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

C. LOREN BUCK AND BRIAN M. BARNES
Institute of Arctic Biology and Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, Alaska 99775

Received 27 July 1999; accepted in final form 2 February 2000

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 (T_a)-dependent patterns of core body temperature (T_b), metabolic rate (TMR), and metabolic fuel use, as determined by respiratory quotient (RQ). At T_a 0 to −16°C, T_b remained relatively constant, and TMR rose proportionally with the expanding gradient between T_b and T_a, increasing >15-fold from a minimum of 0.0115 ± 0.0021 ml O_2·g⁻¹·h⁻¹ at T_a 0°C to 0.85 ± 0.013 ml O_2·g⁻¹·h⁻¹ at T_a 16°C. At T_a 16–20°C, T_b increased with T_a; however, TMR did not change significantly from T_b 0 to 12°C, indicating temperature-independent inhibition of metabolic rate. The overall change in TMR from T_a 4 to 20°C equates to a Q_{10} of 2.4, but within this range of T_b, Q_{10} changed from 1.0 to 14.1. During steady-state torpor at T_a 4 and 8°C, RQ averaged 0.70 ± 0.013, indicating exclusive lipid catabolism. At T_a 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 T_a values <0°C, this relationship supports the hypothesis that availability of nonlipid metabolic fuels limits torpor duration in hibernating mammals; for T_a 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
METHODS

Animals. Arctic ground squirrels (S. p. kennicottii) were live-trapped in July in the northern foothills of the Brooks Range, Alaska, near the Atigun 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 x 30.5 x 20.3 cm) at a photoperiod of 12:12 h light-dark cycle and T₀ 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 T₀, temperature-sensitive radiotransmitters (model VMH-BB, Minimitter, 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-cest 0.1°C against a precision mercury thermometer in a range. Beforehand, transmitters were calibrated to the near-cest 0.1°C against a precision mercury thermometer in a range. Beforehand, transmitters were calibrated to the near-cest 0.1°C against a precision mercury thermometer in a range. Beforehand, transmitters were calibrated to the near-cest 0.1°C against a precision mercury thermometer in a range. Beforehand, transmitters were calibrated to the near-cest 0.1°C against a precision mercury thermometer in a range.

Respirometry. Rates of oxygen consumption and carbon dioxide production were recorded during steady-state torpor for eight adult animals (4 male, 4 female) at T₀ = -16, -8, -4, 0, 4, 8, 12, 16, and 20°C (each ±0.5°C). Metabolic measures were not recorded from all eight animals at T₀ = -8–20°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 T₀ of each subject animal had decreased to below 30°C during entry into a torpor bout. TMR was measured of animals within their nests at T₀ values decreased to below 30°C during entry into a torpor bout. TMR began 4 days after T₀ of each subject animal had decreased to below 30°C during entry into a torpor bout.

RESULTS

Thermoregulatory patterns during torpor at differing T₀. Patterns of thermoregulatory heat production of arctic ground squirrels hibernating at different T₀ values depended on whether T₀ was higher or lower than 0°C. At T₀ values <0°C, all animals increased rates of thermogenesis to maintain a relatively constant T₀. At T₀ values >0°C, animals maintained minimal levels of heat production as T₀ varied with T₀, 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 T₀ values <0°C, T₀ averaged -0.42 ± 0.12°C and did not change significantly with changing T₀ (P = 0.071, N = 8, n = 24; Fig. 1). The lowest T₀ recorded was -1.97°C at T₀ = -8°C. From its minimum value of 0.0115 ± 0.0012 ml O₂·g⁻¹·h⁻¹ at T₀ = 4°C, TMR increased 15.8-fold to 0.182 ± 0.0244 ml O₂·g⁻¹·h⁻¹ at -16°C (Fig. 1). Between T₀ = -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 0.841 O₂/g carbohydrate equivalent.

In a parallel experiment using a separate group of wild-captured arctic ground squirrels of mixed sex, the maximum torpor bout length (TBL) of animals left undisturbed within their nests was determined for T₀ = -16, -8, -4, 0, 4, 10, 16, and 20°C. Surgeries to implant temperature-sensitive radiotransmitters 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 T₀ was <30°C as measured by radiotelemetry. The longest bout of torpor for each individual was recorded as its TBL for each T₀ and averaged with values of TBL of other animals.

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 T₀ 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 T₀. Patterns of thermoregulatory heat production of arctic ground squirrels hibernating at different T₀ values depended on whether T₀ was higher or lower than 0°C. At T₀ values <0°C, all animals increased rates of thermogenesis to maintain a relatively constant T₀. At T₀ values >0°C, animals maintained minimal levels of heat production as T₀ varied with T₀, 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 T₀ values <0°C, T₀ averaged -0.42 ± 0.12°C and did not change significantly with changing T₀ (P = 0.071, N = 8, n = 24; Fig. 1). The lowest T₀ recorded was -1.97°C at T₀ = -8°C. From its minimum value of 0.0115 ± 0.0012 ml O₂·g⁻¹·h⁻¹ at T₀ = 4°C, TMR increased 15.8-fold to 0.182 ± 0.0244 ml O₂·g⁻¹·h⁻¹ at -16°C (Fig. 1). Between T₀ = -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 0.841 O₂/g carbohydrate equivalent.

In a parallel experiment using a separate group of wild-captured arctic ground squirrels of mixed sex, the maximum torpor bout length (TBL) of animals left undisturbed within their nests was determined for T₀ = -16, -8, -4, 0, 4, 10, 16, and 20°C. Surgeries to implant temperature-sensitive radiotransmitters 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 T₀ was <30°C as measured by radiotelemetry. The longest bout of torpor for each individual was recorded as its TBL for each T₀ and averaged with values of TBL of other animals.

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 T₀ 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.
positively and linearly correlated with \( \Delta T \) \((r^2 = 0.95, P < 0.001, N = 8, n = 24)\) but not \( T_b \) \((r^2 = 0.008, P = 0.685, N = 8, n = 24)\). At \( T_a \) values >0°C, \( T_b \) increased linearly with \( T_a \) \((r^2 = 0.99, P < 0.001, N = 9, n = 35; \text{Fig. 1})\). However, TMR did not differ significantly between \( T_a \) 4 and 12°C \((P > 0.05, N = 9, n = 24)\), even though over this range \( T_b \) increased on average 7.9°C. TMR at \( T_a \) 16°C \((0.0179 \pm 0.005 \text{ ml O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1})\) and \( T_a \) 20°C \((0.0466 \pm 0.007 \text{ ml O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1})\) were significantly higher compared with TMR at \( T_a \) 14°C \((P < 0.05)\). In a stepwise linear regression including \( T_b \), \( T_a \), and \( \Delta T \), the increase in TMR from \( T_a \) 4 to 20°C was significantly correlated only to \( T_b \) and is best described with an exponential regression \((r^2 = 0.56, P < 0.001, N = 9, n = 35)\). The overall increase in steady-state TMR from \( T_a \) 4 to 20°C reflects a \( Q_{10} \) of 2.4; however, \( Q_{10} \) varied within this range of \( T_a \) values from 1.10 to 14.1 (Table 1).

Conductance of torpid arctic ground squirrels in nests at \( T_a \) values ≤0°C averaged 0.012 ± 0.0005 ml \( O_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1} \cdot ^\circ\text{C}^{-1} \) and was not significantly correlated to either \( T_a \) \((r^2 = 0.013, P = 0.597, N = 9, n = 24)\) or \( T_b \) \((r^2 = 0.114, P = 0.106, N = 9, n = 24)\). At \( T_a \) values >0°C, conductance was significantly greater than at \( T_a \) values ≤0°C \((P = 0.001)\) and averaged 0.043 ± 0.009 ml \( O_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1} \cdot ^\circ\text{C}^{-1} \). Conductance at \( T_a \) values >0°C was not significantly correlated to either \( T_a \) \((r^2 = 0.270, P = 0.026, N = 11, n = 43)\) or \( T_a \) \((r^2 = 0.173, P = 0.012, N = 11, n = 43)\).

**Influence of a nest.** We compared \( T_b \) and TMR of eight torpid arctic ground squirrels hibernating with and without nest material at \( T_a \) 4 to −8°C. As indicated by \( \Delta T \) either greater or less than 1°C, three of eight animals with or without nests had increased thermoregulatory heat production at \( T_a \) 0°C \((\Delta T = 1.17, 1.24, \text{and 1.27°C})\), all had at \( T_a \) −4 and −8°C \((\Delta T = 3.38–7.98°C)\), and none had at 4°C \((\Delta T = 0.16–0.80°C)\). There was no difference in TMR of animals hibernating with a nest vs. without a nest at \( T_a \) 4°C \((P = 0.559, N = 8, n = 16)\) and 0°C \((P = 0.286, N = 8, n = 16)\). At \( T_a \) values <0°C, TMR of animals hibernating without a nest was significantly higher than within a nest at the same \( T_a \) (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 \( T_a \) −4°C (mean conductance in a nest = 0.1107 ± 0.0021 ml \( O_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1} \cdot ^\circ\text{C}^{-1} \), without a nest = 0.0170 ± 0.0011 ml \( O_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1} \cdot ^\circ\text{C}^{-1} \); \( P < 0.01, N = 8, n = 16)\) and at \( T_a \) −8°C (mean conductance in a nest = 0.0124 ± 0.0003 ml \( O_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1} \cdot ^\circ\text{C}^{-1} \), without a nest = 0.0195 ± 0.0008 ml \( O_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1} \cdot ^\circ\text{C}^{-1} \); \( P < 0.001, N = 8, n = 16)\).

**Fuels of metabolism.** To identify the metabolic fuels used during torpor, we calculated the RQ for ground squirrels hibernating at different \( T_a \). Mean RQ of 0.70 ± 0.013 at \( T_a \) 4 and 8°C increased at lower and higher \( T_a \) values, reaching significantly higher values of 0.86 ± 0.021 at \( T_a \) −16°C \((P < 0.05)\) and 0.88 ± 0.085 at \( T_a \) 20°C \((P < 0.05; \text{Fig. 1})\). At \( T_a \) values <0°C, RQ positively correlated with TMR \((r^2 = 0.409, P < 0.001, N = 8, n = 24)\) but not \( T_b \) \((r^2 = 0.017, P = 0.540, N = 8, n = 24)\). At \( T_a \) values >0°C, RQ was positively correlated with \( T_a \) \((r^2 = 0.523, P < 0.001, N = 8, n = 35)\), and addition of TMR or \( T_b \) in a stepwise regression model did not significantly improve the fit of the regression model.

**TBL.** Maximum TBL of undisturbed animals was longest at \( T_a \) 0°C, averaging 15.1 days, and did not change significantly between \( T_a \) −4 and 4°C \((P > 0.05; \text{Fig. 2})\). Compared with \( T_a \) at 0°C, TBL was significantly shorter at \( T_a \) values less than or equal to −8°C and ≥10°C. To investigate interrelationships of TBL to TMR, \( T_b \), and RQ at differing \( T_a \), we performed linear stepwise regression analyses. Averages of TBL of undisturbed arctic ground squirrels hibernating at \( T_a \) −16 to 20°C were regressed with averages of TMR, \( T_b \), and RQ from animals monitored for respirometry at the corresponding \( T_a \) (with the exception of comparing

### Table 1. \( T_b \), \( T_a \), and average TMR of arctic ground squirrels during steady-state torpor and corresponding \( Q_{10} \) values of TMR between different \( T_b \) measurements

<table>
<thead>
<tr>
<th>Group</th>
<th>( T_a ), °C</th>
<th>( T_b ), °C</th>
<th>( \text{TMR, ml} )</th>
<th>( O_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1} )</th>
<th>( Q_{10}(1–2) )</th>
<th>( Q_{10}(2–3) )</th>
<th>( Q_{10}(3–4) )</th>
<th>( Q_{10}(4–5) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>8</td>
<td>12</td>
<td>16</td>
<td>20</td>
<td>0.0115</td>
<td>0.0115</td>
<td>0.0137</td>
</tr>
<tr>
<td>2</td>
<td>4.66</td>
<td>8.22</td>
<td>12.56</td>
<td>17.11</td>
<td>20.73</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(\( T_a \) ambient temperature; \( T_b \) average body temperature; TMR, maximum torpid metabolic rate.)
DISCUSSION

Arctic ground squirrels hibernating in ambient temperatures ranging from substantially subfreezing conditions to room temperature showed changing interrelationships among Tb, TMR, and RQ that revealed differing physiological responses to heterothermy as Tb and requirements for thermoregulatory heat production changed. At Ta values <0°C, metabolic rate increased proportionately with decreasing Ta while Tb remained constant. At Ta values >0°C, Tb increased parallel with Ta while TMR remained minimal and constant until Ta and Tb increased above 12°C. These relationships are similar to and extend those described for other hibernating mammals held either below or above their lower critical Ta, the Ta at which heterothermic mammals begin to defend their Tb (1, 25, 29, 51). Metabolic fuel use also changed over this range of Ta as indicated by RQ. This uncoupling of metabolic rate from Tb and change in sources of energy over a wide range of Ta provides an opportunity to investigate alternative hypotheses of what limits torpor duration in heterothermic mammals and thus the function of periodic arousal episodes. The lack of an overall relationship between TBL and TMR or apparent rate of carbohydrate use does not support exclusive hypotheses related to depletion of metabolic fuels (14, 18).

Thermoregulation during torpor. The lower critical Ta for torpid arctic ground squirrels was close to 0°C. Although arctic 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 subzero 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>7.67 ± 1.33</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).
tributed 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 congers (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 Tm decreases $\leq$0°C. These energetic adjustments, in addition to their ability to supercool core body temperatures to near $\sim$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 Tm $\leq$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 Tm. In contrast, conductance increases with increased TMR in marsupial hibernators, which lack brown adipose tissue (BAT) and use shivering thermogenesis at Tm 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 Tm may be related to the different mechanisms of thermogenesis in rodent and marsupial hibernators.

At Tm $>0°C$, thermoregulatory heat production was absent, as indicated by minimum TMR and AT, 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 Tm 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 Tm 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 Tm 4–12°C and did not significantly increase until Tm and Tb were $\geq$16°C (Fig. 1); thus a thermal neutral zone between Tm 0 and 16°C exists for torpid arctic ground squirrels. TMR remained constant over this range despite the passive increase in Tb with Tm, averaging 0.67°C greater than Tm, indicating that hibernating arctic ground squirrels use temperature-independent mechanisms of metabolic inhibition. Although the overall increase in TMR from Tm 4 to 20°C is represented by a Q10 of 2.4, Q10 across these Tm values ranges from 1.0 (Tm 4–8°C) to 14.1 (Tm 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 Tm is also apparent in hibernating alpine marmots, Marmota marmota, (1) and eastern pygmy possums, Cercartetus nanus (51). Suppressing TMR over this range of Tb maintains maximum energy savings of torpor over a range of Tm values, although Tm $>0°C$ occur only over a brief part of the hibernation season in the northern distribution of this species (9). At higher Tm values (16–20°C), temperature-independent mechanisms are apparently overcome, thus resulting in exceptionally high Q10 values of TMR from Tm 16 to 20°C.

Metabolic fuels of hibernation. RQ results in this study suggest that, over an extended range of Tm, 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 Tm values $<$0°C, rising from 0.70 to 0.86 from Tm 4 to $\sim$16°C, and positively correlated to Tb at Tm values $>$8°C, rising from 0.70 to 0.88 at Tm 20°C. We do not believe these shifts in RQ with Tm represented transitory changes in respiratory exchange, because our respirometry measurements were made on animals during steady-state conditions (constant Tb 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 Tm 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 Tm 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 glycerol 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 glycerol 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 glycerol 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 are not 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, 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, Tₜ, 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 Tₜ for reestablishing homeostasis of some metabolism-linked process that goes awry at low Tₜ (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 Tₜ 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 offer support for both the metabolic rate and body temperature hypotheses, depending on whether Tₐ 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 and Tₜ above 0°C (Fig. 2), which has been experimentally demonstrated in arctic ground squirrels to be linked to Tₜ 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 Tₜ remains relatively constant is not.

Arousal episodes may serve alternative functions as Tₜ 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 $T_a > 0{\degree}C$ to maintain brain functions. A singular correlate to TBL may still be hypothesized, however, if regional heterothermy occurs in hibernators at $T_a < 0{\degree}C$. A 2–3$^\circ$C difference between core and neck temperatures occurs in torpid arctic ground squirrels at $T_a = –4{.}3^\circ$C (2). This is likely due to the anterior location (subscapular and periarterial) 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 $T_a = –16^\circ$C resulting in $T_b$ of 0$^\circ$C but brain temperatures near 10$^\circ$C would result in TBL equivalent to $T_a$ of 10$^\circ$C, when brain temperatures are also near 10$^\circ$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).

We thank Andrée Porchet and Sharon Loy for help with animals, Brett Luick for design and software for respiratory measures, and Øivind Toien and Alison York for reading the manuscript. This study was funded by grants from the University of Alaska’s Center for Arctic Climate and Global Change and Arctic System Science and the American Heart Association (to C. L. Buck) as well as by National Science Foundation Grant OPP-9819540 (to B. M. Barnes).

REFERENCES


