept at an average constant T_a of $20.6 \pm 1.1^\circ\text{C}$ (average 15 min, two sample t -test, $P = 0.501$). However, it took animals significantly longer (average 51 min) to reach maximum HR at $T_a = 5.7^\circ\text{C}$ (two sample t -test, $P < 0.05$). The time taken to reach maximum \dot{V}_{O_2} did not differ significantly from the time taken to reach maximum HR during all active arousals (paired t -test, $P = 0.85$).

Energetic costs of rewarming. The cost of rewarming from torpor by *N. Gouldi* kept at constant T_a of 5.7°C was 1.33 ± 0.39 kJ ($n = 5$), which made up an average 62% (range

46%–75%) of total energy expenditure over the torpor bout (measured from the peak before entry through to arousal, indicated by arrows in Fig. 1). When exposed to increasing T_a the total energy expenditure during a torpor bout was reduced by 53% from 2.12 ± 0.31 kJ ($T_a = 5.7^\circ\text{C}$, $n = 5$) to 1.0 ± 0.27 kJ ($n = 4$). In addition, exposure to increasing T_a also reduced the cost of arousal by 68% to 0.42 ± 0.08 kJ ($n = 4$). When only passive rewarming was considered, energy expenditure was 0.23 ± 0.21 kJ ($n = 4$) and contributed only 34% to the total cost of arousal (0.70 ± 0.22 kJ, $n = 4$), which included both passive and active portions.

DISCUSSION

Torpor imposes enormous demands on the cardiovascular system, especially during rewarming from low T_b when HR must increase dramatically from less than 10 beats/min to over 700 beats/min in a short time frame. Our study is the first to quantify HR during passive rewarming from torpor and demonstrates the relationship between HR, \dot{V}_{O_2} , and T_{sub} during entry into torpor and throughout passive and active arousal. As animals entered into torpor after an induced partial arousal, HR and \dot{V}_{O_2} decreased simultaneously followed by a drop in T_{sub} , and this progressed in a qualitatively similar pattern to other hibernating species (19). Torpor reentry was characterized by the onset of regular apneas and a return to the episodic breathing pattern typical of hibernating bats. As anticipated, there was a similar exponential pattern of increase in both HR and \dot{V}_{O_2} during passive rewarming that corresponded to rising T_{sub} . However, these values represented an intermediate be-

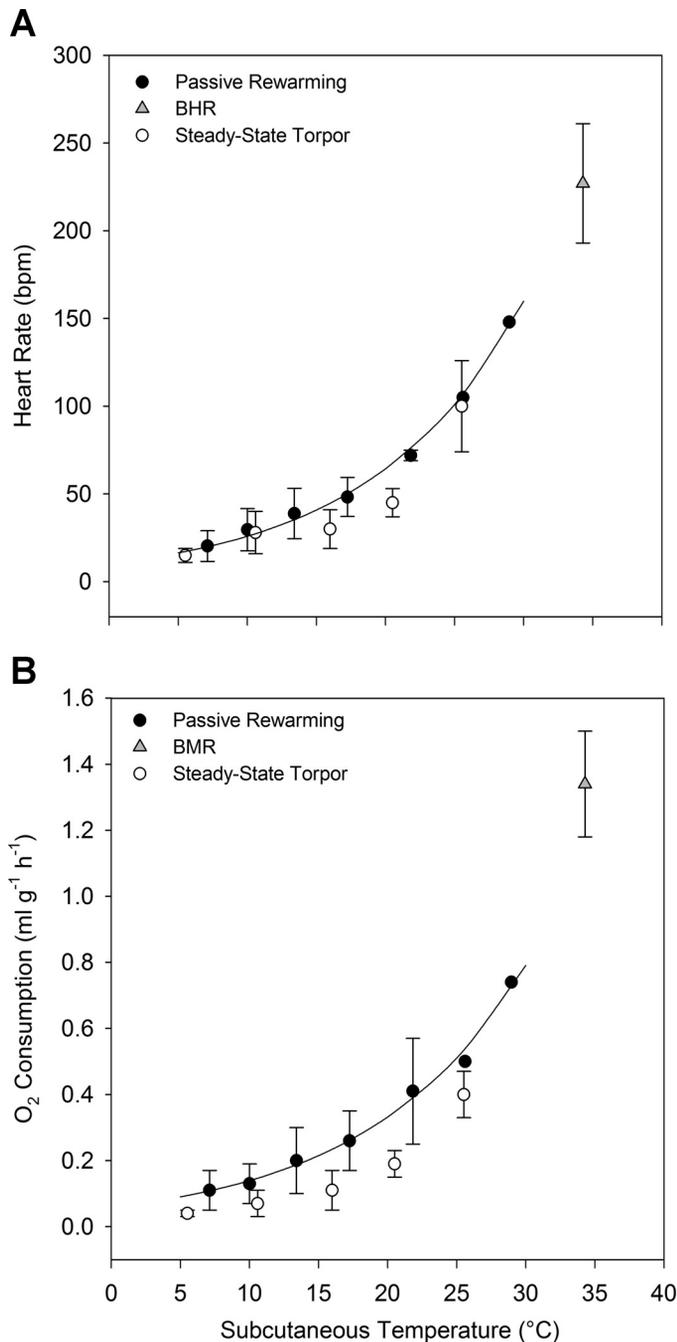


Fig. 5. A: average HR against T_{sub} for steady-state thermoconforming *N. gouldi* (open circles), during passive rewarming (filled circles), and BHR (grey triangle): $\text{HR} = 10.413 \times 1.096^{T_{\text{sub}}}$. B: average \dot{V}_{O_2} against corresponding T_{sub} and BMR (symbols are as for A): $\dot{V}_{\text{O}_2} = 0.058 \times 1.091^{T_{\text{sub}}}$. Both HR and \dot{V}_{O_2} showed a curvilinear response to increasing T_{sub} during passive rewarming and steady-state torpor. Data points for passive rewarming beyond 20°C are averages taken from a single animal.

tween active arousal and steady-state minimum values during torpor. When animals actively aroused, HR and \dot{V}_{O_2} increased at a substantially greater rate than at the same T_{sub} during both entry into torpor and passive rewarming.

Our study is also the first to present BHR for an Australian insectivorous bat. Previous investigations report minimum HR of bats ranging from 128 to 235 beats/min in species weighing between 56 and 825 g ($T_a = 19\text{--}35^\circ\text{C}$) (1, 13, 14). BHR

recorded for *N. gouldi* fell within this range at 227 beats/min, even though animals were much smaller (9.9 g) and was 50% of the BHR predicted from the allometric equation $\text{HR} = 816 W^{-0.25}$ (40). BMR reported in our study ($1.34 \pm 0.16 \text{ ml O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$) was averaged over the same period as BHR and was indistinguishable from previously reported values for *N. gouldi* of $1.22 \text{ ml O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ (7).

HR showed a similar qualitative pattern to \dot{V}_{O_2} during both passive rewarming and entry into torpor, changing exponentially with T_{sub} . However, at each T_{sub} interval during passive rewarming HR and \dot{V}_{O_2} fell slightly above corresponding values when animals were in steady-state torpor, and this difference was even greater for torpor entry. It has been well documented that during steady-state torpor in hibernators metabolic inhibition maintains HR and metabolism at low levels (32). Although there is still an effect of temperature on cardiac and metabolic function, steady-state torpor values fall below what would be expected if temperature was the primary driving force (26). It is not known, however, how important metabolic inhibition is during entry into torpor or at which point inhibition is initiated. In ground squirrels (*Callospermophilus lateralis*) depression of mitochondrial respiration did not occur until animals were in deep torpor, and this suggests that active mitochondrial inhibition may not be critical during entrance into hibernation (16). Our results support this interpretation and indicate that the effects of metabolic inhibition may be relatively small early in torpor entry and increase as torpor entry proceeds and animals become deeply torpid. Furthermore, our data suggest that as animals begin to passively rewarm, metabolic inhibition is withdrawn resulting in higher \dot{V}_{O_2} and HR values that increase in a temperature-dependent fashion ($Q_{10} \sim 2.5$). Because rewarming is a transitional period, the removal of metabolic inhibition would facilitate the onset of active arousal once the animal is warmed to a critical T_b . We would expect, however, that if temperature did not continue to rise to a critical value for arousal, as was the case with the imposed T_a profile, animals would have remained torpid, and metabolic inhibition would have been restored, reducing \dot{V}_{O_2} and HR to steady-state values.

When bats were rewarmed from 7°C after torpor bouts <24 h, the critical T_{sub} inducing arousal was 19.5°C , which was comparable to that found for *N. geoffroyi* exposed to a similar T_a profile ($T_{\text{sk}} = 21.4^\circ\text{C}$) (37) and free ranging *Eptesicus fuscus* passively rewarmed during the hibernation season ($T_{\text{sk}} = 17.9^\circ\text{C}$) (9). At the onset of active arousal there was a doubling of \dot{V}_{O_2} when animals rewarmed from both the constant T_a and following passive rewarming. However, the immediate increase in HR at active arousal was substantially higher in bats that were passively rewarmed compared with those at low T_a . This clear difference in cardiac capacity is likely directly related to temperature of the heart at the corresponding T_{sub} values. Although hibernators' hearts are well adapted to maintain coordinated activity at low T_b , low temperatures still impede many aspects of cardiac function. For example, isolated hearts of *Myotis lucifugus* showed a limited scope for HR increase at low temperatures but clear improvement in scope above 20°C (18). In addition, contractility of isolated myocardium of a number of hibernators declined rapidly below 15°C , with maximum contractility observed between 15.5 and 24°C (4, 28, 29). As the critical $T_{\text{sub}}/T_{\text{sk}}$ for active arousal in a number of temperate bat species is around

this 20°C threshold, it suggests that rewarming the heart before active arousal may be beneficial to reduce physiological stress of arousal. The importance of the heart as a thermogenic organ during arousal has been demonstrated in a number of hibernating species (15), and therefore maximizing cardiac performance at the start of active arousal would clearly be advantageous. Also, considering that early stage arousal from low T_b likely imposes the greatest demands on the cardiovascular system, warming the heart before active arousal may reduce mechanical stresses as well. As such, we suggest that optimal T_b for peak cardiac capacity may also influence the critical threshold temperatures for active arousal following passive rewarming.

Because the cost of arousal increases with the time taken to arouse (17), it would be worthwhile for bats to increase HR to maximal levels as quickly as possible during active arousal to shorten arousal time. During arousal the risk of oxidative stress is at its highest as metabolism increases dramatically, and peak concentrations of ROS have been shown to correspond to periods of maximum $\dot{V}O_2$ during rewarming (5). Although hibernators possess mechanisms to combat oxidative stress, such as upregulation of antioxidants (20), it is possible that during arousal these defenses may not be entirely sufficient. As such, reducing arousal times through passive rewarming may also help to minimize exposure to ROS and/or increase efficiency of antioxidant defenses.

Passive rewarming resulted in substantial energy savings compared with the cost of active arousal at a low T_a . When this is extrapolated to fat usage, ~ 16.2 mg less fat is required on average when bats are exposed to increasing T_a than when forced to actively arouse. Free-ranging male *N. gouldi* were shown to employ torpor twice per day on 80% of tracking days during late spring and regularly rewarmed passively from torpor (35). We exposed bats to similar thermal conditions and demonstrate that a single arousal (including passive rewarming) requires the metabolism of ~ 18 mg of fat in this species. Therefore, the overall extrapolated cost of rewarming in these animals would equate to ~ 36 mg of fat per day for the two arousals. This amounts to a savings of >225 mg of fat per week compared with the cost of wholly active arousal. In addition, the savings attributed to passive rewarming in this study mirror those shown for other bat species in captivity (*N. geoffroyi*, 37) and in the wild (*Eptesicus fuscus*, 9).

Measurements of the energy savings attributed to torpor use and costs of arousal are crucial to the study of bat biology because torpor is so widely used by these animals. HR has been suggested as a viable method for predicting $\dot{V}O_2$ in the field, and this has been supported by a study on torpid and resting bats (6). Because HR can provide information regarding energy expenditure over small time scales, such as the rewarming phase of torpor, and this is the most energetically expensive phase of torpor, one aim of our study was to examine whether or not a strong correlation exists between HR and $\dot{V}O_2$ for both passive and actively rewarmed bats. Our data show a strong positive correlation for both active ($P < 0.01$, $r^2 = 0.94$) and passive arousal ($P < 0.05$, $r^2 = 0.74$) with no overlap between the two states (Fig. 6). This suggests that HR may be a good predictor of $\dot{V}O_2$ during these phases, but that they must be distinguished from one another, indicating the importance of simultaneous measurement of T_{sk} and T_a .

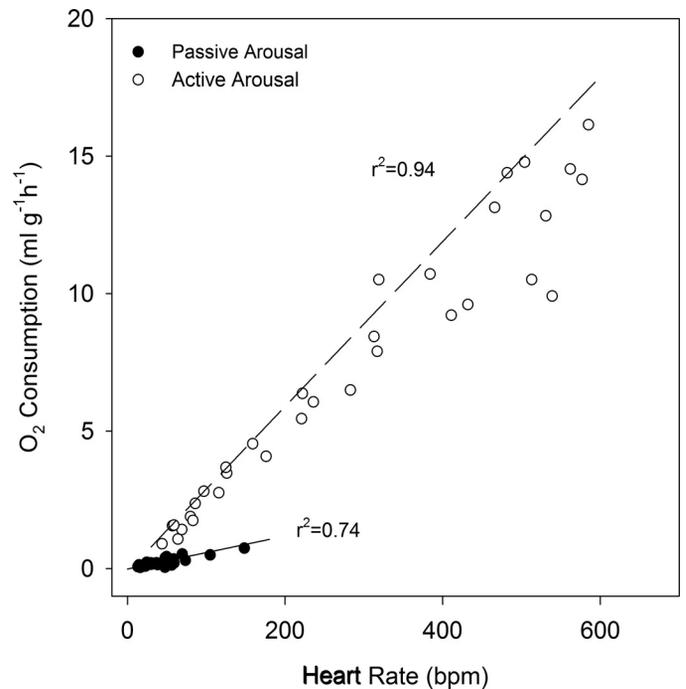


Fig. 6. $\dot{V}O_2$ as a function of HR during active arousal (open circles) and passive arousal (filled circles) for *N. gouldi*. Dashed line represents the regression equation for active arousal [$\dot{V}O_2 = 0.03(\text{HR}) - 0.11$, $r^2 = 0.94$, $P < 0.01$], and the solid line represents the regression equation for passive arousal [$\dot{V}O_2 = 0.005(\text{HR}) - 0.016$, $r^2 = 0.74$, $P < 0.05$].

Perspectives and Significance

Passive rewarming is used by many bat species to reduce energetic costs of arousal from torpor (3, 23, 30, 43). Our study demonstrates the importance of temperature on cardiac capacity during this phase and the possible role this plays in signaling arousal from torpor and reducing stress on the cardiovascular system. We suggest that the ability to rewarm passively may enable individuals to raise T_b to optimal levels for maximal cardiac performance and swift arousal without the need for excessive energy expenditure initially. We also show that metabolic inhibition may not be fully applied until late-stage entry into torpor and that it is at least partially withdrawn during passive rewarming as a response to temperature increases and as a precursor to active arousal. Unfortunately, little information exists regarding changes in metabolism associated with torpor use under natural conditions, including fluctuating T_a . We show a strong correlation between HR and MR throughout different phases of arousal. Therefore, we suggest further study to examine the possibility of HR telemetry as a means of predicting MR in free-ranging heterothermic animals. The extensive and flexible use of torpor in bats has been suggested to play a vital role in long-term survival of these animals (31), and as such the understanding of energy use in the wild has strong implications for their future management and survival.

ACKNOWLEDGMENTS

We thank Clare Stawski and Daniella Rojas for help capturing bats. We also thank Daniella Rojas and Philip Currie for constructive comments on the manuscript.

GRANTS

Funding for this project was provided by the University of New England, the Australian Department of Education, and the Australian Research Council.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: S.E.C. and F.G. conception and design of research; S.E.C. and K.N. performed experiments; S.E.C. and K.N. analyzed data; S.E.C. and F.G. interpreted results of experiments; S.E.C. prepared figures; S.E.C. drafted manuscript; S.E.C., K.N., and F.G. edited and revised manuscript; S.E.C., K.N., and F.G. approved final version of manuscript.

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