

Interrelations between metabolic rate and body temperature during entry into daily torpor in *Sminthopsis macroura*

Xiaowei Song, Gerhard Körtner and Fritz Geiser

Department of Zoology, University of New England, Armidale, NSW 2351, Australia

Introduction

Torpor of heterothermic endotherms is characterised by a substantial decrease of metabolic rate (MR) and body temperature (T_b). However, physiological mechanisms causing the reduction of MR during torpor are poorly understood. Although in many species MR during steady-state torpor can be well explained by the effect of the lowered T_b on biochemical reactions (Tucker 1965; Snapp & Heller 1981), it has been suggested that during torpor entry a temperature-independent metabolic inhibition may be involved (Malan 1986; Geiser 1988; Storey & Storey 1990).

Sminthopsis macroura (Dasyuridae: Marsupialia) is a small nocturnal insectivorous marsupial that lives in arid and semiarid areas of Australia. It regularly displays spontaneous (food *ad lib.*) or induced (food withheld) daily torpor over a range of air temperatures (T_a) (Geiser & Baudinette 1985). The steady-state MR above the set point for T_b (T_{set}) of a torpid animal is largely determined by T_b while below the T_{set} , MR is a function of the difference between T_b and T_a (ΔT) (Song *et al.* 1995). Simultaneous recordings of changes of T_b and MR during torpor entry of *S. macroura* have not been reported. In the present paper, we investigated how the T_b and MR are interrelated during entrance into daily torpor in *S. macroura* and how the cooling rates at various stages of the entry are related to MR.

Material and Methods

Eight adult male *S. macroura* were obtained from a breeding colony at La Trobe University, Melbourne. They were maintained individually in cages at the University of New England at a photoperiod of 12L:12D (lights on 0600-1800 hours) and T_a of $20 \pm 2^\circ\text{C}$. Body mass (BM) averaged about 25 grams. Food (mixture of dog and cat food) and water were provided *ad libitum*. At least 7 days before the first measurement, small temperature-sensitive transmitters (Mimimitter Model X-M, 2 grams, calibrated to the nearest 0.1°C) were surgically implanted into the peritoneal cavity.

Animals were placed into 0.5L respiratory chambers for about 24 hours to simultaneously measure T_b and rate of oxygen consumption (VO_2). Food and water were not available during measurements. The flow rate of dry air through the respirometer was set to about 200mL min^{-1} and measured with a mass flowmeter (FMA 5606, Omega, Stamford). Analog outputs from the flowmeter, oxygen analyser (Ametek Applied Electrochemistry S-3A/1, Pittsburgh), transmitter receiver and digital thermometer (Omega

DP116) for measuring T_a were interfaced to a personal computer. Data acquisition and processing were performed with software written by B. Lovegrove, T. Ruf and G. Körtner. VO_2 values were calculated according to equation 3a from Withers (1977). Values for the resting metabolic rate during normothermia (RMR), basal metabolic rate (BMR) and steady-state metabolic rate during torpor (TMR) were determined (see Song *et al.* 1995 for details). A linear decrease of BM throughout each experiment was assumed for calculation of mass-specific MR. Steady-state TMRs were obtained from torpor bouts longer than 2 hours when both TMR and T_b were minimal. During entry into torpor no steady-state MR value can be obtained directly from the measurements due to the mixing of the gases in the respiratory chamber. Therefore time-corrected instantaneous VO_2 was calculated (Bartholomew *et al.* 1981) at T_a 25°C and 18°C (above the T_{set}), as well as at T_a 10°C (below the T_{set}). Rates of cooling of living individuals were compared with those of a dead individual using temperature-sensitive transmitters. Q_{10} s for MR at different T_b s were calculated according to the equation: $Q_{10} = (MR_1 / MR_2)^{(10 / T_{b1} - T_{b2})}$.

Results

Torpor in *S. macroura* often started in the dark phase, between midnight and lights on in the morning. The entrance into torpor was initiated by a drop of MR from active or resting levels, which was followed by a decrease of T_b (Fig. 1). At T_a s of 25 and 18°C, T_b and MR decreased more or less concurrently during most of the entry phase (Fig. 1a, b). While torpor entry was occasionally interrupted by sudden increases in MR, the MR increase that was most regularly observed occurred when MR was $0.86 \pm 0.04 \text{ mL} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$, which approximates the BMR of $0.88 \pm 0.09 \text{ mL} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ (range: 94% to 109% of BMR). Within $42 \pm 15 \text{ min}$ at T_a 25°C and $39 \pm 13 \text{ min}$ at T_a 18°C, MR decreased by over 86% from the active to the BMR level. During this initial drop of MR, T_b fell by only $1.5 \pm 0.6^\circ\text{C}$ at T_a 25°C and $1.9 \pm 1.0^\circ\text{C}$ at T_a 18°C. The reduction of MR below the BMR level to the steady-state TMR accounted for only a small fraction of the overall reduction in MR, although the T_b decreased by a further $6.2 \pm 1.7^\circ\text{C}$ at T_a 25°C and $11.5 \pm 2.3^\circ\text{C}$ at T_a 18°C.

When MR had reached its minimum after about 3 hours, T_b was still $0.5 \pm 0.3^\circ\text{C}$ at T_a 25°C and $0.9 \pm 0.7^\circ\text{C}$ at T_a 18°C higher than the final minimum values (T_{bmin}). The Q_{10} between BMR and this beginning of the minimum TMR was 3.7 ± 1.3 at T_a 25°C and 3.4 ± 1.7 at T_a 18°C. Usually T_{bmin} was reached within another hour (Fig. 1a, b), and the Q_{10} between BMR and TMR, when both TMR and T_b had reached steady-state minima, was 2.9 ± 1.1 at T_a 25°C and 2.7 ± 0.9 at T_a 18°C.

At T_a 10°C, the time course of the reduction of MR and T_b during torpor entry followed different patterns (Fig. 1c). MR declined to a low undershoot value of $0.5 \pm 0.2 \text{ mL} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ within the initial 52 ± 17 min, which was about 60% of the BMR and 50% of the steady-state TMR, which was reached after a gradual increase of MR after about another 1.5 hours. Over the same time interval T_b usually showed a continuous decline. When MR dropped from active or resting MR to the BMR level, T_b decreased by $3.1 \pm 1.8^\circ\text{C}$. The reduction of MR from BMR to the MR undershoot was accompanied by a $6.3 \pm 3.2^\circ\text{C}$ drop in T_b (Q_{10} was 2.5 ± 0.9) (Fig. 1c).

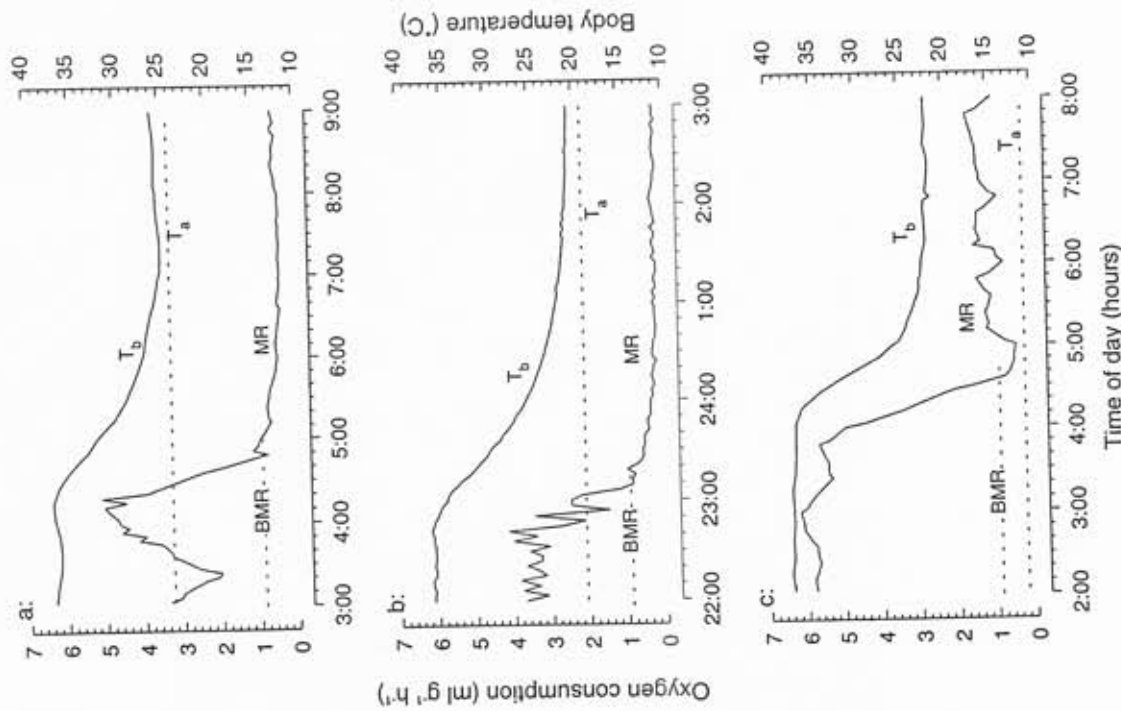


Fig. 1. Simultaneous record of the instantaneous rate of oxygen consumption (VO_2) and body temperature (T_b) during entry into daily torpor of a *Sminthopsis macroura* at air temperatures (T_a) of 25°C (a), 18°C (b) and 10°C (c).

As in all physical bodies the cooling rate during entrance into daily torpor in *S. macroura* was proportional to ΔT . However, unlike the pure passive exponential cooling that was observed in a dead individual of the same BM as the living animals, the log-transformed ΔT did not fit a linear model, but showed three different phases (Fig. 2). At all T_a s, during the initial entry phase when MR was still relatively high, the animal cooled slowly. In the second phase, rates of cooling accelerated as MR fell below 60% and 70% of the pre-entry value at T_a 10°C and 18°C, respectively. During the third phase cooling rates were reduced. At T_a 10°C, the reduced cooling rate was largely caused by the thermoregulatory increase of TMR (Fig. 2a). However, at T_a s 18 and 25°C cooling rates were also reduced, although MR continued to decline during this phase (Fig. 2b) and it appeared that overall insulation had improved when MR approached TMR. Due to the heat production and, most likely, a smaller conductance, rates of cooling of living individuals were less than half of that of the dead animal.

Discussion

Our study shows that even during entrance into daily torpor most of the reduction of MR can be explained by cessation of normothermic thermoregulation and temperature effects. However, during initial entry a fraction of MR reduction may be caused by temperature-independent factors.

It has been proposed that the reduction of MR during entrance into torpor of heterothermic endotherms consists of two separate processes. One is the drop of MR from RMR to BMR, caused mainly by the abandonment of normothermic thermoregulation (not to be confused with metabolic inhibition), with no obvious change in T_b . The other is a further drop of MR below BMR, due to a substantial decrease of T_b (Withers 1992), until a new equilibrium is reached (Bartholomew 1982). *Smithithopsis macroura* always initiated torpor by a rapid reduction of MR that was followed by a fall of T_b supporting this interpretation (Nicol *et al.* 1992). Of course, the two processes of MR reduction cannot be clearly separated since MR is not reduced in a step-wise fashion from active or resting level to BMR because MR is affected by the fall of T_b from the beginning of entrance. This gradual decrease in MR was further illustrated by the cooling curves of *S. macroura* during torpor entry. Although cooling during torpor entry in mammals and birds has been described to be a pure Newtonian cooling curve (Bartholomew 1982; Lasiewski & Lasiewski 1967), the log transformed ΔT over time did not fit a linear model but rather showed three phases. This result shows that in contrast to a dead animal or a physical object with no metabolic heat production, cooling during entry into daily torpor in *S. macroura* is not only determined by physical phenomena, but also related to the level of MR and the alteration of conductance. Rates of cooling decreased when the thermoregulatory TMR increased at the low T_a . However, cooling rates were also reduced at high T_a when TMR was approached. This appears to be caused by a decreased conductance because of a decreased respiratory rate and a decreased peripheral circulation, and an increased pelage insulation.

The Q_{10} for the reduction of MR below BMR during torpor entry in *S. macroura* after both TMR and T_b had reached steady-state values was between 2 and 3, which is typical for temperature effects on rates of enzymatic reactions. This illustrates that the lowered T_b is an important factor in causing the reduction of MR during torpor entry. This applied also to the MR undershoot at low T_a , suggesting a consistent influence of T_b

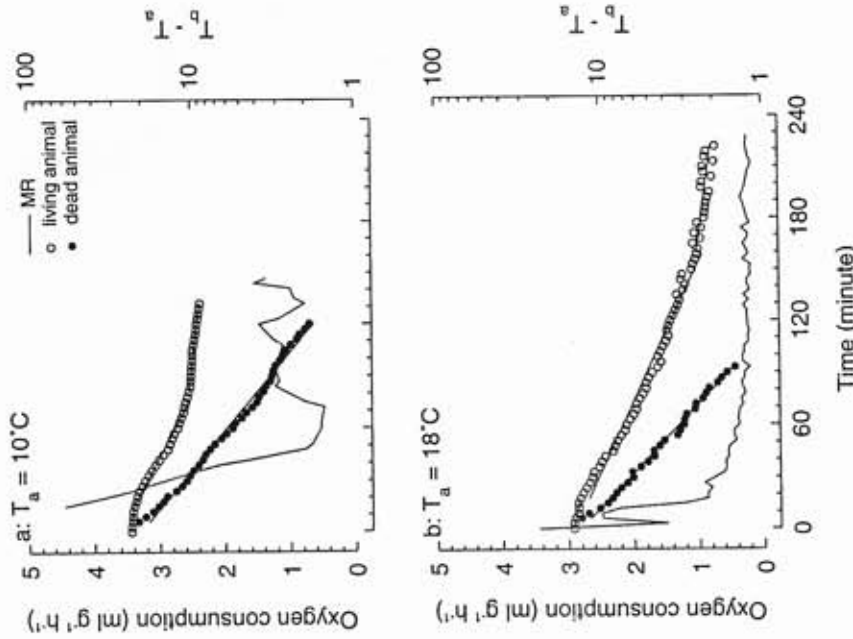


Fig. 2. Cooling rates (open circles) plotted as log-scaled temperature gradient (ΔT) between the animal and its environment during entry into daily torpor of *Smithithopsis macroura* at air temperatures of 10°C (a) and 18°C (b). The line represents the metabolic rate (MR) and closed circles indicate the linear cooling rate of a dead individual. Linear regressions were fitted by the method of least squares.

on MR reduction at T_a s both above and below the T_{set} . While MR is affected by T_b , T_b itself can be precisely controlled during the whole entry phase (Heller *et al.* 1977), indicated by the brief periodic increases of MR which occurred throughout the entry phase. In addition, T_b regulation was also demonstrated by the fact that during torpor entry of *S. macroura* at T_a below the T_{set} , TMR was approached at a stage where a ΔT was maintained with an increased MR. These observations are consistent with the theory of sliding T_{set} for T_b during entry into hibernation (Heller & Colliver 1974; Heller *et al.* 1977).

While temperature effects can explain the steady-state TMR, the minimum TMR was reached when T_b was still in the process of decline. The Q_{10} for the MR reduction between BMR and the start of minimum TMR was slightly above the range for biological reactions. This indicates that the combination of cessation of regulatory thermogenesis and temperature effects on biochemical reactions is not sufficient to explain all of the reduction of MR during early entrance into daily torpor in *S. macroura*. However, when the minimum TMR was reached, the core T_b was less than 1°C above T_{bmin} , and it is possible that most tissues had already reached the temperature minimum. Nevertheless, it is most likely that some additional temperature-independent processes are involved during early entrance into torpor (Malan 1986, 1993; Geiser 1988; Storey & Storey 1990; Milsom 1993; Guppy *et al.* 1994).

Our study shows that entrance into daily torpor is a complex process that involves transient changes of several physiological variables. Detailed information from other species, particularly hibernators, would be useful to investigate further the interrelations between these variables during torpor entry.

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