Thermal relations of metabolic rate reduction in a hibernating marsupial

XIAOWEI SONG, GERHARD KÖRTNER, AND FRITZ GEISER
Zoology, University of New England, Armidale, New South Wales 2351, Australia

Song, Xiaowei, Gerhard Körtner, and Fritz Geiser. 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 (T_b), the differential (ΔT) between T_b and air temperature (T_a), or thermal conductance (C). Above the critical T_a during torpor (T_{tc}) of 4.8 ± 0.7°C, where the T_b was not regulated, the steady-state MR was an exponential function of T_b ($r^2 = 0.92$), and the overall Q_{10} was 3.3. However, larger Q_{10} values were observed at high T_b values during torpor, particularly within the thermoneutral zone ($Q_{10} = 9.5$), whereas low Q_{10} values were observed below T_b 20°C ($Q_{10} = 1.9$). The ΔT did not change over T_a 5–20°C, although MR fell, and therefore the two variables were not correlated. Below the T_{tc}, T_b was regulated at 6.1 ± 1.0°C and MR increased proportionally to ΔT. Our study suggests that MR in torpid *C. nanus* is largely determined by temperature effects and metabolic inhibition. In contrast, ΔT explains MR only below the T_{tc} and C appears to affect MR only indirectly via changes of T_b, suggesting that ΔT and C play only a secondary role in MR reduction during hibernation.

body temperature; *Cercartetus nanus*; oxygen consumption; thermoregulation; torpor

MANY SMALL MAMMALS reduce energy expenditure in response to cold and/or food shortage by becoming torpid. The physiological mechanisms causing the reduction of metabolic rate (MR) during torpor have evoked much controversy. Originally it was proposed that MR is reduced by the effect of lowered body temperature (T_b) on tissue metabolism because the Q_{10} for MR reduction between normothermia and torpor in many species is between 2 and 3, which is typical for the temperature dependence of biochemical reactions (23, 27). However, MRs below that for temperature effects and consequently Q_{10} values well above 2–3 have been observed in a number of species (6, 15, 20). This was attributed to a temperature-independent metabolic inhibition, which may contribute to the low MR in addition to the effect of low T_b (6, 18, 19, 26). More recently, it was proposed that the thermal differential (ΔT) between T_b and air temperature (T_a) and low thermal conductance (C) should be considered as alternative explanations for the low MR during torpor (12, 13, 24).

A detailed study on interrelations between physiological variables during daily torpor of the marsupial *Sminthopsis macroura* suggests that the steady-state MR is determined by different physiological responses above and below the set point (T_{set}) for T_b that is defended by proportional thermoregulation (14, 25). At T_a above the T_{set}, MR is largely a function of T_b, supporting the Q_{10} effect interpretation (25). In contrast, below the T_{set}, 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 T_b ~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 T_a (17), making a detailed analysis on the effect of T_a on steady-state T_b 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 T_b as low as 2°C (3, 7). This species was selected because it displays torpor over a wide range of T_a, which allows a systematic analysis of the interrelations between physiological variables during hibernation. We measured simultaneously steady-state MR and T_b during torpor at T_a from 1 to 30°C and determined how MR, T_b, 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 T_a values below 15°C, animals were acclimated at T_a 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.

MR, determined as rate of oxygen consumption ($\dot{V}O_2$), 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 (Ametek 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 continuously monitored with mass flowmeters (FMA-5606; Omega, Stamford, CT).

For long-term records of T_b, 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 T_a 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.

T_a 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 flowmeter, oxygen analyzer, transmitter receiver, and thermocouples were interfaced via an analog to digital converter (DT100 logger, Data Electronics). Readings were taken every 3 min. $\dot{V}O_2$ values (STPD) were calculated according to *equation* 3*A* of Withers (28).

Animals were considered to be torpid when their T_b was lower than 32°C. At those T_b values, MRs were always <75% of the resting MR (RMR) at the same T_a, except for two measurements from one individual within the thermoneutral zone (TNZ) for which MRs were 80 and 88% of basal MR (BMR). MR during torpor (TMR) was measured at a constant T_a set between 5 and 30°C. Each measurement lasted over a complete torpor bout (1–20 days, depending on the T_a). In addition, cooling experiments were conducted during the light phase, to determine the set point for T_b. When the TMR had stabilized at T_a 6°C, T_a was reduced in 1°C steps, until an increase in TMR and no further decline of T_b were observed. This T_a was then maintained until the steady-state TMR and T_b had been measured. TMR was obtained by calculating the mean of the consecutive 40 lowest $\dot{V}O_2$ values (i.e., over 2 h), and corresponding T_b and T_a were determined.

RMR was measured at constant T_a values ranging from 1 to 30°C in the light phase in normothermic individuals whose T_b values were >32.0°C. To determine the TNZ and the BMR, RMR was also measured between 0930 and 1700. After animals had been in the chambers for at least 1 h, T_a 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 $\dot{V}O_2$ values (i.e., over 30 min). The means of the corresponding 10 T_b and T_a 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 $C = MR/(T_b - T_a)$. Q_{10} values for MR at different T_b were calculated according to the equation $Q_{10} = (MR_1/MR_2)^{(10/T_{b1} - T_{b2})}$.

Data are presented as means ± SD. *N* indicates the number of animals, and *n* indicates the total number of observations. Data obtained from the same individual at the same T_a were averaged for statistical analyses. Differences between means were examined using a Student's *t*-test. Regressions were determined by the method of least squares. Paired comparisons of regressions were conducted using Student's *t*-test (31). Selection of the appropriate regression models (linear or exponential) was made by comparing the coefficient of determination (r^2) for the linear model with the r^2 for the regression of the predicted *y*-value for the exponential model versus the measured *y*-value. Direct comparison of the r^2 values for the linear regression and the exponential regression cannot be made because the total sum of squares for both models differ and equality of the total sum of squares is essential if r^2 values are to be compared (4).

The lower critical temperature (T_{lc}) of each normothermic individual was determined by the intercept of two regressions fitted through the split data set of RMR vs. T_a, whereby the smallest sum of the residual sum of squares for the two regressions determined the best fit (30). Similarly, the critical air temperature during torpor (T_{tc}), below which animals begin to thermoregulate, was determined from the split data set of TMR versus T_a for each individual. This procedure was also applied in the determination of regression lines in the Van't Hoff-Arrhenius plot, in which the two-line fit showed the smallest sum of the residual sum of squares (30). Because ΔT appeared to be constant at low T_a values above the T_{tc} but was temperature dependent at high T_a values, we analyzed the correlation between T_a and ΔT during torpor above the T_{tc} by fitting the whole data set with two regressions, starting at 30°C. Data were separated from the T_a at which the linear regression for the lower ΔT values became insignificant.

RESULTS

The MR and T_b 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 T_b. The steady-state TMR was usually reached after 3–5 h (Fig. 1). Torpor bout duration varied with T_a. Below T_a 25°C, torpor bouts usually lasted for several days (Fig. 1*A*). Above T_a 25°C, torpor bouts were usually shorter than 1 day, and T_b and TMR during steady-state torpor were more variable than at low T_a values (Fig. 1*B*). Torpor was periodically interrupted by spontaneous arousal, which often occurred in the afternoon, several hours before lights off. Arousal was characterized by a MR overshoot and a rise of T_b, followed by postarousal with RMR and normothermic T_b of ~35°C, usually lasting for only a few hours (Fig. 1).

Normothermia. The TNZ of normothermic *C. nanus* ranged from T_a 28.7 ± 0.9 to 32.9 ± 0.7°C, and the BMR was 0.66 ± 0.17 $ml \cdot g^{-1} \cdot h^{-1}$ (body mass = 36.0 ± 7.5 g, $N = 7$, $n = 29$, Fig. 2*A*). The RMR increased linearly with a decreasing T_a ($r^2 = 0.86$, $P < 0.001$) between the T_{lc} and T_a 5°C (Fig. 2*A*). Below T_a 5°C, MR was more variable and increased at a higher rate because of the intensive shivering required for heat production (Fig. 2*A*). These values and corresponding T_b values were excluded from regression analyses.

T_b of normothermic individuals at rest both below and above the TNZ was relatively stable and independent of T_a (Fig. 2*B*). Below the T_{lc}, mean T_b was 33.9 ± 0.6°C ($N = 8$, $n = 80$), and within the TNZ mean T_b was 34.3 ± 0.4°C ($N = 7$, $n = 29$, Fig. 2*B*). ΔT ($T_b - T_a$) was negatively correlated with T_a both below the T_{lc} ($r^2 = 0.99$, $P < 0.001$) and within the TNZ ($r^2 = 0.80$, $P < 0.001$, Fig. 2*C*).

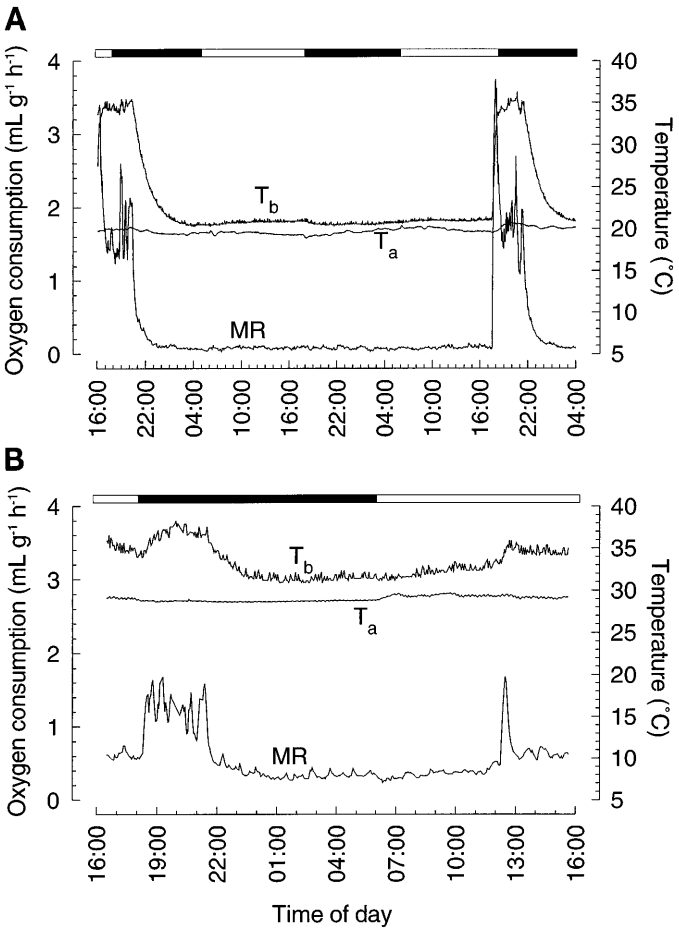

Fig. 1. Fluctuations of metabolic rate (MR) and body temperature (T_b) of a *Cercartetus nanus* at air temperatures (T_a) of 20°C (*A*) and 29°C (*B*). Dark bar indicates dark phase.

Above the T_{lc}, C increased significantly with rising T_a ($r^2 = 0.75$, $P < 0.001$, Fig. 2*D*). Below the T_{lc}, 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. 2*D*). 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. 2, *A* 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} ($N = 7$, $n = 7$), 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. 2*A*) 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. 2*B*). 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 ($N = 6$, $n = 7$).

ΔT also decreased with T_a above the T_{tc} ($r^2 = 0.26$, $P < 0.001$, Fig. 2*C*). However, this was mainly due to a significant relationship between ΔT 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}, ΔT was not correlated with T_a ($r^2 = 0.0003$, $P > 0.05$, mean ΔT = 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 T_a (Fig. 2*D*). At the T_{tc}, the minimum C was 0.023 ± 0.012 ml·g^{-1}·h^{-1}·°C^{-1} ($N = 7$, $n = 7$), which was significantly lower than the minimum C of normothermic individuals in the same range of T_a ($P < 0.01$, *t*-test, Fig. 2*D*).

At T_a values below the T_{tc}, TMR increased with decreasing T_a ($r^2 = 0.61$, $P < 0.01$, Fig. 2*A*). 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. 2*B*). ΔT increased with a decreasing T_a ($r^2 = 0.66$, $P < 0.01$, Fig. 2*C*). 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. 2*D*). Moreover, the C at 1.3°C was similar to the mean C of resting individuals below the T_{lc}, 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. 3*A*). A linear fit was clearly inappropriate, although it was statistically significant ($r^2 = 0.77$, $P < 0.001$, Fig. 3*A*). 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_b of 20.2°C provided the best fit. Below T_b 20.2°C, Q_{10} was 2.1; above T_b 20.2°C, Q_{10} was 3.7 (Fig. 3*B*).

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

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. 5*A*), as during normothermia ($r^2 = 0.03$, $P > 0.05$, Fig. 5*A*). The Q_{10} for MR between normothermic and hibernating thermoregulating animals during hibernation and during normothermia at a T_a of 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 ΔT

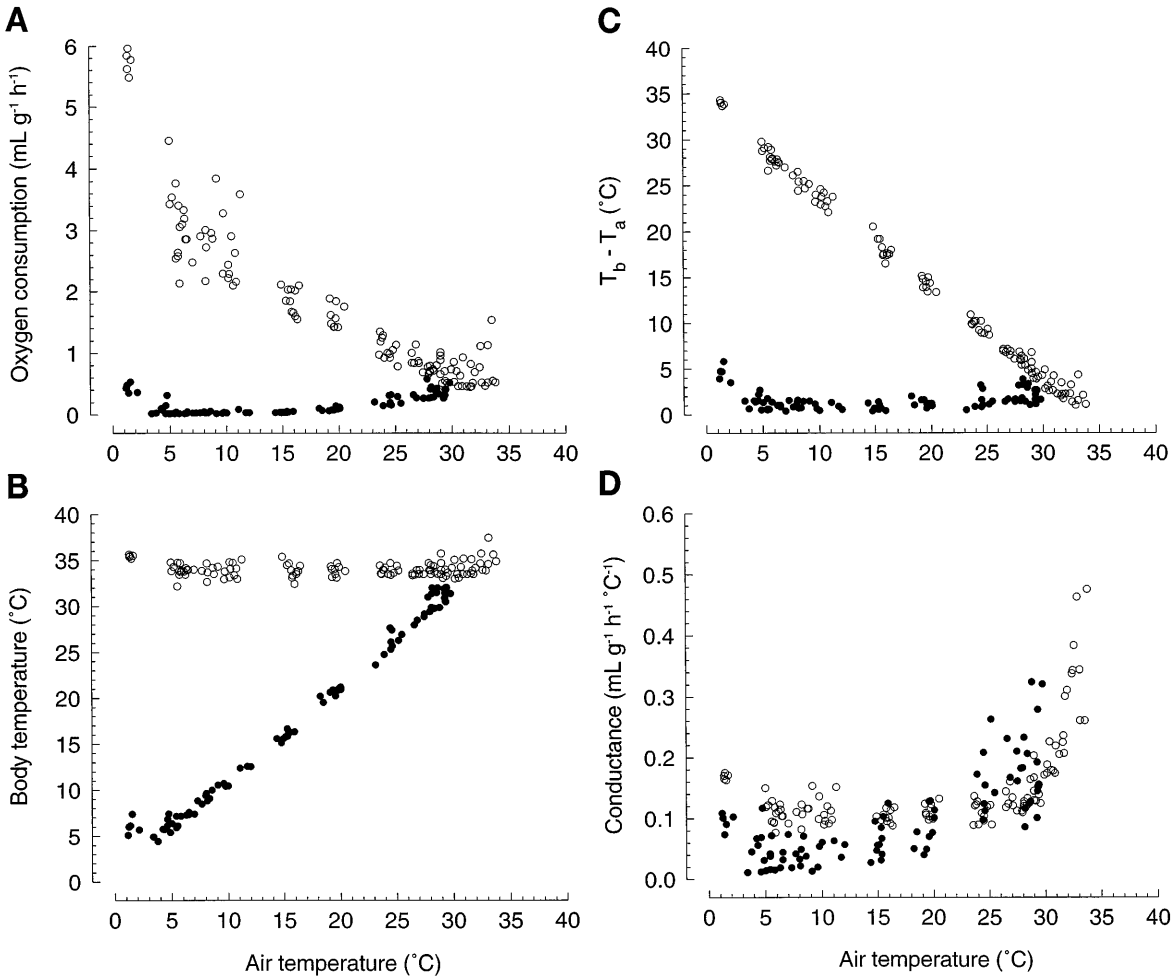

Fig. 2. Effect of T_a on MR measured as rate of oxygen consumption (*A*), T_b (*B*), temperature differential (ΔT) between T_b and T_a (*C*), and apparent thermal conductance (*C*; *D*) during hibernation (●) and normothermia (○) in *C. nanus*. Mean body mass was 36.2 ± 5.8 g. Regression equations ($y = a + b \times T_a$) for physiological variables are shown in Table 1.

($r^2 = 0.91$, $P < 0.001$, Fig. 5*B*). Both the slope and the intercept for the regression of MR vs. ΔT in hibernating and normothermic animals were indistinguishable ($P > 0.05$, *t*-test, Fig. 5*B*). Below the T_{tc}, TMR was also positively related to C ($r^2 = 0.59$, $P < 0.01$, Fig. 5*C*), in contrast to the situation during normothermia where RMR showed no linear relationship with C ($r^2 = 0.04$, $P = 0.05$, Fig. 5*C*).

DISCUSSION

Our study clearly shows that a change of T_a below and above the T_{tc} resulted in an entirely different response of T_b and MR in torpid *C. nanus*. Above the T_{tc}, T_b passively followed T_a, and TMR was an exponential function of T_b. Below the T_{tc}, 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 T_{tc}, 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. ΔT below T_{tc} was not different from that of RMR vs. ΔT below T_{lc}, 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 T_{lc} 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 ($y = a + b \times T_a$) for physiological variables in Fig. 2*

y	*a*	*b*	r^2	*P*
Torpor, above the T_{tc} ($N = 8$, $n = 76$)				
log TMR	−1.991 ± 0.038	0.052 ± 0.002	0.90	<0.001
T_b	0.527 ± 0.171	1.046 ± 0.009	0.99	<0.001
ΔT	0.529 ± 0.171	0.046 ± 0.009	0.26	<0.001
C	−0.029 ± 0.040	0.008 ± 0.002	0.17	<0.001
Torpor, below the T_{tc} ($N = 7$, $n = 11$)				
TMR	0.557 ± 0.080	−0.094 ± 0.024	0.61	<0.01
T_b	5.81 ± 0.796	0.074 ± 0.217	0.0004	0.74
ΔT	5.81 ± 0.706	−0.926 ± 0.217	0.66	<0.01
C	0.103 ± 0.017	0.007 ± 0.005	0.08	0.22
Normothermia, below the T_{lc} ($N = 8$, $n = 80$)				
RMR	3.689 ± 0.090	−0.106 ± 0.005	0.86	<0.001
T_b	33.947 ± 0.157	−0.005 ± 0.008	0.004	0.56
ΔT	33.947 ± 0.157	−1.005 ± 0.008	0.99	<0.001
C	0.076 ± 0.013	0.003 ± 0.001	0.16	<0.001
Normothermia, in the TNZ ($N = 7$, $n = 29$)				
RMR	1.921 ± 0.532	−0.041 ± 0.018	0.14	<0.05
T_b	30.215 ± 2.507	0.129 ± 0.083	0.05	0.13
ΔT	30.227 ± 2.506	−0.872 ± 0.083	0.80	<0.001
C	−1.000 ± 0.131	0.040 ± 0.004	0.75	<0.001

Values for *a* and *b* are means ± SE. TMR, metabolic rate (MR) during torpor; ΔT, thermal differential between body temperature (T_b) and air temperature (T_a); C, thermal conductance; T_{lc}, lower critical temperature during normothermia; T_{tc}, critical T_a 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 T_a 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 T_a values. Although the C of thermoregulating animals during hibernation at low T_a 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 T_b with T_a in torpid *C. nanus* above T_{tc} clearly differed from that of thermoregulating individuals. In this T_a range, TMR was an exponential function of T_b, suggesting that the reduction of TMR is dependent on T_b. It has been well documented that reaction rates of enzymes in vitro also show exponential relationships with temperature with a typical Q_{10} 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 Q_{10} of 2.2–2.7 (Fig. 6) for MR decline from normothermic to torpid thermoregulating animals at the same T_a suggests that even this response to temperature is caused by the reduction of T_b, as has been reported in other hibernators (5, 14).

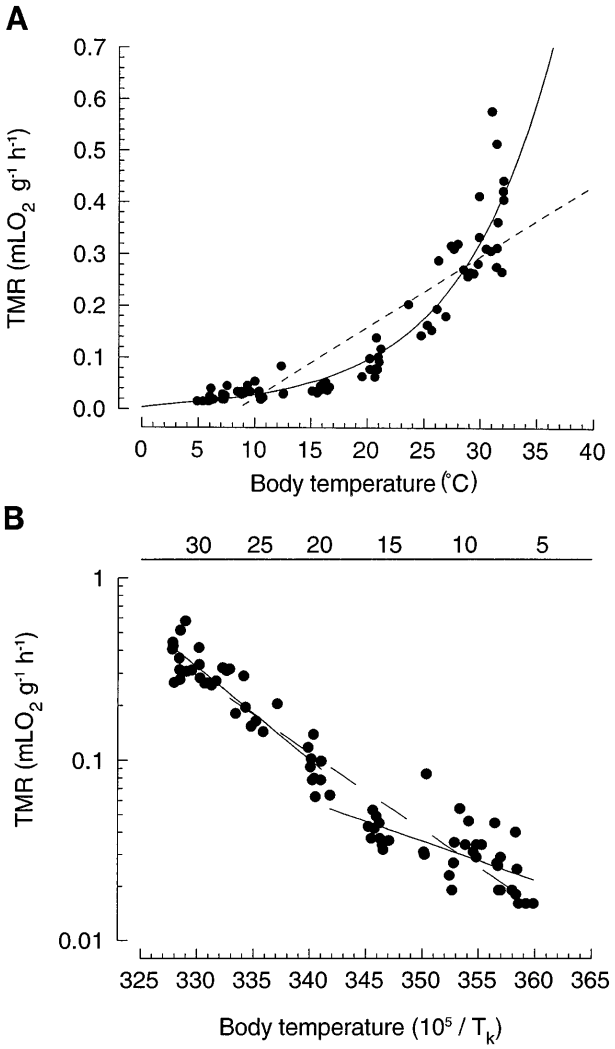

Fig. 3. MR during torpor (TMR) as a function of T_b above the critical air temperature during torpor (T_{tc}) in hibernating *C. nanus*. *A*: both linear regression (dashed line) (TMR = −0.116 + 0.014 T_b, $r^2 = 0.77$, $P < 0.001$) and exponential fit (solid line) (log TMR = −2.02 + 0.05 T_b, $r^2 = 0.92$, $P < 0.001$) were significant. However, the exponential fit provided the appropriate model, because r^2 for the regression of the predicted TMR for the exponential model vs. measured TMR ($r^2 = 0.89$) was larger than that for the linear model. *B*: slope of the regression of TMR vs. T_b (solid lines) in a Van't Hoff Arrhenius plot was steeper at T_b values ≥ 20.21°C [log TMR = 16.209 ± 1.17 − 0.0506 ± 0.004 × $10^5/T_k$, $r^2 = 0.85$, $P < 0.001$, number of animals (N) = 8, number of observations (n) = 39] than at T_b values ≤ 20.21°C (log TMR = 6.88 ± 1.304 − 0.0238 ± 0.004 × $10^5/T_k$, $r^2 = 0.51$, $P < 0.001$, $N = 8$, $n = 38$). T_k, absolute temperature in kelvin. Dashed line shows regression when values were fitted with 1 regression (log TMR = 13.394 ± 0.522 − 0.0422 ± 0.002 × $10^5/T_k$, $r^2 = 0.91$, $P < 0.001$, $N = 8$, $n = 76$). The 2-line fit was better than the single-line fit because the former had the smallest sum of residual sum of squares.

Table 2. *T_a, T_b, and MR in Cercartetus nanus during different physiological states and the corresponding Q_{10} values derived from the MR and T_b measurements*

	State			
	1) Normothermic in the TNZ	*2*) Torpid in the TNZ	*3*) Torpid	*4*) Torpid at the T_{tc}
T_a	30.5	29.0	19.8	5.0
T_b	34.3	31.4	21.0	6.1
MR, ml	0.655	0.341	0.106	0.022
	Q_{10} values			
	Q_{10} (*1–2*)=9.5	Q_{10} (*2–3*)=3.1	Q_{10} (*3–4*)=2.8	
	Q_{10} (*1–3*)=3.9	Q_{10} (*2–4*)=3.0		
	Q_{10} (*1–4*)=3.3			

T_a and T_b in °C; MR in ml·g^{-1}·h^{-1}. TNZ, thermoneutral zone.

Although the T_b was lowered with TMR, the overall Q_{10} 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 Q_{10} values were observed between BMR and TMR at higher T_b. This is different from daily heterotherms in which MR reduction appears to be largely a function of lowered T_b (6, 25), strongly suggesting that temperature effects alone are not sufficient to explain all of the reduction of TMR during hibernation in *C. nanus*. Q_{10} 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).

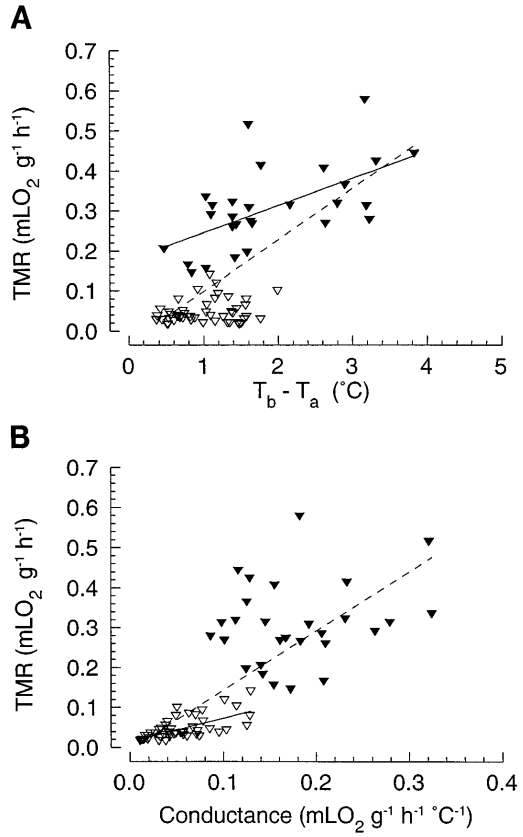

Fig. 4. TMR of torpid, nonthermoregulating *C. nanus* as a function of ΔT between T_b and T_a (*A*) and apparent C at T_c values above the T_{tc} (*B*). Regression equations (through whole data set, dashed line) were as follows: TMR = −0.03 + 0.129 ΔT (r^2 = 0.48, $P < 0.001$, N = 8, n = 76); TMR = 0.104 + 0.326 C (r^2 = 0.14, $P < 0.001$, N = 8, n = 76). In low-T_a range (5–20°C, ▽), TMR was not related to ΔT [TMR = 0.031 + 0.015 ΔT (r^2 = 0.03, P = 0.14, N = 8, n = 48)], but was significantly correlated with C (▽, solid line) [TMR = 0.014 + 0.59 C (r^2 = 0.43, $P < 0.001$, N = 8, n = 48)]. In high-T_a range (23–30°C, ▼, solid line), TMR was significantly correlated with ΔT [TMR = 0.177 + 0.068 ΔT (r^2 = 0.34, $P < 0.001$, N = 8, n = 28)], but was not related to C [TMR = 0.319 − 0.057 C (r^2 = 0.01, P = 0.49, N = 8, n = 28)].

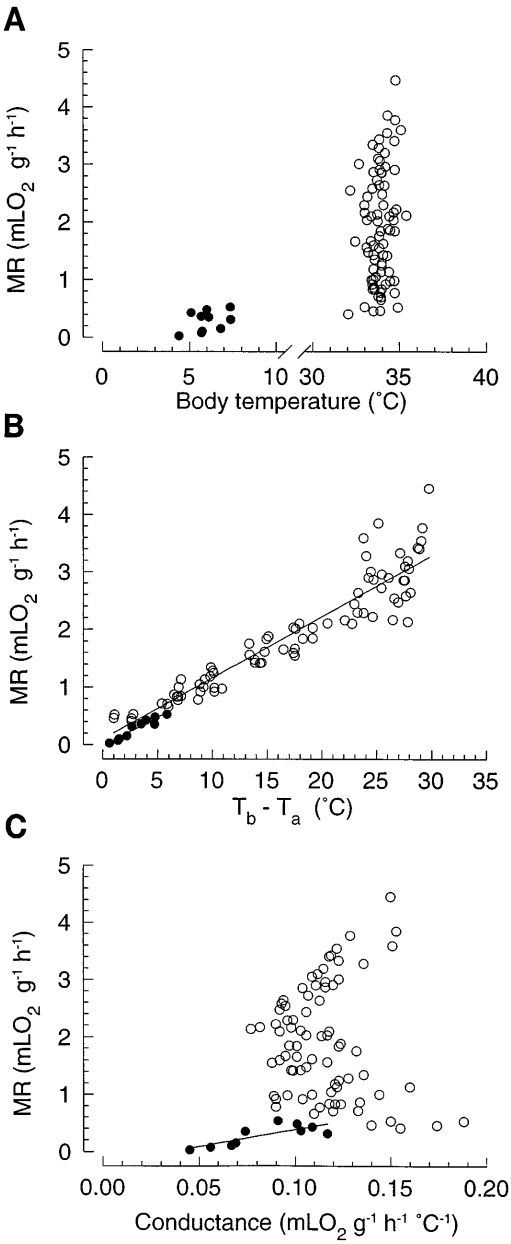

Fig. 5. TMR (●, N = 8, n = 11) below T_{tc} (thermoregulating) and resting MR (RMR) of normothermic individuals (○, N = 8, n = 80) below the lower critical temperature (T_{lc}) as a function of T_b (*A*), ΔT between T_b and T_a (*B*), and apparent C (*C*). Neither TMR nor RMR of thermoregulating *C. nanus* was related to T_b: TMR = −0.191 + 0.079 T_b (r^2 = 0.07, P = 0.23); RMR = −8.67 + 0.313 T_b (r^2 = 0.03, P = 0.07). Both TMR and RMR were significantly related to ΔT: TMR = −0.032 + 0.101 ΔT (r^2 = 0.91, $P < 0.001$); RMR = 0.096 + 0.106 ΔT (r^2 = 0.88, $P < 0.001$). TMR was also positively related to C [TMR = −0.208 + 5.89 C (r^2 = 0.59, $P < 0.01$)], whereas RMR was not related to C [RMR = 2.41 − 3.89 C (r^2 = 0.04, P = 0.05)].

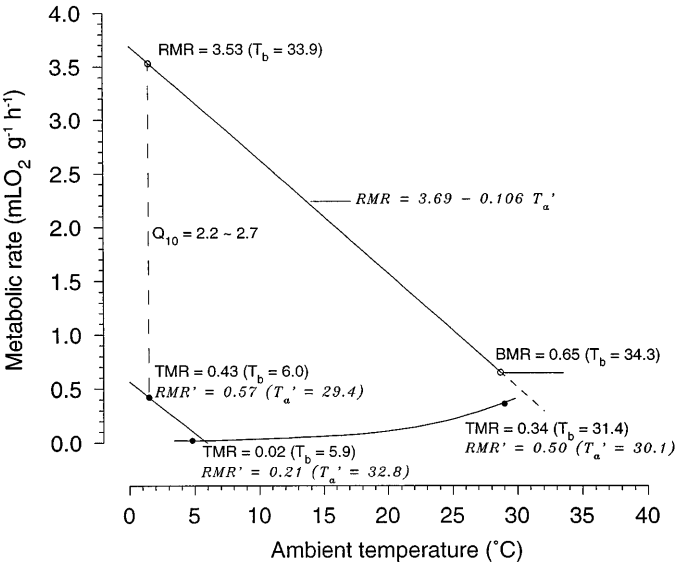

Fig. 6. Comparison between TMR and predicted RMR (RMR′) for these TMR given the same differential between T_b and T_a. RMR′ was calculated from the equation RMR′ = 3.69 − 0.106 T_a'. T_a' was determined by using the formula $T_a' = T_b' - (T_{b1} - T_{a1})$, where T_b' is the normothermic value and T_{b1} and T_{a1} are the values during torpor at which TMR was measured. Examples for measurements below the set point, at T_{tc}, and in the thermoneutral zone showed that all derived RMR′ values were significantly larger than the TMR. Q_{10} values for MR between thermoregulating animals during normothermia and during torpor were calculated for both measured data (Q_{10} = 2.7) and data derived from regressions of MR vs. T_a given in Fig. 2*A* (Q_{10} = 2.2).

Because Q_{10} values over the range of 2–3 were observed in *C. nanus* only at higher T_b values, especially in the TNZ, it seems that Q_{10} values for MR differ at different T_b values (6, 15), and the metabolic inhibition may be more pronounced during the transient state between normothermia and torpor when T_b is high (8, 19). The use of metabolic inhibition at low T_b 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 Q_{10}, of some enzymes of hibernators than those of daily heterotherms (8, 21).

Interestingly, *C. nanus* displayed torpor in the TNZ. The TMR of torpid individuals dropped by nearly 50% from BMR, whereas T_b dropped by only 3°C and therefore a very large Q_{10} 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 T_b 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 T_b at the onset of hibernation in *C. nanus*.

Above the T_{tc}, the ΔT was stable over a wide range of T_a 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 all were significantly higher (33%, 10-fold, 47%, respectively) than the measured values (Fig. 6).

In the high-T_a range (T_a 23–30°C), C was high and did not change with the fall of TMR (Figs. 2*D* and 4*B*). This implies that while thermogenesis was inhibited, heat loss was probably also facilitated via a high C at high T_b values. Although in the low T_a range TMR was positively related to C (Fig. 4*B*), 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 T_b and TMR. This interpretation is supported by the finding that exposure to He-O_2 (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 T_b from reaching the set point during torpor, which would induce an increase of TMR.

The increase of TMR with T_a during torpor above T_{tc} also differs from the thermoregulatory response of normothermic individuals above T_{lc}, although both show an increase of C. Above the T_{lc}, ΔT decreased whereas C showed a steep increase with T_a to avoid overheating and maintain T_b at a normothermic level. In contrast, the increase of C during torpor above T_{tc} was accompanied by a rise in both T_b and TMR. A high C at high T_a values during torpor is not used for maintenance of a constant T_b but appears to reduce T_b 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 T_{tc}, but does not satisfactorily explain TMR above the T_{tc}. C does not appear to affect TMR directly, but may be important at high T_a values at which C is high, most likely to dump heat, and also when the T_{set} is approached when C is minimal, perhaps to delay onset of thermoregulation.

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Address for reprint requests: F. Geiser, Zoology, Univ. of New England, Armidale, NSW 2351, Australia.

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