Thermal relations of metabolic rate reduction in a hibernating marsupial

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Thermal relations of metabolic rate reduction in a hibernating marsupial. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R2097–R2104, 1997.—We tested whether the reduction of metabolic rate (MR) in hibernating Cercartetus nanus (Marsupialia, 36 g) is better explained by the reduction of body temperature (Tb), the differential (ΔT) between Tb and air temperature (Ta), or thermal conductance (C). Above the critical Tb during torpor (Tb5°C) and water were withhold during measurements. The mean body mass of the animals was 36.2 ± 5.8 g.

MR, determined as rate of oxygen consumption (V̇O2), was measured in 0.5-l respirometry chambers fitted with a water-absorbing cardboard insert within a temperature-controlled cabinet (±0.5°C). A two-channel system was used to measure two individuals simultaneously. Air from the respirometry chambers was measured in 3-min intervals for 27 min, and then solenoid valves were switched to reference air (outside) for 3 min. Oxygen content of the air was measured with an oxygen analyzer (Ameked Applied Electrochemistry S-3A/11, Pittsburgh, PA). The flow rate (~200 ml/min during normothermia and ~100 ml/min during steady-state torpor) of dry air was maintained at 200 ml/min during normothermia and at 100 ml/min during steady-state torpor. A detailed study on interrelations between physiological variables during daily torpor of the marsupial Smimhopsis macroura suggests that the steady-state MR is determined by different physiological responses above and below the set point (T̄set) for Tb that is defended by proportional thermoregulation (14, 25). At Ta above the T̄set, MR is largely a function of Tb, supporting the Q10 effect interpretation (25). In contrast, below the T̄set, MR is determined by ΔT as during normothermia (25). However, it is likely that interrelations between variables differ between hibernators (species that show prolonged torpor and generally have Tb ~5°C) and daily heterotherms (species that show shallow daily torpor). It has been proposed that metabolic inhibition may play a particularly important role in hibernators because their reduction of MR is much more pronounced than in daily heterotherms (6, 9, 19).

To provide conclusive results on what determines MR reduction during hibernation, detailed measurements of the relevant physiological variables over a wide temperature range are required. In the past, such measurements were often difficult to interpret because most of the experiments were conducted on sciurid rodents, which enter torpor only at low Ta (17), making a detailed analysis on the effect of Ta on steady-state Tb and MR and the interrelations between these variables difficult.

The experimental animal for the present study was the eastern pygmy-possum (Cercartetus nanus), which is a small marsupial hibernator that shows torpor bouts lasting up to 4 wk and Tb as low as 2°C (3, 7). This species was selected because it displays torpor over a wide range of Ta, which allows a systematic analysis of the interrelations between physiological variables during hibernation. We measured simultaneously steady-state MR and Tb during torpor at Ta from 1 to 30°C and determined how MR, Tb, ΔT, and C of torpid individuals are interrelated and how they differ from those in normothermic individuals.

MATERIAL AND METHODS

Eight adult C. nanus (4 females; 4 males) were caught near Dorrigo, New South Wales, Australia (30°22′ S, 152°45′ E). Animals were held individually in cages (30 × 22 × 14 cm) containing sawdust and bedding material. For the measurements at Ta values below 15°C, animals were acclimated at Ta 10°C. The photoperiod was 12 h light, 12 h dark, with lights on from 0600 to 1800. Animals were fed on apples, walnuts, sunflower seeds, and a mixed paste of baby cereal and honey, supplemented with calcium and vitamins and water. Food and water were withheld during measurements. The mean body mass of the animals was 36.2 ± 5.8 g.

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air was continuously monitored with mass flowmeters (FMA-5606; Omega, Stamford, CT).

For long-term records of Tb, small wax-coated temperature-sensitive transmitters (Mini-Mitter model X-M, accuracy ±0.1°C, −1.5 g) were implanted intraperitoneally under isoflurane anesthesia. Transmitters were calibrated to the nearest 0.1°C against a precision thermometer in a water bath between 0 and 40°C before and after experiments. After the surgery, the animals were allowed at least 7 days for recovery at Tfc 22 ± 1°C. An antenna consisting of a ferrite rod was placed underneath each respirometry chamber and multiplexed to a receiver. The transmitter signal was transformed to a square-wave signal after background noise was subtracted.

Ta in the respirometry chamber was measured to the nearest 0.1°C by a calibrated thermocouple inserted −1 cm into the metabolic chamber. Analog outputs from the flowmeters, oxygen analyzer, transmitter receiver, and thermocouples were interfaced via an analog to digital converter (DT100 logger, Data Electronics). Readings were taken every 3 min. VO2 values (STPD) were calculated according to the equation C

Tb had been measured. TMR was obtained by calculating the mean of the consecutive 40 lowest VO2 values (i.e., over 2 h), and corresponding Ta and Tb were determined.

RMR was measured at constant Ta values ranging from 1 to 30°C in the light phase in normothermic individuals whose Tb values were > 32.0°C. To determine the TNZ and the BMR, RMR was measured between 0930 and 1700. After animals had been in the chambers for at least 1 h, RMR was increased from 27 to 35°C in 1.5°C increments lasting for ~2 h each. RMR values were obtained from the mean of the 10 lowest consecutive VO2 values (i.e., over 30 min). The means of the corresponding 10 Tb and Ta readings were also calculated. BMR was determined as the mean of the 30-min minimum of normothermic individuals within the TNZ. Animals were weighed before and after each measurement. A linear decrease of body mass throughout each measurement was assumed for calculation of mass-specific MR.

The mass-specific apparent C, which is a measure of all kinds of heat loss including respiratory evaporation, was calculated using the equation C = MR(Tb − Ta). Q10 values for MR at different Tb were calculated according to the equation Q10 = (MR(MR2)/(MR(MR2)−Ta)), with 0.9 to 32.9°C, usually lasting for only a few hours (Fig. 1).

Normotherm. The TNZ of normothermic C. nanus ranged from Ta 28.7 ± 0.9 to 32.9 ± 0.7°C, and the BMR was 0.66 ± 0.17 ml·g−1·h−1 (body mass = 36.0 ± 7.5 g, N = 7, n = 29, Fig. 2A). The RMR increased linearly with a decreasing Tb (r2 = 0.86, P < 0.001) between the Tlc and Ta 5°C (Fig. 2A). Below Ta 5°C, Tb was more variable than at low Tb values (Fig. 1B). Tb was periodically interrupted by spontaneous arousal, which often occurred in the afternoon, several hours before lights out. Arousal was characterized by a MR overshoot and a rise of Tb, followed by postarousal with RMR and normothermic Tb of ~35°C, usually lasting for only a few hours (Fig. 1).

RESULTS

The MR and Tb of Cercartetus nanus showed pronounced fluctuations between high values during normothermia and low values during torpor (Fig. 1). Torpor usually started in the dark phase, after animals had been in the respirometry chambers for several hours. Entrance into torpor was initiated by a rapid decrease of MR, followed by a gradual decline in Tb. The steady-state MR was usually reached after 3–5 h (Fig. 1). Torpor bout duration varied with Ta. Below Ta 25°C, torpor bouts usually lasted for several days (Fig. 1A). Above Ta 25°C, torpor bouts were usually shorter than 1 day, and Tb and TMR during steady-state torpor were more variable than at low Tb values (Fig. 1B). Tb was periodically interrupted by spontaneous arousal, which often occurred in the afternoon, several hours before lights out. Arousal was characterized by a MR overshoot and a rise of Tb, followed by postarousal with RMR and normothermic Tb of ~35°C, usually lasting for only a few hours (Fig. 1).

Normothermia. The TNZ of normothermic C. nanus ranged from Ta 28.7 ± 0.9 to 32.9 ± 0.7°C, and the BMR was 0.66 ± 0.17 ml·g−1·h−1 (body mass = 36.0 ± 7.5 g, N = 7, n = 29, Fig. 2A). The RMR increased linearly with a decreasing Tb (r2 = 0.86, P < 0.001) between the Tlc and Ta 5°C (Fig. 2A). Below Ta 5°C, Tb was more variable and increased at a higher rate because of the intensive shivering required for heat production (Fig. 2A). These values and corresponding Tb values were excluded from regression analyses.

Tb of normothermic individuals at rest both below and above the TNZ was relatively stable and independent of Ta (Fig. 2B). Below the Tlc, mean Tb was 33.9 ± 0.6°C (N = 8, n = 80), and within the TNZ mean Tb was 34.3 ± 0.4°C (N = 7, n = 29, Fig. 2B). ΔT (Tb − Ta) was negatively correlated with Tb both below the Tlc (r2 = 0.99, P < 0.001) and within the TNZ (r2 = 0.80, P < 0.001, Fig. 2C).
Above the $T_{tc}$, $C$ increased significantly with rising $T_a$ ($r^2 = 0.75$, $P < 0.001$, Fig. 2D). The $T_{tc}$ C also showed a positive relationship with $T_a$, but the slope of the regression was much shallower ($r^2 = 0.16$, $P < 0.001$, Fig. 2D). The C at $T_a$ 5.8 ± 0.5°C was 0.110 ± 0.015 ml·g$^{-1}$·h$^{-1}$·°C$^{-1}$ ($N = 7$, $n = 14$). At low $T_a$ values, $C$ fluctuated markedly, consistent with variations of RMR (Fig. 2A and D).

Torpor, C. nanus displayed torpor during all measurements at $T_a$ values ranging from 5 to 30°C. During steady-state torpor, two different physiological responses to a change of $T_a$ were observed. Animals showed proportional thermoregulation only below the $T_{tc}$ of 4.8 ± 0.7°C ($N = 7$, $n = 87$, Fig. 2). The minimum $T_b$ at the $T_{tc}$ was 5.9 ± 0.7°C, and the corresponding minimum TMR was 0.019 ± 0.003 ml·g$^{-1}$·h$^{-1}$·°C$^{-1}$ ($N = 7$, $n = 7$), which was 0.6% of the RMR at the same $T_a$.

Over the $T_a$ values ranging from the $T_{tc}$ to 30°C, TMR was positively related to $T_a$ (Fig. 2A) and was better described by an exponential regression ($r^2 = 0.90$) than a linear fit ($r^2 = 0.74$). In this $T_a$ range, $T_b$ linearly correlated with $T_a$ ($r^2 = 0.99$, $P < 0.001$, Fig. 2B). The animals that entered torpor within the TNZ (above $T_a$ 28.7°C) had a TMR of 0.341 ± 0.085 ml·g$^{-1}$·h$^{-1}$ ($N = 6$, $n = 7$) and a $T_b$ of 31.4 ± 0.5°C ($N = 6$, $n = 7$).

$\Delta T$ also decreased with $T_a$ above the $T_{tc}$ ($r^2 = 0.26$, $P < 0.001$, Fig. 2C). However, this was mainly due to a significant relationship between $\Delta T$ and $T_a$ at high $T_a$ from 23 to 30°C ($r^2 = 0.14$, $P < 0.05$). Below $T_a$ 20°C to the $T_{tc}$, $\Delta T$ was not correlated with $T_a$ ($r^2 = 0.0003$, $P > 0.05$, mean $\Delta T = 1.9 ± 0.9°C$), although in this $T_a$ range TMR showed a significant decline ($r^2 = 0.58$, $P < 0.001$, $N = 8$, $n = 48$).

Above the $T_{tc}$, the $C$ of torpid animals decreased with decreasing $T_a$ ($r^2 = 0.61$, $P < 0.01$, Fig. 2A). $T_b$ was maintained relatively stable at 6.1 ± 1.0°C ($N = 7$, $n = 11$), which was 27.4°C lower than the normothermic value, and $T_b$ was not correlated with $T_a$ ($r^2 = 0.0004$, Fig. 2B). $\Delta T$ increased with a decreasing $T_a$ ($r^2 = 0.66$, $P < 0.01$, Fig. 2C). The $C$ at $T_a$ 1.3 ± 0.6°C (the lowest $T_a$ measured) was 0.099 ± 0.013 ml·g$^{-1}$·h$^{-1}$·°C$^{-1}$ ($N = 5$, $n = 5$). The regression coefficient for $C$ vs. $T_a$ below the $T_{tc}$ was not significant ($r^2 = 0.08$, $P > 0.1$, Fig. 2D). Moreover, the $C$ at 1.3°C was similar to the mean $C$ of resting individuals below the $T_{tc}$ although the TMR was only ~10% of RMR at the same $T_a$ (Fig. 2, A and D).

Above the $T_{tc}$ at which animals in steady-state torpor showed no proportional thermoregulation, TMR was an exponential function of $T_b$ ($r^2 = 0.92$, $P < 0.001$, Fig. 3A). A linear fit was clearly inappropriate, although it was statistically significant ($r^2 = 0.77$, $P < 0.001$, Fig. 3A). The $Q_{10}$ for the reduction of steady-state MR over the $T_a$ from the TNZ to the $T_{tc}$ was 3.3. However, $Q_{10}$ varied at different $T_a$ values (Table 2). When data were presented and analyzed in an Arrhenius plot, two linear regressions with a transition at $T_a$ 20.2°C provided the best fit. Below $T_a$ 20.2°C, $Q_{10}$ was 2.1; above $T_a$ 20.2°C, $Q_{10}$ was 3.7 (Fig. 3B).

The relationships between TMR and $\Delta T$ and between TMR and $C$ measured above the $T_{tc}$ differed at lower ($T_{tc}$ to 20°C) and higher (23 to 30°C) $T_a$ values (Fig. 4). In the lower $T_a$ range, the TMR was not correlated with $\Delta T$ ($r^2 = 0.03$, $P > 0.1$, Fig. 4A). The lack of a relationship between TMR and $\Delta T$ below $T_a$ 20°C, where TMR declined with $T_a$ (Fig. 2A), was most likely explained by the significant relationship between $C$ and TMR ($r^2 = 0.43$, $P < 0.001$, Fig. 4B). In the higher $T_a$ range, TMR was significantly related to $\Delta T$ ($r^2 = 0.34$, $P < 0.001$, Fig. 4A). This was most likely explained by the fact that $C$ was not correlated with TMR ($r^2 = 0.01$, $P > 0.1$, Fig. 4B).

In the $T_a$ range below the $T_{tc}$ where $T_b$ was regulated and relatively stable, TMR of hibernating individuals was not correlated with $T_b$ ($r^2 = 0.07$, $P > 0.1$, Fig. 5A), as during normothermia ($r^2 = 0.03$, $P > 0.05$, Fig. 5A). The $Q_{10}$ for MR between normothermic and hibernating thermoregulating animals during hibernation and during normothermia at a $T_a$ 1.5°C was 2.2 for values derived from regressions and 2.7 for measured values (Fig. 6). Below the $T_{tc}$, TMR was a linear function of $\Delta T$.
Both the slope and the intercept for the regression of MR vs. DT in hibernating and normothermic animals were indistinguishable (P > 0.05, t-test, Fig. 5B). Below the Tt, TMR was also positively related to C (r^2 = 0.59, P < 0.01, Fig. 5C), in contrast to the situation during normothermia where RMR showed no linear relationship with C (r^2 = 0.04, P = 0.05, Fig. 5C).

**DISCUSSION**

Our study clearly shows that a change of T_a below and above the Tt resulted in an entirely different response of T_b and MR in torpid C. nanus. Above the Tt, T_b passively followed T_a, and TMR was an exponential function of T_b. Below the Tt, T_b was defended by metabolic thermogenesis and, therefore, TMR was inversely related to T_a. These two different thermal responses of TMR are known to occur in other heterothermic species (14, 16, 25). This suggests that the TMR of heterothermic endotherms in the two T_a ranges are due to different physiological responses.

Thermoregulation during hibernation. Below the Tt, the core T_b of C. nanus was regulated at ~6°C, and metabolic heat production increased with an increasing cold load. This ability of proportional thermoregulation during torpor is one of the principal differences between torpor in endotherms and that in ectotherms (17). As in C. nanus, the onset of thermoregulation of hibernating mammals is stimulated when the set point for T_b is approached (5, 14). Because the regression of TMR vs. DT below Tt was not different from that of RMR vs. DT below Tlc, it appears that the physiological processes underlying thermoregulation at low T_a values during hibernation are similar to those during normothermia (12), although the set point for T_b differs substantially between normothermia and torpor (5, 9).

Nevertheless, it seems that the C contributes to thermoregulation during hibernation and normothermia in a different way. Below the Tlc to about T_a 5°C, C of normothermic resting individuals decreased slightly with T_a, which ensures a minimum heat loss to keep a constant T_b in the cold. In contrast, in torpid thermoregulating individuals during hibernation, C increased substantially together with increasing thermogenesis. Because the posture and position of the animals at low T_a indicated that they were trying to minimize heat...
Table 1. Regression equations (y = a + b × Tₐ) for physiological variables in Fig. 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Equation</th>
<th>Standard Error</th>
<th>R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torpor, above Tₜ₝ (N = 8, n = 76)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log TMR</td>
<td>-1.991 ± 0.038</td>
<td>0.052 ± 0.002</td>
<td>0.90</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tₜ₝</td>
<td>0.527 ± 0.171</td>
<td>1.046 ± 0.009</td>
<td>0.99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ΔT</td>
<td>0.529 ± 0.171</td>
<td>0.046 ± 0.009</td>
<td>0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C</td>
<td>-0.029 ± 0.040</td>
<td>0.008 ± 0.002</td>
<td>0.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Torpor, below Tₜ₝ (N = 7, n = 11)</td>
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<tr>
<td>TMR</td>
<td>0.557 ± 0.080</td>
<td>-0.094 ± 0.024</td>
<td>0.61</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tₜ₝</td>
<td>5.81 ± 0.796</td>
<td>0.074 ± 0.217</td>
<td>0.0004</td>
<td>0.74</td>
</tr>
<tr>
<td>ΔT</td>
<td>5.81 ± 0.706</td>
<td>-0.926 ± 0.217</td>
<td>0.66</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C</td>
<td>0.103 ± 0.017</td>
<td>0.007 ± 0.005</td>
<td>0.08</td>
<td>0.22</td>
</tr>
<tr>
<td>Normothermia, above Tₜ₝ (N = 8, n = 80)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RMR</td>
<td>3.689 ± 0.090</td>
<td>-0.106 ± 0.005</td>
<td>0.86</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tₜ₝</td>
<td>33.947 ± 0.157</td>
<td>-0.005 ± 0.008</td>
<td>0.004</td>
<td>0.56</td>
</tr>
<tr>
<td>ΔT</td>
<td>33.947 ± 0.157</td>
<td>-1.005 ± 0.008</td>
<td>0.99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C</td>
<td>0.076 ± 0.013</td>
<td>0.003 ± 0.001</td>
<td>0.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Normothermia, in the TNZ (N = 7, n = 29)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RMR</td>
<td>1.921 ± 0.532</td>
<td>-0.041 ± 0.018</td>
<td>0.14</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Tₜ₝</td>
<td>30.215 ± 2.507</td>
<td>0.129 ± 0.083</td>
<td>0.05</td>
<td>0.13</td>
</tr>
<tr>
<td>ΔT</td>
<td>30.227 ± 2.506</td>
<td>-0.872 ± 0.083</td>
<td>0.80</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C</td>
<td>-1.000 ± 0.131</td>
<td>0.040 ± 0.004</td>
<td>0.75</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values for a and b are means ± SE. TMR, metabolic rate (MR) during torpor; ΔT, thermal differential between body temperature (Tₜ) and air temperature (Tₐ); C, thermal conductance; Tₜ₝, lower critical temperature during normothermia; Tₜ₝, critical Tₜ during torpor; RMR, resting MR; N, number of animals; n, number of observations.

loss, it is most likely that shivering and the increased respiratory and circulatory activities, which were concomitant with the increasing MR for thermoregulation, resulted in an unavoidable increase in C. This explanation also applies to normothermic animals at Tₜ below 5°C, in which C increased significantly with the onset of shivering, suggesting that shivering thermogenesis is very important in marsupials because they appear to lack brown adipose tissue. Of course, changes of C with thermoregulation in torpor could also involve changes in peripheral circulation caused by elevation in blood pressure and, accordingly, increased blood distribution into the periphery during thermoregulation at low Tₜ values. Although the C of thermoregulating animals during hibernation at low Tₜ values was similar to that during normothermia, the TMR was only a fraction of RMR. This demonstrates that a C below that of normothermic individuals is not a prerequisite either for a low TMR or for thermoregulation during hibernation. It has been shown previously that a low C is also not a prerequisite for a low TMR during daily torpor (10, 25).

Reduction of TMR. The decrease of TMR and Tₜ with Tₜ below Tₜ₝ clearly differed from that of thermoregulating individuals. In this Tₜ range, TMR was an exponential function of Tₜ, suggesting that the reduction of TMR is dependent on Tₜ. It has been well documented that reaction rates of enzymes in vitro also show exponential relationships with temperature with a typical Q₁₀ of 2–3 (1, 8, 22). Because energetic processes of a living organism rely on enzyme-catalyzed reactions, the principle of thermodynamics should act as a general law that governs the biochemical processes of all kinds of animals (19). Our observation and in vivo observations for other heterothermic endotherms (6, 23) as well as ectotherms support the above interpretation. Furthermore, a Q₁₀ of 2.2–2.7 (Fig. 6) for MR decline from normothermic to torpid thermoregulating animals at the same Tₜ suggests that even this response to temperature is caused by the reduction of Tₜ, as has been reported in other hibernators (5, 14).
Although the Tb was lowered with TMR, the overall Q₁₀ for TMR reduction between normothermic and hibernating C. nanus was slightly larger than 3, reflecting a combined effect of temperature and metabolic inhibition. Furthermore, much higher Q₁₀ values were observed between BMR and TMR at higher Tb. This is different from daily heterotherms in which MR reduction appears to be largely a function of lowered Tb (6, 25), strongly suggesting that temperature effects alone are not sufficient to explain all of the reduction of TMR during hibernation in C. nanus. Q₁₀ values above the range of 2–3 have also been reported in other species (6, 15, 20) and suggest a synergistic effect of metabolic inhibition and temperature effects on TMR (6, 18, 26).

### Table 2. Ta, Tb, and MR in Cercartetus nanus during different physiological states and the corresponding Q₁₀ values derived from the MR and Tb measurements

<table>
<thead>
<tr>
<th>State</th>
<th>Ta (°C)</th>
<th>Tb (°C)</th>
<th>MR (ml·h⁻¹)</th>
<th>Q₁₀ (1–2)</th>
<th>Q₁₀ (2–3)</th>
<th>Q₁₀ (3–4)</th>
<th>Q₁₀ (4–5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Normothermic in the TNZ</td>
<td>30.5</td>
<td>34.3</td>
<td>0.655</td>
<td>9.5</td>
<td>3.1</td>
<td>2.8</td>
<td>3.3</td>
</tr>
<tr>
<td>2) Torpid in the TNZ</td>
<td>29.0</td>
<td>31.4</td>
<td>0.341</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) Torpid</td>
<td>19.8</td>
<td>21.0</td>
<td>0.106</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4) Torpid at the Ttc</td>
<td>5.0</td>
<td>6.1</td>
<td>0.022</td>
<td></td>
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</tbody>
</table>

Ta and Tb in °C; MR in ml·g⁻¹·h⁻¹. TNZ, thermoneutral zone.

**Fig. 4.** TMR of torpid, nonthermoregulating C. nanus as a function of ΔT between Tb and Ta (A) and apparent C at Tc values above the Ttc (B). Regression equations (through whole data set, dashed line) were as follows: TMR = −0.03 + 0.129 ΔT (r² = 0.48, P < 0.001, N = 8, n = 76); TMR = 0.104 + 0.326 C (r² = 0.14, P < 0.001, N = 8, n = 76). In low-Ta range (5–20°C, ▽), TMR was not related to ΔT [TMR = 0.031 + 0.015 ΔT (r² = 0.03, P = 0.14, N = 8, n = 48)], but was significantly correlated with C (▽, solid line) [TMR = 0.014 + 0.59 C (r² = 0.43, P < 0.001, N = 8, n = 48)]. In high-Ta range (23–30°C, ▽, solid line), TMR was significantly correlated with ΔT [TMR = 0.177 + 0.068 ΔT (r² = 0.34, P < 0.001, N = 8, n = 28)], but was not related to C [TMR = 0.319 − 0.057 C (r² = 0.01, P = 0.49, N = 8, n = 28)].

**Fig. 5.** TMR (●, N = 8, n = 11) below Ttc (thermoregulating) and resting MR (RMR) of normothermic individuals (○, N = 8, n = 80) below the lower critical temperature (Tlc) as a function of Tb (A), ΔT between Tb and Ta (B), and apparent C (C). Neither TMR nor RMR of thermoregulating C. nanus was related to Tfc: TMR = −0.191 + 0.079 Tb (r² = 0.07, P = 0.23); RMR = −8.67 + 0.313 Tb (r² = 0.03, P = 0.07). Both TMR and RMR were significantly related to ΔT: TMR = −0.032 + 0.101 ΔT (r² = 0.91, P < 0.001); RMR = 0.096 + 0.106 ΔT (r² = 0.88, P < 0.001). TMR was also positively related to C [TMR = −0.208 + 5.89 C (r² = 0.59, P < 0.01)], whereas RMR was not related to C [RMR = 2.41 − 3.89 C (r² = 0.04, P = 0.05)].
The use of metabolic inhibition at low Tb values also appears to be more pronounced in small than in large hibernators, because the extent of metabolic reduction was mass dependent (6). The apparent metabolic inhibition in hibernators could be caused by a number of mechanisms, including respiratory acidosis and pH alterations, reversible phosphorylation, and switching to different metabolic pathways (11, 18, 26). However, part of the substantial reduction of TMR during hibernation may also be explained by the higher activation energy, and thus Q10, of some enzymes of hibernators.

Interestingly, C. nanus displayed torpor in the TNZ. The TMR of torpid individuals dropped by nearly 50% from BMR, whereas Tb dropped by only 3°C and therefore a very large Q10 was observed (Table 2). This is clear evidence for largely temperature-independent metabolic inhibition in endotherms. This result also shows that hibernation is not always initiated by cessation of heat production for normothermic thermoregulation and the subsequent lowered Tb as commonly accepted (2, 29). It suggests that metabolic inhibition may play a very important role in the transition from normothermic thermoregulation to MR reduction during entry into hibernation (6, 15, 18, 19). This point of view is further supported by the observation that the decline of MR was faster than that of Tb at the onset of hibernation in C. nanus.

Above the Ttc, the ΔT was stable over a wide range of Ta in which TMR showed a significant decline. This lack of a correlation between the two variables demonstrates that TMR is not downregulated in proportion to ΔT. This is further supported by the observation that the calculated TMR values derived from RMR values using the reduction of ΔT alone were significantly higher (33%, 10-fold, 47%, respectively) than the measured values (Fig. 6).

In the high-Ta range (Ta 23–30°C), C was high and did not change with the fall of TMR (Figs. 2D and 4B). This implies that while thermogenesis was inhibited, heat loss was probably also facilitated via a high C at high Tb values. Although in the low Ta range TMR was positively related to C (Fig. 4B), it seems that C did not cause the TMR decline but changed passively as a consequence of the changing TMR. This may include a significantly decreased peripheral circulation and a decreased respiratory heat loss (10) in association with the low Tb and TMR. This interpretation is supported by the finding that exposure to He-O2 (helium-oxygen) resulted in a fall of TMR but not a change of C. In this case a lowered peripheral circulation appeared to compensate for the direct effect of the more conductive medium on C (10). However, the lowered C at the low TMR may prevent Tb from reaching the set point during torpor, which would induce an increase of TMR.

The increase of TMR with Ta during torpor above Ttc also differs from the thermoregulatory response of normothermic individuals above Ttc although both show an increase of C. Above the Ttc, ΔT decreased whereas C showed a steep increase with Tb to avoid overheating and maintain Tb at a normothermic level. In contrast, the increase of C during torpor above Ttc was accompanied by a rise in both Tb and TMR. A high C at high Ta values during torpor is not used for maintenance of a constant Tb but appears to reduce Tb and thus TMR.

Our study provides clear evidence of metabolic inhibition during mammalian hibernation. It supports the view that the reduction of MR during hibernation is largely caused by temperature effects and metabolic inhibition. The ΔT appears to determine the steady-state MR below the Ttc, but does not satisfactorily explain TMR above the Ttc. C does not appear to affect TMR directly, but may be important at high Ta values at which C is high, most likely to dump heat, and also when the Tset is approached when C is minimal, perhaps to delay onset of thermoregulation.

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Fig. 6. Comparison between TMR and predicted RMR (RMR') for these TMR given the same differential between Tb and Ta. RMR' was calculated from the equation RMR' = 3.69 - 0.106 Tb. Tb was determined by using the formula Tb = Tb(1 - Ta/Ta1), where Tb1 is the normothermic value and Ta1 and Ta2 are the values during torpor at which TMR was measured. Examples for measurements below the set point, at Tb1 and in the thermoneutral zone showed that all derived RMR' values were significantly larger than the TMR. Q10 values for MR between thermoregulating animals during normothermia and during torpor were calculated for both measured data (Q10 = 2.7) and data derived from regressions of MR vs. Ta given in Fig. 2A (Q10 = 2.2).

Because Q10 values over the range of 2–3 were observed in C. nanus only at higher Ta values, especially in the TNZ, it seems that Q10 values for MR differ at different Tb values (6, 15), and the metabolic inhibition may be more pronounced during the transient state between normothermia and torpor when Tb is high (8, 19). The use of metabolic inhibition at low Tb values also appears to be more pronounced in small than in large hibernators, because the extent of metabolic reduction is mass dependent (6). The apparent metabolic inhibition in hibernators could be caused by a number of mechanisms, including respiratory acidosis and pH alterations, reversible phosphorylation, and switching to different metabolic pathways (11, 18, 26). However, part of the substantial reduction of TMR during hibernation may also be explained by the higher activation energy, and thus Q10, of some enzymes of hibernators than those of daily heterotherms (8, 21).

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