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Metabolic Rate and Thermolabile Properties of Ognev's Great Tube-nosed Bat *Murina leucogaster* in Response to Variable Ambient Temperature

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The winter-resident Korean bats, *Murina leucogaster ognevi*, show a circadian cycle of thermoregulation and locomotion in summer, as do other bat species in temperate regions. They are most active between dusk and dawn with body temperature (T_b) of 35-40℃, and are usually torpid in their roost sites for the rest of day with their T_b close to ambient temperature (T_a) of around 15℃. The present study was conducted to determine thermogenic and thermolabile properties of the heterothermic bats that would influence their daily feeding activities and, ultimately, their energy conservation strategy. Testing on active male *Murina*, resting metabolic rate (RMR, gauged by oxygen consumption rate) at the lower limit of thermoneutral zone (31℃) was 2.0 L $kg^{-1}h^{-1}$. The regression slope of RMR below the thermoneutral zone (an index of metabolic thermal sensitivity) was - 0.38 L $kg^{-1}h^{-1}℃^{-1}$. The metabolic rate at the roost T_a (15℃) was 4.5 times the lowest RMR in the active state, but becomes nearly zero in the torpid state. This implies that by being torpid during daytime (between dawn and dusk), the individual bats would save about 4.7 kcal each day in mid-summer. Interspecific comparisons of thermal metabolic response over a mass scale suggest that the smaller bats show a relatively higher metabolic rate in thermoneutral zone and a greater thermal sensitivity of metabolism, which follows the general principle seen in homeothermic metabolism. Thermolabile features in metabolic responses seem to be fairly common for these bats in conditions other than a fully active state. Types of thermolabile responses and their energetic significance are discussed.

Many species of bats in temperate regions have a circadian cycle of locomotory and thermoregulatory activities in summer. They are most active between dusk and dawn for feeding, but go into torpor in their roost sites during daytime (Pearson, 1947; Altringham, 1996). Bats regulate their body temperature (T_b) at 35-40℃ in the active state but decrease T_b close to the ambient temperature (T_a) of their habitat (ca. 10-15 ℃) during dormancy (Altringham, 1996; Choi et al. in press). Locomotory activity requires high T_b, which demands continual thermogenesis in the cool environment (O'Farrell and Studier, 1970). Heterothermy therefore allows them to reduce the energetic cost of such activities (O'Farrell and Studier, 1970; Altringham, 1996). Because energy saved this way can be diverted to other activities (e.g., mating and territory defence; Altringham, 1996), daily torpidity would be advantageous for their survival and, ultimately, for their fitness. Thus, to assess the adaptive significance of heterothermy as an energy-saving mechanism, we must first know their metabolic rate at the habitat temperature both in an active and in torpid states. Furthermore, since there is a continuum in the metabolic response between 'fully' active and 'fully' torpid states, it is also important to consider intermediate responses between the two extremes that would affect their total energy expenditure (O'Farrell and Studier, 1970). Thermolability would be very natural in heterotherms which stay in conditions other than a fully active state. A number of studies have reported the importance of heterothermic properties of bats for survival in a variable environment (e.g., fluctuating temperature and/or food availability; O'Farrell and Studier, 1970; Schmidt-Nielsen, 1990; Morris et al., 1994). Such issues, however, have

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been poorly addressed with Korean bats, and in particular, thermolabile features have been underappreciated in previous studies (Morris et al., 1994).

In a typical homeothermic response, the rate of energy metabolism remains lowest over a thermoneutral zone (TNZ) but increases with decreasing T_a (Bartholomew, 1982). We can define thermal characteristics of the metabolic curve with various parameters including position of TNZ, metabolic rate in TNZ, and a slope of the increasing phase of the metabolic curve below the lower limit of TNZ. These parameters allow us to envisage thermal adaptations of animals that rule their feeding activities and their thermoregulatory physiology and behavior. In addition, the parameters, as metabolic indices, can be used for comparative studies.

In this study, we have addressed the thermogenic capacity of locally abundant Ognev's great tube-nosed bats, *Murina leucogaster ognevi*, by examining the resting metabolic rate of individuals in active and dormant states. Our goal was to find thermal characteristics of the metabolic curve of this species and compare them with those of other species reported previously. We attempted to estimate the amount of energy saved daily by these bats through heterothermy. Our thermolabile response results highlight the importance of energetic studies in understanding thermoregulatory responses in bats.

Materials and Methods

Subjects

We captured *Murina* males from a natural limestone cave in Chiak Mt. (37° 20′ N, 128° 10′ E) between July and late August 1997. Torpid bats were easily collected by hand at their roost sites, and flying bats were captured using bird nets (1.5 m × 3 m) spread along their roost areas. We wore thick cotton gloves to prevent bats from biting, as well as to minimize heat transfer from our hands to the animals in subsequent T_b measurements. Cloacal temperatures of individuals were measured within 30 s of captivity in the cave using a sensing tip of a 40 AWG duplex copper-constantan thermocouple connected to an Omega HH-73T digital thermometer. Air temperatures of the bats' roost areas were also measured with the thermocouple thermometer. We carried eleven bats to our laboratory, weighed and housed in two wire-mesh cages (20 cm × 20 cm × 40 cm) in a cool, humid room (ca. 12℃). The bats were supplied with water ad libitum, and were used for the metabolic experiments within 24 h of captivity.

Metabolic rate

Oxygen consumption rates of individual bats were measured to gauge resting metabolic rate (RMR) at variable T_a. Experimental procedures for metabolic measures followed general protocols of Stack and Rossi (1988) and Choi et al. (1994). All metabolic trials were conducted in a 1 L chamber thermally isolated by a water jacket around it. The chamber temperature was controlled to within ± 1℃ by coolant circulated through the jacket and was read with a thermocouple sensor inserted through the top of the chamber. The animals were allowed to perch in the normal (vertical) posture on a wire-mesh wall installed around the inside chamber. Oxygen consumption was measured with an automated, open-circuit Oxymax Equal Flow System (Columbus Ins) at a flow rate of 500 mL/min. Incurrent air flow was controlled by an air supply regulator of the system (0-2450 mL/min capacity). The excurrent subsample of air (100-150 mL/min) was diverted through a couple of drier columns to the Columbus O_2 sensor. Analog signals of the sensors were transmitted through an analog-to-digital converter and then to an IBM 486 compatible computer. The gas analyzers were calibrated on each day of experiment.

All experiments were initiated at a chamber temperature of 37℃. In preliminary experiments, this temperature was found to be an upper limit of TNZ of this species. After the subject was introduced in the chamber, we allowed an equilibration period of about 30 min for the oxygen readings being stabilized. Chamber temperature was then lowered down to 5℃ at a rate of 0.5℃/min. The RMR in terms of oxygen consumption rate (L h^{-1}) was determined using the following equation (Hill, 1972; Chappel and Roverud, 1990):

$$RMR = \text{flowrate}(pO_2[\text{in}] - pO_2[\text{ex}])/(1 - pO_2[\text{ex}]),$$

where pO_2[in] is the proportion of oxygen in the incurrent air (0.2093) and pO_2[out] the proportion of oxygen in the excurrent air. Heat production rate (kcal h^{-1}) was calculated by converting the volume of oxygen consumed to heat unit (i.e., oxygen consumption rate × 4.8). RMR and heat production rate were corrected to standard temperature (0℃) and pressure (760 mmHg), and were divided by body mass of that subject to obtain mass-specific values. All data from morphological and metabolic measures are presented as mean ± 1 standard error of the mean (SEM), unless otherwise noted.

Results

Air temperature of the bats' roost places in the cave was very stable, ranging between 13° and 16℃ over the study period. The bats stayed in cracks or holes of the cave wall in the torpid state during daytime, and flew out actively at dusk and dawn. Cloacal temperatures were 15-17℃ ($n = 5$) in torpor, but were 34-40℃ in flight ($n = 6$). The cloacal temperatures might be slightly higher than actual values at least

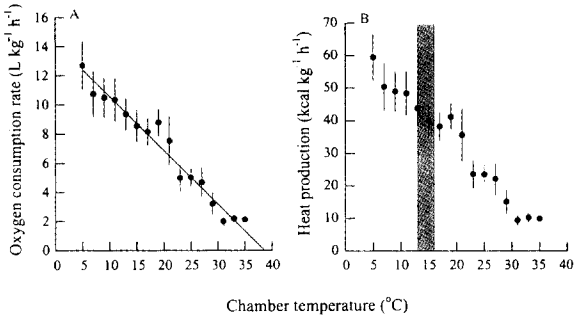

Fig. 1. Changes in resting metabolic rate (A) and heat production rate (B) as a function of ambient temperature (T_a) in the active *Murina leucogaster* ($n = 4$). A regression line for metabolic data below the lower limit of thermoneutral zone (31℃) is, RMR=14.33-0.38 T_a. A stippled vertical bar in B represents the T_a range for the study period. All points are means, and vertical bars represent ±1 SEM.

for T_b reading even within 30 s would threaten them to raise metabolism.

Four out of eleven *Murina* (7.8 g±0.3) showed typical endothermic responses in this study. Changes in their average RMR are shown as a function of T_a in Fig. 1, and thermal characteristics of the metabolic curve are summarized in Table 1. The lowest RMR was 2.0 L $kg^{-1}h^{-1}$ at 31℃ T_a, and this temperature is considered the lower limit of TNZ for these bats. The upper limit of the TNZ was not defined in this study. Temperature at zero metabolic rate, which often corresponds to T_b of homeothermic animals at rest (Bartholomew, 1982), was 37.7℃ (Table 1). At 15℃ (the average T_a of the cave), RMR and heat production rate were 9.02 L $kg^{-1}h^{-1}$ and 43.30 kcal $kg^{-1}h^{-1}$, respectively (Table 1). The regression slope of the metabolic curve below TNZ that indicates thermal sensitivity of the animal was -0.38 L $kg^{-1}h^{-1}℃^{-1}$.

Thermolability of the *Murina* in metabolic responses to variable T_a occurred in a quite high proportion (64% of the eleven subjects). Based on metabolic curve patterns obtained, thermolabile responses were grouped in four distinct types (Fig. 2). The first case depicts the completely torpid state of the bats with gradual decrease in RMR to zero as T_a decreased down to 5℃ (Fig. 2A, $n = 2$). Initiation of dormancy was often interrupted by an abrupt arousal at T_a right below the TNZ (Fig. 2B, $n = 2$; and Fig. 2C, $n = 2$). The sharp metabolic rise in this arousal was much greater than the slope of metabolic increase

Table 1. Metabolic rate, heat production, and thermal characteristics of *Murina leucogaster* in response to variable ambient temperature.

Properties	Values
Lower limit of TNZ (℃)	31
Temperature at zero RMR (℃)	37.7
RMR at 31℃ (L $kg^{-1}h^{-1}$)	2.00±0.29[a]
RMR at 15℃ (L $kg^{-1}h^{-1}$)	9.02±1.09[a]
Heat at 15℃ (kcal $kg^{-1}h^{-1}$)	43.30±4.99[a]
Slope of RMR at 15℃ (L $kg^{-1}h^{-1}$degree^{-1})	-0.38[b]

$n = 4$; TNZ: thermoneutral zone; RMR, resting metabolic rate
[a], mean ± 1SEM; [b], regression slope below TNZ

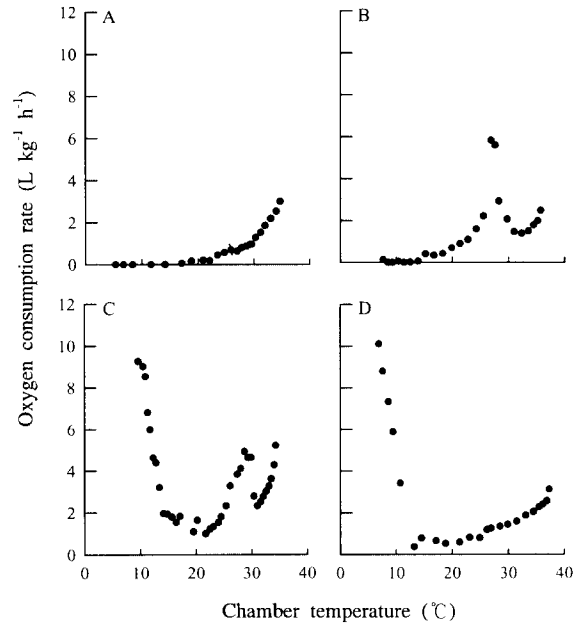

Fig. 2. Thermolabile responses of resting metabolic rate to variable ambient temperatures in *Murina leucogaster*. Among seven bats, types A, B, and C were seen from two bats, respectively, and type D from one.

below TNZ in the active bats in Fig. 1. Dormancy was restored as T_a further decreased in some cases (2B), or a rapid arousal was retriggered at T_a's below 13-15℃ in others (2C). In one case, metabolic response decreased like the torpid case, but then turned to a rapid arousal against further stress of low temperature (Fig. 2D, $n = 1$).

Discussion

The general metabolic pattern of the active *Murina* is similar to the response patterns of other species with 6-17 g mass (Herreid and Schmidt-Nielsen, 1966; O'Farrell and Studier, 1970). The lowest RMR of the *Murina* (2.0 L $kg^{-1}h^{-1}$) is comparable to that of 7-9 g fringed myotis, *Myotis thysanodes* (1.74; O'Farrell and Studier, 1970), but is about 33% greater than the basal metabolic rate of 16 g big brown bats, *Eptesicus fuscus* (ca. 1.5; Herreid and Schmidt-Nielsen, 1966). The lower limit of TNZ of our bats (31℃) is also similar to that of the myotis bats (32.5 ℃), but is 4℃ lower than that of the big brown bats (35℃). This means that over a temperature range down to 32℃, the thermoregulatory cost remains minimal in the *Murina* and the myotis, whereas it reaches about 35% of the lowest level in the big brown bats. The metabolic thermal sensitivity below TNZ in the *Murina* falls between those of the fringed myotis (-0.43) and the big brown bats (-0.28). From these interspecific comparisons, a scaling trend on metabolism is evident: the smaller the body size, the

higher the metabolic rate in TNZ and the greater the thermal sensitivity of metabolism. Because smaller animals have a relatively greater surface/volume ratio and thus higher rate of heat loss compared to large ones at low temperature, this trend in bats follows the general principle seen in homeothermic metabolism (Bartholomew, 1982). In active bats, T_b is maintained at about 2-3℃ higher than T_a while changing in parallel with T_a within TNZ (Herreid and Schmidt-Nielsen, 1966). Judging from this observation, T_b of our bats at the lower limit of TNZ (31℃) would be about 34℃, and this T_b seems to ensure their minimal T_b for homeothermic function such as flight. It is well documented that the T_b required for powerful flight ranges from 28 to 32℃ in most temperate-resident bats (summarized in Table 8 by Studier and O'Farrell, 1972; Choi et al. in press).

Estimation of the amount of energy saved by bats through heterothermy appears to be fairly straightforward because, as seen in Fig. 2A, the nearly zero RMR at the bats' roost temperature (15℃) could reduce their thermoregulatory cost by almost 100% if they retire into torpor. Specifically, heat production rate approaches zero in the fully torpid bats at or below 15℃ T_a, but reaches 43 kcal $kg^{-1}h^{-1}$ in the active bats at this T_a (Table 1). This indicates that if the bats are homeothermic as are other mammals even during daytime, they must continually utilize nutrient fuels for thermoregulation. If each daytime (between dawn and dusk) is approximately 14 h in August, individual bats would spend an additional 4.7 kcal each day merely for thermogenesis. This is the minimal energy required. If the bats fly around during this time, they must keep up an even higher metabolic rate for locomotion and ultrasonic production for echolocation. The cost of flight and foraging demands 3 to 20 times RMR for an insectivorous bat (Altringham, 1996). Thus, considering nocturnal flight would secure bats a higher chance of predator avoidance, the optimal foraging strategy for the bats might be to emerge right after dusk, to feed on insects as effectively as possible, and to stay torpid in the cave for the rest of the day.

However, the dichotomy of fully active versus fully torpid states used for estimation of thermoregulatory cost looks oversimplied. Metabolic responses to temperature were fairly variable in these heterothermic bats as seen in Fig. 2B - D. Although systematic analyses were not pursued in this study, factors such as health, status of energy reserves, captivity conditions, time of day or season may be important causes for the thermogenic variation (see Studier and O'Farrell, 1972; Morris et al. 1994). Total amount of energy spent for a short-burst arousal on the way to torpor and/or a very rapid metabolic augmentation even at further thermal stress (Figs. 2B and 2C) must be significant for the energy conservation strategy of the bats. Based on these observations, the level of metabolism at the roost temperature may predict whether bats go into deep torpor or not. Considering the four types of metabolic responses (Fig. 2), torpor comes always when metabolic rate becomes zero at T_a of 13-15℃. This T_a is the average roost temperature of the bats, and may be the neural threshold for thermogenic activity. If the bats stay in a thermal environment similar to their roosts, they may turn off the neural processes to shut down the costly metabolism, or keep processes at the minimal level that would allow them to effectively retrigger endothermic processes for immediate responses (e.g, flight and predator avoidance). To comprehend the energetic significance of metabolic fluctuation in detail in these animals, it would be worth examining metabolic rates, T_b and muscle tremor (e.g., by electromyography) simultaneously at variable temperatures, to determine shivering and non-shivering thermogenesis at specific T_b's

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