

Research



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Physiology

Warm bodies, cool wings: regional heterothermy in flying bats

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Many endothermic animals experience variable limb temperatures, even as they tightly regulate core temperature. The limbs are often cooler than the core at rest, but because the large locomotor muscles of the limbs produce heat during exercise, they are thought to operate at or above core temperature during activity. Bats, small-bodied flying mammals with greatly elongated forelimbs, possess wings with large surfaces lacking any insulating fur. We hypothesized that during flight the relatively small muscles that move the elbow and wrist operate below core body temperature because of elevated heat loss. We measured muscle temperature continuously in the small fruit bat *Carollia perspicillata* before and during wind tunnel flights, and discretely in diverse bats at rest in Belize. We found that bats maintained high rectal temperatures, but that there was a steep proximal-to-distal gradient in wing muscle temperature. Forearm muscles were 4–6°C cooler than rectal temperature at rest and approximately 12°C cooler during flights at an air temperature of 22°C. These findings invite further study into how bats and other endotherms maintain locomotor performance in variable environments, when some muscles may be operating at low temperatures that are expected to slow contractile properties.

1. Introduction

Endotherms such as birds and mammals maintain high and relatively constant core body temperatures in the face of often extreme variation in environmental temperature. However, the temperature of peripheral body regions is more variable (regional heterothermy). Such variation is in part a consequence of heat exchange with a thermally variable environment, but it also reflects adaptations to minimize heat loss in cool environments [1]. Although cooling peripheral tissues reduces overall heat loss in the cold, the function of these tissues can be affected. For example, muscle function is tightly tied to operating temperature: as temperature decreases, so do rate-dependent contractile properties [2]. Thus, variable limb muscle temperatures may have performance costs for locomoting animals, and/or put selective pressure on the temperature sensitivity of physiochemical processes within limbs. We previously reported that an extensor muscle in the forearm of the bat *Carollia perspicillata* has a lower than expected thermal sensitivity of contractile rates, and have hypothesized that this is an adaptation to routine operation below core body temperature [3].

One would expect that regional cooling disproportionately affects those regions of the body with large surface-area-to-volume ratios, including the limbs and the locomotor muscles within them. Indeed, skin temperatures are depressed relative to core temperature in the unfurred feet of Arctic mammals, the limbs of sloths and bat feet during flight, among other examples [1,4–6]. In these cases, whether cooling of the affected tissues would influence locomotion is unclear. The assumption in the literature has been that locomotor muscles warm with exercise. This idea is supported by some studies in humans that

find increases in muscle temperature with exercise in the large muscles of the thighs [7], although the extent of warming depends on starting muscle temperature, duration of exercise and cold exposure [8]. Increased muscle temperature with exercise at an air temperature of 25°C has been seen in the much smaller laboratory rat [9]. Seals and penguins swimming in cold water also have been found to maintain high temperatures in major locomotor muscles [10,11].

None of these studies, however, examined small distal muscles that may be important in locomotion but are relatively more exposed than the major locomotor muscles. Bats represent a morphological and locomotor extreme among mammals, possessing wings composed of poorly insulated forelimb structures (figure 1). The muscles of the arm, forearm and hand contribute to the flight stroke but are separated from the environment by only a thin layer of skin and are vulnerable to convective and radiative heat loss during flight. This morphology, as well as thermal imaging showing low wing-surface temperatures, suggest that the distal muscles may operate at considerably colder temperatures than the large muscles in the trunk that power flight [12,13]. No direct measurements of muscle temperature during flight have been documented. Here, we assess the extent of regional heterothermy during flight in bats by measuring appendicular muscle temperatures in the laboratory and the field.

2. Methods

(a) Experimental approach

We measured wing muscle temperature (T_m) in bats continuously during flight and discretely in bats at rest. Continuous measurements of T_m were achieved by inserting custom-made thermocouples (Type-K, 0.076 mm wire) subcutaneously on the surface of the pectoralis, biceps and forearm extensor muscles of the bat wing, and allowing instrumented bats to fly in a wind tunnel. We also designed thermocouple probes to allow discrete measurements of muscle temperature. These discrete measurements were made by inserting a custom-made hypodermic thermocouple probe into the muscles of interest in *C. perspicillata* at rest in the laboratory, and in the same muscles in bats in the field (see electronic supplementary material for details of thermocouple construction and calibration). Data were analysed in R using the R Stats Package and DescTools [14,15].

(b) Laboratory measurement of muscle temperature

Laboratory-bred *Carollia perspicillata* were housed in the Animal Care Facility at Brown University under a reversed 12 h:12 h dark:light cycle and provided with food and nectar ad libitum. Study animals were adults, male and female, with a mean body mass of 18.0 ± 1.2 g ($n = 4$). The individuals were trained to fly in an open return wind tunnel with a 0.9 m long, 0.55 m square test section.

Bats were anaesthetized with isoflurane and the thermocouples inserted through small openings in the skin created with a 25-gauge needle, then sutured in place at the point of insertion. All thermocouple leads were anchored to the back with a single suture. Once instrumented, the bats were allowed to recover from anaesthesia, offered fruit juice and banana, and then were introduced into an approximately 5 m s^{-1} airflow in the wind tunnel [16,17]. Temperature was recorded continuously using a PowerLab 16S/P data acquisition system (ADInstruments). The bats made intermittent landings and were encouraged back into flight with the wave of a hand. Rectal

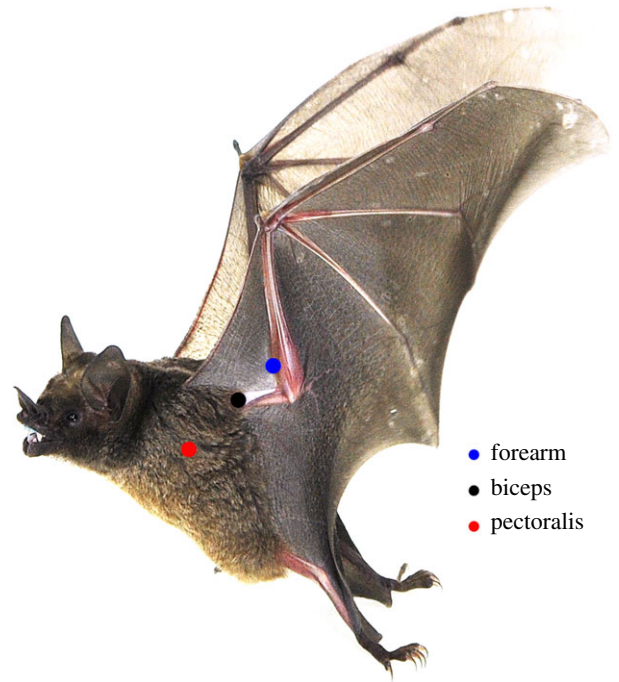


Figure 1. Many of the muscles of the bat wing are clearly visible through the skin. The placements of thermocouples for continuous temperature measurement are indicated by coloured dots.

temperature (T_r) was measured manually before and after flight. The implanted thermocouples were removed under manual restraint. Mean T_m was calculated by averaging measurements over the period beginning 100 s after flight initiation and ending when the flight ended, 5 to 9 min later. The hypodermic and continuous probes were compared by making simultaneous measurements in two bats hand-held in still air immediately after wind-tunnel flights, and three bats hand-held in a 5 m s^{-1} flow (see electronic supplementary material).

(c) Field measurement of muscle temperature

Bats were captured in mist nets and harp traps at Lamanai Archaeological Reserve and Lamanai Outpost Lodge near Indian Church, Belize, during the dry season, 24 April–7 May 2017 and 30 April–4 May 2018. We measured T_m discretely by inserting hypodermic thermocouple probes into the muscles of interest (pectoralis, biceps and forearm extensors) and reading the temperature with a hand-held Keithley thermometer soon after capture in ground-level mist nets or at rest after several hours in holding bags. T_m measurements made soon after capture and after several hours in bags were statistically indistinguishable and thus were analysed together.

3. Results

(a) Laboratory

Mean air temperature in the wind tunnel was $21.6 \pm 0.6^\circ\text{C}$. Pre- versus post-flight T_r did not differ significantly (paired t -test, $p = 0.375$); the combined mean was $39.0 \pm 0.3^\circ\text{C}$. Before flight in the wind tunnel, pectoralis and biceps temperatures were close to T_r but the forearm muscles were significantly cooler (ANOVA, $F_{3,12} = 15.81$, $p < 0.001$, with *post hoc* Tukey's HSD) (table 1; electronic supplementary material, table S1). Biceps and forearm temperatures began to fall immediately after the bats were introduced to the tunnel, stabilizing after approximately 100 s (figure 2).

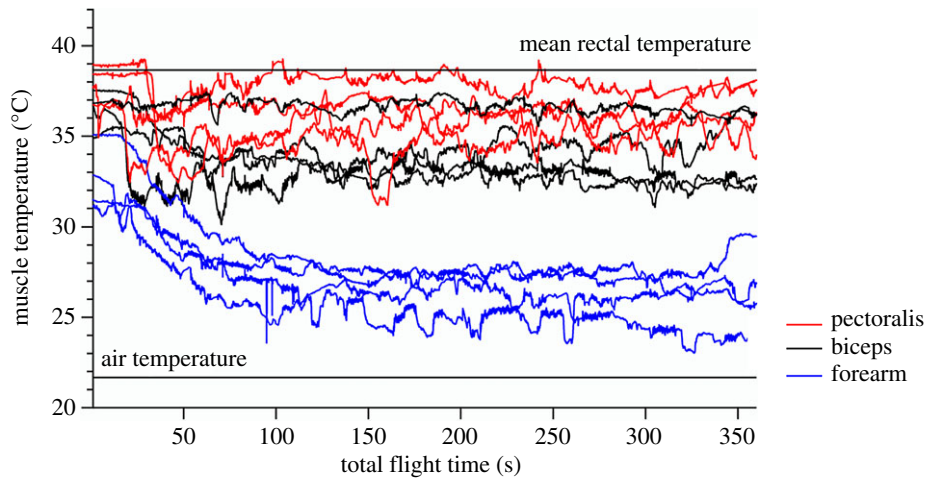


Figure 2. Continuous muscle temperature measurements for all bats, with longer flights abbreviated. Mean post-flight rectal temperature is an indication of core body temperature.

Table 1. Mean \pm s.e.m. temperature measurements for *Carollia perspicillata* in the laboratory ($n = 4$) and for bats sampled in the field ($n = 71$). Vertical bars indicate groups of muscle temperatures that did not differ significantly (Tukey's HSD; $p < 0.05$). For laboratory measurements, post-flight rectal temperatures were used to calculate temperature differentials. Wind tunnel muscle temperatures are mean values calculated from 100 s into the flight to the end of flight. Field data are pooled over all individuals and species and each mean $T_r - T_m$ differed significantly from zero (Dunnett's test, $p < 0.001$ for all comparisons) and from the others (ANOVA with Tukey's HSD, $p < 0.001$). Mean air temperature was $21.7 \pm 0.3^\circ\text{C}$ in the wind tunnel and $29.8 \pm 0.2^\circ\text{C}$ in the field. Asterisks indicate significant differences from rectal temperature based on Dunnett's test; *, $p < 0.05$; **, $p < 0.003$; details in electronic supplementary material, table S1.

site	temperature ($^\circ\text{C}$)		temperature difference rectal – muscle ($^\circ\text{C}$)		
	laboratory pre-flight	wind tunnel in flight	laboratory pre-flight	wind tunnel in flight	pooled field
rectal	39.4 ± 0.6	38.7 ± 0.3	—	—	—
pectoralis	38.0 ± 0.5	36.3 ± 0.8	1.4 ± 0.6	2.4 ± 0.7	$1.1 \pm 0.2^{**}$
biceps	36.8 ± 0.5	34.3 ± 1.0	$2.6 \pm 0.8^*$	$4.4 \pm 1.0^{**}$	$2.6 \pm 0.2^{**}$
forearm	33.3 ± 1.0	27.1 ± 0.6	$6.1 \pm 1.0^{**}$	$11.6 \pm 0.4^{**}$	$4.2 \pm 0.2^{**}$

Mean T_m during flight differed significantly from T_r (ANOVA, $F_{3,12} = 46.64$, $p < 0.001$, electronic supplementary material, table S1). Mean T_r differed significantly from mean biceps and forearm but not pectoralis temperatures (Tukey's HSD: $p = 0.005$, $p < 0.001$, $p = 0.15$, respectively) and forearm temperature was significantly lower than either pectoralis or biceps temperature ($p < 0.001$, table 1). Forearm temperature, on average, did not exceed 27°C during flights once T_m had stabilized, and was 11.6°C cooler than T_r . The simultaneous comparisons of the subcutaneous probes with the hypodermic probes revealed no significant differences between the two measurement types (paired t -test, $p = 0.18$, electronic supplementary material, figure S1).

(b) Field

We sampled 71 individuals from 17 bat species (electronic supplementary material, table S2). We detected no phylogenetic signal in our data (electronic supplementary material, table S3), so we pooled the data from all bats. Air temperatures ranged from approximately 25 to 35°C . Mean T_r for all bats studied was $39.4 \pm 0.1^\circ\text{C}$. Mean $T_r - T_m$ values were all significantly different from zero (Dunnett's test, $p < 0.001$) and are shown in table 1 (see electronic supplementary material, figure S2 for breakdown by species). We found a significant relationship between $T_r - T_m$, air temperature, and body

mass for the three muscles (pectoralis, biceps and extensors) (ANCOVA, $F_{6,206} = 32.2$, $p < 0.001$, electronic supplementary material, figure S3 and table S4). When we subdivided the data by muscle and performed linear regressions we found a significant negative effect of air temperature on the rectal – biceps temperature differential ($F_{1,69} = 10.5$, $R^2 = 0.13$, $p = 0.002$, estimated coefficient: -0.25) and on the rectal – forearm temperature differential ($F_{2,68} = 8.71$, $R^2 = 0.2$, $p < 0.001$, estimated coefficient: -0.31). There was a significant negative effect of body mass on the rectal – forearm temperature differential ($R^2 = 0.2$, $p < 0.001$, estimated coefficient: -0.031) (electronic supplementary material, table S5).

4. Discussion

Our measurements of muscle temperature in both laboratory and field demonstrate substantial regional heterothermy in the muscles of the bat wing. Bat wings exhibit a remarkable proximal-to-distal, warm-to-cold gradient for the range of environmental conditions in which we measured muscle temperatures. In our wind tunnel flights, air temperatures, flight speeds and durations resembled those observed during foraging flights of the same species under natural conditions, suggesting that the decrease in distal T_m we report is likely directly ecologically relevant [17]. Even in warm

environmental conditions in the field, our measurements suggest that bats regularly experience lower muscle temperatures in the distal wing than in the proximal wing or the core.

Altering peripheral temperatures has long been recognized as a thermoregulatory strategy in endotherms. Lightly insulated structures such as bat wings have been suggested to function in dumping heat during flight under warm conditions [5,18], though in cool conditions surface temperatures of the wings and feet during flight are close to air temperature [5,13]. The extent to which this cooling affects the small, distal muscles of the limbs has not, to our knowledge, been previously quantified in any animal, but our data indicate that cooling occurs during, or even as a result of, locomotor activity in bats. We conclude that keeping these muscles close to body temperature may be difficult given the small amounts of heat produced by the muscles, limited heat transfer via blood flow, and heat loss to the environment via convection and radiation [13]. Mechanisms to maintain the temperature of these muscles are possible, but might impose an unsustainable metabolic burden.

The extent to which muscles experience regional heterothermy in flying bats is striking because bat flight requires high limb cycling frequencies driven by fast and coordinated contraction [19,20] of the warm pectoralis and the cooler muscles distal to the shoulder. These distal muscles likely must also contract and relax at the wingbeat frequency, but temperature effects in the muscles may impair locomotor function. At the muscle temperatures we measured in our wind tunnel experiments, rate-dependent contractile processes in muscle would be expected to be substantially reduced [2]. Such decreases in contractile rates could interfere with the flight stroke. Indeed, Carpenter [5] found that during wind tunnel flights at low temperatures, African fruit bats developed a performance deficit after flight initiation even though normal core temperatures were maintained, possibly as a result of distal wing muscles cooling during flight.

We hypothesize that compensatory mechanisms have evolved to allow sustained flight in bats that fly with cool wing muscles. Among the possible mechanisms are altered flight mechanics, changes in muscle recruitment, and changes in the thermal dependence of contractile properties. In regard to the latter possibility, a forearm extensor muscle in *C. perspicillata* has lower thermal dependence than expected based on data from

other mammalian skeletal muscles, consistent with a functional adaptation to habitually low temperatures in this muscle [3]. We suggest that a thermoregulatory trade-off exists between the performance benefits of keeping distal muscles warm and the energetic benefit of conserving energy by allowing distal regions to cool. The bats we have studied partially circumvent performance deficits by lowering the temperature sensitivity in the muscles that experience low and variable temperatures, but whether similar physiological mechanisms are used by other animals is not known.

As our data illustrate, endotherms must sometimes contend with thermal challenges to particular tissues, such as locomotor muscles, even if core body temperature is unchanged. This finding warrants a reassessment of muscle temperature variation and its functional implications in endotherms. When muscles cool, performance deficits can occur [5,21]. The degree to which adaptations have evolved to mitigate such deficits in animals that regularly experience muscle cooling is a fertile avenue for further research.

Ethics. Approvals: Brown University IACUC nos 1803000340, 1603000199, 19-01-0012; Belize Forestry Department Scientific Research and Collecting Permits WL/2/1/18(15), WL/2/1/17(13). We followed USDA standards and those for capture and handling of wild bats by the American Society of Mammologists [22].

Data accessibility. Data are available on Dryad: <https://doi.org/10.5061/dryad.6h661t9> [23].

Authors' contributions. A.D.R., R.L.M. and S.M.S. conceived the study. A.D.R. and R.L.M. collected laboratory data and drafted the manuscript. A.D.R. collected and analysed field and laboratory data. All authors critically revised the manuscript, gave final approval for publication and agree to be held accountable for the content of this article.

Competing interests. We declare we have no competing interests.

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