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Thermogenic Capacity at Subzero Temperatures: How Low Can a Hibernator Go?

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ABSTRACT

Hibernation in mammals is a physiological and behavioral adaptation to survive intervals of low resource availability through profound decreases in metabolic rate (MR), core body temperature (T_b), and activity. Most small mammalian hibernators thermoconform, with T_b approximating ambient temperature (T_a); arctic species are an exception, since they must actively defend what can be large thermal gradients between T_b and T_a. Here we compare the thermogenic capacity of the arctic ground squirrel (*Urocitellus parryii*) to that of the golden-mantled ground squirrel (*Callospermophilus lateralis*), a temperate-zone montane hibernator. We allowed animals to reenter torpor at sequentially lower T_a's and found that arctic ground squirrels maintained steady state torpor at T_a's as low as $-26°C$, through a 36-fold increase in torpid MR (TMR), compared to their minimum TMR, exhibited at a T_a of 0°C. Golden-mantled ground squirrels are able to maintain steady state torpor at T_a's at least as low as $-8°C$, through a 13.5-fold increase in MR, compared to their minimum TMR at a T_a of 2°C. In a second experiment, torpid animals were exposed to continuously decreasing T_a's (0.25°C/30 min); individuals of both species increased their metabolism while remaining torpid at low T_a's (as low as $-30°C$ for arctic ground squirrels

and $-10°C$ for golden-mantled ground squirrels). Although the capacity to hibernate at subfreezing T_a's is not unique to arctic ground squirrels, their large body size, greater torpid metabolic scope, and previously ascribed capacity to supercool allow them to occupy much colder hibernacula for prolonged seasons of hibernation.

Introduction

Seasonality of environments, which encompasses annual cycles of temperature, precipitation, and resource availability, is a selective force that has led to the evolution of a variety of molecular, physiological, and behavioral adaptations in indigenous species. One of the most intriguing adaptations of animals to seasonal environments is mammalian hibernation, characterized by extremely reduced metabolic rate (MR; as low as 1%–5% of the basal rate; Geiser and Ruf 1995), body temperature (T_b), and activity (reviewed in Boyer and Barnes 1999; Storey 2000; Carey et al. 2003; Geiser 2004). Mammalian hibernation is a geographically and taxonomically widespread phenomenon that allows animals to survive periods of low resource availability by providing significant savings in energy use as they subsist on hoarded and/or endogenous energy stores (Geiser 2004; McKechnie 2014).

A majority of hibernation research has been conducted on sciurids, ground squirrels and marmots, held in the laboratory and exposed to above-freezing ambient temperatures (T_a's; reviews: Davis 1976; Geiser and Baudinette 1990; Geiser 2004), with relatively few published investigations examining the physiology of hibernation at T_a's lower than 0°C (Geiser and Kenagy 1988; Barnes 1989; Buck and Barnes 2000; Karpovich et al. 2009). Hibernators rely on temperature-independent metabolic inhibition, in addition to the temperature-dependent or Q_{10} effects associated with decreased T_b, to reduce MR during torpor (reviewed in Geiser 2004). In most species hibernating at $T_a \geq T_b$ set-point, torpid MR (TMR) decreases with decreasing T_a in a Q_{10}-dependent manner (Hammel et al. 1968; Geiser and Kenagy 1988; Snyder and Nestler 1990; Geiser 2004), although arctic ground squirrels (*Urocitellus parryii*) also utilize temperature-independent metabolic inhibition, enabling them to maintain a constant, low TMR over a T_a and T_b range of 0°–16°C (Buck and Barnes 2000). This relationship of decreasing TMR with decreasing T_b holds true for most hibernators so long as the animal's T_b remains near T_a. However, as T_a approaches and decreases below the T_b set-point, the animal must either increase its TMR to maintain $T_b \geq T_b$ set-

point or arouse from torpor; failing these, the animal will die (Geiser and Kenagy 1988; Geiser et al. 1990; Arnold et al. 1991; Buck and Barnes 2000).

Although sciurids have been used extensively to study hibernation in the laboratory, comparatively little is known about hibernation and hibernacula conditions of sciurids in the wild (literature summary in table 1). Of these studies, the majority include soil temperatures from a single season and one small section of the species' overall distribution; only two sciurid species have been shown to experience overwinter soil temperatures $\leq -5°$C: the arctic ground squirrel and the Alaska marmot (*Marmota browerii*). These two species are also the only representatives of the Arctic, where continuous permafrost constrains the depth of hibernacula, so that these animals predictably experience subfreezing soil temperatures across much of their hibernation season (Carl 1971; Buck and Barnes 1999b; Lee et al. 2009). Given the limited information about hibernaculum conditions available on species living in the temperate zone, it is unclear whether they are routinely subjected to subzero soil temperatures for extended periods of time within their current range distributions.

Since few species are known to hibernate at subzero T_a's, very few captive studies of hibernators have challenged animals with T_a's substantially below freezing and analyzed their corresponding torpid thermogenic capacity. Laboratory studies of arctic ground squirrels reveal that they have a tremendous capacity to increase metabolism and generate heat during torpor and arousals at low T_a (Buck and Barnes 2000; Karpovich et al. 2009). Field measurements of arctic ground squirrel hibernacula have shown that T_a during winter can decrease to as low as $-23.4°$C (Buck and Barnes 1999b), which is lower than the T_a's that hibernators have been subjected to in captive investigations ($-16°$C; Buck and Barnes 2000). Thus, the maximum MR arctic ground squirrels can maintain during steady state torpor remains unknown. Laboratory studies conducted at subzero T_a's on hibernating golden-mantled ground squirrels (*Callospermophilus lateralis*), a temperate-zone species, have produced

conflicting results with respect to their thermogenic capacity during torpor. Geiser and Kenagy (1988) found that all golden-mantled ground squirrels increase metabolism and generate heat to prevent the potential deleterious effects associated with T_b falling below the T_b set-point, whereas Wit and Twente (1983) found that not all individuals were able to increase torpid thermogenesis and that, when exposed to subfreezing T_a's ($-1°$ to $-2°$C), a subset of animals either aroused or died. Arctic ground squirrels and golden-mantled ground squirrels are two sciurid species that have demonstrated some capacity for thermogenic torpor, making these species good candidates for an analysis of thermogenic capacity during torpor.

Here we report the thermogenic capacity of hibernating arctic ground squirrels during steady state torpor and compare their response to that of golden-mantled ground squirrels, which may only rarely be exposed to subfreezing T_a's. We anticipated that both species would be capable of defending large thermal gradients between T_b and T_a. We hypothesized that differences between species in their capacity to maintain a thermal gradient should principally be a function of differences in size and thus thermal inertia, such that the smaller golden-mantled ground squirrels should exhibit a greater increase in MR with more moderate decreases in T_a, but that the two species would exhibit similar maximum TMRs and similar metabolic scopes. We predicted that arctic ground squirrels, given their larger size, more northerly distribution, and use of subzero hibernacula, would be able to maintain torpor at lower T_a's than golden-mantled ground squirrels. To test our hypotheses, we utilized standardized protocols whereby we exposed animals to progressively lower T_a's while concurrently measuring both MR and T_b.

Material and Methods

Study Species and Husbandry

The arctic ground squirrel is distributed from northeastern Russia throughout Alaska and northwestern Canada (Iwen 1999).

Table 1: Published values for burrow/soil temperature and minimum torpid metabolic rate (TMR)

Species	Burrow/soil temperature (°C)	Minimum TMR (mL O$_2$/(g*h))	Sources
Callospermophilus lateralis	-2 to 4.9	.045	Snap and Heller 1981; Healy et al. 2012; C. L. Frank, personal communication
Callospermophilus saturatus	2	.017	Kenagy et al. 1989; Geiser et al. 1990
Ictidomys tridecemlineatus	-1	.02	Kisser and Goodwin 2012; C. L. Buck, personal observation
Marmota browerii	Min.: -15.0, mean: -7.3	NA	Lee et al. 2009
Marmota marmota	0	.013	Arnold et al. 1991
Marmota flaviventris	5–7	.022	Florant and Heller 1977; Florant et al. 2000
Marmota monax	1.9	.032	Lyman 1958; Ferron 1996
Urocitellus columbianus	-2	NA	Young 1990
Urocitellus parryii	Min: -23.4, mean: -8.9	.01	Buck and Barnes 1999b, 2000
Urocitellus richardsonii	-2.6	.02	Wang 1978; Michener 1992

Note. Previously published values of North American hibernating sciurids and the corresponding soil temperatures. Min = minimum; NA = not available.

Eighteen (5 female, 13 male) arctic ground squirrels (*Urocitellus parryii*) were either live-trapped north of the Brooks Range, Alaska, near the Atigun River (68°27′N, 149°21′W, elevation 812 m), and transported to the University of Alaska Anchorage vivarium or born in captivity to mothers that were live-trapped near the Atigun River. The golden-mantled ground squirrel (*Callospermophilus lateralis*) lives in the temperate zone (between the Tropic of Cancer and the Arctic Circle in the Northern Hemisphere), and its range includes the montane regions of western North America and extends south through southern New Mexico (Howell 1938). Eight (4 female, 4 male) golden-mantled ground squirrels were live-trapped for this experiment in Red Feather Lakes, Larimer County, Colorado (40.8°N, 105.59°W, elevation 2,531 m), and transported to the University of Alaska Anchorage.

Before experiments, all animals were maintained individually in metal cages (48 cm × 31 cm × 30 cm; Unifab, Kalamazoo, MI) on a 12L : 12D photoperiod and at a T_a of 20° ± 2°C. Animals were provided cotton batting for nesting (Perfect Fit, McDonald, Tukwila, WA), food (Mazuri Rodent Chow, Brentwood, MO), and water ad lib. In the fall of each experimental year, we moved animals into environmental chambers maintained at a T_a of 2° ± 1°C with an 8L : 16D photoperiod. As animals began to exhibit bouts of torpor, determined during daily observation, we transferred them and their nests into plastic tubs (43 cm × 27 cm × 19 cm for arctic ground squirrels, 41 cm × 25 cm × 18 cm for golden-mantled ground squirrels; Nalgene, Rochester, NY), removed all food and water, and allowed them to resume torpor. All work was approved by the University of Alaska Anchorage Institutional Animal Care and Use Committee (protocol 175424–2).

Body Temperature (T_b)

To record T_b, we surgically implanted temperature-sensitive radiotransmitters (~7 g; model TA10TA-F40-LF, Data Sciences International, St. Paul, MN) into the peritoneal cavity of animals at least 2 mo before initiation of metabolic measurements. Briefly, animals were anesthetized with isoflurane, and under aseptic conditions a 3-cm incision was made along the animal's midline through the cutaneous and muscle layers; the gas-sterilized transmitter was placed inside the peritoneal cavity. The muscle and subcutaneous layers were closed with chromic gut and polydioxanone sutures, respectively, and the skin was subsequently glued (Vetbond, 3M, St. Paul, MN). After surgery, animals were returned to their wire cages at a T_a of 20° ± 2°C, where they remained until being moved to an environmental chamber (2° ± 1°C). Before surgery, all transmitters were calibrated to 0.1°C with a mercury thermometer at 0.0°, 35°, and 39°C. Transmitters were activated once hibernation began and animals were moved to tubs. The T_b was recorded every 10 min.

Respirometry

Rates of oxygen consumption were recorded concurrently from four animals with an automated two-channel system that alternated between channels every 5 min (adapted from Tøien 2013). During measurements, the tubs housing animals were covered with closed-foam gasket–sealed polycarbonate lids. Excurrent air was drawn from the chambers through flow meters, after which a subsample passed through a dual-gas flow multiplexer (a modified RM-8, Sable Systems International, Las Vegas, NV) that switched air streams between a pair of animals and calibration gases. A subsample was then dried with Nafion dryers (Perma Pure, Toms River, NJ) in a reflux mode before being analyzed for O_2 and CO_2 content with an Oxilla II dual-channel O_2 analyzer and two CA-10A CO_2 analyzers (Sable Systems International). Immediately before each recording, the CO_2 and O_2 analyzers were span and zero calibrated (using the standard of 20.94% O_2 in air, soda lime, and a calibration gas with 0.5% CO_2 in air). At the beginning of each recording, and every hour throughout, a reference air sample was collected from inside the animal holding chamber. Samples of zero-CO_2 air and span gas were automatically collected every 3 h. The rate of oxygen consumption was calculated according to the principles of the Haldane transformation (Haldane 1912), with corrections as outlined in Tøien (2013). The rate of chamber air flow was measured and maintained at either 200 ± 10 mL/min (low flow) or 2,500 ± 10 mL/min (high flow) with mass flow controllers (Flowbar8, Sable Systems International, and Brooks 5850E, 5-L/min range, Coastal Instruments, Burgaw, NC, respectively). Computer-controlled baselining units (Sable Systems International) were used to automatically switch from low to high chamber flow when animals aroused and from high to low flow when animals went into torpor, on the basis of O_2 depletion thresholds of 1.3% and 0.08%, respectively (as diagrammed in fig. 15 of Tøien 2013). All respirometry data were collected, corrected for drift, and analyzed with LabGraph (Tøien 2013). Efficacy of system performance was assessed by burning a known mass of ethanol within the respirometry chamber before measurements began, halfway through the temperature protocols, and again after completion of experiments.

Steady State Torpor

In the fall, at the beginning of the hibernation season, all animals (18 arctic ground squirrels, 8 golden-mantled ground squirrels) were moved into environmental chambers, and respirometry measurements were initiated after all animals had exhibited at least one bout of torpor. To ensure that animals were in steady state torpor, we conducted all measurements between days 1 and 18 of a torpor bout (T_b remained constant [±0.5°C] for 2 or more hours before recording). At low flow, data were not used until after 6 h (3.26 complete air changes), to ensure that measured gas concentrations accurately reflected animal metabolism; at high flow, the duration was reduced to 2 h (13.6 complete air changes). Mean rates of oxygen consumption (i.e., TMRs) were determined for individual animals during steady state torpor over 6-h periods.

Between experimental temperatures, all animals were weighed to the nearest gram (CW11–2EO, Ohaus, Pinebrook, NJ) and physically disturbed to induce an arousal, so that all animals were euthermic before exposure to the next T_a. Arctic ground squirrels were randomly divided into two groups, each of which experienced a different T_a exposure protocol: protocol 1 included T_a's ranging from 2° to −20°C in 2°C increments; animals in protocol 2 were exposed to T_a's of 2° and 0°C before we decreased the T_a to −10°C and then −20°C, after which T_a was decreased in 2°C increments until animals either failed to enter torpor or were unable to maintain low T_b or until a maximum MR was obtained (as indicated by successive T_a's eliciting the same MR). Golden-mantled ground squirrels were exposed to the temperatures from protocol 1 after their initial torpor bout; however, in accordance with our animal care protocol, golden-mantled ground squirrels were removed from the cold room after measurements were made at −8°C. Animals were at each T_a for at least 24 h before being aroused for the next experimental T_a.

Ramping

All ramping experiments were conducted in the year after steady state torpor experiments (i.e., in the subsequent hibernation season). Once all animals had undergone at least one bout of torpor at 0°C, we began the ramping protocols as follows. Seven arctic ground squirrels (6 male, 1 female) were aroused from torpor at 0°C, weighed, and then placed at −20°C, where they again entered steady state torpor. While T_b and MR were recorded simultaneously, these animals were then subjected to gradually decreasing T_a's (set-point decreased by 0.25°C every 30 min, except at −25.5°C, which was maintained for an hour to allow animals to acclimate) until animals exhibited an arousal. We defined arousal as an increase in MR, as measured by oxygen consumption, of 0.1 mL $O_2/(g*h)$ for five consecutive decreases in T_a. This definition allowed us to differentiate between incremental increases in MR associated with the progressively increasing temperature gradients and the actual attempt to arouse from low T_b while excluding the MR "overshoots" (when an animal increased MR for a brief period of time that did not manifest as an increase in T_b) that the animals exhibited during the protocol. We followed a similar protocol with four golden-mantled ground squirrels (2 male, 2 female) in steady state torpor at 0°C. The starting T_a's for both species were chosen on the basis of results obtained from our steady state torpor work. Mean MRs for ramping were selected from the last 10 min before the set-point was again adjusted. The T_b and T_a we report for the ramping experiment are the last temperatures recorded before the change in T_a.

Data Analysis

All data presented are means ± SEM, unless otherwise noted. We used linear mixed effects models in R (REML function in lmerTest package, ver. 3.0.1, RStudio), with individual ID included as a random effect to examine the rate at which steady state mass-specific TMR changed in response to changing T_a. For arctic ground squirrels we included only data collected between 0° and −24°C in our model (the linear portion of the response; see "Results"), whereas for golden-mantled ground squirrels we included data collected between 2° and −8°C. To determine whether the TMR of arctic ground squirrels had reached a plateau, we compared the MRs at −24° and −26°C, using a paired t-test. We compared the maximum TMRs between species by using a Student's t-test. For all tests, we concluded that results were statistically significant when $P \le 0.05$.

Results

Steady State Torpor

Ground squirrels used in these experiments were able to maintain a low, constant T_b indicative of steady state torpor at all temperatures tested. In response to exposure to incrementally decreasing subzero T_a's, torpid squirrels of both species increased MRs and continued to defend an increasingly large thermal gradient between T_a and T_b (fig. 1).

Arctic ground squirrels maintained low T_b's and steady state torpor at T_a's as low as −26°C (4 of 5 animals exposed to −26°C). At this T_a, the four animals maintained a TMR of 0.36 ± 0.01 mL $O_2/(g*h)$, a value not significantly different from the TMR of six animals torpid at −24°C (0.37 ± 0.01 mL $O_2/(g*h)$; paired t-test, $P = 0.3$; fig. 1). Arctic ground squirrels displayed a 36-fold increase in TMR from 0° to −24°C (from 0.01 ± 0.00 mL $O_2/(g*h)$ at 0°C to 0.37 ± 0.01 mL $O_2/(g*h)$ at −24°C). The relatively high TMR at −26°C (0.36 ± 0.01 mL $O_2/(g*h)$) enabled these arctic ground squirrels to defend a ~25.5°C thermal gradient and maintain a mean core T_b of −0.5° ± 0.3°C. The change in TMR from −24° to 0°C was −0.014 mL $O_2/(g*h)/$°C. Of the five animals exposed to −26°C, one animal entered torpor briefly (2.6 h, minimum T_b: −0.4°C) before initiating an arousal; this animal was unable to fully arouse and died during the attempt (maximum T_b achieved: 3.0°C). As a result of this fatality, no animals were exposed to T_a's lower than −26°C during the steady state torpor measurements.

Golden-mantled ground squirrels maintained steady state torpor at T_a's as low as −8°C, the lowest T_a they were subjected to, given the constraints of our animal care protocol. The highest TMR recorded from golden-mantled ground squirrels occurred in animals torpid at −8°C (0.29 ± 0.01 mL $O_2/(g*h)$). This TMR is significantly lower than the maximum TMR recorded from arctic ground squirrels at −26°C (Student's t-test; $P = 0.002$), but the TMR of golden-mantled ground squirrels had not yet plateaued. The golden-mantled ground squirrels were able to elevate their MR by 13.5-fold (from 0.02 ± 0.001 mL $O_2/(g*h)$ at 2°C to 0.29 ± 0.01 mL $O_2/(g*h)$ at −8°C) as they maintained above-freezing T_b at the subzero T_a's tested. Golden-mantled ground squirrels exhibited their lowest T_b (0.0° ± 0.2°C) when hibernating at −4°C, thus establishing a ~4°C temperature gradient between their core and the envi-
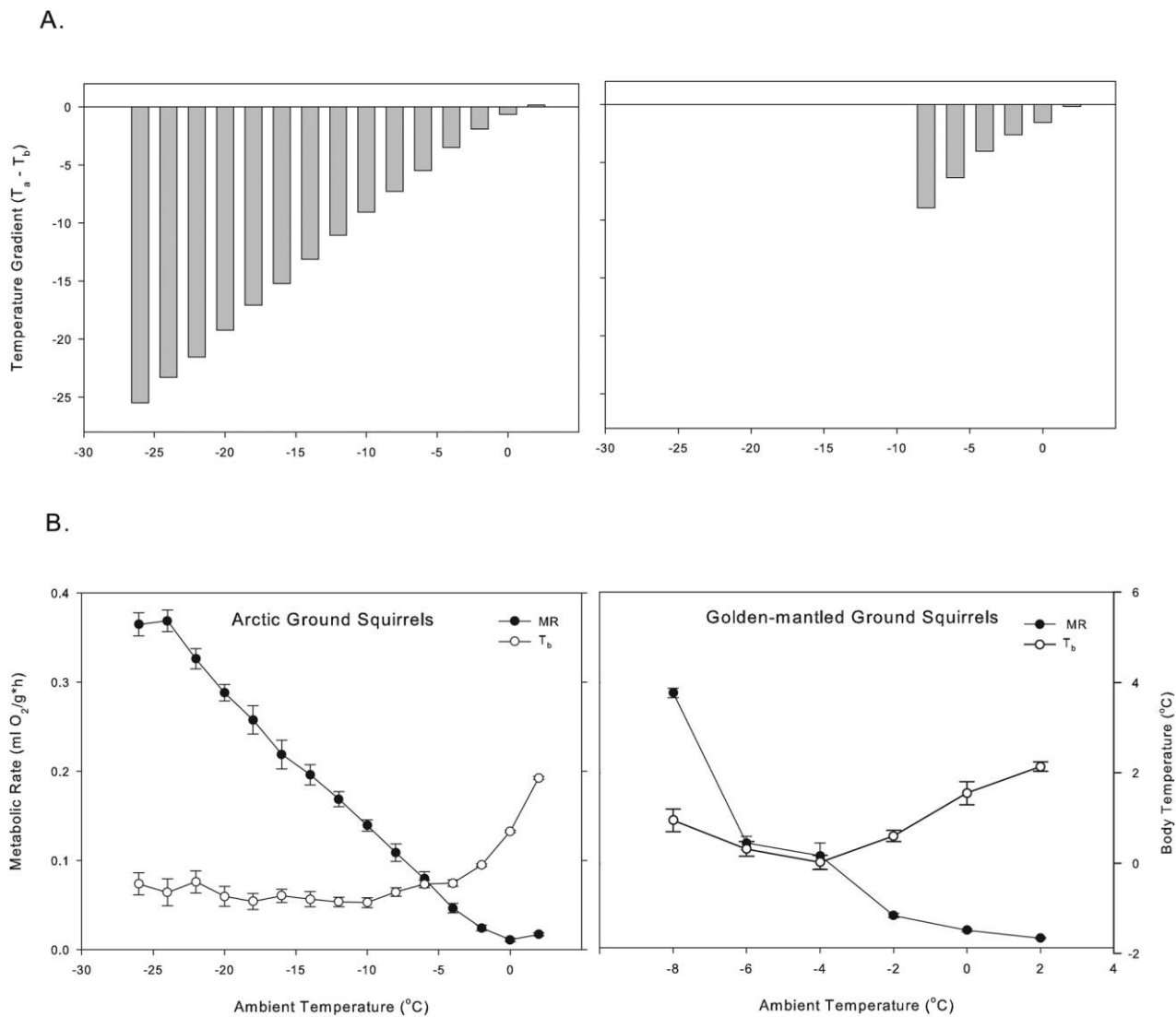
A.

B.

Figure 1. *A*, Mean temperature gradients of animals (*left*, arctic ground squirrels; *right*, golden-mantled ground squirrels) in steady state torpor at experimental ambient temperatures (T_a). *B*, Effect of T_a on steady state torpid metabolic rate (MR; mean $\pm$ SEM) in ground squirrels. The number of arctic ground squirrels ranged from 4 to 18 individuals at each temperature. The number of golden-mantled ground squirrels ranged from 2 to 8 individuals at each temperature. T_b = body temperature.

ronment. However, while they reached their T_b nadir at $-4°C$, the largest gradient golden-mantled ground squirrels defended occurred at T_a of $-8°C$ ($9.0° \pm 0.3°C$). The change in MR from T_a's of $-8°$ to $-2°C$ was -0.024 mL $O_2/(g*h)/°C$.

Animals were weighed before the steady state recordings had begun and during every induced arousal. For arctic ground squirrels undergoing the first steady state protocol (T_a's from $2°$ to $-20°C$ in $2°C$ increments), 11 arousals were induced and animals lost 154 ± 11 g of initial mass (fall: 750 ± 32 g, spring: 596 ± 30 g, a loss of $\sim$21%) over the 104 days of the experiment. Over the same time course, arctic ground squirrels in the second protocol (T_a's of $2°$, $0°$, $-10°$, $-20°$, $-22°$, $-24°$, and $-26°C$) underwent six induced arousals and lost 220 ± 23 g of initial body mass (fall: 783 ± 19 g, spring: 563 ± 23 g, a loss of $\sim$28%). The two golden-mantled ground squirrels that were

subjected to all T_a's in the steady state protocol lost a total of 85 and 55 g (fall: 247 and 222 g, spring: 162 and 167 g, a loss of $\sim$34% and $\sim$25% of initial body mass, respectively) over five induced arousals and 84 days of the experiment.

Ramping

Individuals from both species were able to defend a thermal gradient between torpid T_b and T_a without immediately arousing when challenged with incrementally lower subzero T_a's. Before the metabolic measurements for the ramping experiment, arctic ground squirrels spontaneously entered torpor at $0°C$; these animals were aroused and weighed and reentered torpor at $-20°C$ immediately before the ramping protocol began. The mean T_a at which arctic ground squirrels alarm-

aroused was $-25.9° \pm 1.1°C$ (range: $-23.0°$ to $-30.0°C$). The average TMR just before arousal was 0.29 ± 0.02 mL $O_2/(g*h)$, ranging from 0.20 to 0.38 mL $O_2/(g*h)$ (table 2). The golden-mantled ground squirrels that entered torpor at a T_a of 0°C were then exposed to progressively lower T_a's; the mean T_a that induced arousal was $-6.3° \pm 1.8°C$ (range: $-3.0°$ to $-10.0°C$). TMRs just before arousal averaged 0.12 ± 0.06 mL $O_2/(g*h)$ and ranged from 0.01 to 0.26 mL $O_2/(g*h)$ (table 2).

Discussion

In this study, we investigated the thermogenic responses of two hibernating ground squirrel species exposed to subfreezing temperatures during steady state torpor. We found that arctic ground squirrels exhibited a 36-fold increase in TMR as they defended a thermal gradient of ~25.5°C between T_b and T_a, at a T_a as low as $-26°C$, below the lowest published soil/hibernacula temperature for this species (table 1). We suggest that the maximum TMR we measured in arctic ground squirrels was very close to the maximum TMR they are capable of, as MR exhibited an abrupt plateau between $-24°$ and $-26°C$. This was supported by our finding that torpid squirrels subjected to steadily decreasing T_a aroused at $-25.9° \pm 1.1°C$, although one individual continued to hibernate at $T_a = -30.0°C$. The maximum TMR of 0.37 ± 0.01 mL $O_2/(g*h)$ is close to their basal MR (0.4–0.61 mL $O_2/(g*h)$; Scholander et al. 1950; Withers et al. 1979) but is lower than the 0.51–0.84 mL $O_2/(g*h)$ reported for resting MR of wild-caught

arctic ground squirrels (Sheriff et al. 2013). We also found that golden-mantled ground squirrels were capable of increasing their TMR by at least 13.5-fold as they defended a thermal gradient of ~9°C between T_b and T_a while maintaining steady state torpor at T_a's as low as $-8°C$ (the lowest T_a tested). The highest TMR measured for the golden-mantled ground squirrels, like that of the arctic ground squirrels, was also well below their published basal MR (0.29 ± 0.01 vs. 0.73 mL $O_2/(g*h)$; Snapp and Heller 1981). Arctic ground squirrels, however, exhibited lower minimum T_b, lower minimum TMR, a greater torpid metabolic scope, a smaller increase in TMR with decreasing T_a, and the capacity to maintain torpor at significantly lower T_a's, compared to golden-mantled ground squirrels.

The golden-mantled ground squirrels utilized in our study maintained T_b above 0°C while hibernating at subfreezing temperatures, contrary to Wit and Twente (1983). Animals were able to remain in steady state torpor at T_a's as low as $-8°C$, and one animal aroused only when T_a reached $-10°C$; this value is substantially lower than the T_a of $-2°C$ that resulted in death for a subset of animals in Wit and Twente (1983). It is likely that the discrepancy between our findings and previously published results represents the difference in protocols utilized and not a real difference in thermal tolerance of the animals themselves. Our steady state protocol allowed animals to fully arouse before subjecting them to a lower T_a, and our ramping protocol was much more gradual than what animals underwent in previously published work, where torpid animals were moved directly from an above-zero T_a to a below-freezing T_a (Wit and Twente 1983).

Although both arctic ground squirrels and golden-mantled ground squirrels maintained torpid T_b's within a fairly narrow range throughout the experiments, the two species differed slightly in response to subzero T_a's. While arctic ground squirrels' T_b during torpor remained relatively constant with decreasing T_a, abdominal T_b in golden-mantled ground squirrels increased by ~0.9°C between T_a's of $-4°$ and $-8°C$ (from $-0.08° \pm 0.16°C$ to $0.95° \pm 0.25°C$, $P = 0.08$). Throughout the hibernation cycle, T_b is closely monitored and regulated by the hypothalamus; as the hypothalamus is heated or cooled, the MR of the animal is suppressed or increased, ensuring that hypothalamic temperature does not deviate significantly from its setpoint (Heller and Hammel 1972; Mills and South 1972; Florant and Heller 1977). However, thermogenic animals exhibit regional heterothermy during torpor (Barnes 1989), and therefore it is unclear whether the observed difference in abdominal T_b reflects a difference in hypothalamic set-point or was due to differences in heat transfer from the brown adipose tissue in the thoracic region. The arctic ground squirrels in our experiment maintained a minimum abdominal T_b ($-0.94° \pm 0.11°C$) slightly higher than those previously published for captive animals hibernating in outdoor enclosures ($-2.9°C$; Barnes 1989) or for free-living animals hibernating in the wild ($-2.0°$ to $-0.9°C$; Buck et al. 2008; Williams et al. 2012); however, our values are similar to those reported by Barnes (1989) for a single captive animal hibernating in environmental chambers ($-1.3°C$).

Table 2: Results from ramping experiment

Animal number	T_a of arousal (°C)	MR at arousal
Golden-mantled ground squirrels:		
GMGS 1	−10	.26
GMGS 2	−3	.02
GMGS 3	−9	.18
GMGS 4	−3	.01
Means	−6.3 ± 1.8	.12 ± .10
Arctic ground squirrels:		
AGS 10–17	−23	.20
AGS 10–04	−25	.23
AGS 08–15	−24	.34
AGS 09–13	−23	.29
AGS 08–34	−28	.38
AGS 09–06	−28	.30
AGS 08–28	−30	.32
Means	−25.9 ± 1.1	.29 ± .02

Note. Maximum metabolic rates (MR; reported as oxygen consumption in mL $O_2/(g*h) \pm$ SEM) from minimum ambient temperature (T_a) at which animals continued to exhibit torpor as determined via a temperature-ramping experiment that involved exposing animals to progressively decreasing T_a's during a single torpor bout. Individual golden-mantled ground squirrels are presented as their animal number (GMGS#), as are the arctic ground squirrels (AGS#).

We presume that the increase in MR with decreasing T_a observed in this study directly reflects an increase in rates of nonshivering thermogenesis. In hibernators, nonshivering thermogenesis is activated via norepinephrine's effects on uncoupling protein-1 (UCP-1) in brown adipose tissue (Cannon and Nedergaard 2004, 2011). Animals hibernating at temperatures below their hypothalamic set-point may be particularly dependent on heat generated by brown adipose tissue. While electromyographic activity from shivering has been observed at T_b's as low as 4°C (Tøien et al. 2001), shivering is not thought to be an effective heat generator at this temperature (Kitao and Hashimoto 2012). Brown adipose tissue, on the other hand, upregulates UCP-1 upon cold exposure, and the highest levels are found in hibernators that are actively generating heat (Barger et al. 2006), indicating functionality at very low temperatures.

In this study, we established an upper limit to the TMR for the arctic ground squirrel of 0.37 ± 0.01 mL O_2/(g*h) (a 36-fold increase from lowest TMR; fig. 1). Evidence supporting this as a maximum TMR in this species comes from the apparent plateau in MR between animals torpid at T_a's of −24° and −26°C. Interestingly, this maximum TMR is substantially lower than what these animals are capable of generating during an arousal (Karpovich et al. 2009). We were fortuitously able to record MR during arousals from two animals that were torpid at T_a −26°C; these animals demonstrated maximum arousal MRs of 3.24 and 3.44 mL O_2/(g*h) (a 323- and 343-fold increase, respectively, over the lowest mean TMR). These maximum arousal MRs are very similar to what Karpovich et al. (2009) found for arctic ground squirrels arousing from torpor at −12°C (3.40 ± 0.18 mL O_2/(g*h)) as well as being similar to peak levels resulting from stimulated arousals of arctic ground squirrels torpid at 2°C (Tøien et al. 2001), indicating that this is the maximum MR for thermogenesis during arousal in this species. It is possible that the discrepancy between maximum TMR and the MRs achieved during arousals is due to a threshold effect; that is, animals may be able to increase their TMR only so much before the norepinephrine concentrations elicit an arousal. Once norepinephrine levels enabling nonshivering thermogenesis reach the threshold, an arousal is initiated, the T_b set-point of the hypothalamus is reset to euthermic levels (∼37°C), and MR increases accordingly (Florant and Heller 1977). Thus, alarm arousals might simply be a consequence of the elevated norepinephrine levels in both the hypothalamus and the brown adipose tissue associated with increased thermogenesis during torpor at low T_a. While this is a speculative hypothesis, there is some evidentiary support, in that injections of norepinephrine directly into the hypothalamus and injections into the periphery both induce arousal from torpor (intrahypothalamic injection: Beckman and Satinoff 1972; intraperitoneal injection: Twente and Twente 1978).

We observed differences in the TMRs generated from each protocol; the steady state torpor protocol resulted in higher TMRs, compared to those from the ramping protocol (fig. 1; table 2). Interestingly, we did see a convergence in the data from the two approaches onto a single value for the lower T_a limit of hibernation for each species. For the arctic ground squirrel, both experiments found a T_a limit close to −26°C; the maximum MR of torpor plateaued between T_a's of −24° and −26°C (0.37 ± 0.01 mL O_2/(g*h); fig. 1) in the first experiment, and animals aroused at a mean T_a of −25.9° ± 1.1°C during the ramping experiment (table 2). The golden-mantled ground squirrels were more responsive to decreasing T_a and aroused from torpor at a mean T_a of −6.3° ± 1.8°C (range: −3° to −10°C; table 2) during the ramping experiment, while the steady state protocol was terminated at −8°C. The difference in lower T_a limit between species might reflect the thermal inertia gained from the larger body mass of the arctic ground squirrels, which were 316% heavier than golden-mantled ground squirrels at peak adiposity. Thermal inertia may also help explain why TMR was lower in the ramping experiment, as this inertia could result in a lag between changes in T_a and hypothalamic T_b and thus delay the metabolic response.

In addition to the increased thermogenic load animals incurred as a result of subfreezing T_a's in our study, they also underwent frequent induced arousals. This combination of elevated TMR and induced arousals resulted in a significant body mass loss (17%–33%) over 104 days of hibernation for the arctic ground squirrels. For a comparison, free-living adult female arctic ground squirrels lose 30% of their body mass over the course of the 237 ± 2.2-d hibernation season (Buck and Barnes 1999a), which includes ∼15 spontaneous arousals (Buck et al. 2008). The two golden-mantled ground squirrels that were exposed to the full range of T_a's in the steady state protocol lost 34.4% and 24.8% of their fall body mass over five induced arousals and 84 days of hibernation; this compared to ∼29% body mass loss over ∼232 days of hibernation and ∼20 arousals for a free-living individual (Healy et al. 2012). The rapid body mass loss our animals experienced over a short period of time is indicative of the cost of arousals for a hibernator in addition to the increased TMR incurred at low T_a's. At T_a's above the T_b set-point, 86% of overwinter energy expenditure is accounted for by arousals (Wang 1978); as T_a decreases, the relative cost of arousals actually decreases because of the increased metabolic load of thermogenic torpor (Karpovich et al. 2009). Although soil temperatures measured in the Arctic can be as low as −23.4°C, soils at most hibernacula typically do not freeze solid at a depth of ∼1 m until late October (Buck and Barnes 1999b); therefore, arctic ground squirrels need only be thermogenic during deep torpor for a portion (5–7 mo; Buck et al. 2008) of the hibernation cycle.

Hibernation functions as an energy conservation strategy that enables survival during periods of low resource availability. Arctic ground squirrels were able to maintain torpor at T_a's as low as −26°C, which is only ∼3°C colder than what they are known to experience in the field (−23.4°C; table 1). Although golden-mantled ground squirrels have a temperate-zone distribution, we found that they remain at a T_b of ∼0°C and have the capacity to increase MR and maintain steady state torpor in T_a's at least as low as −8°C. Interestingly, this is also only a few degrees cooler than the minimum winter soil

temperatures these animals are known to experience in the wild (−4.9°C; table 1). Our results are consistent with the hypothesis that the physical environment plays an important role in shaping the hibernation phenotype of hibernating sciurids. However, we recognize the severe limitations of inferring adaptation on the basis of two-species studies (reviewed in Garland and Adolph 1994), and we encourage further study on the thermogenic capacity of hibernators during deep torpor, so that multispecies comparisons can be made.

Acknowledgments

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