

Energetics of arousal episodes in hibernating arctic ground squirrels

Shawna A. Karpovich · Øivind Tøien ·
C. Loren Buck · Brian M. Barnes

Received: 25 June 2008 / Revised: 10 February 2009 / Accepted: 15 February 2009 / Published online: 11 March 2009
© Springer-Verlag 2009

Abstract Arctic ground squirrels overwintering in northern Alaska experience average soil temperature of -10°C . To examine energetic costs of arousing from hibernation under arctic compared to temperate conditions, captive ground squirrels were maintained in ambient temperatures (T_a) of 2, -5 and -12°C . Rates of oxygen consumption and carbon dioxide production were used to estimate metabolic rate and fuel use during the three phases of arousal episodes: rewarming, euthermia, and recooling. Respiratory quotient comparisons suggest exclusive use of lipid during rewarming and mixed fuel use during euthermia. Animals rewarming from torpor at $T_a -12^{\circ}\text{C}$ took longer, consumed more oxygen, and attained higher peak rates of oxygen consumption when compared to 2°C . T_a had no significant effect on cost or duration of the euthermic phase. Animals re-cooled faster at -12°C than at 2°C , but total oxygen consumption was not different. T_a had no significant effect on the total cost of arousal episodes when all three phases are included. Arousal episodes account for 86% of estimated costs of a complete hibernation cycle including torpor when at 2°C and only 23% at -12°C . Thus, due to the higher costs of steady-state metabolism during torpor, proportional metabolic costs of

arousal episodes at T_a characteristic of the Arctic are diminished compared to relative costs of arousals in more temperate conditions.

Keywords Oxygen consumption · Ground squirrel · *Spermophilus* · Metabolic rate · Arousal · Arctic

Abbreviations

T_a	Ambient temperature
T_b	Body temperature
MR	Metabolic rate
BMR	Basal metabolic rate
RQ	Respiratory quotient

Introduction

Hibernating ground squirrels alternate between extended bouts of torpor (1–3 weeks) characterized by profound hypometabolism and low body temperatures and short arousal episodes (1–2 days) (Twente and Twente 1965; Barnes and Ritter 1993). Arousal episodes include three phases: rewarming, euthermia, and recooling. During rewarming, metabolic heat production, originating from non-shivering thermogenesis in brown adipose tissue (BAT) and muscular shivering, increase to elevate core body temperature (T_b) from minimum levels during torpor to euthermic, or normothermic levels. The 10–15 h interbout euthermic phase consists of limited activity, while animals usually remain inside their nests (Lyman 1948; Daan et al. 1991). The recooling phase begins when metabolic rate (MR) decreases below levels necessary for homeothermy, and T_b gradually declines (Heldmaier et al. 2004) to a new set point level that

Communicated by H. V. Carey.

S. A. Karpovich · Ø. Tøien · B. M. Barnes (✉)
Institute of Arctic Biology, University of Alaska Fairbanks,
Fairbanks, USA
e-mail: brian.barnes@uaf.edu

S. A. Karpovich
Alaska Department of Fish and Game, Fairbanks, USA

C. L. Buck
Biological Sciences Department, University of Alaska
Anchorage, Anchorage, USA

usually approximates ambient temperature in above freezing conditions. The universal presence of regular arousal episodes among small hibernating mammals suggests a fundamental limit to the length of time torpor can be sustained before a return to high T_b and metabolic rate is required (Willis 1982).

Energetics of hibernation in ground squirrels and other small mammals held at ambient temperatures (T_a) representative of temperate or alpine environments where soils do not freeze have been well investigated. Thermal conforming animals in steady-state torpor maintain T_b 0.5–2°C above T_a and have very minimal rates of metabolism, as low as 0.01 ml O_2 g^{-1} h^{-1} or 1–2% of basal metabolic rate (BMR) (Geiser 1988, 2004; Buck and Barnes 2000). Metabolic rate during rewarming can reach levels that are several times BMR and during euthermia are typically maintained above basal rates due to the requirement for thermoregulation. Arousal episodes are reported to be the most energetically costly component of hibernation for ground squirrels overwintering in above freezing conditions and are estimated to represent the great majority of the energy expended over the entire heterothermic season. There are few reported measurements of metabolic rate during hibernation under freezing conditions, however. Two studies (Heller and Colliver 1974; Geiser and Kenagy 1988) included slightly subzero temperatures, and one study (Buck and Barnes 2000) measured metabolic rate, respiratory quotient, and T_b during steady-state torpor at T_a 's to -16°C . None of these studies included the energetics of arousal episodes.

Arctic ground squirrels (*Spermophilus parryii*) overwintering in northern Alaska experience winter-long average soil temperatures at hibernacula depth (1 m) of -10°C and minima of -23°C (Buck and Barnes 1999), and they adopt minimum core T_b as low as -2.9°C (Barnes 1989). These environmental conditions require that arctic ground squirrels are continuously thermogenic during most of the hibernation season. In the present study, we contrasted the metabolic costs of arousal episodes in arctic ground squirrels hibernating in above freezing conditions to costs during hibernation at $T_a < 0^\circ\text{C}$ within the range of those experienced by animals in their natural burrows. We hypothesized that energetic costs and duration of the rewarming phase would increase with decreasing T_a and that animals would maintain interbout euthermia for shorter durations at lower T_a to minimize energetic costs. Finally, using data on the increased cost of maintaining steady-state torpor at $T_a < 0^\circ\text{C}$ from Buck and Barnes (2000) in combination with field data on duration of torpor bouts (Buck et al. 2008; Barnes et al. unpublished), we calculated the proportional costs of arousal episodes within the entire heterothermic season.

Materials and methods

Animals

Arctic ground squirrels (*S. p. kenocottii*, 10 adult males, 2 adult females) were captured in August near Toolik Lake, Alaska ($68^\circ38'\text{N}$, $149^\circ38'\text{W}$) and transported by truck to the University of Alaska Fairbanks where they were housed individually in cages within environmental chambers. Animals were maintained on a 12L:12D photoperiod and in a T_a of 2°C and provided water and ad lib Masuri Rodent Chow supplemented with sunflower seeds, carrots, and apples.

At least 1 month prior to the start of hibernation, temperature-sensitive radiotransmitters (Minimitter Inc., Sunriver, OR) were implanted into the peritoneal cavity of each animal (body mass range 653–943 g). Before implant, transmitters were sealed in heat shrink tubing and triple coated in Elvax (Minimitter Inc.), creating a package weight of approximately 17 g, calibrated to the nearest 0.1°C at 0 and 20°C against a precision mercury glass thermometer, and gas sterilized (Long et al. 2007). Surgeries were performed under isoflurane anesthesia. Signals from the radiotransmitters were received using a model RA1010 receiver with a computer interface for data acquisition (Data Sciences International, Saint Paul, MN). T_b and an index of locomotor activity, indicated by movements of the animal in its cage relative to two orthogonally mounted bar antennae, were recorded each 5 min.

Animals were randomly assigned to experimental treatment groups of T_a 2, -5 or -12 (each $\pm 2^\circ\text{C}$) and maintained in hanging wire cages ($28 \times 49 \times 20$ cm) with cotton batting for nest construction. Food and water were removed after hibernation began. Prior to metabolic trials, animals had been hibernating at each experimental temperature for at least 14 days. Metabolic rate and respiratory quotient (RQ) during arousal episodes were estimated by open flow respirometry (details below). Ground squirrels that had been torpid continuously for 6–11 days were placed individually into sealed lexan chambers ($42 \times 22 \times 20$ cm) positioned over a radio receiver. Metabolic chambers contained 3–5 cm of wood chips and 85 g of cotton batting as nesting material. No food or water was available within the chamber. Transfer of the animal from cage to chamber initiated an arousal as discerned by sustained increases in oxygen consumption and T_b .

For measuring rates of oxygen consumption and carbon dioxide production, room air was pulled through the metabolic chamber with a vacuum pump at 2 l min^{-1} , or 0.3 followed by 3 l min^{-1} ; the low to high flow rate sequence was used for better resolution of rates of oxygen consumption during early rewarming. Flow rate was

determined by a two different mass flowmeters; model HFM-229H, 0–0.3 l/min and AFSC-10 K, 0–10 l/min, respectively (Teldyne Hastings-Raydist, Hampton, VA). Flow meters were calibrated by measuring weight loss of a cylinder of compressed, dry air as its contents passed through the flow meter (Tøien et al. 2001).

Excurrent air was drawn through calcium chloride to remove moisture prior to measurements of flow or gas concentrations. A subsample was passed through an oxygen analyzer (Applied Electrochemistry model S-3A, Thermo Instruments Division, Pittsburgh, PA) and then through an infrared carbon dioxide analyzer (Beckman model 864, Fullerton, CA). Each 2–10 h, CO₂ and O₂ analyzers were calibrated with ambient air and the CO₂ analyzer additionally with a span gas of 1% CO₂.

The analyzers were connected to a 12-bit AD converter (Labmaster TM-40 PGL, Scientific Solutions Inc., Solion, OH). Averages of flow rate, CO₂%, O₂%, and room and metabolic chamber temperatures were logged onto a computer each minute. With 9,600 times oversampling the resolution was better than 0.0005% O₂ and 0.0001% CO₂. The data acquisition software (modified version of LabGraph, Tøien 1992) linearized the output from the CO₂ analyzer and automatically corrected data from both gas analyzers for baseline and span drift by linear interpolation.

Metabolic rate, represented by mass specific rate of oxygen consumption (ml g⁻¹ h⁻¹), was calculated by the data acquisition software using the following equations to compensate for respiratory volume change according to the principles of the Haldane transformation (Haldane 1912; Wagner et al. 1973).

$$\text{O}_2 \text{ consumption} = ((\text{Flow}_I \times \text{FI}_{\text{O}_2}) - (\text{Flow}_E \times \text{FE}_{\text{O}_2}))/\text{BM}$$

$$\text{Flow}_I = \text{Flow}_E \times (1 - \text{FE}_{\text{O}_2} - \text{FE}_{\text{CO}_2}) / (1 - \text{FI}_{\text{O}_2} - \text{FI}_{\text{CO}_2})$$

Flow_I = air flow (STPD, l h⁻¹) entering chamber, Flow_E = air flow (STPD, l h⁻¹) exiting chamber, FI_{O₂} = fraction of O₂ entering chamber, FE_{O₂} = fraction of O₂ exiting chamber, FI_{CO₂} = fraction of CO₂ entering chamber, and FE_{CO₂} = fraction of CO₂ exiting chamber, BM = body mass (kg). Temperature of the metabolic chamber was measured with a 30-ga copper-constantan thermocouple that was threaded through the lid of the chamber, extending 1–2 cm into the chamber and connected to a thermocouple amplifier (AD595, Analog devices Inc., Norwood, MA) and linearized in software (Tøien 1992). The resolution after digitizing was 0.05°C and further improved to 0.01°C by oversampling. It was calibrated in software against an NIST traceable reference thermometer in a temperature controlled water bath or stirred ice bath at 20 and 0°C. The integrity of the respirometry system was assessed every 30 days or less

during data collection by burning known amounts of 100% ethanol in the respirometry chamber with a small lamp. The stoichiometric equation of ethanol combustion was used to verify mass loss of an ethanol lamp placed inside the metabolic chamber, allowed to burn until it extinguished, and reweighed after complete washout of chamber. The ethanol burn also provided a check of the calibration gas for the CO₂ analyzer because the RQ of burning ethanol is 0.6667. Oxygen consumption during ethanol burns deviated by 2.2% (0.025 ± 0.006 l/h), and RQ deviated by 1.8% (mean RQ of ethanol trials = 0.6783 ± 0.0116).

Data analysis

We delimited the beginning of the rewarming phase of arousal as when animals were moved into the metabolic chamber and the end as when *T_b* increased to ≥30°C. The recooling phase began when *T_b* decreased and remained ≤30°C and ended when *T_b* fell to ≤5°C. The duration of the euthermic phase was thus defined as from *T_b* ≥ 30°C to first *T_b* ≤ 30°C (Barnes and Ritter 1993). Metabolic rate and *T_b* during euthermia were averaged from the first *T_b* > 35.0°C to first *T_b* < 35.0°C, including and excluding periods of elevated MR associated with movement, as detected by activity units registered by the receiver. *T_b* > 35°C was chosen for the average non-active MR rate instead of *T_b* > 30°C to exclude depression in MR at the beginning of the recooling phase and decreases in MR associated with transient decreases in *T_b*, as occurred in Fig. 1c.

The phases of each arousal episode (rewarming, euthermia, and recooling) were analyzed to determine average duration, average MR, and total oxygen consumed. Respiratory quotient was measured during rewarming and euthermia and was arcsine square root transformed prior to statistical analysis. Respiratory quotient values during recooling are not reported because these were considered to be unreliable as MR decreased rapidly while flow rates of air remained high. Peak (maximum) MR, *T_b* at initiation of trial and duration from initiation of trial to peak MR during rewarming were determined. Total costs of arousal episodes were calculated by summing the oxygen consumption of each phase. The proportional cost of arousal episodes as part of a complete cycle of hibernation that includes the torpor bout was estimated. Costs of torpor were calculated by multiplying mean MR during torpor (Buck and Barnes 2000) by the average torpor bout durations from field animals (Buck et al. 2008; Barnes et al. unpublished) that correspond to the three *T_a* treatments (2, –5 and –12°C) used in this study.

Data obtained from the three *T_a* treatments were compared using the SAS general linear model for analysis of unbalanced designs. Pair-wise comparisons were obtained

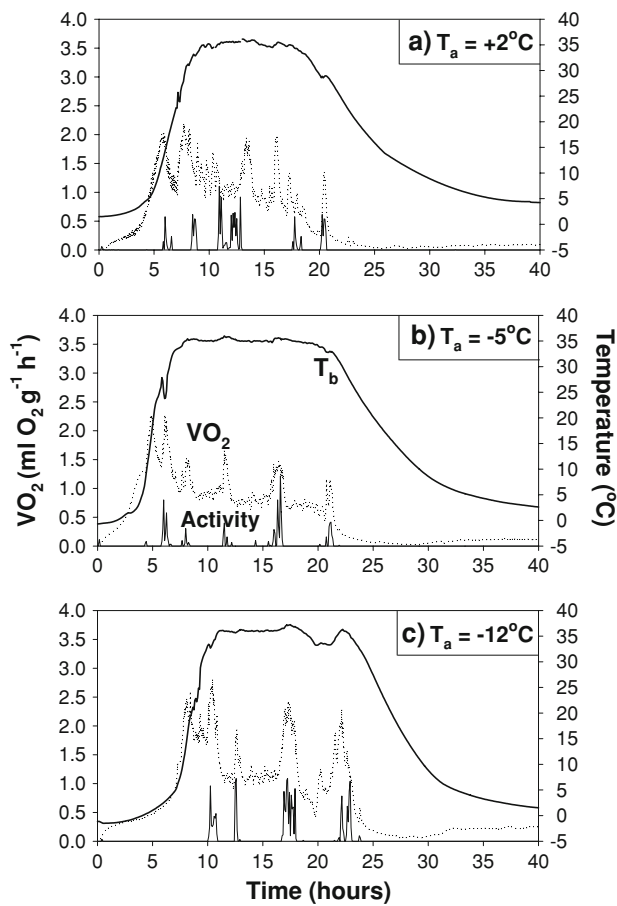


Fig. 1 Individual recordings of metabolic rate (MR), core body temperature (T_b), and activity during an arousal episode in representative arctic ground squirrels hibernating at ambient temperatures (T_a) **a** 2°C, **b** -5°C, and **c** -12°C

using least square means (Zar 1996). Results of statistical comparisons of metabolic parameters did not change when data were reanalyzed on a mass-independent basis using the general linear model with mass included as an independent variable (Hayes and Shonkwiler 1996). Each mean contains 1–2 females; interactions and covariation between sex and the parameters measured had no significant effect on the general linear model. Values are reported as mean \pm standard errors. For measurements of resting MR at each T_a , 30-min averages of rates of oxygen consumption were calculated, when MR was stable at minimal levels and not associated with activity. The corresponding 30-min average of T_b was also recorded during each minimum MR measurement.

Results

Patterns of rewarming, euthermia, and recooling during arousal episodes in arctic ground squirrels were generally similar at the three T_a 's (Fig. 1).

Rewarming phase

Body temperatures of torpid animals at the initiation of arousal differed among T_a 's, with T_b approximating ambient at T_a 2°C and averaging -0.17°C at T_a -5 and -1.44°C at T_a -12°C (Table 1). During rewarming, T_a had a significant effect on both duration of the phase and the intensity of metabolism. Rewarming from the initiation of arousal to a T_b of 30°C took 45% longer (2.5 h increase) at T_a -12°C than at 2°C ($P = 0.018$), while time to T_b 30°C at -5°C was intermediate and not significantly different from the other T_a groups (Table 1). However, when the time to rewarm from a T_b of 5 to 30°C was compared, there was no difference among T_a groups ($P = 0.12$; average for all groups 2.29 ± 0.13 h).

Compared to animals hibernating at 2°C, time from the initiation of arousal to when MR reached peak levels was 53% (2.2 h increase) longer at -5°C ($P = 0.034$) and 67% (2.8 h) longer for animals at T_a -12°C ($P = 0.002$). Peak MR also averaged 28% higher at T_a -12°C than in 2°C ($P = 0.002$) and 42% higher at -12°C than at -5°C ($P = 0.002$). Animals rewarming at T_a -12°C consumed 42% more total oxygen to reach T_b 30°C than animals rewarming at T_a 2°C ($P = 0.006$); total oxygen consumed at T_a -5°C for rewarming was intermediate and not significantly different from the other groups (Fig. 2a).

Respiratory quotient during the thermogenesis of rewarming did not differ among groups and averaged 0.71–0.72 (Table 1).

Euthermic phase

There were no differences among groups in either duration of interbout euthermia, calculated as the amount of time with $T_b > 30^\circ\text{C}$, or in total oxygen consumed during that time (Table 1; Fig. 2a). Average MR, excluding intervals with activity, did differ. Animals consumed 47% more oxygen while resting at T_a -12°C as compared to at 2°C ($P = 0.005$) (Table 1). T_b did not differ among groups when averaged either with or without intervals of activity. Respiratory quotient during interbout euthermia (0.77–0.79) was higher than during rewarming in all T_a groups ($P = 0.002$ –0.03) (Table 1).

Recooling phase

Ground squirrels reentered torpor more rapidly and sustained a higher MR while recooling at low compared to high T_a . It took approximately one-third as long at T_a -12°C ($P = 0.005$) and one-half as long at T_a -5°C for animals to recool to a T_b of 5°C, compared to in animals at T_a 2°C. Average MR during recooling at T_a -12°C was twofold higher than at 2°C ($P = 0.014$), but when duration

Table 1 Metabolic rate (MR) and body temperature (T_b) during hibernation in arctic ground squirrels at ambient temperatures (T_a) of 2, -5 and -12°C

T_a (°C)	T_b (°C)	Duration (h)	Average MR (ml g ⁻¹ h ⁻¹)	Total O ₂ (ml g ⁻¹)	Time to peak MR (h)	Peak MR (ml g ⁻¹ h ⁻¹)	RQ
Initial rewarming phase							
2	2.37 ± 0.38a	5.65 ± 0.51a	0.96 ± 0.06	5.42 ± 0.28a	4.25 ± 0.30a	2.65 ± 0.22a	0.72 ± 0.01
-5	-0.17 ± 0.24b	7.04 ± 0.44a,b	0.91 ± 0.05	6.41 ± 0.38a,b	6.49 ± 0.41b	2.39 ± 0.15a	0.71 ± 0.01
-12	-1.44 ± 0.40b	8.20 ± 0.89b	0.94 ± 0.08	7.71 ± 0.34b	7.08 ± 0.65b	3.40 ± 0.18b	0.71 ± 0.01
Interbout euthermia							
2	35.38 ± 0.53	14.75 ± 2.23	0.94 ± 0.10a	13.87 ± 2.31	b	b	0.79 ± 0.02
-5	35.49 ± 0.30	17.49 ± 2.09	1.06 ± 0.07a	18.36 ± 2.47	b	b	0.78 ± 0.01
-12	35.60 ± 0.32	14.58 ± 2.74	1.38 ± 0.05b	20.12 ± 3.22	b	b	0.77 ± 0.02
Initial recooling phase							
2	30	20.15 ± 2.39a	0.06 ± 0.01a	1.21 ± 0.24	b	b	b
-5	30	10.65 ± 1.94b	0.09 ± 0.01a,b	0.96 ± 0.26	b	b	b
-12	30	7.68 ± 0.63b	0.12 ± 0.01b	0.92 ± 0.26	b	b	b
Steady-state torpor ^a							
2	2.2	230	0.014 ± 0.001a	3.22	b	b	0.72 ± 0.02a
-5	-0.5	389	0.053 ± 0.004b	20.67	b	b	0.77 ± 0.01b
-12	-1.5	470	0.134 ± 0.008c	62.98	b	b	0.82 ± 0.02c

Values are mean ± SE. For each table cell, $n = 5-11$, mean = 6.2. Values for average MR during euthermia do not include intervals when animals were moving

Significant differences among T_a groups are denoted with different letters

^a Values for T_b , MR, and RQ during torpor are from Buck and Barnes (2000); duration of torpor bouts is from field data (Buck et al. 2008; Barnes et al. unpublished)

^b Not applicable or not available

of the recooling phase is considered, total oxygen consumed during the recooling phase did not differ among groups (Fig. 2a).

Total and proportional costs of an arousal episode

The total amount of oxygen consumed over all three phases of the arousal episode was 20.50 ± 2.44 ml O₂ g⁻¹ for T_a 2°C, 25.73 ± 2.43 ml O₂ g⁻¹ for T_a -5°C, and 28.75 ± 2.53 ml O₂ g⁻¹ for T_a -12°C and did not significantly differ among groups ($P = 0.399$). These averages do not include increases in metabolism associated with activity and the energy required for warming between T_b 30 and 35°C, as phases of the arousal episode were defined as $\leq T_b$ 30°C for rewarming and $T_b > 35°C$ for euthermia.

To calculate the total cost of a cycle of hibernation composed of an arousal episode followed by a torpor bout, we combined the average costs of an arousal episode at each T_a with the average metabolic costs of steady-state torpor in arctic ground squirrels at the same T_a 's from Buck and Barnes (2000) multiplied by the average duration of torpor bouts at the same T_a s as measured in field animals (from Buck et al. 2008; Barnes et al. unpublished) (Table 1). These results predict the total average cost of a

hibernation cycle in equivalencies of oxygen consumed as 23.7 ml O₂ g⁻¹ at 2°C, 46.4 ml O₂ g⁻¹ at -5°C, and 91.7 ml O₂ g⁻¹ at -12°C. Animals would consume 95% more oxygen during one hibernation cycle at T_a -5°C and 191% more at T_a -12°C, compared to a hibernation cycle at T_a 2°C (Fig. 2a). In this model the cost of the arousal episode in proportion to the total hibernation cycle decreases at subzero T_a 's due to the relative increase in oxygen consumed that is necessary for thermoregulation during torpor. At T_a 2°C rewarming represents 23%, euthermia 58%, recooling 5% (total for the arousal episode 86%), and torpor 14% of the hibernation cycle, compared to at T_a -5°C rewarming 14%, euthermia 39%, recooling 2% (total 55%), and torpor 45%, and at T_a -12°C, rewarming 9%, euthermia 17%, recooling 1% (total 27%), and torpor 73% (Fig. 2b).

Discussion

This study investigates the energetic costs of arousal episodes in arctic ground squirrels hibernating under ambient conditions representative of the Arctic. The population of arctic ground squirrels from which animals for this study were drawn regularly experience soil temperatures during

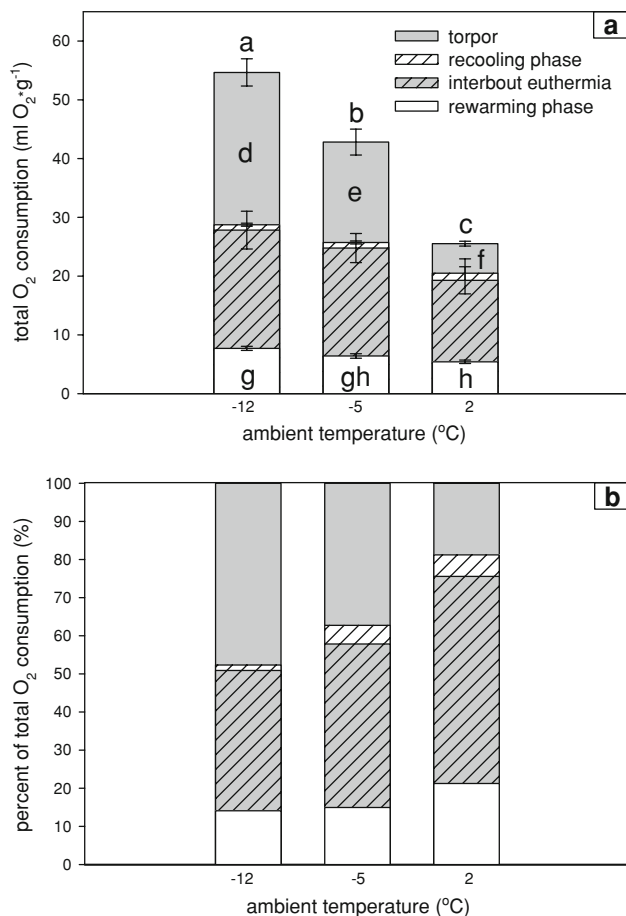


Fig. 2 Effect of ambient temperature (T_a) on each phase of hibernation expressed as totals associated with a single hibernation cycle, one arousal episode and one torpor bout (a) and as proportion of the total oxygen consumption for the hibernation season (b). Unlike letters on the bars represent statistically significant differences

winter that average between -5 and $-13^\circ C$ over the 7 month period of October–April, with minima to $-23^\circ C$ (Buck and Barnes 1999); these conditions are extreme compared to those reported for hibernacula of other hibernators. Minimum soil temperatures reported for burrows of other ground squirrels and marmots remain mostly above freezing even in alpine and northern plains environments (Fig. 3). In these non-arctic environments, thick snow cover can insulate hibernacula that may be deeper than those of arctic ground squirrels, which are limited to depths of 70–100 cm by permafrost and typically have only 12–35 cm of snow cover (Buck and Barnes 1999). Winter air temperatures in the Arctic are also very low, averaging $-25^\circ C$ October–April, near the site where animals were collected.

Extreme thermal conditions of the Arctic present a metabolic challenge not only for arousing animals rewarming to euthermia, but also for animals in torpor that maintain significant thermal gradients between

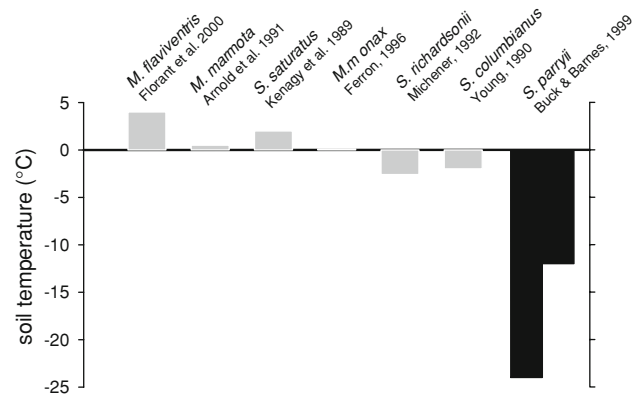


Fig. 3 Minimum overwinter hibernacula temperatures (gray bars) reported for the Sciurids *Marmota flaviventris* (Florant et al., 2000), *M. marmota* (Arnold et al., 1991), *S. saturatus* (Kenagy et al., 1989), *M. monax* (Ferron, 1996), *S. richardsonii* (Michener, 1992), *S. columbianus* (Young, 1990) and minimum and mean overwinter hibernacula temperatures (black bars) for *S. parryii* (Buck and Barnes 1999) near Toolik Lake, Alaska

temperatures of the body and that of the surrounding soil over torpor bouts of 2–3 weeks in length (Barnes and Ritter 1993). Buck and Barnes (2000) demonstrated that MR during steady-state torpor increases proportionately as T_a is decreased below $0^\circ C$; MR during torpor at $T_a -5$ and $-12^\circ C$ are 2.8- and 8.6-fold higher, respectively, than minimal values measured during steady-state torpor at $T_a 2^\circ C$ (Table 1).

Arousing under arctic conditions

Even though animals hibernating at subzero T_a in the present study began the rewarming phase of an arousal episode with elevated MR compared to animals at $2^\circ C$, it took them longer to arouse. This was partly due to the greater rise in temperature required to reach T_b $30^\circ C$ for animals beginning the rewarming process from a lower T_b , but it was also due to a protracted early rewarming as T_b warmed to about $5^\circ C$ as compared to animals in a T_a of $2^\circ C$. This was especially evident in animals at $T_a -12^\circ C$ (Fig. 1c). This could be due to a low capacity for heat production at low T_b or a lesser degree of responsiveness of animals with subzero T_b to the stimulus to arouse. Once animals were actively rewarming, peak rates of thermogenesis and overall costs of rewarming were highest in animals at $T_a -12^\circ C$, as they worked to produce the largest gradient between T_b and T_a among groups, although rates of rewarming from $T_b 5$ to $30^\circ C$ did not differ among groups. Rate of energy expenditure and overall costs of arousal have also been demonstrated to increase with decreasing T_a in the eastern pygmy-possum, *Cercartetus nanus*, hibernating over a T_a range of 0 – $30^\circ C$ (Song et al. 2000).

Euthermy

Average T_b during the euthermic phase of arousals was about 35.5°C and did not differ among T_a groups. This T_b is representative of the diurnal minimum reached each night in free-living arctic ground squirrels resting in their burrows during their active season (Long et al. 2005). Duration of the euthermic phase averaged 15.6 h and was not shorter at low T_a 's, which is contrary to our prediction that ground squirrels minimize costs of arousal episodes in colder conditions by shortening their time at high T_b . Duration of the euthermic phase in hibernating golden-mantled ground squirrels, *S. lateralis*, whose rates of heat loss and energetic costs of arousal episodes were increased by shaving dorsal fur, were also not different from non-shaved controls (Kauffmann et al. 2004). These results argue that there is an inviolate duration of euthermy that is required for arousal episodes. The functional significance of arousal episodes in hibernators remains controversial, but most hypotheses cite a requirement of high T_b to enable a physiological or neurobiological mechanism to proceed that is slowed or inhibited in torpor by low T_b (Galster and Morrison 1975; Trachsel et al. 1991; Barnes et al. 1993; Van Breukelen and Martin 2001; Prendergast et al. 2002). A requirement for a minimum duration of euthermy during arousal episodes is consistent with the need from arousals for a specific product that results from a temperature-dependent rate and time dependent process. Average resting metabolic rate during euthermia in aroused arctic ground squirrels was between 2 and 2.7 times that of BMR, which is estimated at 0.40–0.61 O₂ g⁻¹ h⁻¹ (Scholander et al. 1950; Withers et al. 1979) for this species. This elevation in metabolism in part reflects requirements for thermoregulation to maintain constant T_b at T_a that were below the lower critical temperature of 6–10°C for arctic ground squirrels (Erikson 1956).

Total oxygen consumption during the euthermic phase including periods of activity did not differ statistically among T_a groups, although there was a trend of energetic costs increasing with decreasing T_a . When resting MR in the absence of detected movement was compared, average rates increased with decreasing T_a , as was expected as animals defended larger thermal gradients. Arctic ground squirrels mostly sleep during the euthermic phase of arousals with movement related to grooming and nest repair during short periods of wake (Daan et al. 1991).

Recooling phase

During reentry into torpor, animals cooled faster at low compared to high T_a , a result that also occurred in shaved ground squirrels that were presumed to have had greater rates of heat loss (Kauffmann et al. 2004). These are not

unexpected results unless entry into torpor is considered to be a regulated process that follows a similar time-course regardless of T_a , as may be an assumption of the “sliding T_b set point” hypothesis. This hypothesis (Florant and Heller 1977; Ortman and Heldmaier 2000) suggests that hibernators control rates of change in T_b during cooling as they defend setpoint values that decrease regularly to a new minimum level that is defended during torpor. Rate of setpoint change during entry into torpor, however, may be affected by T_a and be reduced more quickly at lower temperatures to allow for a suppression of thermogenesis and faster cooling. Alternatively, the rate of decrease in the setpoint may be faster than the rate of T_b change in all groups and thus not influence cooling rates in this range of T_a .

Animals recooling in T_a -5 and -12°C anticipated the need for thermogenesis during torpor by increasing MR when T_b was approximately 5°C, well before animals neared the freezing point. Initiation of thermogenesis was evident for animals recooling only at subzero T_a values (Fig. 1b, c), while no thermogenesis was apparent at T_a 2°C (Fig. 1a). Consequently, even though the duration of the recooling phase was extended at T_a 2°C, the total oxygen consumed was not different than at -5 and -12°C.

Respiratory quotient

The RQ when averaged over hours is an indicator of the type of fuel that is being oxidized through metabolism. The RQ of 0.71 measured during rewarming in all groups is consistent with a nearly exclusive use of fat as substrate for thermogenesis as animals aroused from torpor to T_b 30°C. Rewarming during arousal is initiated through non-shivering thermogenesis in BAT which primarily uses free-fatty acids as fuel (Wilson et al. 1987; Isler et al. 1987). Shivering thermogenesis is present at a T_b of 5°C and higher during arousal in arctic ground squirrels (Tøien et al. 2001), and although shivering can be fueled either by carbohydrates or fatty acids in mammals (Haman 2006), from RQ values in the present study shivering also appears to almost exclusively use fatty acids as substrate. There was no transient rise in RQ seen during rewarming in this or in a previous study in this species (Tøien et al. 2001) to suggest that arousing arctic ground squirrels release stored CO₂, as reported during arousals in the golden-mantled ground squirrel (Snapp and Heller 1981) and after daily torpor in deer mice (Nestler 1990). Due to the rapid decline in metabolic rate and an inability to switch to lower flow rates, we were unable to record gas exchanges with the high accuracy needed for reliable RQ measurements during the recooling phase. As a ratio, RQ is more sensitive to the accuracy of the measurements than O₂ consumption alone, and RQs at low rates of oxygen consumption will

contribute equally to the average, compared to oxygen consumption where low values will contribute less to the average. Thus we were not able to determine whether a transient decrease in RQ occurred to indicate CO₂ storage during reentry into torpor. Transient changes in RQ during arousal and recooling are thought to reflect a respiratory acidosis and its reversal that may be involved in the suppression of thermogenesis and metabolism during entry into torpor and arousal (Malan 1993; Snapp and Heller 1981).

Measures of RQ during the euthermic phase rose significantly to values of 0.77–0.79 that were sustained over 15 h, indicating the recruitment of protein and/or carbohydrates as metabolic fuels. Ground squirrels fast during the heterothermic season of hibernation and do not replace metabolized substrates through dietary intake; this may be an issue for maintaining glucose homeostasis during hibernation. Since rates of gluconeogenesis in the liver are greatly slowed or inhibited at temperatures representative of torpor (Staples and Hochachka 1998), one function of arousal episodes may be to replenish carbohydrates stores (Galster and Morrison 1970). Galster and Morrison (1975) demonstrated that arctic ground squirrels increase blood glucose and liver and muscle glycogen levels during arousal episodes. Gluconeogenesis through increased rates of protein catabolism occurs, as well as from glycerol released from fat metabolism, which may explain the increase in RQ measured in the present study. RQ also rises significantly during steady-state torpor in arctic ground squirrels hibernating at subzero T_a 's. This is thought to reflect the increased utilization of glucose to support the continuously elevated levels of metabolism necessary to maintain T_b above -2.9°C (Buck and Barnes 2000).

Total and proportional costs of arousal episodes

Arousal episodes while hibernating under arctic, subfreezing conditions were not demonstrated to be energetically more costly compared to those occurring under more moderate conditions over the temperature ranges compared in this study. This conclusion follows from measuring the time and energy required by animals undergoing induced arousals under captive conditions and the assumption these do not differ from patterns associated with spontaneous arousals under natural conditions. This emphasizes that the major costs of arousal episodes in hibernating mammals result from the duration of the euthermic phase. To consider the costs of arousal episodes in the context of a complete hibernation cycle that includes the torpor bout, values of MR during torpor and lengths of torpor bouts were taken from other studies of arctic ground squirrels that compared animals across the same T_a range as the present study (Buck and Barnes 2000; Buck et al. 2008; Barnes et al.

unpublished). In the field at subzero T_a 's, the length of the torpor bout in free-living arctic ground squirrels increases (9.5 days at T_a 2°C , 16 days at T_a -5°C , and 19.5 days at T_a -12°C), as does the calculated cost per day of torpor due to increasing costs of thermoregulation as measured in captive animals (Table 1). Even though costs of individual arousal episodes do not differ with decreasing T_a 's, their relative cost within a single hibernation cycle and their overall cost as a proportion of the entire heterothermic season diminish with decreasing T_a due to the higher costs of steady-state metabolism during torpor. At T_a -12°C , arousal episodes are estimated to represent only 27% of total costs. This contrasts with previous estimates under non-arctic conditions that emphasize the relative costs of arousals. At T_a 5°C , Wang (1978) attributed 83% of the energetic cost of the hibernation season to arousals in Richardson's ground squirrels. Strijkstra (1999) calculates arousal episodes to comprise 86% of the energetic cost of the hibernation season in European ground squirrels at T_a 3 – 20°C . If the hibernation season is considered as the total time animals remain sequestered underground in their hibernacula, for male arctic ground squirrels the pre-emergent euthermic interval, a 2–3 week period in spring when animals undergo reproductive maturation, while remaining in their burrows at high T_b , is also a significant energetic cost that is estimated to represent up to 50% of entire hibernation season (Barnes 1996).

Acknowledgments This study was supported by grants from the NSF (9819540 and 0732755), the US Army Medical Research (W81XMH-06) and the Institute of Arctic Biology. Animals were maintained according to the Animal Care and Use Committee regulations of the University of Alaska Fairbanks.

References

- Arnold W, Heldmaier G, Ortmann S, Pohl H, Ruf T, Steinlechner S (1991) Ambient temperatures in hibernacula and their energetic consequences for alpine marmots (*Marmota marmota*). *J Therm Biol* 16:223–226
- Barnes BM (1989) Freeze avoidance in a mammal: body temperatures below 0°C in an arctic hibernator. *Science* 244:1521–1616
- Barnes BM (1996) Relationships between hibernation and reproduction in male ground squirrels. In: Geiser F, Hulbert AJ, Nicol SC (eds) Adaptations to the cold: 10th international hibernation symposium. University of New England Press, Armidale, pp 71–80
- Barnes BM, Ritter D (1993) Patterns of body temperature change in hibernating arctic ground squirrels. In: Carey C, Florant GL, Wunder BA, Horwitz B (eds) Life in the cold: ecological, physiological, and molecular mechanisms. Westview Press, Boulder, pp 119–130
- Barnes BM, Omtzigt C, Daan S (1993) Hibernators periodically arouse in order to sleep. In: Carey C, Florant GL, Wunder BA, Horwitz B (eds) Life in the cold: ecological, physiological, and molecular mechanisms. Westview Press, Boulder, pp 555–558

- Buck CL, Barnes BM (1999) Temperatures of hibernacula and changes in body composition of arctic ground squirrels over winter. *J Mammal* 80:1264–1276
- Buck CL, Barnes BM (2000) Effects of ambient temperature on metabolic rate, respiratory quotient, and torpor in an arctic hibernator. *Am J Physiol* 279:R255–R262
- Buck CL, Breton A, Tøien Ø, Barnes BM (2008) Overwinter body temperature patterns of free-living arctic ground squirrels (*Spermophilus parryi*). In: Lovegrove BG, McKechnie AE (eds) Hypometabolism in animals: torpor, hibernation and cryobiology. University of KwaZulu-Natal, Pietermaritzburg, pp 317–326
- Daan S, Barnes BM, Strijkstra AM (1991) Warming up for sleep?—ground squirrels sleep during arousals from hibernation. *Neurosci Lett* 128:265–268
- Erikson H (1956) Observations on the metabolism of arctic ground squirrels (*Citellus parryi*) at different environmental temperatures. *Acta Physiol Scand* 36:66–74
- Ferron J (1996) How do woodchucks (*Marmota monax*) cope with harsh winter conditions? *J Mammal* 77:412–416
- Florant GL, Heller HC (1977) CNS regulation of body temperature in euthermic and hibernating marmots (*Marmota flaviventris*). *Am J Physiol* 232:R203–R208
- Florant GL, Hill V, Ogilvie MD (2000) Circadian rhythms of body temperature in laboratory and field marmots (*Marmota flaviventris*). In: Heldmaier G, Klingenspor M (eds) Life in the cold: eleventh international hibernation symposium. Springer, Berlin, pp 223–231
- Galster W, Morrison P (1970) Cyclic changes in carbohydrate concentrations during hibernation in the arctic ground squirrel. *Am J Physiol* 218:1228–1232
- Galster W, Morrison P (1975) Gluconeogenesis in arctic ground squirrels between periods of hibernation. *Am J Physiol* 228:325–330
- Geiser F (1988) Reduction of metabolism during hibernation and daily torpor in mammals and birds: temperature effect or physiological inhibition? *J Comp Physiol B* 158:25–37
- Geiser F (2004) Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annu Rev Physiol* 66:239–274
- Geiser F, Kenagy GJ (1988) Torpor duration in relation to temperature and metabolism in hibernating ground squirrels. *Physiol Zool* 61:442–449
- Haldane JS (1912) Methods of air analysis. Charles Griffin & Co Ltd., JB Lippincott Co., Philadelphia
- Haman F (2006) Shivering in the cold: from mechanisms of fuel selection to survival. *J Appl Physiol* 100:1702–1708
- Hayes JP, Shonkwiler JS (1996) Analyzing mass-independent data. *Physiol Zool* 69:974–980
- Heldmaier G, Ortmann S, Elvert R (2004) Natural hypometabolism during hibernation and daily torpor in mammals. *Respir Physiol Neurobiol* 141:317–329
- Heller HC, Colliver GW (1974) CNS regulation of body temperature during hibernation. *Am J Physiol* 227:583–589
- Isler D, Hill HP, Meier MK (1987) Glucose metabolism in isolated brown adipocytes under beta-adrenergic stimulation. Quantitative contribution of glucose to total thermogenesis. *Biochem J* 245:789–793
- Kauffmann AS, Paul MJ, Zucker I (2004) Increased heat loss affects hibernation in golden-mantled ground squirrels. *Am J Physiol Integr Physiol* 287:R167–R173
- Kenagy GJ, Sharbaugh SM, Nagy KA (1989) Annual cycle of energy and time expenditure in a golden-mantled ground squirrel population. *Oecologia* 78:269–282
- Long RA, Martin TJ, Barnes BM (2005) Body temperature and activity patterns in free-living arctic ground squirrels. *J Mammal* 86:312–322
- Long RA, Hut RA, Barnes BM (2007) Simultaneous collection of body temperature and activity data in burrowing mammals: a new technique. *J Wildl Manage* 71(4):1375–1379
- Lyman CP (1948) The oxygen consumption and temperature regulation of hibernating hamsters. *J Exp Zool* 109:55–78
- Malan A (1993) Temperature regulation, enzyme kinetics, and metabolic depression in mammalian hibernation. In: Carey C, Florant GL, Wunder BA, Horwitz B (eds) Life in the cold: ecological, physiological, and molecular mechanisms. Westview Press, Boulder, pp 241–252
- Michener GR (1992) Sexual differences in over-winter torpor pattern of Richardson's ground squirrels in natural hibernacula. *Oecologia* 89:397–406
- Nestler JR (1990) Relationship between respiratory quotient and metabolic rate during entry to and arousal from daily torpor in deer mice (*Peromyscus maniculatus*). *Physiol Zool* 63:406–413
- Ortmann S, Heldmaier G (2000) Regulation of body temperature and energy requirements of hibernating Alpine marmots (*Marmota marmota*). *Am J Physiol Regul Integr Comp Physiol* 278:R698–R704
- Prendergast BJ, Freeman DA, Zucker I, Nelson RJ (2002) Periodic arousal from hibernation is necessary for initiation of immune responses in ground squirrels. *Am J Physiol Regul Integr Comp Physiol* 282:R1054–R1062
- Scholander PF, Hock R, Walters V, Irving L (1950) Adaptation to cold in arctic and tropical mammals and birds in relation to body temperature, insulation and basal metabolic rate. *Biol Bull* 99:259–271
- Snapp BD, Heller HC (1981) Suppression of metabolism during hibernation in ground squirrels (*Citellus lateralis*). *Physiol Zool* 54:297–307
- Song X, Körtner G, Geiser F (2000) Temperature selection and energy expenditure in the marsupial hibernator *Cercartetus nanus*. In: Heldmaier G, Klingenspor M (eds) Life in the cold: eleventh international hibernation symposium. Springer, Berlin, pp 119–126
- Staples JF, Hochachka PW (1998) The effect of hibernation status and cold-acclimation on hepatocyte gluconeogenesis in the golden-mantled ground squirrel (*Spermophilus lateralis*). *Can J Zool* 76:1734–1740
- Strijkstra AM (1999) Energy expenditure during hibernation in European ground squirrels (*Spermophilus citellus*). In: Periodic euthermia during hibernation in the European ground squirrel: causes and consequences. PhD dissertation, University of Groningen, Haren, The Netherlands
- Tøien Ø (1992) Data acquisition in thermal physiology: measurements of shivering. *J Therm Biol* 17:357–366
- Tøien Ø, Drew KL, Chao ML, Rice ME (2001) Ascorbate dynamics and oxygen consumption during arousal from hibernation in arctic ground squirrels. *Am J Physiol* 281:R572–R583
- Trachsel L, Edgar DM, Heller HC (1991) Are ground squirrels sleep deprived during hibernation? *Am J Physiol Regul Integr Comp Physiol* 260:R1123–R1129
- Twente JW, Twente JA (1965) Effects of core temperature upon duration of hibernation of *Citellus lateralis*. *J Appl Physiol* 20:411–416
- Van Breukelen F, Martin SL (2001) Translational initiation is uncoupled from elongation at 18° C during mammalian hibernation. *Am J Physiol Reg Integr Comp Phys* 281:R1374–R1379
- Wagner JA, Horwath SM, Dahms TE, Reed S (1973) Validation of open-circuit method for the determination of oxygen consumption. *J Appl Physiol* 34:859–863
- Wang LCH (1978) Energetics and field aspects of mammalian torpor: the Richardson's ground squirrel. In: Wang LCH, Hudson JW (eds) Strategies in the cold. Academic Press, New York, pp 109–145

- Willis JS (1982) The mystery of the periodic arousal. In: Lyman CP, Willis JS, Malan A, Wang LCH (eds) Hibernation and torpor in mammals and birds. Academic Press, New York, pp 92–101
- Wilson S, Thurlby PL, Arch JR (1987) Substrate supply for thermogenesis induced by the beta-adrenoceptor agonist BRL 26830A. *Can J Physiol Pharmacol* 65:113–119
- Withers PC, Casey TM, Casey KK (1979) Allometry of respiratory and haematological parameters of arctic mammals. *J Comp Physiol* 64:343–350
- Young PJ (1990) Hibernating patterns of free-ranging Columbian ground squirrels. *Oecologia* 83:504–511
- Zar JH (1996) *Biostatistical analysis*, 3rd edn. Prentice-Hall Inc., Simon and Schuster