Effects of ambient temperature on metabolic rate, respiratory quotient, and torpor in an arctic hibernator

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Buck, C. Loren, and Brian M. Barnes. Effects of ambient temperature on metabolic rate, respiratory quotient, and torpor in an arctic hibernator. Am J Physiol Regulatory Integrative Comp Physiol 279: R255–R262, 2000.—Arctic ground squirrels (Spermophilus parryii) overwinter in hibernacula conditions that are substantially below freezing. During torpor, captive arctic ground squirrels displayed ambient temperature (Ta)-dependent patterns of core body temperature (Tb), metabolic rate (TMR), and metabolic fuel use, as determined by respiratory quotient (RQ). At Tb 0 to –16°C, Tb remained relatively constant, and TMR rose proportionally with the expanding gradient between Tb and Ta, increasing >15-fold from a minimum of 0.0115 ± 0.0012 ml O$_2$ · g$^{-1}$ · h$^{-1}$. At Ta 0–20°C, Tb increased with Ta; however, TMR did not change significantly from Tb 0 to 12°C, indicating temperature-independent inhibition of metabolic rate. The overall change in TMR from Tb 4 to 20°C equates to a Q10 of 2.4, but within this range of Tb, Q10 changed from 1.0 to 14.1. During steady-state torpor at Tb 4 and 8°C, RQ averaged 0.70 ± 0.013, indicating exclusive lipid catabolism. At Tb –16 and 20°C, RQ increased significantly to >0.85, consistent with recruitment of nonlipid fuels. RQ was negatively correlated with maximum torpor bout length. For Tb values <0°C, this relationship supports the hypothesis that availability of nonlipid metabolic fuels limits torpor duration in hibernating mammals; for Tb values >0°C, hypotheses linked to body temperature are supported. Because anterior body temperatures differ from core, overall, the duration torpor can be extended in hibernating mammals may be dependent on brain temperature.

hibernation; metabolism; arctic ground squirrel; metabolic fuel

detailed measurements of body temperature and metabolism over a wide range of ambient temperatures, including levels below which hibernators begin to regulate their body temperature (51), and investigations at biochemical and molecular levels to identify unique or differential gene regulation related to energy metabolism across the stages of hibernation (6). The hypometabolic and hypothermic state in hibernators cannot be sustained for more than several weeks, and instead, torpor is regularly interrupted by arousals to euthermia (58). The functional significance of these arousal episodes is not known, but hypotheses generally relate to either metabolic rate and metabolic fuel-dependent processes or, alternatively, body temperature-dependent processes. Separating effects of metabolic rate and body temperature on frequency of arousals and thus torpor bout length requires investigations over a wide range of ambient temperature (Ta) over which metabolic rate and body temperature become uncoupled.

Although ample comparative data have been published on metabolic rate during torpor (TMR) and metabolic fuel use of mammalian hibernation during torpor at Ta values >0°C, only a few studies have been done at Ta slightly below 0°C (1, 25, 29) and none in the conditions that prevail in hibernacula in the arctic (−5 to −25°C) (2, 9). To determine the energetic costs and substrate use associated with hibernation under arctic conditions, we measured core body temperature (Tb), TMR, and respiratory quotient (RQ) of arctic ground squirrels (Spermophilus parryii) in steady-state torpor at subzero Tb values (Spermophilus parryii) in steady-state torpor at subzero Ta values from 0 to −16°C. To further investigate relationships between Tb, TMR, and Ta and to investigate temperature-independent inhibition of metabolic rate in hibernating arctic ground squirrels, we extended measurements to Ta values of 0–20°C. To examine how torpor bout length changes in response to changes in Tb, Tb, TMR, and metabolic fuel use, in parallel experiments we measured frequency of arousal episodes in a group of undisturbed arctic ground squirrels at Ta values ranging from −16 to 20°C.

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METHODS

Animals. Arctic ground squirrels (S. p. hennicottii) were live-trapped in July in the northern foothills of the Brooks Range, Alaska, near the Aitigun River (68° 38’ N latitude) and transported to the animal holding facility at the University of Alaska Fairbanks. Animals were maintained individually in metal cages (45.7 × 30.5 × 20.3 cm) at a photoperiod of 12:12 h light-dark cycle and Ta of 5 ± 2°C before the beginning of the experiment. Food (Mazuri Rodent Chow, carrots, and sunflower seeds) and water were provided ad libitum.

Body temperature. To record Tb, temperature-sensitive radio transmitters (model VMH-BB, Miniminiter, Sunriver, OR) were surgically implanted into each animal’s peritoneal cavity at least 1 mo before the start of metabolic measurements. For surgery, animals were anesthetized with methoxyflurane. Beforehand, transmitters were calibrated to the near vicinity at least 1 mo before the start of metabolic measurements.

Respirometry. Rates of oxygen consumption and carbon dioxide production were recorded during steady-state torpor for eight adult animals (4 male, 4 female) at Ta = −16, −8, −4, 0, 4, 8, 12, 16, and 20°C (each ±0.5°C). Metabolic measures were not recorded from all animals at Ta = −8 to −20°C because not all animals continued to hibernate at these relatively high temperatures. Animals were tested after they had been in hibernation for at least 1 mo, and measurements of TMR began 4 days after Tb of each subject animal had decreased to below 30°C during entry into a torpor bout. TMR began 4 days after Tb of each subject animal had decreased to below 30°C during entry into a torpor bout. Tb averaged 1.97°C at Ta = 0°C, Tb averaged 0.0004°C at Ta = 1.97°C at Ta = 0°C, Tb averaged 0.0004°C at Ta = 8°C, Tb averaged 0.52°C at Ta = 8°C, Tb averaged 0.52°C at Ta = 16°C, Tb averaged 0.12°C and 0.12°C at Ta = 16°C, Tb averaged 0.12°C and 0.12°C at Ta = 20°C, Tb averaged 0.42°C at Ta = 20°C, Tb averaged 0.42°C at Ta = 24°C, Tb averaged 10.22°C at Ta = 24°C, Tb averaged 10.22°C at Ta = 26°C, Tb averaged 0.33°C at Ta = 26°C, Tb averaged 0.33°C at Ta = 30°C as measured by radiotelemetry. The lowest Tb of each subject animal was 30°C as measured by radiotelemetry. The lowest Tb of each subject animal was 30°C as measured by radiotelemetry. The lowest Tb of each subject animal was 30°C as measured by radiotelemetry. The lowest Tb of each subject animal was 30°C as measured by radiotelemetry.

Data are presented as means ± SE. Statistical evaluations of multiple group comparisons were completed with a one-way ANOVA and pairwise comparisons with a Tukey’s test. A Student’s t-test was used for between-group comparisons, and nonnormally distributed data were analyzed with a Mann-Whitney’s rank sum test (66). A simple linear regression model was used to test for significant correlations between variables. For regression analysis, TMR results for Ta values >0°C were log transformed to meet the assumptions of equal variance. We compared the coefficient of determination (r²) to select between linear and curvilinear models. RQ data were transformed (arcsin square root transformation) before analysis to meet the assumptions of normality for parametric tests. In reporting sample sizes, N represents the number of animals and n the number of measurements. Because the regression analyses violate assumptions of independence and may underestimate α due to pseudoreplication, differences were considered significant at P < 0.01. Differences were considered significant at P < 0.05 for nonregression analyses.

RESULTS

Thermoregulatory patterns during torpor at differing Ta. Patterns of thermoregulatory heat production of arctic ground squirrels hibernating at different Ta values depended on whether Ta was higher or lower than 0°C. At Ta values <0°C, all animals increased rates of thermogenesis to maintain a relatively constant Tb. At Ta values >0°C, animals maintained minimal levels of heat production as Tb varied with Ta (Fig. 1). At Ta = 0°C, three of eight animals had increased rates of thermoregulatory heat production as indicated by a greater ΔT (Tb − Tbvar), which ranged from 1.20 to 1.26°C, and an elevated TMR, averaging 0.020 ± 0.0027. These values were significantly greater than corresponding values in the remaining five animals of AT averaging 0.52°C (P < 0.05) and TMR averaging 0.014 ± 0.0004 (P < 0.05). From these results, we considered torpid animals to be actively thermoregulating if ΔT was >1°C.

At Ta values <0°C, Tb averaged −0.42 ± 0.12°C and did not change significantly with changing Ta (P = 0.071, N = 8, n = 24; Fig. 1). The lowest Tb recorded was −1.97°C at Ta = −8°C. From its minimum value of 0.0115 ± 0.0012 ml O₂·g⁻¹·h⁻¹ at Ta = 4°C, TMR increased 15.8-fold to 0.182 ± 0.0244 ml O₂·g⁻¹·h⁻¹ at −16°C (Fig. 1). Between Ta = −4 and −16°C, TMR was the product of the RQ and TMR to protein free rates of carbohydrate utilization by relating RQ to proportions of carbohydrate consumed (32). This proportion was multiplied by the TMR and converted using 0.84 l O₂/g carbohydrate equivalent.

In a parallel experiment using a separate group of wild caught adult arctic ground squirrels of mixed sex, the maximum torpor bout length (TBL) of animals left undisturbed within their nests was determined for Ta = −16, −8, −4, 0, 4, 10, 16, and 20°C. Surgeries to implant temperature-sensitive radio transmitters were completed at least 1 mo before hibernation using the same protocol as with animals in the metabolism experiment. TBL was calculated as the number of hours Tb was <30°C as measured by radiotelemetry. The longest bout of torpor for each individual was recorded as its TBL for each Ta and averaged with values of TBL of other animals.

Because the regression analyses violate assumptions of independence and may underestimate α due to pseudoreplication, differences were considered significant at P < 0.01. Differences were considered significant at P < 0.05 for nonregression analyses.

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positively and linearly correlated with ΔT (r² = 0.95, P < 0.001, N = 8, n = 24) but not Tb (r² = 0.008, P = 0.685, N = 8, n = 24). At Ta values >0°C, ΔT averaged 0.61 ± 0.12°C and was not significantly correlated to TMR (P = 0.184, N = 8, n = 35).

At Ta values >0°C, Tb increased linearly with Ta (r² = 0.99, P < 0.001, N = 9, n = 35; Fig. 1). However, TMR did not differ significantly between Ta 4 and 12°C (P > 0.05, N = 9, n = 24), even though over this range Tb increased on average 7.9°C. TMR at Ta 16°C (0.0179 ± 0.005 ml O₂ · g⁻¹ · h⁻¹) and Ta 20°C (0.0466 ± 0.007 ml O₂ · g⁻¹ · h⁻¹) were significantly higher compared with TMR at Ta 4°C (mean conductance in a nest was significantly higher than within a nest at the corresponding Ta (with the exception of comparing Ta 0°C and 4°C; Table 1). In a stepwise linear regression including Tb, Ta, and ΔT, the increase in TMR from Ta 4 to 20°C was significantly correlated only to Tb and is best described with an exponential regression (r² = 0.56, P < 0.001, N = 9, n = 35). The overall increase in steady-state metabolic rate from Ta 4 to 20°C reflects a Q₁₀ of 2.4; however, Q₁₀ varied within this range of Ta values from 1 to 14.1 (Table 1).

Conductance of torpid arctic ground squirrels in nests at Ta values <0°C averaged 0.012 ± 0.0005 ml O₂ · g⁻¹ · h⁻¹ · °C⁻¹ and was not significantly correlated to either Ta (r² = 0.013, P = 0.597, N = 9, n = 24) or Tb (r² = 0.114, P = 0.106, N = 9, n = 24). At Ta values >0°C, conductance was significantly greater than at Ta values <0°C (P = 0.001) and averaged 0.043 ± 0.009 ml O₂ · g⁻¹ · h⁻¹ · °C⁻¹. Conductance at Ta values >0°C was not significantly correlated to either Ta (r² = 0.270, P = 0.026, N = 11, n = 43) or Ta (r² = 0.173, P = 0.012, N = 11, n = 43).

**Influence of a nest.** We compared Tb and TMR of eight torpid arctic ground squirrels hibernating with and without nest material at Ta 4 to −8°C. As indicated by ΔT either greater or less than 1°C, three of eight animals with or without nests had increased thermoregulatory heat production at Ta 0°C (ΔT = 1.17, 1.24, and 1.27°C), all had at Ta −4 and −8°C (ΔT = 3.38–7.98°C), and none had at 4°C (ΔT = 0.16–0.80°C). There was no difference in TMR of animals hibernating with a nest vs. without a nest at Ta 4°C (P = 0.559, N = 8, n = 16) and 0°C (P = 0.286, N = 8, n = 16). At Ta values <0°C, TMR of hibernating without a nest was significantly higher than with a nest at the same Ta (32.3% at −4°C and 35.8% at −8°C; P < 0.05, N = 8, n = 32). This difference can be attributed to significantly higher conductance for animals without a nest at both Ta −4°C (mean conductance in a nest = 0.0107 ± 0.001 ml O₂ · g⁻¹ · h⁻¹ · °C⁻¹, without a nest = 0.0170 ± 0.001 ml O₂ · g⁻¹ · h⁻¹ · °C⁻¹, P < 0.001, N = 8, n = 16) and at Ta −8°C (mean conductance in a nest = 0.0124 ± 0.0003 ml O₂ · g⁻¹ · h⁻¹ · °C⁻¹, without a nest = 0.0195 ± 0.0008 ml O₂ · g⁻¹ · h⁻¹ · °C⁻¹, P < 0.001, N = 8, n = 16).

**Fuels of metabolism.** To identify the metabolic fuels used during torpor, we calculated the RQ for ground squirrels hibernating at different Ta. Mean RQ of 0.70 ± 0.013 at Ta 4 and 8°C increased at lower and higher Ta values, reaching significantly higher values of 0.86 ± 0.021 at Ta −16°C (P < 0.05) and 0.88 ± 0.085 at Ta 20°C (P < 0.05; Fig. 1). At Ta values <0°C, RQ positively correlated with TMR (r² = 0.409, P < 0.001, N = 8, n = 24) but not Tb (r² = 0.017, P = 0.540, N = 8, n = 24). At Ta values >0°C, RQ was positively correlated with Tb (r² = 0.523, P < 0.001, N = 8, n = 35), and addition of TMR or Tb in a stepwise regression model did not significantly improve the fit of the regression model.

**TBL.** Maximum TBL of undisturbed animals was longest at Ta 0°C, averaging 15.1 days, and did not change significantly between Ta −4 and 4°C (P > 0.05; Fig. 2). Compared with Ta at 0°C, TBL was significantly shorter at Ta values less than or equal to −8°C and ≥10°C. To investigate interrelationships of TBL to TMR, Tb, and RQ at differing Ta, we performed linear stepwise regression analyses. Averages of TBL of undisturbed arctic ground squirrels hibernating at Ta −16 to 20°C were regressed with averages of TMR, Tb, and RQ from animals monitored for respirometry at the corresponding Ta (with the exception of comparing Ta 0°C and 4°C; Table 1).
Thermoregulation during torpor. The lower critical
Ta for torpid arctic ground squirrels was close to 0°C. Although
ground squirrels can supercool their abdomen to −2.9°C, head and neck temperatures remain ≥0°C (2), which requires increased levels of heat production at Ta < 0°C. As Ta decreased substantially below 0°C, arctic ground squirrels continued steady-state torpor while increasing rates of thermogenesis. In similar conditions, other hibernating species either freeze (63) or return to euthermic Tb in an “alarm arousal” (30). At Ta −16°C, TMR was almost 16-fold higher than minimum values at Ta > 0°C. Ta values of −16°C and lower are relevant to hibernating arctic ground squirrels. Minimum hibernacula temperatures in burrows on the North Slope of Alaska during winter range from −18 to −25°C (2, 38) and averaged −8.9°C October through April at burrow sites near where the experimental animals were captured (9). Because the lower critical temperature of euthermic arctic ground squirrels is 6–10°C (11), animals overwintering in sub-zero burrows must be continuously thermogenic whether they are torpid or euthermic. An animal in an average temperature burrow of −9°C without a nest would maintain a TMR 10-fold higher than if hibernating at Ta > 0°C (0.1 vs. 0.01 ml O₂ · g⁻¹ · h⁻¹); presence of a nest would lower this to a 6.5-fold increase in TMR and decrease lower critical temperature of euthermic animals to near 0°C (11). These results illustrate the substantially elevated energetic costs of hibernating under arctic conditions encountered by arctic ground squirrels compared with hibernators dis-

**Table 2. TMR, RQ, and TBL in arctic ground squirrels hibernating at differing Ta.** Corresponding rates of carbohydrate use are derived from RQ and TMR, assuming protein-free metabolism.

<table>
<thead>
<tr>
<th>Ta, °C</th>
<th>TMR, ml O₂ · g⁻¹ · h⁻¹</th>
<th>RQ</th>
<th>Proportion of Carbohydrate</th>
<th>Carbohydrate Use, g × 10⁶</th>
<th>Maximum TBL, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>−16</td>
<td>0.180 ± 0.008</td>
<td>0.86 ± 0.02</td>
<td>0.76</td>
<td>163.1</td>
<td>6.75 ± 0.62</td>
</tr>
<tr>
<td>−8</td>
<td>0.089 ± 0.003</td>
<td>0.79 ± 0.01</td>
<td>0.56</td>
<td>60.7</td>
<td>9.44 ± 0.85</td>
</tr>
<tr>
<td>−4</td>
<td>0.041 ± 0.004</td>
<td>0.77 ± 0.01</td>
<td>0.46</td>
<td>22.4</td>
<td>13.57 ± 2.26</td>
</tr>
<tr>
<td>0</td>
<td>0.016 ± 0.001</td>
<td>0.74 ± 0.02</td>
<td>0.28</td>
<td>5.4</td>
<td>15.01 ± 0.55</td>
</tr>
<tr>
<td>4</td>
<td>0.012 ± 0.000</td>
<td>0.70 ± 0.02</td>
<td>0</td>
<td>0</td>
<td>13.5 ± 0.94</td>
</tr>
<tr>
<td>8</td>
<td>0.012 ± 0.001</td>
<td>0.70 ± 0.02</td>
<td>0</td>
<td>0</td>
<td>13.5 ± 0.94</td>
</tr>
<tr>
<td>20</td>
<td>0.047 ± 0.006</td>
<td>0.88 ± 0.03</td>
<td>0.81</td>
<td>45.2</td>
<td>5.33 ± 0.33</td>
</tr>
</tbody>
</table>

Values are means ± SE. Proportion of carbohydrate was derived from respiratory quotient (RQ), assuming metabolism of carbohydrate (glucose) and lipid (glycerol tripalmitate) (32). Carbohydrate use is expressed per g per h. Maximum torpor bout length (TBL) was derived from a separate group of ground squirrels kept at the corresponding ambient temperature (Ta).
distributed in lower latitudes, where burrow temperatures only briefly or never decrease below freezing (1, 31, 40, 65). Arctic ground squirrels meet these thermoregulatory and energetic challenges in part through a larger body size compared with other hibernating congeners (maximum body size 1.5 kg vs. <0.6 kg in other Spermophilus species; Refs. 9, 39), which offers S. parryii a proportionally greater ability to store fat combined with a lower mass specific metabolic rate and thus an ability to fast longer than smaller species (41). Moreover, minimum TMR in this species is among the lowest measured in hibernating endotherms (26) and does not begin to rise until Tₐ decreases ≤0°C. These energetic adjustments, in addition to their ability to supercool core body temperatures to near −3.0°C, are extreme physiological and behavioral adaptations in an arctic resident mammal that are commensurate with the extremes of the arctic environment.

At Tₐ 0°C, conductance in torpid arctic ground squirrels was minimal and similar to that in other hibernating rodents (49) and did not change as TMR increased with decreasing Tₐ. In contrast, conductance increases with increased TMR in marsupial hibernators, which lack brown adipose tissue (BAT) and use shivering thermogenesis at Tₐ less than the lower critical temperature (50, 51). Song et al. (51) suggest that, in marsupials, increased conductance during shivering is due to increased peripheral circulation. Rodents rely on nonshivering thermogenesis during hibernation and are not known to shiver during steady-state torpor; therefore, different patterns of conductance with respect to Tₐ may be related to the different mechanisms of thermogenesis in rodent and marsupial hibernators.

At Tₐ >0°C, thermoregulatory heat production was absent, as indicated by minimum TMR and ΔT, and heat loss was facilitated by conductance that significantly increased compared with subfreezing temperatures. Values for conductance calculated for torpid arctic ground squirrels without a nest at Tₐ 0 and 4°C were lower by 64 and 55%, respectively, than values predicted by Snyder and Nestler (49), based on measures of TMR of smaller animals hibernating at Tₐ 6°C. The lower than predicted conductance could be explained by the larger animals used in this study (~200 g greater mass) and greater insulative properties of the fur.

TMR of arctic ground squirrels was minimal at Tₐ 4–12°C and did not significantly increase until Tₐ and Tₐ were ≥16°C (Fig. 1); thus a thermal neutral zone between Tₐ 0 and 16°C exists for torpid arctic ground squirrels. TMR remained constant over this range despite the passive increase in Tₐ with Tₐ, averaging 0.67°C greater than Tₐ, indicating that hibernating arctic ground squirrels use temperature-independent mechanisms of metabolic inhibition. Although the overall increase in TMR from Tₐ 4 to 20°C is represented by a Q₁₀ of 2.4, Q₁₀ across these Tₐ values ranges from 1.0 (Tₐ 4–8°C) to 14.1 (Tₐ 16–20°C; Table 1). Temperature effects on tissue and whole organism rates of metabolism normally have Q₁₀ values of 2–3 (47). Several hibernating species show Q₁₀ values of metabolic rate well above 3 during the entry phase of torpor (23, 28, 50, 51), suggesting that temperature-dependent and -independent metabolic inhibition of metabolism may have additive effects (36, 54). Temperature independence during steady-state torpor over wide ranges of Tₐ is also apparent in hibernating alpine marmots, Marmota marmota, (1) and eastern pygmy possums, Cercartetus nanus (51). Suppressing TMR over this range of Tₐ maintains maximum energy savings of torpor over a range of Tₐ values, although Tₐ >0°C occur over only a brief part of the hibernation season in the northern distribution of this species (9). At higher Tₐ values (16–20°C), temperature-independent mechanisms are apparently overcome, thus resulting in exceptionally high Q₁₀ values of TMR from Tₐ 16 to 20°C.

Metabolic fuels of hibernation. RQ results in this study suggest that, over an extended range of Tₐ, metabolic fuel use during steady-state torpor can vary. Hibernators are reported to use fat as the exclusive metabolic substrate during torpor (48, 52); however, previous researchers used a narrow range of ambient temperatures in their investigations. An RQ value of 0.70 indicates exclusive fat catabolism and 1.0 exclusive carbohydrate catabolism. RQ values between 0.70 and 1.0 can reflect either catabolism of protein or mixed fuel use (32). RQ of arctic ground squirrels during torpor was positively correlated to TMR at Tₐ values <0°C, rising from 0.70 to 0.86 from Tₐ 4 to −16°C, and positively correlated to Tₐ at Tₐ values >8°C, rising from 0.70 to 0.88 at Tₐ 20°C. We do not believe these shifts in RQ with Tₐ represented transitory changes in respiratory exchange, because our respirometry measurements were made on animals during steady-state conditions (constant Tₐ and TMR) on the fourth day of a torpor bout, and values represent averages over a 1-h interval. Although changes in RQ may reflect retention or release of carbon dioxide rather than shifts in substrate use, and resulting changes in relative acidosis of blood have been linked to metabolic inhibition during hibernation, these changes occur over short time intervals (<16 min) during entry into or arousal from torpor (33, 36, 43) and therefore are unlikely to have contributed to the differences in RQ reported here.

Why do metabolic substrates shift away from exclusively lipids at low and high Tₐ values? Upward shifts in RQ during torpor likely reflect addition of carbohydrate rather than protein to the utilization of fat, since unchanging plasma urea and nonprotein nitrogen levels suggest that protein is not catabolized during torpor (20, 44, 67) and rates of gluconeogenesis are low in hepatocytes isolated from hibernators (21). An increase in glucose use in arctic ground squirrels at subzero temperatures may be related to increases in TMR supporting thermogenesis (67). Increased carbohydrate needs would follow from elevated activity of heart muscle, erythrocytes, and brain (all glucose-utilizing tissues) associated with supporting higher rates of metabolism at lower Tₐ and, in addition and proba-
bly most significantly, glucose requirements to support increased nonshivering thermogenesis in BAT (12, 27). BAT mass increases three- to fourfold in arctic ground squirrels before hibernation, through hypertrophy and hyperplasia (7), and, in rats, glucose uptake by BAT increases 50- to 100-fold after cold exposure or norepinephrine injection (35, 59). Increased glucose uptake in thermogenic BAT supplies oxaloacetate (via conversion to pyruvate) during citrate acid cycle oxidation (10).

Glucose utilization during torpor is indicated by progressive decreases in blood glucose and liver glycogen concentrations over the torpor bout, which are replenished during arousal episodes (18, 20). Much of this replacement carbohydrate may be derived from protein in arctic ground squirrels, which lose 30–40% of their lean body mass over the hibernation season (8, 19). Gluconeogenesis from amino acids is required because glyceral release from the metabolism of triacylglycerols is insufficient to provide for glucose requirements during torpor when metabolic rates are low. Zimmerman (67) estimated that TMR of arctic ground squirrels would have to reach 0.2 ml O₂·g⁻¹·h⁻¹ of only fat oxidation for gluconeogenesis from glyceral to prevent depletion of glycogen. TMR this high would be reached at Tₐ of approximately −20°C (Fig. 1). These temperatures do occur in hibernacula of arctic ground squirrels, but they are not sustained for long intervals (9), suggesting an additional need for gluconeogenesis from muscle tissue over the majority of the hibernation season. Nonetheless, higher rates of glyceral release and its combustion as carbohydrate may contribute to the upward shift of RQ as TMR increases at lower Tₐ values.

These arguments for an increase in RQ at Tₐ values <0°C cannot altogether explain its increase at Tₐ values >8°C. At Tₐ 12°C, RQ was significantly higher compared with Tₐ at 4–8°C, without a concomitant increase in TMR and thermogenesis. In addition, overall increases in RQ and TMR at Tₐ values >0°C are not proportional to changes at Tₐ <0°C. At Tₐ values >8°C, RQ is most directly related to changes in Tₐ b, suggesting a changing use of metabolic fuels as tissue temperatures rise, but for unknown reasons.

**TBL and the function of arousal episodes.** The changing relationships among Tₐ, Tₐ b, TMR, and the use of metabolic fuels in hibernating arctic ground squirrels provides an opportunity to examine alternative hypotheses of what limits duration of torpor in mammals and thus the functional significance of arousal episodes. The many hypotheses for the physiological function of arousal episodes fall into two general categories: metabolic rate and body temperature. Metabolic rate hypotheses associate arousals with a requirement of high Tₐ b for reestablishing homeostasis of some metabolism-linked process that goes awry at low Tₐ b (17, 23). For example, metabolic fuels used for maintenance metabolism, e.g., glucose (20) or fatty acids (14), may be depleted during torpor due to low temperature inhibition of enzymes linked to their release or synthesis, requiring arousals for their replenishment in the blood and tissues. Alternatively, metabolic by-products, e.g., water (16) or urea (45), may accumulate during torpor when kidney filtration and liver function are diminished or absent (15), and hibernators must arouse to remove them. Metabolism-linked hypotheses assume that the rate of depletion or accumulation of the critical substance(s) is proportional to the metabolic rate during torpor. Although there is some experimental evidence supporting specific aspects of these hypotheses in individual species, no consensus has been reached for acceptance of the general metabolic rate hypothesis (23, 24, 62). Body temperature hypotheses alternatively center on low tissue temperature inhibition of other homeostatically regulated processes that are not directly linked to metabolic rate. For example, hibernators with cold brains may not be able to sleep, yet sleep debt could continue to accumulate during torpor, albeit at a reduced rate (13, 57). Hibernators may be forced to warm periodically to maintain sleep balance (4, but see Refs. 34, 55). Other body temperature hypotheses specify a requirement for periodic high brain temperatures for memory consolidation or maintenance (42, 56), for preservation of neuronal connections in the central nervous system (34, 46, 55), a need of high Tₐ b for maintenance of opiate system suppression of metabolism (61), for reproductive development (3), or a requirement of high tissue temperatures for gene expression, e.g., transcription or translation, and thereby a generalized need for periodic euthermia for homeostasis of gene products such as mRNA or proteins (37). These hypotheses assume that the need for arousals develops at a rate proportional to body or brain temperature during hibernation, but not necessarily in proportion to whole body metabolic rate.

The changes in TBL found in the present study support offer support for both the metabolic rate and body temperature hypotheses, depending on whether Tₐ b is above or below 0°C, but overall we hypothesize TBL in arctic ground squirrels depends on brain temperature. At Tₐ <0°C, TBL decreases concomitantly with increasing rates of metabolism, which is consistent with the metabolic hypothesis (25). The general reciprocal relationship between changing RQ and TBL across the full range of Tₐ supports the specific hypothesis that depletion of nonlipid fuels, e.g., glucose, limits how long torpor can be sustained before an arousal is required (20). However, the observed decrease in TBL at Tₐ >0°C, when TMR and estimated rates of glucose metabolism remain minimal, is not consistent with the metabolic hypothesis.

The reciprocal decrease of TBL with increasing Tₐ b above 0°C (Fig. 2), which has been experimentally demonstrated in arctic ground squirrels to be linked to Tₐ b and not to season (5) and is similar in other hibernating mammals (25, 58), is most supportive of the body temperature hypotheses, whereas the decrease in TBL at Tₐ <0°C while Tₐ b remains relatively constant is not.

Arousal episodes may serve alternative functions as Tₐ b and requirements for thermoregulation vary. Thus arctic ground squirrels may arouse more frequently at Tₐ <0°C to replenish glycogen stores and more fre-
quently at $T_a > 0°C$ to maintain brain functions. A singular correlate to TBL may still be hypothesized, however, if regional heterothermy occurs in hibernators at $T_a < 0°C$. A 2–3°C difference between core and neck temperatures occurs in torpid arctic ground squirrels at $T_a = -4.3°C$ (2). This is likely due to the anterior location (subcapsular and periartrial) of the largest stores of BAT, which are the major thermogenic tissues maintaining gradients between $T_b$ and subzero $T_a$. This regional heterothermy may increase as $T_a$ decreases and the requirement for thermogenesis increases. An increase in heterothermy at $T_a = -16°C$ resulting in $T_b$ of 0°C but brain temperatures near 10°C would result in TBL equivalent to $T_a$ of 10°C, when brain temperatures are also near 10°C. Resolving causal relationships between brain temperature and TBL should be amenable to experiments that vary brain temperature separately from $T_b$ and metabolic rate (30).

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