ENERGETICS OF HIBERNATION IN THE ARCTIC GROUND SQUIRREL

(*SPERMOPHILUS PARRYII KENICOTTI*)

By

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A

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Abstract

Arctic ground squirrels (*Spermophilus parryii kenicotti*) hibernate underground; experiencing burrows temperatures of -5 to -25°C. Hibernation consists of 7-9 months of torpor interrupted every 2-3 weeks by 10-20 h arousal episodes with high body temperatures and metabolic rates. Metabolic rates and body temperatures were measured during arousal episodes under temperate and arctic conditions. No difference in cost of arousal episodes was detected. Yet, when the cost of thermogenesis during torpor was included, proportional cost of arousal episodes decreased from 86 to 27% comparing temperate to arctic conditions. The relationship between metabolic rate and body temperature was examined during the recooling phase of hibernation. As animals entered torpor, body temperature was allowed to either decrease naturally or was experimentally altered at different points along the recooling curve. The changes in metabolic rate can be explained by changes in body temperature and no sign of temperature-independent metabolic suppression was detected.

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Introduction

Hibernation in Arctic Ground Squirrels

The extreme environment represented by winter in the Arctic has captivated

biologists for generations. From a physiological point of view, adaptations allowing

animals to survive during periods with very low temperatures, short day lengths, and

poor food availability are particularly fascinating for the challenges to limits of design

and regulation of body temperature patterns and energetics. This thesis examines

hibernation, one strategy utilized to survive the winter months.

The arctic ground squirrel (*Spermophilus parryii kenicotti*) with a range that

extends north to the Arctic Ocean is the northern most hibernating rodent (Hall 1981).

Arctic ground squirrels overwintering near Toolik Lake, Alaska, experience minimum

soil temperatures at hibernacula level of -8 to -23°C (Buck and Barnes 1999a). Unique

adaptations that allow *S. p. kenicotti* to survive these extreme conditions during

hibernation include their ability to decrease core body temperatures to -2.9°C (Barnes

1989) and adopt minimal torpid metabolic rates as low as 0.01 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ (Buck and

Barnes 2000). Mechanisms that allow metabolic rate to be depressed below basal levels

are the subject of ongoing controversy.

Several decades of studies on energetics of hibernation in ground squirrels have

been conducted under temperate conditions, with only two studies at slightly sub-zero

temperatures (Heller and Coliver 1974; Geiser and Kenagy 1988), and no studies,

besides those conducted at the Institute of Arctic Biology (Barnes 1989, Buck and Barnes 1999a,b, and 2000, Barnes et al. unpublished), have been conducted under arctic conditions.

Arousal Episodes

Animals spend 7-9 months in frozen burrow systems, alternating between 2-3 week bouts of hypothermia and hypometabolism (torpor) and 10-20 h arousal episodes with high body temperatures and metabolic rates (Twente and Twente 1965; Barnes and Ritter 1993). Arousal episodes include 3 phases: rewarming, interbout euthermia and recooling. The rewarming phase is characterized by an increase in metabolic rate followed by an increase in body temperature from torpid levels to levels equivalent to euthermic nocturnal minima (Lyman 1948, Wang 1978, Daan et al. 1991). During interbout euthermia, animals are either alert or asleep, and they maintain high body temperatures (35 to 37°C) and elevated metabolic rates. The recooling phase is characterized by an abrupt decrease in metabolic rate followed by a more gradual decrease in body temperature, thus returning the animals to torpor.

The universal presence of arousal episodes among hibernating mammals suggest a limit to the length of time torpor can be sustained before a return to high body temperature and metabolic rate is required (Willis 1982). The cause of this limit in torpor duration and the functional significance of arousals remain unclear. Many current hypotheses associate arousals with the requirement for a high body temperature

to maintain a metabolism-linked process that is inhibited at low temperatures (French 1985; Geiser et al. 1993). Some examples are homeostasis of plasma glucose (Galster and Morrison 1975), fatty acids (Dark and Ruby 1993), body water (Fisher and Manery 1967), and nitrogen (Pengelley and Fisher 1961). Metabolic rate hypotheses assume that the rate of depletion or accumulations of these substances are proportional to the metabolic rate during torpor.

Although the adaptive function of arousal episodes remains uncertain, Chapter 1 quantifies the energetic costs associated with each phase of arousal episodes comparing ambient temperatures that are representative of both temperate and arctic environments. These data are then combined with torpid metabolic rate measurements (Buck and Barnes 2000) and field torpor bout lengths (Barnes et al. unpublished) to examine the total costs of hibernation contrasting temperate and arctic conditions.

Recooling phase

As ground squirrels enter torpor, core body temperature drops to just above ambient or the minimum attainable temperature of -2.9°C (Barnes 1989), and metabolic rate reaches levels as low as 1% of minimal resting levels (Buck and Barnes 2000). Explanations of the mechanisms by which hibernators depress metabolic rate during the recooling phase and torpor are controversial. Hypotheses fall into two categories: temperature-dependent or temperature-independent metabolic suppression. Temperature-dependent metabolic suppression suggests that the central nervous set

point for thermoregulation is adjusted downward. This permits body tissues to cool, after which metabolic reaction rates slow passively due to the lowered tissue temperature effect as dictated by slowed kinetics of reaction rates (Snapp and Heller 1981; Heldmaier and Ruf 1992; Hosken and Withers 1997). The temperature-dependent hypothesis is also known as the Q_{10} effect (Schmidt-Neilsen 1997). Hypothesis of temperature-independent metabolic suppression suggests that first metabolism is actively suppressed, and then set point and body temperature fall as a consequence of decreasing heat production (Malan 1993; Snyder and Nestler 1990). In Chapter 2, we first defined the appropriate starting point for the measurement of recooling as the individual's minimal metabolic rate, most likely associated with sleep (Wang 2000). Next, we examined the relationship between body temperature and metabolic rate to determine if the decline in body temperature alone is sufficient to explain the degree of metabolic suppression during the recooling phase.

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Chapter One

Energetics of Hibernation under Arctic Conditions

Prepared for submission to the *Journal of Comparative Physiology B: Biochemical Systemic and Environmental Physiology* as "Energetics of Hibernation under Arctic Conditions." By Shawna Karpovich, Øivind Tøien, and Brian M. Barnes.

Abstract. Arctic ground squirrels (*Spermophilus parryii kenicottii*) overwintering near Toolik Lake, Alaska experience minimum soil temperatures at hibernacula level of -8 to -23°C. To determine energetic costs of hibernating under arctic compared to temperate conditions, captive ground squirrels were studied at three ambient temperatures: 2, -5 and -12°C. Rates of oxygen consumption and carbon dioxide production were measured to determine metabolic rate and metabolic fuel use during the three phases of arousal episodes. Values for respiratory quotient suggest exclusive use of lipid during rewarming and mixed fuel use during interbout euthermia. Arctic ground squirrels rewarmed slower, consumed more oxygen, and reached higher peak rates of oxygen consumption at ambient temperature -12°C compared to 2°C; values at -5°C were intermediate. Ambient temperature, however, had no effect on cost or duration of the interbout euthermic phase. Animals recooled faster at -12°C than at 2°C, but rates of oxygen consumption were not different. Ambient temperature had no effect on total cost of arousal episodes, which include all three phases. Arousal episodes accounted for 86% of total hibernation costs at 2°C, 45% at -5°C and only 23% at -12°C.

Abbreviations: T_a = ambient temperature, T_b = body temperature, MR = metabolic rate, BMR = basal metabolic rate, RQ = respiratory quotient

Introduction

The annual cycle of the arctic ground squirrels (*Spermophilus parryii*) consists of a brief active season, with individuals limiting above ground activity to 3-5 months (Carl 1971; Barnes 1996; Buck and Barnes 1999b). Animals spend the remaining 7-9 months in frozen burrow systems (hibernacula), alternating between 2-3 week bouts of hypothermia and hypometabolism (torpor) and 10-20 h arousal episodes with high body temperatures (T_b) and metabolic rates (Twente and Twente 1965; Barnes and Ritter 1993). Arousal episodes include 3 phases: rewarming, interbout euthermia and recooling. During the rewarming phase, metabolic rate (MR) and thermogenesis increase to produce an increase in T_b from the low levels experienced during torpor to euthermic levels. The interbout euthermic phase consists of sleep and activity (Lyman 1948, Daan et al. 1991). The recooling phase is initiated when MR drops below basal levels and T_b declines more gradually. The universal presence of arousal episodes among hibernating mammals suggest a 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 held at ambient temperatures (T_a) >0°C has been well investigated. Animals begin the rewarming phase from steady state torpor with T_b values just above ambient and minimal rates of metabolism, as low as 1% of basal MR or 0.01 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ (Geiser 1988). Upon initiation of the rewarming phase, rates of oxygen consumption and respiration increase, followed by an increase in T_b. Rates of oxygen consumption increase rapidly, reaching a sharp peak and then fall

to euthermic levels; this is accompanied by a more gradual increase in T_b (Wang 1978). Rewarming is accomplished through nonshivering thermogenesis in brown adipose tissue together with active shivering (Galster and Morrison 1975; Tøien et al. 2001). The anterior of the body warms first due to the subscapular location of brown adipose tissue depots and restriction of blood flow from the posterior (Lyman 1948; Barnes 1989). Approximately 2-3 h after initiation of rewarming, there is a rapid increase in rectal temperatures indicating vasodilatation and reperfusion of the posterior (Lyman 1948; Tøien et al. 2001).

Interbout euthermia is the interval during which animals maintain elevated T_b values and metabolic rates. Once rewarming is accomplished, T_b stabilizes at levels that correspond to the nocturnal minima of daily temperature cycles in non-hibernating ground squirrels (Daan et al. 1991). Ground squirrels remain at elevated T_b values for 5 to 25 h. Activity, such as grooming and nest arrangement, occurs during interbout euthermia, but 93% of this time is spent in a curled hibernating posture (Wang 1978) and 66% is dedicated to sleep (Daan et al. 1991). Even though little activity occurs during interbout euthermia, it is the phase with the highest energetic cost. The interbout euthermic phase represents up to 62% of the energetic cost associated with arousal episodes and 52% of the energetic cost of the entire hibernation season (Wang 1978).

The predominant fuel utilized during hibernation under temperate conditions is lipid (Snapp and Heller 1981). However, arctic ground squirrels shift to mixed carbohydrate and lipid use during steady state torpor under arctic conditions (Buck and Barnes 1999a). Arctic ground squirrels show reduced levels of plasma glucose and

tissue glycogen during torpor, which is then replenished during arousal episodes through gluconeogenesis via metabolism of glycerol and protein (Galster and Morrison 1970; Buck and Barnes 1999a).

Arousal episodes, including all three phases, are reported to be the most energetically costly component of hibernation, representing up to 83% of the energy expended over the entire hibernation season (Wang 1978). Most available data on thermoregulatory patterns and energetic costs of hibernation, however, are from experiments conducted at ambient temperatures above 0°C. Only two studies have been at slightly sub-zero temperatures (Heller and Coliver 1974; Geiser and Kenagy 1988), and no studies of the energetics of hibernation have been investigated under arctic conditions. *S. p. kenicotti* overwintering near Toolik Lake, Alaska experience minimum soil temperatures at hibernacula level of -8 to -23°C and adopt minimum core T_b values as low as -2.9°C (Barnes 1989; Buck and Barnes 1999a). Maintenance of minimum attainable T_b values against this thermal gradient together with elevated levels of oxygen consumption measured during steady state torpor at ambient temperatures (T_a) <0°C, (Buck and Barnes 2000) indicate that arctic ground squirrels are continuously thermogenic during most of the hibernation season. This increased energetic cost associated with hibernation under arctic conditions may be expected to increase the total cost of arousal episodes. However, the increased cost associated with steady state torpor under arctic conditions may decrease the proportional cost of arousal episodes over the hibernation season.

We contrasted the cost of arousal episodes under temperate conditions to costs under temperatures equivalent to those experienced by arctic ground squirrels in their natural burrows. Metabolic trials were conducted at three T_a values: 2°C was chosen to represent temperate conditions, and -5 and -12°C as the range of average temperatures in arctic burrows. We hypothesized that, as the gradient between T_b and T_a is increased, metabolic rate would increase resulting in higher energetic costs during arousal episodes at low compared to high T_a values. We also predicted that animals would maintain interbout euthermia for shorter durations at arctic compared to temperate conditions, to offset the cost of enduring a high T_a to T_b gradient. Finally, considering the increased cost of torpor at subzero temperatures (Buck and Barnes 1999b), we hypothesized that the proportional cost of arousal episodes would decrease with decreasing T_a.

Materials and methods

Animals

Arctic ground squirrels (10 male, 2 female, all adult) captured near Toolik Lake, Alaska (68°38'N, 149°38'W) were housed individually in cages kept within environmental chambers at the University of Alaska Fairbanks. Animals were held within a 12L:12D photoperiod at T_a of 2°C and were given water and ad lib Mazuri Rodent Chow (Brentwood, MO) supplemented with sunflower seeds, carrots and

apples. For body temperature measurements, temperature-sensitive radio transmitters (model VMH-BB Mini-Mitter, Bend, OR, resolution of ± 0.2°C) were sealed in heat shrink tubing and triple coated in Elvax (Mini-Mitter, Bend, OR). Transmitter packages were 3.5 x 2.5 cm discs and weighed 17-20 g. Transmitters were calibrated at 0 and 20°C with a temperature controlled water bath and calibrated glass thermometers. Transmitters were sterilized before being surgically implanted into the abdominal cavity. Prior to surgeries, animals were administered gas anesthesia of 1-5% halothane (Halocarbon Laboratories, River Edge, NJ) mixed with medical grade oxygen and delivered at 1 liter·min^{-1}. After the animal became unresponsive to touch, a 5 x 7 cm area of the abdomen was shaved and cleaned, and a 3 cm incision was made along the midline allowing access to the abdominal cavity through the linea alba. Next, the transmitter was inserted and the linea alba and subcutis were closed with absorbable sutures. The skin was closed with a non-absorbable suture, which was removed after 14 d. Animals were allowed a 24 h recovery period in a warm room.

Metabolic rate and respiratory exchange quotient (RQ) during arousal episodes of hibernating ground squirrels were estimated by open flow respirometry. Prior to metabolic trials, animals had been hibernating at each experimental T_a, 2, -5 or -12 each ± 2°C, for at least 14 d. Ground squirrels that had been torpid continuously for 6-11 d were transferred into a sealed lexan chamber (42 x 22 x 20 cm) containing 3-5 cm of wood chips and approximately 85 g of cotton batting nesting material. No food or water was available within the chamber. When handling of the animals during the transfer initiated an arousal, which we discerned by sustained increases in oxygen

consumption and T_b, the chamber was positioned on a radio receiver. Activity was detected as the transmitter signal changed position over the receiver. T_b and locomotor activity data, from the transmitter were recorded every 5 minutes on a PC using Dataquest III software (Data Sciences International, Saint Paul, MN).

For measurements of rates of oxygen consumption and carbon dioxide production, room air was pulled through the metabolic chamber with a vacuum pump at 2 liters·min^{-1} through the whole experiment, or 0.3 followed by 3 liters ·min^{-1}; the low to high flow rate sequence was used for better resolution of rates of oxygen consumption during rewarming. Flow rate was determined by Hastings flow meters (models AFSC-10K 0-10 liters·min^{-1} and 229H 0-300 ml·min^{-1} Teledyne Hastings-Raydist, Hampton, VA) calibrated by Hastings Inc. and verified by measuring weight loss of a cylinder of compressed, dry air as its contents passed through the flowmeter.

Excurrent air was drawn through calcium chloride to remove moisture prior to measurements of flow or gas concentrations. A sub sample was passed through an oxygen analyzer (Ametek S-3A, Sunnyvale, CA, resolution $\pm$ 0.01%), then through a carbon dioxide analyzer (Beckman 415A, Fullerton, CA, resolution $\pm$ 0.05%). Each 2-10 h, the CO_2 and O_2 analyzers were calibrated with ambient air and the CO_2 analyzer with a span gas of approximately 1% CO_2. The respirometry parameters were automatically corrected for baseline and span drift by linear interpolation (LabGraph, Tøien 1992).

Metabolic rate, represented by mass specific rate of oxygen consumption (ml·g^{-1}·h^{-1}), was calculated using the following equations to compensate for respiratory

volume change according to the principles of the Haldane transformation (Haldane 1912, Wagner et al. 1973).

$$O_2 \text{ consumption} = ((Flow_I \cdot F_{IO_2}) - (Flow_E \cdot F_{EO_2}))/BM$$

$$Flow_I = Flow_E \cdot (1 - F_{EO_2} - F_{ECO_2})/(1 - F_{IO_2} - F_{ICO_2})$$

$Flow_I$ = air flow (STPD, liters·h^{-1}) entering chamber, $Flow_E$ = air flow (STPD, liters·h^{-1}) exiting chamber, F_{IO_2} = fraction of O_2 entering chamber, F_{EO_2} = fraction of O_2 exiting chamber, F_{ICO_2} = fraction of CO_2 entering chamber, and F_{ECO_2} = fraction of CO_2 exiting chamber, BM = body mass (kg).

Temperature of the metabolic chamber was measured with a 30-gauge copper-constantan thermocouple that was threaded through the lid of the metabolic chamber, extending 1-2 cm into the chamber and connected to a thermocouple thermometer calibrated at 0 and 20°C. Averages of flow rate, CO_2%, O_2%, and room and metabolic chamber temperatures were logged onto a computer each minute (LabGraph, Tøien 1992). The system as a whole was calibrated every 30 d or less, during data collection via ethanol burns. The stoichiometric equation of ethanol combustion was used to verify weight loss of an ethanol lamp placed inside the metabolic chamber and allowed to burn until it went out. Respiratory Quotient of the ethanol burn was validated in the same manner. The system was 97.72 ± 0.42% accurate (n = 15).

Data analysis

Body temperature and activity data were merged with T_a, O_2 consumption, and CO_2 production via Microsoft Excel. The end of the rewarming phase was defined as the time when T_b rose above 30°C, and the beginning of the recooling phase was when T_b fell and remained below 30°C. Interbout euthermia was thus defined as the duration from T_b >30°C to first T_b <30°C, instances when T_b declined <30°C for short periods, then returned to >30°C were not incorporated into the euthermic phase. These only occurred just prior to recooling, as if animals attempting to recool were disturbed. MR during interbout euthermia was determined as the duration from the first T_b >35°C to last T_b <35°C, excluding the 60 minutes at the end of rewarming phase and periods of elevated MR associated with locomotor activity.

The phases of rewarming, interbout euthermia, and recooling were analyzed to determine average duration, and average MR and total oxygen consumed within each phase. RQ was measured during rewarming and interbout euthermia and was arcsine square root transformed prior to statistical analysis. Peak (maximum) MR, T_b at peak MR, T_b at initiation of trial, and duration from initiation of trial to peak MR during rewarming were also determined. Total and proportional costs were calculated by summing the total oxygen consumption of each phase of arousal episodes together with total oxygen consumption during torpor, which was calculated by multiplying average torpid MR data (Buck and Barnes 2000) with torpor bout durations from field animals

(Barnes et al. unpublished) that correspond to the three T_a values, 2, -5 and -12°C used in this study.

Data obtained at the three T_a values were compared using the SAS general linear model for analysis of unbalanced designs with mass as an independent variable. Pairwise comparisons were obtained using least square means (Zar 1996). Results of statistical comparisons of metabolic parameters did not change when data were re-analyzed on a mass-independent basis using the general linear model with mass included as an independent variable (Hayes and Shonkwiler 1996). Each mean contains one or two females, interactions and covariation between sex and age and the parameters measured were determined to have no significant effect on the general linear model. Values are reported as means ± standard errors.

Results

At the initiation of arousals, core T_b was close to ambient in animals at T_a 2°C, but higher than ambient in animals held at T_a -5 and -12°C, although animals in both of these groups began rewarming with subzero mean T_b values (Table 1). Animals rewarming at T_a -12 and -5°C had initial T_b values that were 3.81°C (P < 0.001) and 2.54°C (P < 0.001) lower than the initial T_b of 2.37°C of animals rewarming at 2°C. An increase in rate of oxygen consumption followed by an increase in T_b was observed in all animals after each was transferred into the metabolic chamber (Fig. 1).

Rewarming ground squirrels took longer and reached higher metabolic rates while arousing at low compared to high T_a values. Duration from initiation of trial to peak MR values was 67% longer at T_a -12°C (P = 0.002) and 53% longer at -5°C (P = 0.034) compared to 2°C (Table 1). Peak MR values were 28% higher (P = 0.002) at T_a -12°C than at 2°C and 42% higher at -12°C than at -5°C (P = 0.002) (Table 1). T_b at the time MR reached peak was 86% higher in animals rewarming at T_a -12°C (P = 0.003) and 47% higher in animals rewarming at -5°C (P = 0.044) compared to 2°C (Table 1). Rewarming to T_b 30°C took 45% longer at T_a -12°C (P = 0.018) than at 2°C, while duration at -5°C was intermediate (Table 1). Animals rewarming at T_a -12°C consumed 38% more total oxygen to reach T_b 30°C than animals rewarming at T_a 2°C (P = 0.006) values at T_a -5°C were intermediate (Fig. 3a).

There was no difference among groups in duration of interbout euthermia, calculated as the amount of time with T_b >30°C, or in average MR, total MR, or in average T_b while animals were euthermic (Table 1, Fig. 3a). Yet, average MR did differ, when intervals associates with activity were excluded. Animals consumed 34% more oxygen while resting at T_a -12°C (P = 0.046) and 12% more at -5°C (P = 0.005) compared to 2°C (Table 1). Average resting T_b was not different than average T_b including values during activity.

Ground squirrels re-entering torpor took less time and sustained a higher MR while recooling at low compared to high T_a values. Animals recooled 162% faster at T_a

-12°C (P = 0.0004) and 89% faster at -5°C (P = 0.0002) compared to 2°C. Average MR during recooling was 100% higher at T_a -12°C (P = 0.014) than at 2°C, but when duration of the recooling phase is considered, total oxygen consumed during the recooling phase did not differ among groups (Fig. 3).

Respiratory quotient was higher during interbout euthermia than during rewarming at all three T_a values (P = 0.002 to 0.03) (Table 1).

Steady state torpor duration values from Barnes et al unpublished (230 ± 16 h at T_a 2°C, 389 ± 17 h at T_a -5°C, and 470 ± 7 h at T_a -12°C, were combined with torpor costs from Buck and Barnes 2000 (Table 1). These data were used together with the cost of the three phases of one arousal episode to calculate the total cost of a hibernation cycle represented by the total cost of one arousal episode and one steady state torpor bout. Total cost of a hibernation cycle was 23.1 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ at 2°C, 45.4 at -5°C, and 85.7 at -12°C. Animals consumed 196% more oxygen during one hibernation cycle at T_a -5°C and 371% more at T_a -12°C compared to a hibernation cycle at T_a 2°C (Fig. 4a). The proportion of the total cost of hibernation during hibernation represented by each phase of the arousal episodes decreased markedly with the addition of total oxygen consumption during torpor. The rewarming phase represents 23%, interbout euthermia 58%, recooling 5% and torpor 14% of the hibernation season at T_a 2°C, compared to 14% rewarming, 39% euthermia, 2% recooling and 45% torpor at T_a -5°C; and 9% rewarming, 17% euthermia, 1% recooling, and 73% torpor at T_a -12°C (Fig. 4b).

Discussion

The relationships between T_b and metabolic rate differ for ground squirrels hibernating under arctic compared to temperate conditions. The population of arctic ground squirrels used in this study experience whole season average soil temperatures at the hibernacula level between -5 and -13°C while hibernating in their natural burrows (Buck and Barnes 1999a). Since arctic ground squirrels exposed to subzero temperatures supercool to a minimum T_b of -2.9°C (Barnes 1989), animals hibernating under arctic conditions are obligated to support a significant gradient between T_b and soil temperature at all times (Fig. 2). This is apparent in Buck and Barnes' (2000) study that shows torpid MR increases proportionately as T_a is decreased below 0°C, at T_a -5 and -12°C are 279% and 857% higher, respectively, than the minimal values measured at 2°C.

Rewarming Phase

Animals begin the rewarming phase with T_b and MR values at the torpid level. The elevated MR associated with thermogenesis during torpor at subzero T_a values did not, however, produce a faster rewarming after arousals were stimulated (Table 1, Fig. 1). Time to reach peak MR and time to reach T_b >30°C took longer at subzero T_a values than at 2°C (Table 1). This is perhaps due to the lower initial tissue temperature in animals rewarming at T_a <0°C. Lower T_b values may slow the increase in heart rate,

rates of ventilation, and rates of nonshivering thermogenesis that are required for warming (Zimmer and Milsom 2001). This is supported by the observation that the greatest lag in rewarming was from initiation to T_b 5°C (Fig. 1). Time to rewarm from T_b 5°C to T_b 30°C was not different between groups.

Once in active rewarming, ground squirrels at T_a -12°C produced significantly higher peak MR values than did animals at 2°C (Table 1). Total oxygen consumption was also elevated, reflecting the greater work needed to overcome the larger gradient between T_a and increasing T_b values (Fig. 3). This indicates that the cost of rewarming at T_a values experienced in arctic hibernacula is elevated above the levels reported in most studies of hibernation, which are performed at T_a >0°C.

At the time animals reach peak MR, T_b is significantly warmer in animals at subzero T_a values than in animals at T_a 2°C. Since rates of oxygen consumption support the level of thermogenesis, one would expect T_b and MR to be strongly linked. Yet this does not seem to be the case, animals reach peak MR with a significantly higher T_b at subzero T_a values (Table 1). Perhaps animals rewarming at subzero T_a values begin to reperfuse the posterior of the body before peak MR is reached causing core T_b to be warmer at that point (Lyman 1948; Barnes 1989; Tøien et al. 2001).

Interbout Euthermia

When animals reached euthermic T_b values, and the gradient between T_a and T_b was sustained, average non-active MR was significantly higher at T_a values <0°C

compared to 2°C. Yet, this increased thermogenic cost was not detected when MR associated with activity was included. The prediction that animals would stay euthermic for shorter periods of time at lower T_a values to offset the greater costs associated maintaining high T_b values was not supported. This could be due to the difficulty associated with measuring natural hibernation patterns under laboratory conditions. In three of 17 trials, T_b fell below 30°C at the end of the euthermic period and then rewarmed for a short time before entering the recooling phase. These data were removed from analysis because it was most likely due to some disturbance. T_b fell and rewarmed in two trials, but not below 30°C, so these intervals were considered part of the normal euthermic period (Fig. 1c). These transient drops in T_b are not seen in the field, and as predicted, interbout euthermia is significantly shorter at T_a -12 than at 2°C during undisturbed hibernation (durations of interbout euthermia in the field: 15 ± 2 h at T_a -12, 17 ± 4 h at T_a -5 and 19 ± 6 h at T_a 2°C; Barnes et al. unpublished). However, there was still no significant difference in cost of interbout euthermia between the three T_a values upon reanalysis of the data using the durations recorded in the field. Also, both the lab and field data fall within the 10-20 h range of published euthermic durations (Twente and Twente 1965; Barnes and Ritter 1993).

Recooling Phase

Upon initiation of recooling, thermogenesis is suppressed, which allows T_b to decrease (Snapp and Heller 1981) (Fig. 1). Recooling durations in this study were

shorter at subzero T_a values than at 2°C (Table 1), and rates of the decrease in T_b were

significantly faster at T_a -12 than at 2°C (P = 0.003). Initiation of thermogenesis during

torpor, indicated by elevated MR values once T_b approached minimum levels, was

evident for animals recooling 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°C and -12°C.

Arousal Episode

The three phases of arousal episodes were combined to determine the total cost

and duration associated for the entire arousal episode. For arousal episodes, duration

was significantly shorter at subzero T_a values, compared to 2°C, but average MR was

higher. This, combined with the lack of significant differences in duration and MR

between T_a values during interbout euthermia, which was the longest and most

expensive phase (Table 1), overcame any differences that would have been predicted

from the greater cost of rewarming or the shorter duration of recooling at T_a values

<0°C (Fig. 3a). The arousal episode data was then reanalyzed using interbout euthermic

durations from field data that, in contrast to this study, are significantly shorter at lower

T_a values (Barnes et al. unpublished). This caused total oxygen consumption during

interbout euthermia to become significantly higher at T_a -12°C compared to at T_a 2°C,

but it did not cause the total oxygen consumed during the entire arousal episodes between T_a values to become significantly different (Fig. 3b).

Even though rewarming had a higher energetic cost under arctic compared to temperate conditions, the other phases of arousal episodes did not differ and costs of the entire arousal episodes do not differ (Fig. 3). Therefore, our hypothesis that arousal episodes would have higher energetic costs at low compared to high T_a values was not supported.

Respiratory Quotient

The utilization and availability of metabolic fuels during rewarming and interbout euthermic phases of hibernation were examined by measuring RQ. Respiratory quotients measured in this study are significantly different comparing phases; averaging 0.71 during rewarming and 0.77 during interbout euthermia. This suggests lipid use during rewarming and mixed fuel use during interbout euthermia. This result supports the hypothesis put forward by Galster and Morrison (1970) that during steady state torpor lipid catabolism occurs coupled to a gradual reduction in blood glucose and liver glycogen. Then as animals undergo an arousal episode, protein catabolism and gluconeogenesis take place along with lipid catabolism to restore plasma glucose and glycogen stores that are depleted during torpor.

Total and Proportional Costs of Hibernation

Total and proportional costs were represented by a single cycle of hibernation (Fig. 4) by incorporating torpid MR measurements from Buck and Barnes (2000) and torpor duration values from Barnes et al. (unpublished). This was calculated by summing the total oxygen consumption a single arousal episode and a single torpor bout. This can be used to represent proportional costs of hibernation, but it is an underestimation of the total costs associated with hibernating in the Arctic because animals hibernating under arctic conditions have longer hibernation seasons than animals hibernating under temperate conditions. Nevertheless, it was considerably more expensive to hibernate under arctic compared to temperate conditions (Fig. 4a).

Arousal episodes are considered to be the most energetically costly component of the hibernation season. At T_a 5°C, Wang (1978) attributes 83% of the energetic cost of the hibernation season to arousals in Richardson's ground squirrels. Strijkstra et al. (1999) calculates arousals to comprise 86% of the energetic cost of the hibernation season in European ground squirrels at T_a values between 3 and 20°C. In arctic ground squirrels at T_a 2°C, 86% of the total hibernation season was also associated with arousal episodes. However, maintenance of thermogenesis during torpor decreased relative cost of arousal bouts by up to 59%. Only 55% of the total cost of the hibernation season could be explained by arousal episodes at T_a -5°C and 27% at -12°C (Fig. 4b).

Acknowledgements

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Table 1.1. Metabolic rate (MR) and body temperature (T_b) parameters during hibernation in arctic ground squirrels at ambient temperatures (T_a) 2, -5 and -12°C. For each table cell, n = 5-11, mean = 6.2. Activity is included in interbout euthermic MR and T_b values. Torpid T_b, MR and RQ values are from Buck and Barnes (2000), torpid duration is from field data (Barnes et al. unpublished). Statistical significance is noted by unlike superscripts.

T_a (°C)	T_b (°C)	duration (h)	average MR ($ml \cdot g^{-1} \cdot h^{-1}$)	time to peak MR (h)	MR ($ml \cdot g^{-1} \cdot h^{-1}$)	T_b at peak MR (°C)	RQ
	(initial)		rewarming phase		(peak)		
2	2.37 ± 0.38^a	5.65 ± 0.51^a	0.96 ± 0.06	4.25 ± 0.30^a	2.65 ± 0.22^a	11.99 ± 0.89^a	0.72 ± 0.01
-5	-0.17 ± 0.24^b	7.04 ± 0.44^{ab}	0.91 ± 0.05	6.49 ± 0.41^b	2.39 ± 0.15^a	17.60 ± 1.91^b	0.71 ± 0.01
-12	-1.44 ± 0.40^b	8.20 ± 0.89^b	0.94 ± 0.08	7.08 ± 0.65^b	3.40 ± 0.18^b	22.31 ± 3.93^b	0.71 ± 0.01
	(average)		interbout euthermia		(average no activity)		
2	35.82 ± 0.21	14.64 ± 2.25	0.94 ± 0.10	*****	0.85 ± 0.14^a	*****	0.78 ± 0.02
-5	35.72 ± 0.54	16.69 ± 1.90	1.03 ± 0.07	*****	0.95 ± 0.07^b	*****	0.79 ± 0.01
-12	36.03 ± 0.35	14.41 ± 2.70	1.18 ± 0.07	*****	1.14 ± 0.05^b	*****	0.75 ± 0.01
			recooling phase				
2	*****	20.15 ± 2.39^a	0.06 ± 0.01^a	*****	*****	*****	*****
-5	*****	10.65 ± 1.94^b	0.09 ± 0.01^{ab}	*****	*****	*****	*****
-12	*****	7.68 ± 0.63^b	0.12 ± 0.01^b	*****	*****	*****	*****
	(mean)		steady state torpor				
2	2.2	230 ± 16^a	0.014 ± 0.001^a	*****	*****	*****	0.72 ± 0.02
-5	-0.5	389 ± 17^b	0.053 ± 0.004^b	*****	*****	*****	0.77 ± 0.01
-12	-1.5	470 ± 7^c	0.134 ± 0.008^c	*****	*****	*****	0.82 ± 0.02
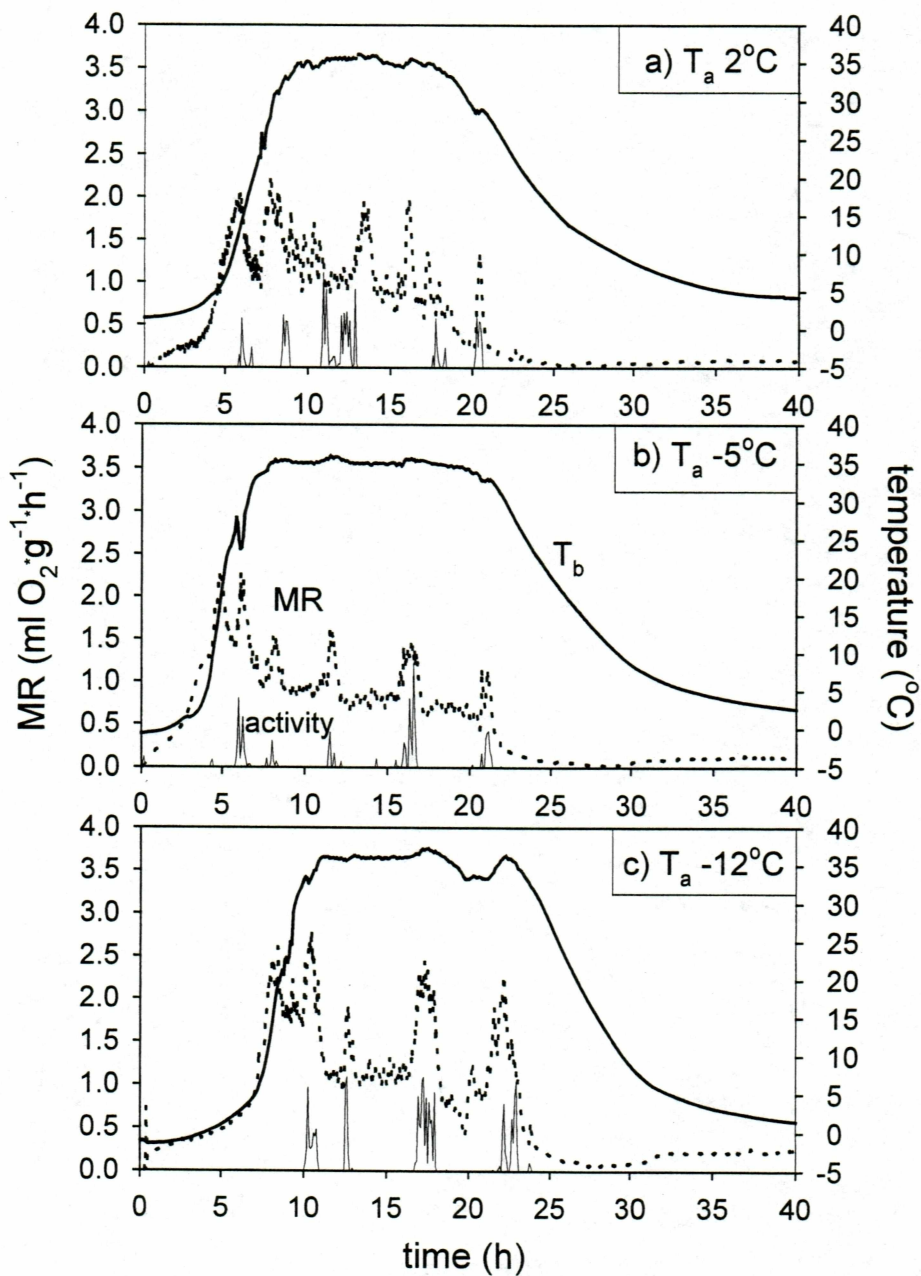
Figure 1.1. Individual recordings of metabolic rate (MR), core body temperature (T_b) and activity during an arousal episode in representative arctic ground squirrels hibernating at ambient temperatures (T_a) (a) 2°C, (b) -5°C, and (c) -12°C.
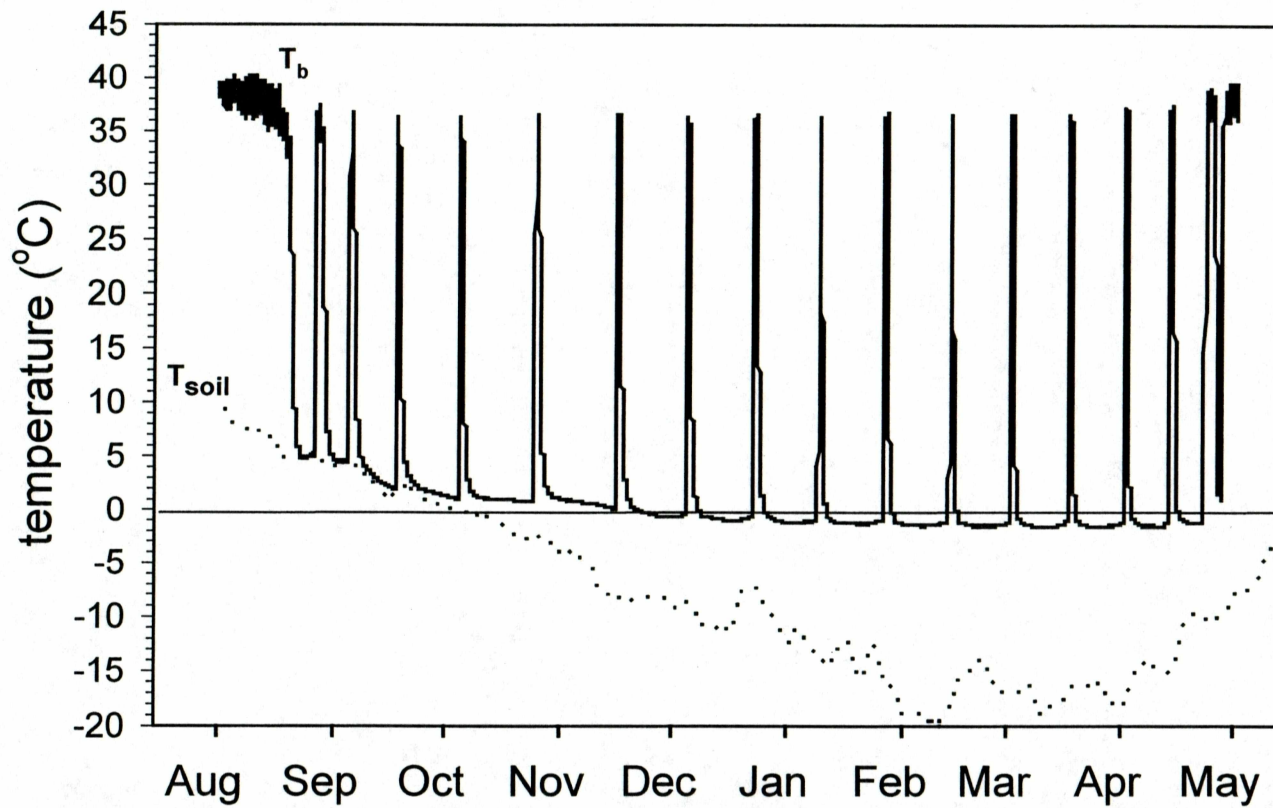
Figure 1.2. Representative recording of overwinter body temperature (T_b) and soil temperature (T_{soil}) near hibernaculum (-1m) in a natural burrow at Toolik Lake Alaska. For reference, the straight horizontal line shows where T_b falls below 0°C. Taken from Barnes et al. unpublished.
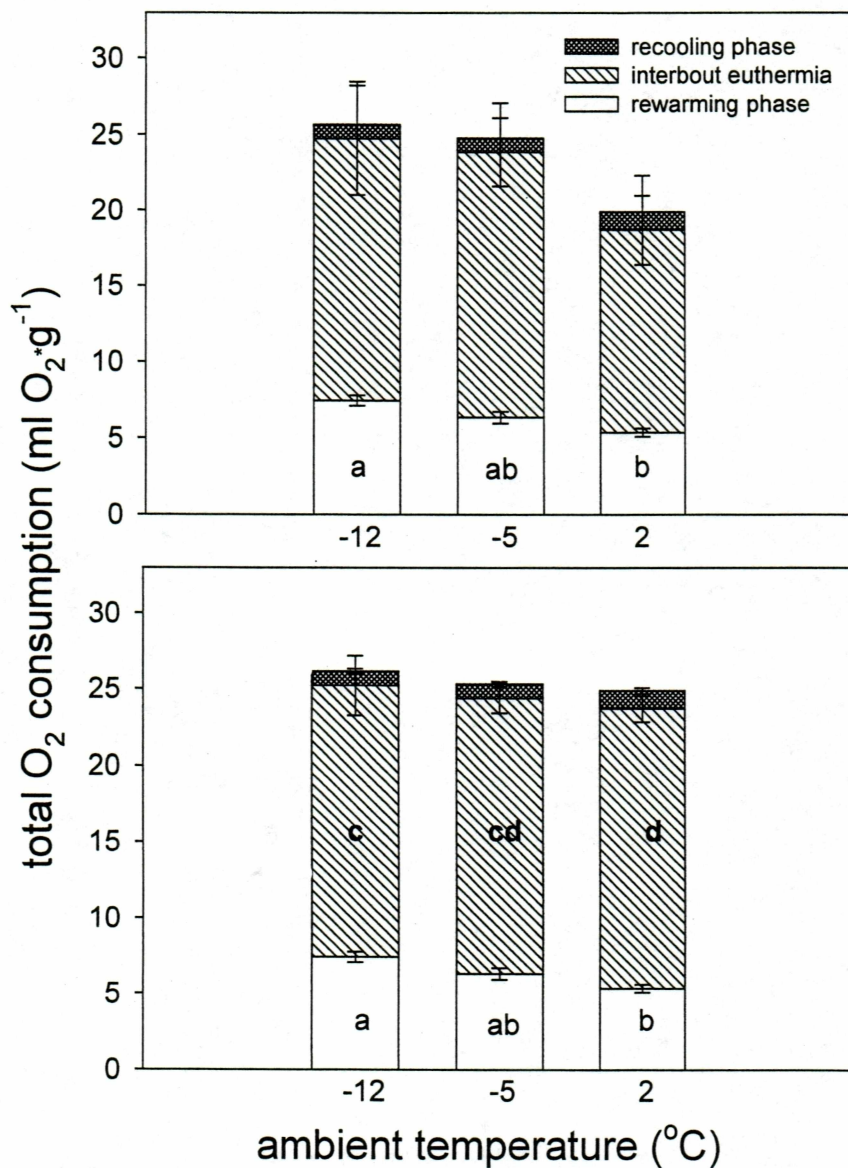
Figure 1.3. Total oxygen consumption during each phase of arousal episodes at ambient temperatures -12, -5 and 2°C, (n = 5,7,5, respectively) with interbout euthermic durations from the lab (upper panel) and from the field (n = 52; lower panel). Unlike letters on the bars represent statistically significant differences.
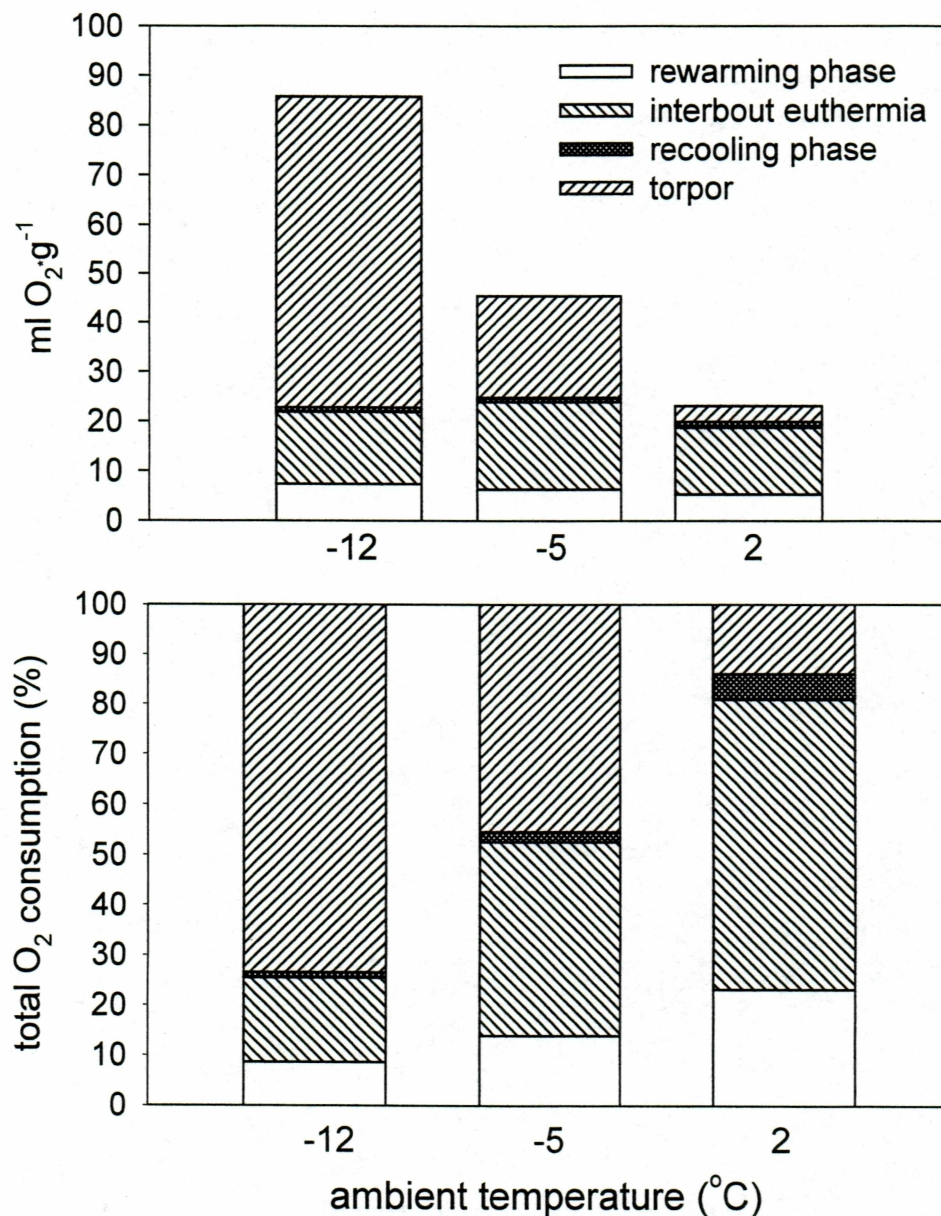
Figure 1.4. Effect of ambient temperature (T_a) on each phase of hibernation expressed as totals associated with a single hibernation cycle, one arousal episode and one torpor bout (upper panel) and as proportion of the total oxygen consumption for the hibernation season (lower panel).

Chapter Two

Metabolic Suppression During the Recooling Phase of Hibernation in the Arctic

Ground Squirrel

Abstract. A quantitative investigation into the relationship between change in body temperature (T_b) and metabolic rate (MR) patterns during entry into torpor was performed at three ambient temperatures. Minimum metabolic rate in euthermic arctic ground squirrels (*Spermophilus parryii kenicotti*) averaged 0.48 ± 0.01 ml $O_2 \cdot g^{-1} \cdot h^{-1}$. During the recooling phase, a significant effect of ambient temperature during the recooling phase was detected on rate of T_b decrease, but there was no effect on rate of MR depression. MR decreased with a temperature coefficient represented by Q_{10} values from 3.1 to 3.6. Even though these values are slightly above the biologically expected range of 2-3, the slopes of the Q_{10} lines were not significantly different than a Q_{10} of 3.

Metabolic rate and T_b measurements during recooling and torpor were also compared while T_b was experimentally warmed. Q_{10} values of 2.6 to 3.9 were observed, but slopes of the Q_{10} lines were not different than a Q_{10} of 3. These results indicate that temperature-dependent and not temperature-independent mechanisms of metabolic suppression are sufficient to explain the decrease in MR during the recooling phase of hibernation.

Keywords: Metabolic suppression, *Spermophilus*, Q_{10}, torpor, BMR

Abbreviations: T_a = ambient temperature, T_b = body temperature, MR = metabolic rate, BMR = basal metabolic rate, Q_{10} = change of rate in chemical reactions associated with a 10°C change in temperature, T_{lc} = lower critical temperature

Introduction

Ground squirrels enter hibernation while asleep (Daan et al. 1991), piloerect, curled into a tight ball with their nose tucked under their tail. Ground squirrels then spend the winter months alternating between prolonged bouts of profound hypothermia and hypometabolism (torpor) and brief arousal episodes with high body temperatures (T_b) and metabolic rates (MR) (Twente and Twente 1965; Barnes and Ritter 1993). Explanations of the mechanisms by which hibernating mammals depress MR during entry or the recooling phase of torpor and steady state torpor are controversial. Hypotheses fall into two general categories: temperature-dependent and temperature-independent metabolic suppression. Temperature-dependent metabolic suppression suggests that entry into torpor begins with a downward adjustment of the set point for thermoregulation. This permits body and tissues to cool, after which decreases in metabolism follow passively due to the Q_{10} effect (Snapp and Heller 1981; Heldmaier and Ruf 1992; Hosken and Withers 1997). Temperature-independent metabolic suppression suggests that first metabolism is actively suppressed, and then body temperature falls as a consequence of decreasing heat production (Malan 1993; Snyder and Nestler 1990). Support for temperature-independent mechanisms would come from observations of metabolic rate and T_b relationships during recooling that produce Q_{10} values >3, the upper limit of what is accepted as the normal physiological range (Geiser 1988; Song et al. 1997).

Arctic ground squirrels (*Spermophilus parryii*) hibernate at ambient temperatures, from room temperature 20°C to -20°C (Buck and Barnes 2000). This wide range in T_a values corresponds to a wide range of MR and T_b values, which offers an opportunity to look for evidence for different mechanisms of metabolic suppression during the recooling phase of hibernation. If temperature independent inhibition of metabolism is occurring, we hypothesized: a) Q_{10} of the recooling phase will be >3; b) during the recooling phase, body tissues will cool at a rate driven by T_a, but metabolic rate will decrease at a constant rate regardless of T_a; and c) increases in T_a, and therefore T_b, during the recooling phase, will have no effect on metabolic rate.

Materials and methods

Animals

Arctic ground squirrels (9 male, 7 female, all adult) captured near Toolik Lake, Alaska (68°38'N, 149°38'W) were housed individually in cages kept within environmental chambers at the University of Alaska Fairbanks. Animals were held within a 12L:12D photoperiod at T_a of 2°C and were given water and ad lib Mazuri Rodent Chow (Brentwood, MO) supplemented with sunflower seeds, carrots and apples. For body temperature measurements, temperature-sensitive radio transmitters (model VMH-BB Mini-Mitter, Bend, OR, resolution of ± 0.2°C) were sealed in heat shrink tubing and triple coated in Elvax (Mini-Mitter, Bend, OR). Transmitter packages

were 3.5 x 2.5 cm discs and weighed 17-20 g. Transmitters were calibrated at 0 and 20°C with a temperature controlled water bath and calibrated glass thermometers. Transmitters were sterilized before being surgically implanted into the abdominal cavity. Before surgeries, animals were administered gas anesthesia of 1-5% halothane (Halocarbon Laboratories, River Edge, NJ) mixed with medical grade oxygen and delivered at 1 liter·min^{-1}. After the animal became unresponsive to touch, a 5 x 7 cm area of the abdomen was shaved and cleaned, and a 3 cm incision was made along the midline allowing access to the abdominal cavity through the linea alba. Next, the transmitter was inserted and, the linea alba and subcutis were closed with absorbable sutures. The skin was closed with a non-absorbable suture, which was removed after 14 d. Animals were allowed a 24 h recovery period in a warm room.

Metabolic rate in ground squirrels before and during the recooling phase was estimated by open flow respirometry. Prior to metabolic trials, animals had been hibernating at each experimental T_a of 5, 2, -5 or -12 each ± 2°C, for at least 14 d. Ground squirrels that had been continuously torpid for 6-11 d were transferred into a sealed lexan chamber (42 x 22 x 20 cm) containing 3-5 cm of wood chips either with or without approximately 85 g of cotton batting nesting material. No food or water was available within the chamber. When handling of the animals during the transfer initiated an arousal, which we discerned by sustained increases in oxygen consumption and T_b, the chamber was positioned on a radio receiver. Activity was detected as the transmitter signal changed position over the receiver. T_b and locomotor activity data,

from the transmitter were recorded every 5 minutes on a PC using Dataquest III

software (Data Sciences International, Saint Paul, MN).

For measurements of rates of oxygen consumption and carbon dioxide

production, room air was pulled through the metabolic chamber with a vacuum pump at

1-2 liters·h^{-1}. Flow rate was determined by a Hastings flow meter (models AFSC-10K

0-10 liters·min^{-1}, Teledyne Hastings-Raydist, Hampton, VA) calibrated by Hastings Inc.

and verified by measuring weight loss of a cylinder of compressed, dry air as its

contents passed through the flowmeter.

Excurrent air was drawn through calcium chloride to remove moisture prior to

measurements of flow or gas concentrations. A sub sample was passed through an

oxygen analyzer (Ametek S-3A, Sunnyvale, CA, resolution $\pm$ 0.01%), then through a

carbon dioxide analyzer (Beckman 415A, Fullerton, CA, resolution $\pm$ 0.05%). Each 2-

10 h, the CO_2 and O_2 analyzers were calibrated with ambient air and the CO_2 analyzer

with a span gas of approximately 1% CO_2. The respirometry parameters were

automatically corrected for baseline and span drift by linear interpolation (LabGraph,

Tøien 1992).

Metabolic rate, represented by mass specific rate of oxygen consumption

(ml·g^{-1}·h^{-1}), was calculated using the following equations to compensate for respiratory

volume change according to the principles of the Haldane transformation (Haldane

1912, Wagner et al. 1973).

$$O_2 \text{ consumption} = ((\text{Flow}_I \cdot \text{FI}_{O_2}) - (\text{Flow}_E \cdot \text{FE}_{O_2}))/BM$$

$$\text{Flow}_I = \text{Flow}_E \cdot (1 - \text{FE}_{O_2} - \text{FE}_{CO_2})/(1 - \text{FI}_{O_2} - \text{FI}_{CO_2})$$

Flow_I = air flow (STPD, liters·h^{-1}) entering chamber, Flow_E = air flow (STPD, liters·h^{-1}) exiting chamber, FI_{O_2} = fraction of O_2 entering chamber, FE_{O_2} = fraction of O_2 exiting chamber, FI_{CO_2} = fraction of CO_2 entering chamber, and FE_{CO_2} = fraction of CO_2 exiting chamber, BM = body mass (kg).

Temperature of the metabolic chamber was measured with a 30-gauge copper-constantan thermocouple that was threaded through the lid of the metabolic chamber, extending 1-2 cm into the chamber and connected to a thermocouple thermometer calibrated at 0 and 20°C. Averages of flow rate, CO_2%, O_2%, and room and metabolic chamber temperatures were logged onto a computer each minute (LabGraph, Tøien 1992). The system as a whole was calibrated every 30 d or less, during data collection via ethanol burns. The stoichiometric equation of ethanol combustion was used to verify weight loss of an ethanol lamp placed inside the metabolic chamber and allowed to burn until it went out. Respiratory Quotient of the ethanol burn was validated in the same manner. The system was 97.72 ± 0.42% accurate (n = 15).

Minimum metabolic rate was measured on 13 arctic ground squirrels, average mass of 727 ± 22 g, during the non-hibernating season in summer. Animals were fasted for 8-10 h before being placed into the metabolic chamber in the dark and without a nest. T_a was initially set to –5, 5, 17.5, 20 or 28°C and programmed to warm or cool by 3 to 5°C every 6 h during 24 h long trials. The pattern of T_a, warm to cold or cold to warm, and the time of day that the trial began were randomized. Measurements to

evaluate the influence of a nest were conducted by repeating trials with 84 ± 4 g nest of cotton batting material present at T_a -12 to 5°C.

For respirometry measurements during recooling into torpor, trials were conducted with either the beginning T_a remaining unaltered or, after the animal began to recool, T_a was raised either abruptly to T_a 20-22°C or gradually ($>1°C·h^{-1}$) from T_a 5 to 35°C.

Data analysis

Data of body temperature and activity were merged with T_a and MR measurements via Microsoft Excel. For measurements of resting MR at each T_a, 30-minute averages of rates of oxygen consumption were calculated, when MR was stable at minimal levels and not associated with activity. Minimum metabolic rate was determined as the average individual minimum MR. The corresponding 30-minute average of T_b was also recorded during each minimum MR measurement. A one-way ANOVA determined that there was no significant difference in MR of animals with and without nesting material between T_a 15 and 30°C. Therefore, simple linear regression lines were fitted to MR values of animals without nesting material between T_a -5 and 15°C, and to measurements for animals with nesting material between T_a -12 and 5°C.

During recooling trials, T_b at each individual's minimum MR determined the beginning of the recooling curve and T_b 5°C was defined as the end of the recooling phase. Slopes of decreasing MR during recooling at different T_a values were compared

by calculating Q_{10} values between T_b individual minimum MR and T_b 17.5°C to exclude MR associated with thermoregulation at lower T_b values. In recooling trials, 30-minute averages of MR corresponding to discrete T_b values were used to estimate Q_{10}. The equation used to calculate Q_{10} was:

$$Q_{10} = (MR_1/MR_2)^{(10/(T_1-T_2))}$$

MR_1 is the metabolic rate at temperature T_1; MR_2 is the metabolic rate at temperature T_2.

Body temperature and MR data were converted to a logarithmic scale before being fitted with simple linear regression lines for T_b and MR reduction curves and calculation of recooling Q_{10} values. For the altered recooling data, slopes of MR change for each individual animal were averaged. Comparisons between slopes were performed with z-tests. Rates of T_b and MR decreases during unaltered recooling were compared using one-way ANOVAs; pairwise comparisons were performed with Tukey tests (Zar 1996). Sex and age were determined to have no significant effect on MR in arctic ground squirrels (Karpovich et al. 2002). All regression lines were highly significant (P <0.0001); log MR vs. T_b 2 to 15°C had a P value of 0.01. All values are reported as means ± standard errors.

Results

Average metabolic rates of euthermic arctic ground squirrels, measured at rest during their non-hibernating season in summer, were not significantly different over the

range of T_a 15 to 28°C, with minimal values occurring between T_a 22 and 25°C (Fig. 1). Average minimum MR of individuals (n = 8) was 0.48 ± 0.01 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ at T_a 24 ± 0.6°C and T_b 33 ± 0.4°C. Metabolic rate increased linearly and proportionately as T_a was decreased from 15 to -5° (Fig. 1). Metabolic rate increased with a slope corresponding to a 21% lower thermal conductance in animals within a nest compared to animals without nesting material (Table 1). Lower critical temperature (T_{lc}), determined by the intercept between the horizontal line representing minimal MR and the linear regression lines calculated from MR data at $T_a \leq 15$°C, was 21.9°C for animals without a nest and 15.3°C for animals within nests (Fig. 1). T_b was significantly higher during metabolic trails conducted at T_a -5 and 28°C, compared to at T_a 10°C, but T_b did not significantly vary at other T_a values and averaged 37 ± 0.1°C. Regression lines representing MR below T_{lc} extrapolated to T_b values of 39.5 to 38.9°C, at zero MR.

Examples of change in T_b and MR as arctic ground squirrels entered torpor at T_a values 2, -5 and -12°C are shown for representative animals in Figure 2. T_b and MR decreased exponentially, with MR rapidly decreasing from elevated levels and T_b falling more gradually. Average rates of decrease in T_b and MR at the three T_a values, calculated from linear regressions of log-transformed data, are shown in Figure 3. Animals cooled significantly faster (P <0.001) at lower compared to higher T_a values; however, rates of change of MR did not differ significantly with T_a (Figs 2 and 3). Average slopes of cooling curves, calculated between the T_b value at each individual's minimum MR (avg. beginning T_b = 33 ± 0.4°C) to T_b 5°C, were 95% and 165% steeper at T_a -5 and -12°C, respectively, compared to at T_a 2°C (Fig. 3, Table 2). Average

slopes of the decrease in MR, calculated from each individual's summer minimum MR to MR at the time T_b reached 15°C, were also steeper at lower compared to higher T_a values, but the slopes were not significantly different (Fig. 3, Table 2).

As animals re-entered torpor at T_a -5 and -12°C, MR decreased rapidly until T_b reached 25°C, when the rate of decrease became more gradual. Metabolic rate began to increase as T_b reached 10°C (Fig. 2). These animals then maintained a higher MR during torpor compared to animals at T_a 2°C.

In figure 4, average log transformed MR values during recooling at each T_a are plotted against T_b values and shown together with lines corresponding to rates of change equal to Q_{10} 2 and 3. Temperature coefficients (Q_{10}) were compared only for pre-thermogenic values above T_b 10°C. Q_{10} values for rate of change in MR versus T_b during recooling were 3.6 at T_a 2 and -5°C and 3.1 at T_a -12°C, but these values did not significantly differ from each other. All three curves were significantly steeper than a slope representing Q_{10} of 2 (P = 0.0002) but not significantly different from a slope representing than Q_{10} of 3 (Fig 4).

In experiments that altered T_a during recooling, changes in MR of arctic ground squirrels were recorded while T_b was either raised or prevented from decreasing during recooling by either slowly (2-3 days; Fig 5 upper panel) or abruptly (2-3 hrs; Fig 5 lower panel) raising T_a. MR increased as T_b increased in torpid animals. This is shown by plotting MR corresponding to integer values of T_b, as T_b was varied between 2 and 30°C (Fig. 5). The average slope of individual regression lines fitted to these data corresponds to a Q_{10} of 2.6.

Metabolic rate data during experiments that altered T_a during recooling was separated based on the findings of Buck and Barnes (2000) that showed no significant change in steady state torpid MR from T_a 0 to 16°C. Results from $T_b \geq 16$°C had a MR versus T_b relationship corresponding to Q_{10} 3.9, and data from $T_b < 16$°C corresponds to a Q_{10} 2.3 (Fig. 6, Table 1). The fitted line for all of the data extrapolates to MR 0.34 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ at T_a 38°C, while for data from $T_b \geq 16$°C the regression line extrapolates to MR 0.49 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ at T_b 38°C (Figs 6 and 7).

Discussion

During torpor, hibernating mammals can decrease MR to 1% of basal metabolic rate (Geiser 1988), but the mechanisms that cause metabolic suppression remain controversial. Geiser (1988) reviewed two theories or categories of mechanisms: strict temperature dependence and temperature independence. Temperature dependence, also known as the Q_{10} effect, hypothesizes that as T_b decreases, kinetic reaction rates of biochemical pathways linked to cellular metabolism slow according to the Van't Hoff rule. This rule states that for each 10°C change in temperature, reaction rates including whole animal metabolic rates change in parallel by a factor or Q_{10} of 2-3 (Schmidt-Neilsen 1997). Therefore, as heterothermic animals enter the recooling phase of hibernation or daily torpor and T_b decreases from 38 to 28°C, the temperature dependent hypothesis predicts that MR would concomitantly decrease by 50% ($Q_{10} = 2$) to 33% ($Q_{10} = 3$) of beginning levels. Further decreases in T_b from 28 to 18°C, from 18 to 8°C,

and finally from 8 to -2°C, the average minimal T_b measured in hibernating arctic ground squirrels (Barnes 1989), would be paralleled by further factorial changes in MR culminating for this 40°C change in T_b in an overall decrease in MR to 6% for a Q_{10} of 2 and 1% for a Q_{10} of 3 of beginning rates of oxygen consumption, assuming only aerobic metabolism. Temperature dependent hypothesis thus assume that passive changes in metabolism will follow passive changes in T_b that result from heterothermic mammals terminating defense of a high thermoregulatory set point for T_b (Snapp and Heller 1981).

The theory of temperature independence of metabolic suppression hypothesizes that MR is actively suppressed through molecular mechanisms. For example respiration and thermogenesis due to futile cycles within the mitochondria of cells may be suppressed by a regulated decrease in mitochondrial proton leak (Barger 2002). These changes at the cellular level are translated to changes at the organ and ultimately at the whole animal level. As a consequence, heat produced from metabolic reactions is reduced, and therefore, T_b decreases passively at a rate directed by the T_a-T_b gradient. Temperature independent hypothesis thus assume that active (not passive) changes in metabolism will precede (not follow) passive changes in T_b. Terminating defense of a euthermic thermoregulatory set point for T_b (Snapp and Heller 1981) is a prerequisite under both hypotheses.

During the recooling phase of hibernating arctic ground squirrels, we looked for evidence of temperature independent metabolic suppression. First, we examined the rate of decrease in both MR and T_b. If MR was being suppressed first and then T_b

followed passively, we hypothesized that there would be no difference in the rate of MR decrease at different T_a values, but that due to a changing $T_b - T_a$ gradient, T_b would cool at a rate directed by T_a. Conversely, if T_b was the force suppressing MR, the rate of decrease in MR should parallel the rate of decrease in T_b. Second, we calculated Q_{10} values during the recooling phase. If the decrease in MR was at a level beyond temperature effect of T_b alone, the relationship between MR and T_b should result in a Q_{10} value greater than 3. Third, we looked at the effect of preventing T_b from recooling during entry into torpor and the effect of rewarming T_b in animals that just completed the recooling phase. If MR was actively suppressed by a mechanism not related to T_b, alteration of T_b should have no effect on MR, which would remain "clamped" at minimum levels.

To examine metabolic suppression during the recooling phase of hibernation, the first step was to determine the appropriate rate of metabolism to compare subsequent decreases to as animals begin the entry into torpor. Use of metabolism associated with activity or thermogenesis, would yield results indicating a high degree of metabolic suppression, although basal metabolism would have been unaffected. Wang and Lee (2000) stressed the importance of comparing equivalent metabolic states at different T_b values; therefore, we measured each individual's minimum MR and then compared deviations from this value as each individual underwent the recooling phase entering into torpor.

Minimum Metabolic Rate

Previous estimates of basal metabolic rate of arctic ground squirrels (Table 3)
average 30% higher than our estimate of 0.48 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ (Fig. 1). Possible
explanations of this disparity could involve differences in body mass and energetic state
of animals. Hock (1960) performed his measurements on a different subspecies of
arctic ground squirrel *S. p. plesius* with a smaller body mass than *S. p. kenicotti*.
Smaller animals in general have higher gram specific metabolic rates (Kleiber 1961).
Also, our MR measurements were collected during 24 h trials with the animals held in
darkness; therefore many of the minimum MR values reported in Fig. 1 likely represent
MR of sleeping animals, which can have 27-29% lower basal metabolic rates than
awake animals (Snapp and Heller 1981). Arctic ground squirrels enter hibernation from
sleep (Daan et al. 1991); therefore, investigations into metabolic suppression during the
recooling phase should be examined using sleeping MR as the starting point. Snapp
and Heller (1981) compared MR of sleeping *S. lateralis* with torpid MR and calculated
a Q_{10} of 2.5, suggesting temperature dependant metabolic suppression. Furthermore,
Erikson (1956) reports "sub-basal" MR values between 0.3 to 0.6 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ on
sleeping arctic ground squirrels at T_a 30°C, values that are comparable to those in the
present study.

Lower critical temperature for *S. parryii* estimated by Scholander et al (1950)
was between 17 and 20°C and was estimated at approximately 25°C by Erikson (1956).
These values encompass our estimate of 21.9°C. Addition of cotton batting nesting

material shifted the lower critical temperature to 15.3°C, which suggests that since

burrow temperatures never warm above 10°C during the summer months (Buck and

Barnes 1999a) arctic ground squirrels are continuously thermogenic while in their

burrows. Chappell (1981) using Scholander's (1950) BMR estimate of 0.61 ml

$O_2 \cdot g^{-1} \cdot h^{-1}$, suggested that nests made of grasses might shift the lower critical

temperature to 5 to 7°C.

Body temperature did not differ significantly from T_a -2 to 25°C, but it was

elevated at T_a -5 and 28°C when compared to T_a 10°C (Fig. 1). This is most likely

associated with increased activity and thermoregulation at the coldest and warmest T_a

values in which experiments were conducted.

T_b and MR reduction during recooling

We hypothesized that if temperature independent mechanisms were responsible

for the reduction in metabolism, during the recooling phase, body tissues would cool at

a rate driven by T_a, but that metabolic rate would decrease at a constant rate regardless

of T_a. Figure 3 offers support for this hypothesis. As expected, due to the larger

gradient between body and ambient temperatures, T_b cools fastest at T_a -12°C

and slowest at 2°C, but there was no difference in the rate of MR depression, suggesting

active metabolic suppression that was not dependent on T_b.

However, the decrease in MR was extremely difficult to describe. Ground

squirrels almost always begin to recool after a bout of activity, which caused a peak in

MR (Fig. 2). This obscures the relationship between T_b and MR at the beginning of the recooling phase. Also, the thermal gradient and therefore the degree of elevation of resting MR during euthermia due to thermogenesis is lowest at T_a 2°C and highest at T_a -12°C. Therefore, at the point where thermogenesis is abandoned, the degree of elevation in MR would cause the MR reduction curve to begin from higher starting points, thus obscuring the shape of the curve. A combination of these two factors could obscure the significant difference between the MR reduction curves at the three T_a values. MR follows the same trend as T_b; this indicates the possibility of some degree of temperature dependent metabolic suppression.

Q_{10} during recooling

The relationship between MR and T_b during the recooling phase produced Q_{10} values above 3, outside of the physiologically expected range. These results also support the active metabolic suppression hypothesis (Fig. 4). However, when the slope of these lines were statistically compared to a slope of 3, there was no difference, suggesting that metabolic suppression is passive, based on changes in T_b alone.

Average T_b during euthermic minimum metabolic rate measurements was 37°C. However, average T_b at the point the recooling curve reached each animal's minimum metabolic rate was only 33°C. This represents a 4°C decrease in T_b before MR begins to decrease that was not included in the Q_{10} calculations. Figure 7 shows the beginning portion of the Q_{10} curves from figure 4 for the six trials performed at T_a -5°C. From the

beginning of the recooling phase to T_b 25°C, the slopes of the lines are steeper than a Q_{10} of 3 (Fig. 7, top panel). The curves were re-plotted with T_b 37°C in place of 33°C, the lines now are either parallel or more shallow in slope when compared to a Q_{10} of 3 (Fig. 7, bottom panel). MR was measured on a real time basis, with minimal lag between changed in gas concentrations and detection of these changes. T_b was measured with transmitters, coated in elvax and implanted into the core of the abdominal cavity, which could have caused a time lag between changes in tissue temperatures and transmitter detection of this change. Correction for a time lag between temperature changes in tissues and detection by the transmitter would decrease the Q_{10} values and offer stronger support for temperature dependent metabolic suppression. We attempted to address this by "clamping" T_b at constant temperatures during the recooling phase.

Analysis of Q_{10} values during recooling produced Q_{10} values above 3, but the slopes of the lines were not significantly different than 3. This could be argued to support both hypotheses. In order to resolve this, we experimentally altered T_a, and therefore T_b, during the recooling phase (Fig. 5). The gradual increase in T_b above a fixed T_a shown in the top panel of Figure 5 is most likely the result of metabolic heat trapped within the nest that did not dissipate due to the small gradient between T_a and T_b. This is not apparent in the trial depicted in the bottom panel because the animal arouses only a few hours after reaching T_a 22°C, where it began to experience the same low T_a to T_b gradient as the animal in the top panel.

In the altered recooling trials, MR did not decrease to minimal levels as it does in Figure 2; instead, MR increases linearly with T_b. The lack of a "clamp" that holds MR at minimum levels is evidence for mechanisms of metabolic suppression that are temperature dependent. Also, the relationship between the altered T_b values and MR values results in a Q_{10} of 2.6, which is within the biologically expected range and suggests that metabolic suppression is passive. The line fitted to the altered recooling data (Fig. 6) extrapolates to T_b 38°C at a MR of 0.34 ml $O_2 \cdot g^{-1} \cdot h^{-1}$, which is lower than our measure of BMR. This is most likely due to the error associated with extrapolation and the small sample size used to draw this line.

Metabolic rate of active ground squirrels between T_a 15 and 28°C were not significantly different (Fig. 1). Consequently, since T_b passively followed T_a during the altered recooling trials (Fig. 5, bottom panel), we split the altered recooling data in half and reanalyzed as T_b <16 or ≥16°C. Log MR plotted against T_b 0 to 15°C had a slope of 0.084 ml $O_2 \cdot g^{-1} \cdot h^{-1} \cdot °C^{-1}$ (Table 1), which was not statistically different from 0. This agrees with Buck and Barnes (2000) study that showed no change in MR of torpid arctic ground squirrels between T_a 0 and 16°C. The Q_{10} of Log MR plotted against T_b 16 to 30°C was 3.2 but had a slope not significantly different than a Q_{10} of 3. The regression line fitted to the data between T_b 16 and 30°C extrapolated to T_b 38°C, at MR of 0.49 ml $O_2 \cdot g^{-1} \cdot h^{-1}$, only 0.01 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ higher than the minimal MR measured on euthermic animals.

Support for active suppression or temperature independent metabolic suppression is marginal at best, even though MR seems to decrease independently of T_b,

and examination of the relationship between MR and T_b produced Q_{10} values above 3 during the recooling phase at the three T_a values. However, the slopes of the regression lines fitted to the MR vs. T_b data are not significantly different than the slope of a line equal to a Q_{10} of 3, indicating that the recooling curve is not different from a relationship that is temperature driven. Also, MR increased as T_b was increased in torpid animals indicating that MR is dependent on body temperature and the relationship between altered T_b and MR resulted in Q_{10} values between 2.6 and 3.2 with slopes, again, not significantly different than a Q_{10} of 3.

Therefore, from this study, we conclude that suppression of metabolic rate during the recooling phase of hibernation in arctic ground squirrels can be explained by changes in T_b alone and is, therefore, temperature dependant. This agrees with Geiser's (1988) review on reduction of metabolism, he calculated Q_{10} values by comparing euthermic and torpid T_b and MR values for 60 mammalian and 8 avian species with body mass ranging from 2 to 9000 g (most below 100 g). He concluded that in nearly all species the reduction in MR could be explained by changes in temperature alone and Q_{10} values above 3 were only observed in small hibernating species.

Acknowledgements

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Table 2.1. Slope and R^2 values for regression lines fitted to: with nest, without nest (Fig. 1), and altered recooling data (Fig. 6). Regressions were significant at the $P < 0.0001$ level, except log MR vs. T_b 2-15°C at $P = 0.01$.

regression coefficients	without nest MR vs. T_a	with nest MR vs. T_a	altered recool Log MR vs. T_b	altered recool Log MR vs. T_b (T_b 2 to 15°C)	altered recool Log MR vs. T_b (T_b 16 to 30°C)
slope (ml $O_2 \cdot g^{-1} \cdot h^{-1} \cdot °C^{-1}$)	-0.028	-0.022	0.095	0.084	0.113
R^2	0.48	0.39	0.63	0.13	0.87

Table 2.2. Slope and R^2 values for regression lines fitted to: rates of decrease in body temperature (T_b), reduction in metabolic rate (MR) (Fig. 3), and Q_{10} values of recooling data (Fig. 4). All regressions were significant at the $P < 0.0001$ level.

T_a (^oC)	rate of T_b decrease		rate of MR reduction		Q_{10}	
	Log T_b vs. time		Log MR vs. time		Log MR vs. T_b	
	slope		slope		slope	
	($^oC \cdot h^{-1}$)	R^2	(ml $O_2 \cdot g^{-1} \cdot h^{-1} \cdot {}^oC^{-1}$)	R^2	(ml $O_2 \cdot g^{-1} \cdot h^{-1} \cdot {}^oC^{-1}$)	R^2
2	-0.078_a	0.91	-0.239	0.83	0.131	0.89
-5	-0.152_b	0.94	-0.335	0.82	0.126	0.88
-12	-0.207_c	0.92	-0.346	0.78	0.110	0.93

*different subscripts denote statistical significance

Table 2.3. Estimations of basal metabolic rate for ground squirrels.

| Author	Descriptor	Estimate based on...	BMR (ml $O^2 \cdot g^{-1} \cdot h^{-1}$)
this study	*S. p. kennicotti*	oxygen consumption	0.48
Scholander et al, 1950	*S. p. kennicotti*	oxygen consumption	0.61
Erikson, 1955	*S. p. kennicotti*	oxygen consumption	0.60
Hock, 1960	*S. p. plesius*	oxygen consumption	0.80
Withers et al., 1979	*S. p. kennicotti*	oxygen consumption	0.40
Kleiber, 1947	Mammals	all mammals	0.67
McNab, 1988	Burrowing grazers	feeding habits	0.40
Hayssen and Lacy, 1985	Eutherians	all eutherians	0.51
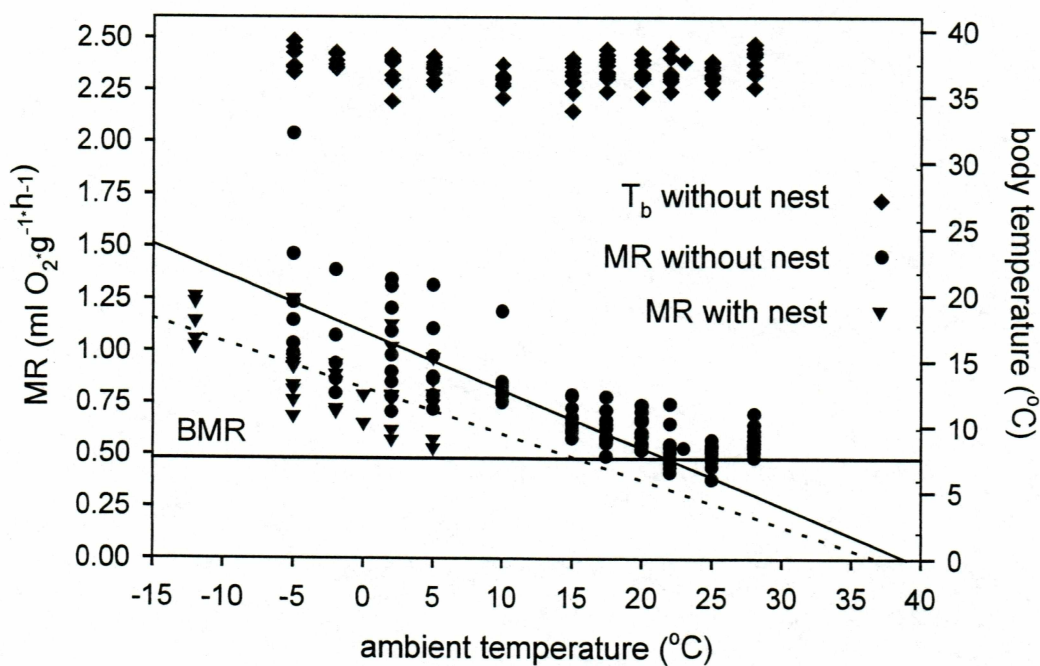
Figure 2.1. Resting metabolic rate (MR) of arctic ground squirrels with and without nesting material during the non-hibernating season, (without nest, n = 6, 6, 9, 9, 10, 13, 11, 10, 12, 1, 13, 11; with nest n = 5, 8, 5, 2, 7, 6, from left to right). Each symbol for MR represents a 30-min. average of consecutive 1-min. recordings of rates of oxygen consumption selected during 6-h trials at each T_a and intervals of no locomotor activity.
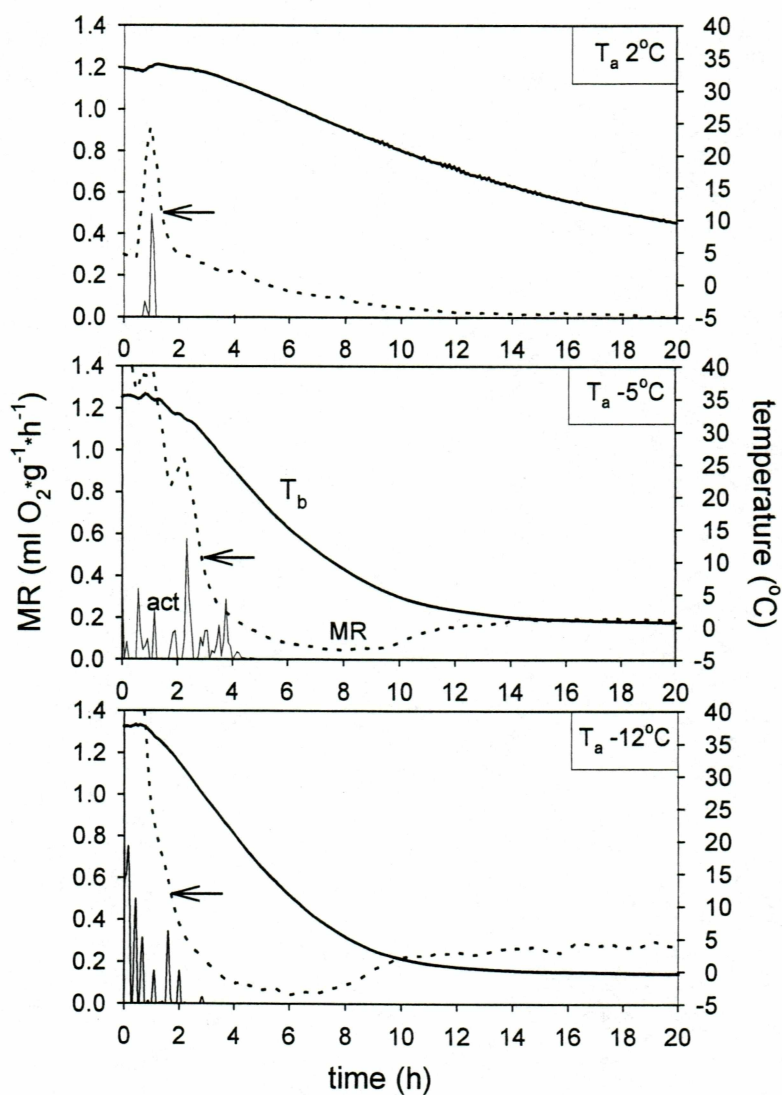
Figure 2.2. Representative recordings of body temperature (T_b, solid line) and metabolic rate (MR, dotted line) during the recooling phase in hibernating arctic ground squirrels at ambient temperatures 2°C, -5°C, and -12°C. Spikes across the x-axis indicate occurrence of activity (act), shown without units. The arrows indicate values of resting minimum metabolic rate as determined during euthermia for each individual.
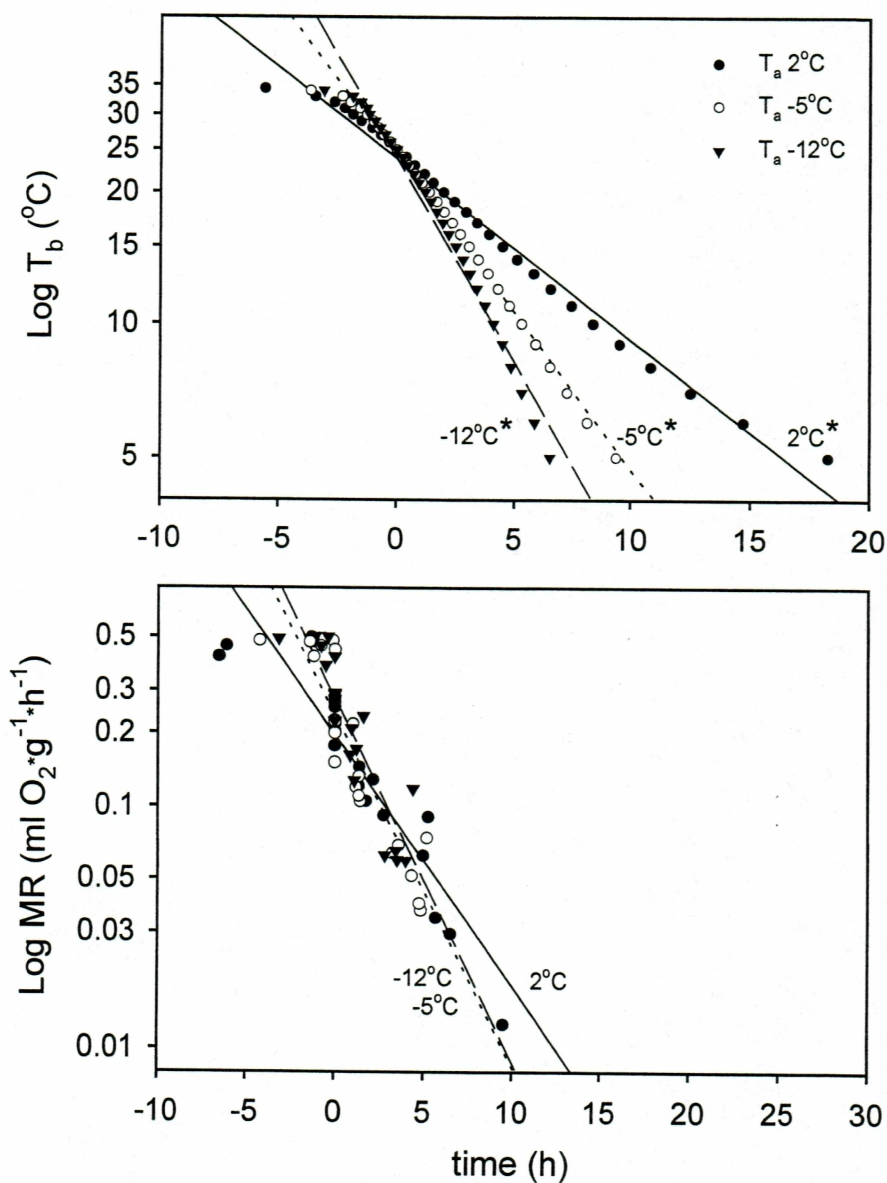
Figure 2.3. Decrease in average body temperature (T_b; upper panel) and average metabolic rate (MR; lower panel) over time during the recooling phase at ambient temperatures (T_a) 2, -5 and -12°C (n = 5, 6, 5). T_b 25°C is shown as time 0. Asterisks indicate statistical significance.
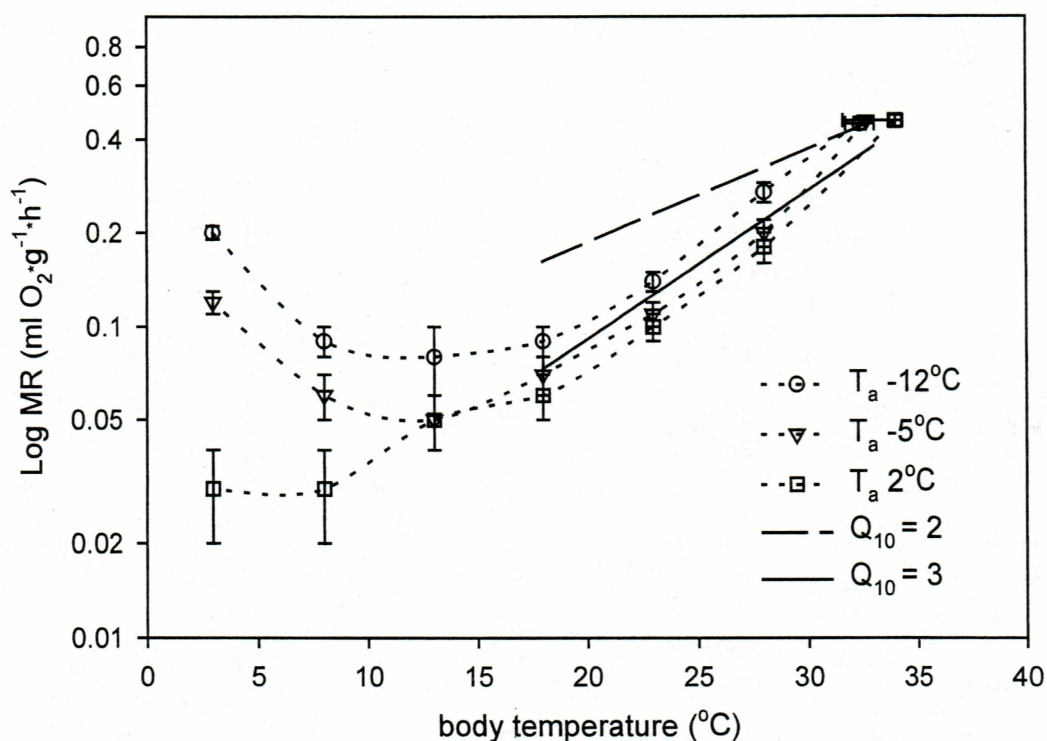
Figure 2.4. Relationship between metabolic rate (MR) and body temperature (T_b)

during the recooling phase at ambient temperatures (T_a) 2 (n = 5), -5 (n = 6) and -12°C

(n = 5). The slopes of the dotted lines represent temperature coefficient (Q_{10}) values of

3.60, 3.57 and 3.14, at T_a 2, -5 and -12°C, respectively. The solid and dashed lines

represent rates of change corresponding to Q_{10} values of 2 and 3. For Ta -5 and -12°C,

values below T_b 17.5°C are elevated due to thermogenesis, therefore no values below T_b

17.5°C were included in the analysis.
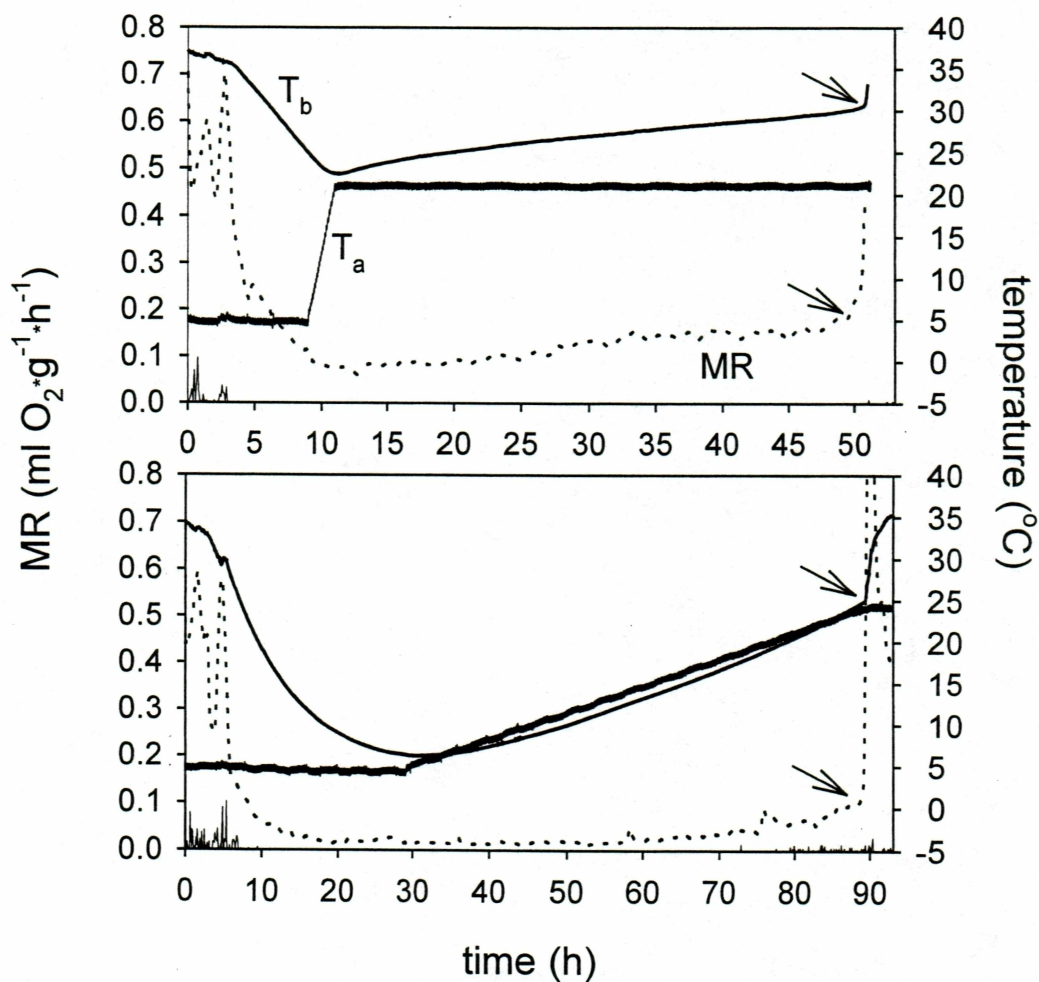
Figure 2.5. Representative recordings of individual body temperature (T_b, solid line), ambient temperature (T_a, heavy solid line), and metabolic rate (MR, broken line), during recooling trials in arctic ground squirrels when T_a was altered. The spikes across the bottom axis show occurrence of activity. Arrows indicate the increase in T_b and MR associated with initiation of an arousal.

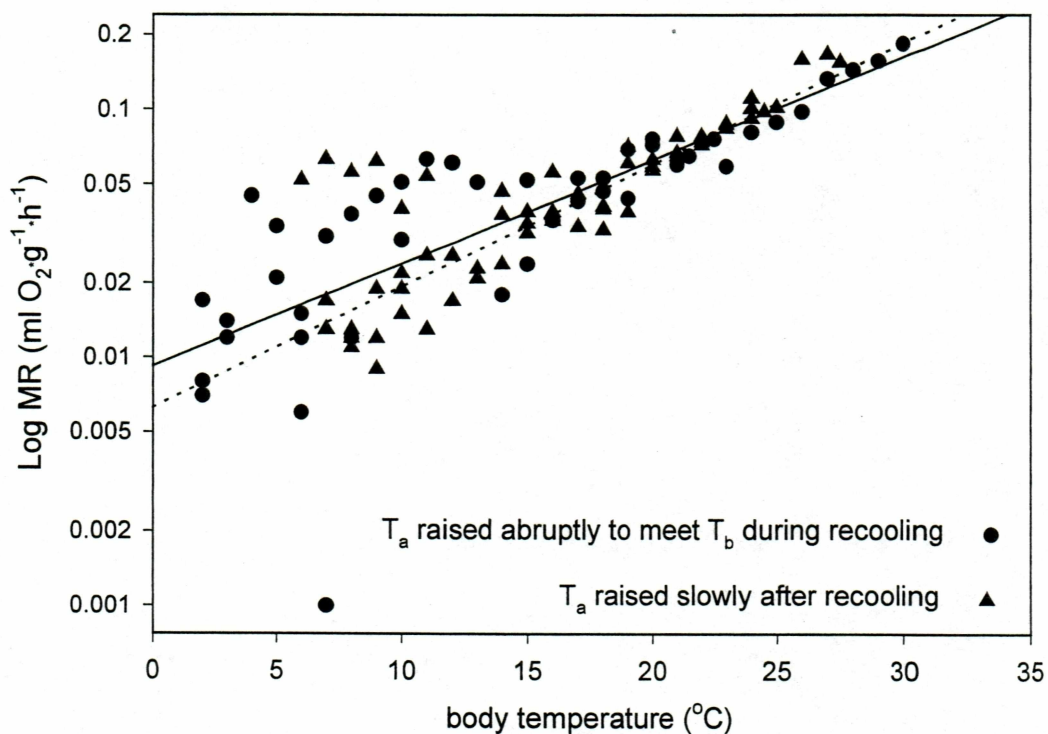Figure 2.6. Relationship between metabolic rate (MR) and body temperature (T_b) during the recooling phase of hibernation in arctic ground squirrels when ambient temperature was altered (n = 8). The broken line represents the regression line for all data and corresponds to a Q_{10} of 2.6. The solid line represents the regression line for data with $T_b \geq 16°C$ and corresponds to a Q_{10} of 3.9.
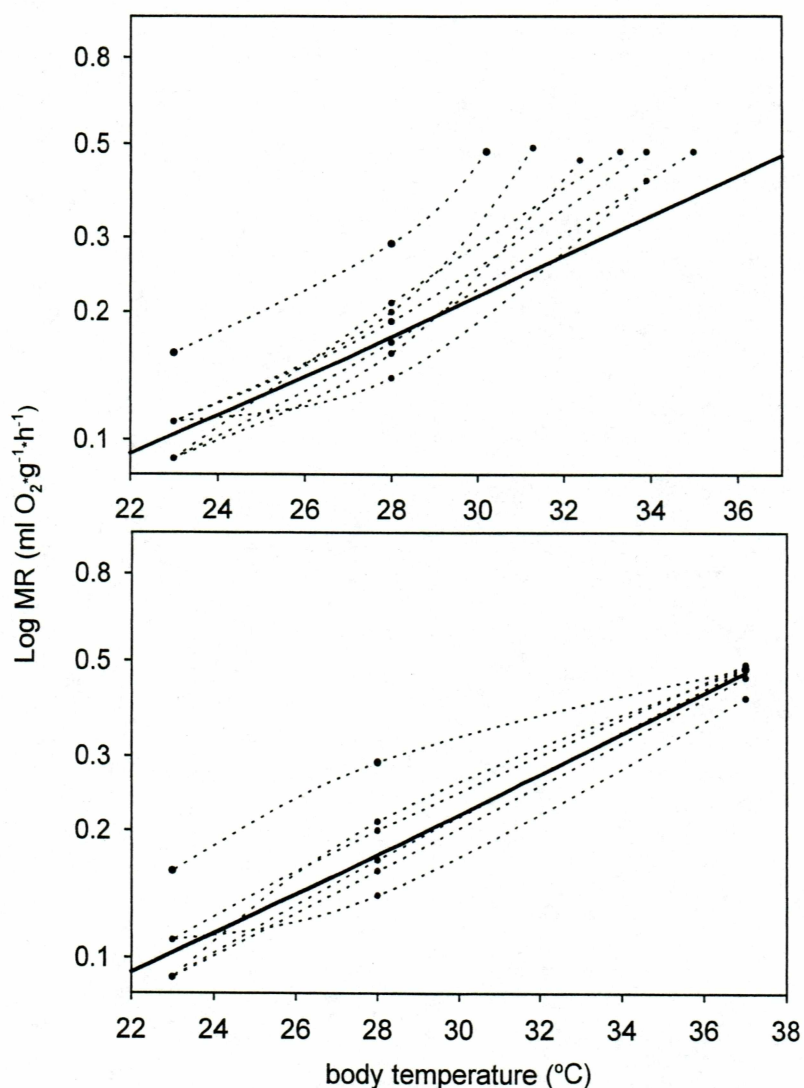
Figure 2.7. Relationship between metabolic rate (MR) and body temperature (T_b) during the beginning of the recooling phase at ambient temperature (T_a) -5°C. Each individual's minimal metabolic rate is paired with the T_b at the time the decrease in MR curve reached each individual's minimum MR during recooling (33 ± 0.04°C; top panel) or the T_b from the summer measurements of euthermic minimum metabolic rate (37 ± 0.1°C; bottom panel). The dotted lines are individual trials and the heavy solid line represents a slope corresponding to a Q_{10} of 3.

Conclusions

Arousal Episodes

Arctic ground squirrels, like temperate hibernating ground squirrels, increase metabolic rates and body temperatures every 2-3 weeks for 10-20 hours throughout the hibernation season. However, unlike temperate species, arctic ground squirrels can face a large gradient between body temperature and subfreezing ambient temperatures during torpor and arousal episodes. We examined energetic costs during the rewarming, interbout euthermic and recooling phases of arousal episodes. Rewarming was more energetically expensive under arctic compared to temperate conditions. Interbout euthermia, without activity, was also more expensive, but activity, which may have included shivering, obscured any statistically significant differences between arctic and temperate conditions. Recooling was much faster under arctic conditions, but thermoregulation at low body temperatures outweighed any significant differences in the energetic cost of recooling between arctic and temperate conditions.

Therefore, there was no difference between arctic and temperate conditions in energetic costs associated with arousal episodes. In spite of the lack of difference in total costs, when considered along with cost of torpor, the proportional costs of arousal bouts decrease considerably under arctic conditions. Maintenance of thermogenesis during torpor decreased relative cost of arousal bouts by up to 59%.

Recooling Phase

Investigation into the timing of the decreases in metabolic rate and body temperature showed body temperature cooling faster under arctic conditions, but no difference in metabolic rate was detected. This offers only weak support for temperature-independent metabolic suppression since the decrease in metabolic rate was difficult to define and was often times obscured by euthermic activity or thermogenesis.

Calculation of Q_{10} values for the relationship between body temperature and metabolic rate during the recooling phase, both with altered and unaltered body temperature recooling curves, resulted in values that were >3, which is outside of the biologically expected range and again suggests metabolic suppression. However comparison of the slope of these curves is not different than a Q_{10} of 3. So, even though the curves are slightly above 3, they do not "behave" differently than a Q_{10} of 3. We therefore concluded that the decrease in metabolic rate during the recooling phase could be explained by the change in body temperature; i.e. recooling is driven by temperature-dependant metabolic suppression.

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