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# Transient peripheral warming accompanies the hypoxic metabolic response in the golden-mantled ground squirrel

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**Summary**

**The hypoxic metabolic response of mammals involves a reversible metabolic suppression, possibly brought about by a reduction in the body temperature set-point. In the present study we tested the hypothesis that this is accompanied by a transient increase in heat loss that facilitates the decline in body temperature and metabolic rate. Peripheral heat distribution was assessed using infrared thermography to measure the surface temperatures of the golden-mantled ground squirrel at three different ambient temperatures (10, 22 and 30°C). During early hypoxic exposure, surface temperatures increased dramatically in the feet, ears and nose, and this increase was more dramatic and prolonged at 22°C than at the other two temperatures. These increases were associated with a fall in metabolic rate. Following this initial increase, surface temperatures decreased back to control values, and at 10°C, the surface temperatures of the eyes and body decreased below normoxic levels. Subsequent normoxic recovery was not accompanied by transient changes in surface temperatures, despite large increases in metabolic rate associated with post-hypoxic shivering and thermogenesis. The temporal changes in surface temperature suggest that peripheral blood flow is initially increased during hypoxia, shifting heat away from the core to the periphery and thus facilitating cooling. These results are consistent with the hypothesis that hypoxia leads to a regulated fall in body temperature.**

Key words: hypoxia-induced hypothermia, body temperature regulation, infrared thermography, regulated heat loss, metabolic depression, golden-mantled ground squirrel.

## Introduction

Hypoxia is well known to reduce metabolism and body temperature in many vertebrates (Wood and Gonzales, 1996), especially small mammals (Frappell et al., 1992; Mortola, 1993). This metabolic suppression is accompanied by the behavioural selection of lower environmental temperatures and an increase in behaviours that appear to facilitate heat loss (e.g. reduced huddling response in neonatal mammals, and adoption of postures conducive to heat loss; Mortola and Feher, 1998). Auto-regulatory responses such as these appear to be geared toward promoting a balance between oxygen supply and oxygen demand. When oxygen supply is limited, autonomic regulatory processes act to lower oxygen demand to match the restricted supply (i.e. decreased metabolism, slower heart rate and lowered body temperature, $T_b$).

The lowered metabolism and body temperature seen in hypoxia (i.e. hypoxia-induced hypothermia; Wood and Gonzales, 1996) are believed to reflect a regulated lowering of body temperature set-point, affecting all thermoeffectors in a coordinated manner. The alternative hypothesis that $T_b$ merely falls as a passive consequence of oxygen lack has been suggested as a possibility (Fewell et al., 1997; Gordon, 1997; Mortola and Gautier, 1995), but there is little supportive evidence for this. The role of heat loss and peripheral blood flow regulation during hypoxia, however, has not received much attention in this context. In order for an endotherm to lower body temperature, body heat must be lost from the core. This requires that perfusion to the peripheral tissues be maintained or increased, to deliver core heat to the periphery and thus more rapidly facilitate conductive, convective and radiative heat transfer. This effector arm for body temperature regulation will produce temporal changes in surface temperature ($T_s$), depending on the deviation of $T_b$ from the regulated set-point. The $T_s$ of a mammal, however, depends on the interactions between ambient temperature ($T_a$), metabolic heat production, insulation and cutaneous blood flow (Klir et al., 1990; Webb et al., 1992). Although fur forms an insulative barrier to heat exchange, changes in blood flow through the cutaneous arterio–venous anastamoses and the capillary beds are still instrumental in regulating body temperature and heat loss (Jänig, 1990), particularly within the thermoneutral zone (TNZ). For the most part, the body surfaces that play the most significant role in regulating heat loss in mammals (i.e. feet, ears and nose) are covered in relatively short fur (Klir and Heath, 1994) and blood flow to

these ‘thermal windows’ is regulated by a hypothalamic integrator.

Hypoxia can act on the regulation of cutaneous blood flow and $T_s$ in two ways: locally and centrally. Local effects can result from the oxygen-limited metabolism of the smooth muscle of arterioles, through the release of metabolically derived vasodilatory substances. Central effects are integrated in the hypothalamus, and are mediated through the sympathetic nervous system acting on the blood vessels themselves, effecting either vasodilation or vasoconstriction (Klir and Heath, 1994; Marshall, 1998). Cutaneous blood flow, and thus $T_s$ and heat loss, are mainly under central sympathetic nervous control (Jänig, 1990), while local factors play a small role under normal conditions. If, however, oxygen demand is to be lowered to match the restricted oxygen supply in hypoxia, one would expect the reductions in $T_b$ and metabolic rate to be accompanied by changes in blood flow that would favour essential tissues (such as heart and brain) at the expense of less essential tissues (such as skin, viscera and muscle; Sidi et al., 1988). If heat dumping occurs as a part of a regulated reduction in $T_b$ during hypoxia, however, this would require that peripheral blood flow to the thermal windows be maintained or increased for heat loss, rather than restricted to conserve oxygen for essential tissues, at least during the early stages of hypoxic exposure. Given these potentially conflicting demands, the aim of the current study was to demonstrate whether peripheral heat dumping occurred during the early period of hypoxia exposure, which would give strong support to the prevailing hypothesis that hypoxia resets body temperature to a new and lower set-point, and would indicate that controlled heat loss at the periphery must play an important role in this process. To determine whether this is so, we measured $T_s$ at ambient temperatures well below (10°C), near the lower critical temperature (22°C), and near the upper critical temperature (30°C) of the TNZ of the golden-mantled ground squirrel (Barros et al., 2001), also to test the hypothesis that the temperatures at which animals can make thermoregulatory related cardiovascular adjustments (i.e. at the lower critical temperature) would be associated with the largest, most prolonged changes in $T_s$ in hypoxia.

## Materials and methods

### *Animals*

Golden-mantled ground squirrels *Spermophilus lateralis* Say 1823 ($N$=21) were collected from a population in Redding, California. They were housed in an environmental chamber at room temperature and fed rat chow *ad libitum* supplemented with sunflower seeds and fruit. All experiments were conducted during the summer months when the squirrels were euthermic and active. Both male and female squirrels were examined in this study and since there was no effect of gender, all the results were pooled together. The experiments complied with UBC and Canadian Council for Animal Care guidelines.

### *Metabolic rate determination*

Metabolic rates were determined using flow-through respirometry. Squirrels were placed in 0.5 l Plexiglass chambers with incurrent gas flow set to 1500 ml min$^{-1}$. Gases (21% $O_2$ or 7% $O_2$) were mixed using a gas mixing flow meter (Cameron GF-3/MP). A subsample (150 ml min$^{-1}$) of the excurrent gas from the respirometer was scrubbed of water vapour and $CO_2$ and analysed for $O_2$ content (Beckman OM-11 Oxygen analyser), while another subsample of gas was scrubbed of water vapour and analysed for $CO_2$ content (Beckman LB-2 $CO_2$ analyser). $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were subsequently calculated using equations from Withers (1977), and the respiratory exchange ratio (RER) was determined as $\dot{V}_{CO_2}/\dot{V}_{O_2}$.

### *Infrared imaging and surface temperature estimation*

One technique for assessing peripheral heat loss and $T_s$, particularly in endotherms, is infrared thermography. This optical technique involves detecting electromagnetic radiation (at wavelengths in the range 8–12 μm), and converting the intensity of this radiation to a greyscale or colour image in the visible range of light. All objects above 0K emit infrared radiation (IR), and the intensity of this radiation is related to the $T_s$ and the emissivity of the body (Speakman and Ward, 1998). This technique has found many diagnostic uses in clinical and veterinary applications where a rapid assessment of limb and peripheral blood flow is desired (Inagaki et al., 1992; Jones, 1998; Turner, 2001).

In the present study, surface temperatures were estimated using an Inframetrics™ thermal imaging camera (Model 522 Imaging Radiometer). In order to obtain accurate surface temperatures and still maintain a sealed respirometer, we experimented with a number of thin, plastic materials that would serve as ‘windows’ through which the animal’s IR could pass to the camera. Polyethylene (a Ziploc© freezer bag stretched onto a hard plastic frame) provided an adequate seal for the respirometer while permitting most IR to pass. We were able to validate the accuracy of the IR estimates through the polyethylene by using materials of known temperature and emissivity. The video output from the thermal imaging camera was recorded on videotape and transferred to a computer using a frame-grabber card (Grab-It Pro™). Grey-scale pictures (256 colours) from the camera were captured at 1 min intervals to provide a redundant number of images for off-line analysis. Subsequently, the grey-scale images were analysed using SigmaScan™ software. Outlines of the eyes, ears, nose, feet and back (flank region) were drawn using the software trace function, and then the average intensity (based on a 0–255 scale) of the enclosed area was calculated and converted to the corresponding surface temperature. Surface temperatures were determined from images every 5 min (see Fig. 1 for a sample image and setup). When the squirrels changed position, so that parts of their bodies became invisible to the camera, the best estimate of surface temperature was determined from other captured images within 2 min of the desired time point.

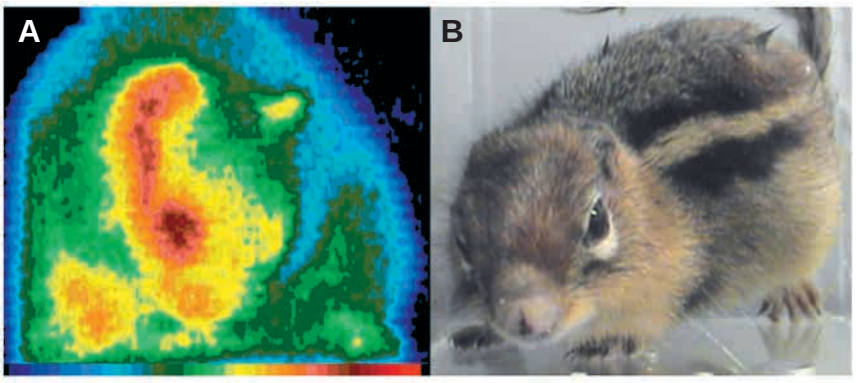


Fig. 1. (A) Infrared thermogram of a golden-mantled ground squirrel. (B) Actual image of a squirrel (not taken simultaneously with A) to demonstrate the angle of the body (but not the head) at which the thermogram was taken. The squirrel was usually facing the camera and, hence, the head and front legs were clearly visible, with the cooler flank region dominating the rear part of the image.

### *Experimental protocol*

Squirrels were placed into the respirometer, which was then placed within a temperature controlled environmental chamber. The animal was allowed to acclimate to the experimental setup for at least 1 h. Ambient temperature was kept at one of three different temperatures, 10°C ($N$=7), 22°C ($N$=7) and 30°C ($N$=7). Metabolic rate and whole body thermograms from the thermal imaging camera were initially recorded every minute for 140 min. After 20 min of recording normoxic baseline values, the oxygen in the respirometer was changed to 7% $O_2$ for the subsequent 60 min, after which the respirometer was returned to 21% $O_2$ and 60 min of recovery from hypoxia was recorded.

### *Data analysis*

After determining $T_s$, we calculated the difference between $T_s$ and $T_a$ ($\Delta T_{s–a}$) at each part of the body to approximate the effect of ambient temperature on regional cutaneous blood flow. We also determined the change in $T_s$ above normoxic, control values at each surface ($\Delta T_s$=hypoxic $T_s$–normoxic $T_s$) and at each temperature, as an estimate of the temporal changes in blood flow occurring during hypoxia. For purposes of analysis, $T_s$, $\Delta T_{s–a}$, $\Delta T_s$, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and RER were averaged over 5 min intervals and expressed as means ± S.E.M. All statistics relating to the infrared thermography were actually performed on the raw $T_s$ data. Variables were analysed using a repeated-measures analysis of variance (ANOVA), with temperature as the factor and time as the repeated measure. *Post hoc* multiple comparisons were made using a Bonferroni test compared against a normoxic, control value at the appropriate temperature. All statistical tests were considered significant at $P$<0.05.

## Results

### *Metabolic rate*

#### *Effects of ambient temperature in normoxia*

$T_a$ had a significant effect on $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ in normoxia, with values at 10°C being significantly higher than values at 22°C and 30°C (Table 1; Fig. 2). Similarly, $\dot{V}_{CO_2}$ was significantly higher at 10°C than at 22°C and 30°C. The RER was not significantly affected by $T_a$ in normoxia, ranging from 0.87–0.90.

#### *Effects of Hypoxia and Recovery*

Upon exposure to 7% $O_2$, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ decreased

Table 1. *Metabolic rates ($\dot{V}_{O_2}$, $\dot{V}_{CO_2}$) and respiratory exchange ratios (RER) of normoxic (time 0), hypoxic (15 and 60 min at 7% $O_2$; transient and steady state values) and normoxic recovery (10 and 60 min post-hypoxia; transient and steady state values) squirrels*

| | $T_a$ (°C) | Normoxia | 15 min hypoxia | 60 min hypoxia | 10 min recovery | 60 min recovery |
|---|---|---|---|---|---|---|
| $\dot{V}_{O_2}$ | 10 | 40.4±4.4 | 27.1±2.3* | 21.2±1.5* | 91.2±10.4* | 45.3±6.2 |
| | 22 | 32.0±1.6 | 23.9±2.1* | 23.4±1.6* | 53.8±6.5* | 39.1±3.5 |
| | 30 | 30.5±1.7 | 29.4±2.3 | 24.8±2.0 | 23.2±1.1* | 28.3±1.7 |
| $\dot{V}_{CO_2}$ | 10 | 36.5±4.0 | 26.5±2.2* | 16.7±1.5* | 72.8±7.1* | 42.0±5.6 |
| | 22 | 28.3±1.7 | 26.7±0.6* | 19.2±0.6* | 43.3±4.8* | 35.2±3.5 |
| | 30 | 25.3±1.1 | 29.1±2.3 | 19.7±0.9* | 14.4±2.2* | 21.3±2.3 |
| RER | 10 | 0.91±0.03 | 0.98±0.04 | 0.79±0.06 | 0.81±0.02 | 0.93±0.02 |
| | 22 | 0.88±0.04 | 1.16±0.11 | 0.83±0.04 | 0.81±0.02 | 0.90±0.02 |
| | 30 | 0.83±0.03 | 1.00±0.08 | 0.81±0.07 | 0.63±0.03* | 0.74±0.04 |

Values are means ± S.E.M. ($N$=7).
*A significant effect compared to normoxic control at a specific temperature ($P$<0.05).

significantly below normoxic levels at 10°C and 22°C, but did not decline significantly at 30°C (Table 1; Fig. 2). Stable values were usually achieved by 40 min of hypoxic exposure at all temperatures. Neither $\dot{V}_{O_2}$ nor $\dot{V}_{CO_2}$ were significantly affected by $T_a$ during hypoxia. The RER increased significantly during the first 10 min of hypoxia at all temperatures, and returned to normoxic levels within 40 min.

Upon returning the respirometer gas to normoxia, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ increased significantly within the first 10–15 min at both 10°C and 22°C (Table 1; Fig. 2). These large increases in $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ accompanied bouts of intense shivering. At 30°C, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ decreased significantly on return to normoxia, remaining lower than control values for up to 20 min. RER was not significantly affected by normoxic recovery at 10°C and 22°C, although it was transiently lower during recovery at 30°C.

### *Surface temperatures*

#### *Effects of ambient temperature in normoxia*

The surface temperatures of all parts of the body were significantly affected by $T_a$ (Table 2), being lowest at 10°C and highest at 30°C. However, $\Delta T_{s-a}$ was highest at 10°C and lowest at 30°C (Fig. 3A), such that a significant linear correlation existed between $\Delta T_{s-a}$ and $T_a$ at each surface of the body except for the nose, where the $\Delta T_{s-a}$ at 30°C was lower than expected and thus the relationship between $\Delta T_{s-a}$ and $T_a$ at the nose was not linear. In general the $\Delta T_{s-a}$ was similar for all surfaces except the eyes where this difference was much higher.

#### *Effects of hypoxia and recovery*

$T_s$, $\Delta T_{s-a}$ and $\Delta T_s$ all changed significantly during hypoxia. At 10°C, the $T_s$ of the nose and feet increased significantly during the first 15 min of hypoxia, whereas the $T_s$ of the eyes and the flank did not. Both of the latter surfaces, as well as the ears, then displayed significant decreases in $T_s$, $\Delta T_{s-a}$ and $\Delta T_s$

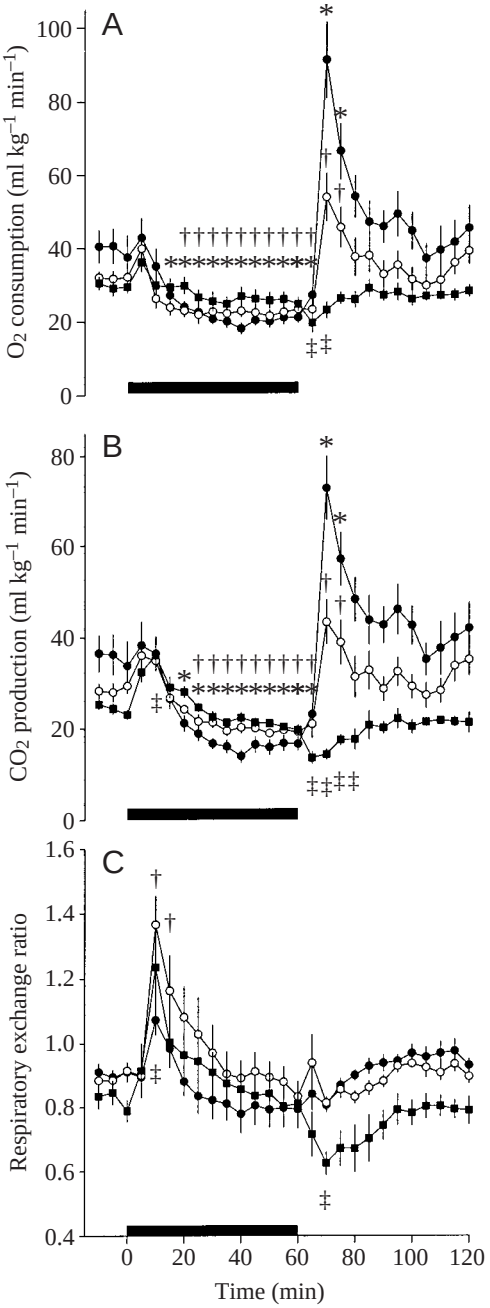


Fig. 2. Metabolic rates of oxygen consumption $\dot{V}_{O_2}$ (A) and $CO_2$ production $\dot{V}_{CO_2}$ (B), and respiratory exchange ratio RER (C) during normoxia, 60 min of hypoxia (black bar) and 60 min of recovery in normoxia. Filled circles denote values at $T_a$=10°C, open circles at $T_a$=22°C, and filled squares at $T_a$=30°C. *Significant difference from normoxic control at 10°C; † significant difference from normoxic control at 22°C; ‡significant difference from normoxic control at 30°C ($N$=7).

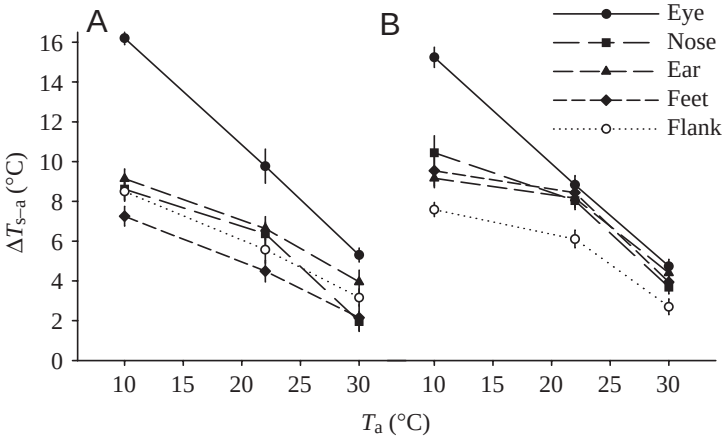


Fig. 3. Temperature differential between surface $T_s$ and ambient temperature $T_a$ ($\Delta T_{s-a}$) as a function of $T_a$ for eyes (filled circles), nose (filled squares), ears (filled triangles), feet (filled diamonds) and flank (open circles) in normoxia (A) and after 15 min of hypoxia (B). A linear relationship between $\Delta T_{s-a}$ and $T_a$ implies minimal specific thermoregulatory adjustments in $T_s$, whereas a departure from linearity at a given $T_a$ implies thermoregulatory adjustments at that surface to either increase or decrease heat loss.

Table 2. *Surface temperatures ($T_s$) of squirrels during normoxia (time 0), hypoxia (15 and 60 min at 7% $O_2$; transient and steady state values) and normoxic recovery (10 and 60 min post-hypoxia; transient and steady state values) at three different ambient temperatures*

| Region | $T_a$ (°C) | Normoxia | 15 min hypoxia | 60 min hypoxia | 10 min recovery | 60 min recovery |
|---|---|---|---|---|---|---|
| Eye | 10 | 25.9±0.5 | 25.2±0.3 | 23.0±0.5* | 23.8±0.7 | 25.2±0.7 |
| | 22 | 32.2±0.8 | 31.0±0.5 | 30.5±0.6 | 30.3±0.5 | 30.9±0.7 |
| | 30 | 35.3±0.4 | 35.0±0.3 | 34.1±0.3 | 33.9±0.4* | 34.1±0.4 |
| Nose | 10 | 19.0±0.5 | 21.0±0.9* | 18.4±0.5 | 18.5±0.4 | 18.8±0.7 |
| | 22 | 28.9±0.5 | 29.7±0.6* | 28.2±0.8 | 28.4±0.8 | 28.4±0.7 |
| | 30 | 31.9±0.4 | 33.7±0.3* | 32.6±0.8 | 32.2±0.6 | 32.7±0.5 |
| Ear | 10 | 19.6±0.6 | 19.8±0.2 | 18.0±0.5* | 18.4±0.4 | 19.5±0.6 |
| | 22 | 28.7±0.6 | 30.3±0.8* | 29.1±0.8 | 28.1±0.5 | 28.0±0.7 |
| | 30 | 33.7±0.5 | 34.2±0.2 | 33.0±0.5 | 32.9±0.5 | 33.6±0.6 |
| Feet | 10 | 17.6±0.7 | 20.1±0.9* | 17.7±0.3 | 17.7±0.3 | 18.1±0.6 |
| | 22 | 26.6±0.5 | 30.6±0.8* | 27.6±0.6 | 27.3±0.8 | 27.8±0.7 |
| | 30 | 32.1±0.4 | 33.7±0.4* | 33.1±0.5 | 32.8±0.3 | 32.7±0.3 |
| Flank | 10 | 18.6±0.4 | 18.0±0.3 | 17.3±0.3* | 17.7±0.4* | 18.7±0.3 |
| | 22 | 27.6±0.6 | 28.0±0.5 | 27.0±0.5 | 26.9±0.6 | 27.4±0.6 |
| | 30 | 32.7±0.4 | 33.5±0.3 | 32.6±0.5 | 32.7±0.3 | 33.0±0.3 |

Values are means ± S.E.M. ($N$=7).
*A significant effect of $T_s$ at a specific $T_a$. $T_a$ had a significant effect on all $T_s$ in all groups, which is not indicated above.

during the last half of the exposure to hypoxia (Table 2; Fig. 4).

At 22°C, the $T_s$ of the nose, ears and feet increased significantly and this increase was sustained for nearly 30 min in hypoxia, whereas the eyes and the flank showed no changes at any time in hypoxia (Figs 4, 5). In some cases, squirrels adopted a heat loss posture, lying with their feet elevated above their bodies (Fig. 5). At 30°C, there were small, but significant increases in $T_s$ in the nose and feet during the first 15 min of hypoxia, but no changes in other body surfaces throughout hypoxia.

On recovery from hypoxia, there was little temporal change in the $T_s$ at most body surfaces and at most $T_a$ values, except for the $T_s$ of the flank and the eyes. At a $T_a$ of 10°C, the $T_s$ of the flank was significantly lower than that seen during the normoxic control period after 10 min of normoxic recovery. This effect, however, was short-lived. At 22°C, the $T_s$ values of all surfaces during recovery were not significantly different from normoxic control values. At 30°C, there was a prolonged and significant decrease in the $T_s$ of the eyes, which lasted well into the recovery period.

Since $\Delta T_{s–a}$ and $\Delta T_s$ (Fig. 6) are calculated from $T_s$ itself, the significant differences in $T_s$ are reflected in these other variables.

## Discussion

### *General*

The results of this study support the hypothesis that hypoxia leads to a regulated decrease in body temperature. Specific ‘thermal windows’ on the surface of the body warmed during the early stages of hypoxia. The increases in surface temperature occurred simultaneously with reductions in metabolic heat production (present study) and $T_b$ (cf. Barros et al., 2001), suggesting that core heat was shifted to the periphery, consistent with the hypothesis that this occurs to facilitate a regulated drop in body temperature. These changes in $T_s$ were dependent on $T_a$, further suggesting that peripheral thermosensors detecting external temperature modulate the coordinated thermoregulatory and metabolic response to hypoxia.

### *Critique of methods*

IR thermography has not seen much experimental application in the study of temporal changes in blood flow. Although it is a useful, non-invasive way to assess surface temperature, it suffers from several disadvantages: (1) the surface of interest must always be available for viewing, (2) the emissivity of the object must be known accurately, and (3) in animals with an insulating layer, the technique only assesses surface temperature and not the actual skin temperature. We feel that we were able to overcome these drawbacks in the present study and that the data we obtained provide a good indication of skin temperature and, hence, useful insight into peripheral blood flow to the various surfaces. We were able to obtain sufficient images of each body surface of interest in each animal throughout each experiment. The emissivity of fur is quite high (Speakman and Ward, 1998) and should not have changed throughout an experiment, making the calculated values of $T_s$ at each of these surfaces relatively accurate. Finally, although $T_s$ could only be assessed at the outer layer of the fur, the nose, ears and feet have a short, coarse fur

covering, and thus our calculated values of $T_s$ for these surfaces should also be an accurate estimate of skin temperature. By contrast, our calculated values of $T_s$ for the flank, which has longer fur and a larger degree of insulation, will be a less accurate indication of flank skin temperature.

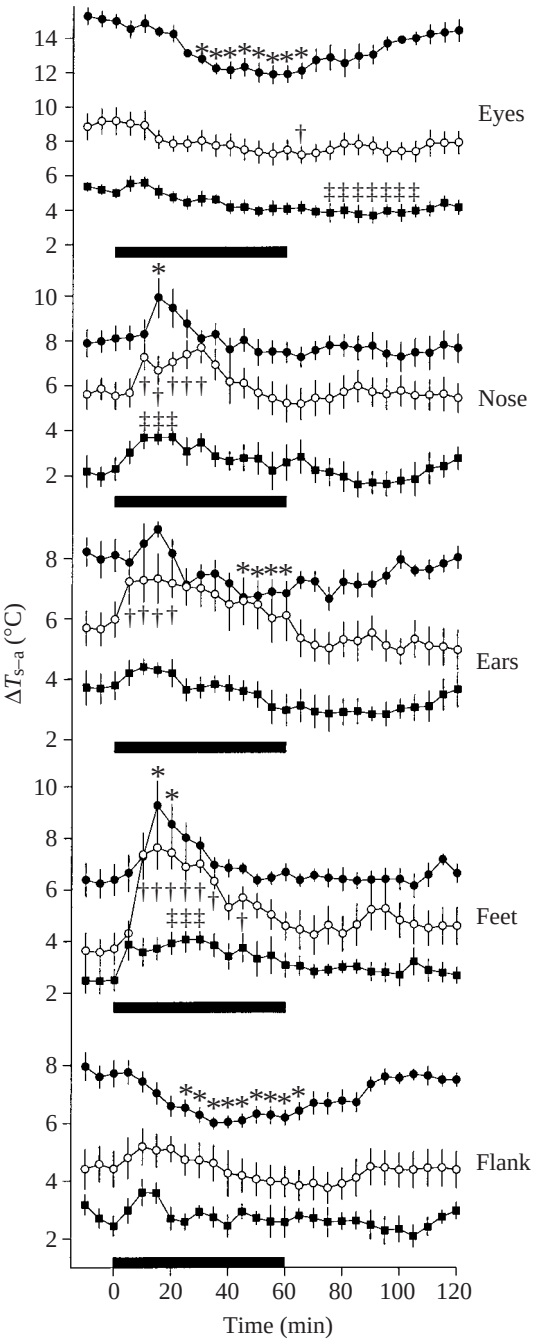


Fig. 4. Temperature differential between surface $T_s$ and ambient temperature $T_a$ ($\Delta T_{s-a}$) during normoxia, 60 min of hypoxia (black bar), and 60 min of recovery in normoxia on different body surfaces. Filled circles denote values at $T_a$=10°C, open circles at $T_a$=22°C, and filled squares at $T_a$=30°C. *Significant difference from normoxic control at 10°C; †significant difference from normoxic control at 22°C; ‡significant difference from normoxic control at 30°C ($N$=7).

Given that our measures of changes in $T_s$ accurately reflect changes in skin temperature, the question then arises as to what extent these changes can be assumed to reflect reflexly mediated changes in blood flow. The major concern when using changes in surface temperature to assess thermoregulatory control is the potential for local tissue hypoxia in the cutaneous vascular bed to alter blood flow *via* non-reflex mechanisms, and also to effect heat loss. We do not believe that this was occurring in our squirrels for several reasons. (1) There were different changes in $T_s$ in response to hypoxia at different ambient temperatures. This suggests that the changes were not just a passive effect of local hypoxia acting on peripheral blood flow but were reflexly mediated and proportional to the magnitude of the need to lose heat. (2) Non-specific increases in local blood flow should occur at most, if not all, body surfaces, and this was not the case. The feet, ears and nose increased in temperature while the temperature of the surface of the flank and eyes either decreased or remained constant. (3) Given that the haemoglobin oxygen affinity in this species is very high, 7% $O_2$ should only produce a small degree of desaturation of arterial blood (Maginniss and Milsom, 1994), which should not lead to local hypoxia severe enough to prompt significant humorally mediated increases in cutaneous blood flow.

Thus, in the present study we measured $T_s$, from which we inferred changes in $T_{skin}$, from which we inferred changes in peripheral blood flow. We then use the latter to assess the extent to which changes in peripheral blood flow and heat transfer to the environment during hypoxia can provide an insight into whether the reductions in $T_b$ are the result of a regulated or a passive process.

### *Hypoxic metabolic response*

One of the most notable results of our study was the observation that the lower the $T_a$ and the higher the starting metabolic rate, the greater the hypoxia-induced fall in metabolism. At the highest ambient temperature (lowest starting metabolic rate), metabolic rate did not change significantly in hypoxia, while at the lowest ambient temperature (highest starting metabolic rate) metabolic rate fell dramatically to the lowest levels recorded during hypoxia (Fig. 2). This strongly suggests that the decrease in metabolism seen in hypoxia was due to the suppression of thermogenic heat production to basal levels combined with a subsequent fall in metabolism due to the decrease in $T_b$. Previous studies have shown that $T_b$ in hypoxia decreases in proportion to $T_a$; colder ambient temperatures lead to lower $T_b$ values (Barros et al., 2001; Wood and Stabenau, 1998). This explanation suggests that $\dot{V}_{O_2}$ did not fall significantly in squirrels at 30°C because they were near the upper end of (but within) the TNZ, where thermogenesis would already be basal, and the fall in $T_b$ would be small. Squirrels at 22°C were near the lower end of (but below) the TNZ and displayed a small but significant reduction in thermogenesis, a larger fall in $T_a$, and a larger fall in metabolism. Squirrels at 10°C were well below the TNZ and thus showed the largest fall in metabolism, due to the removal

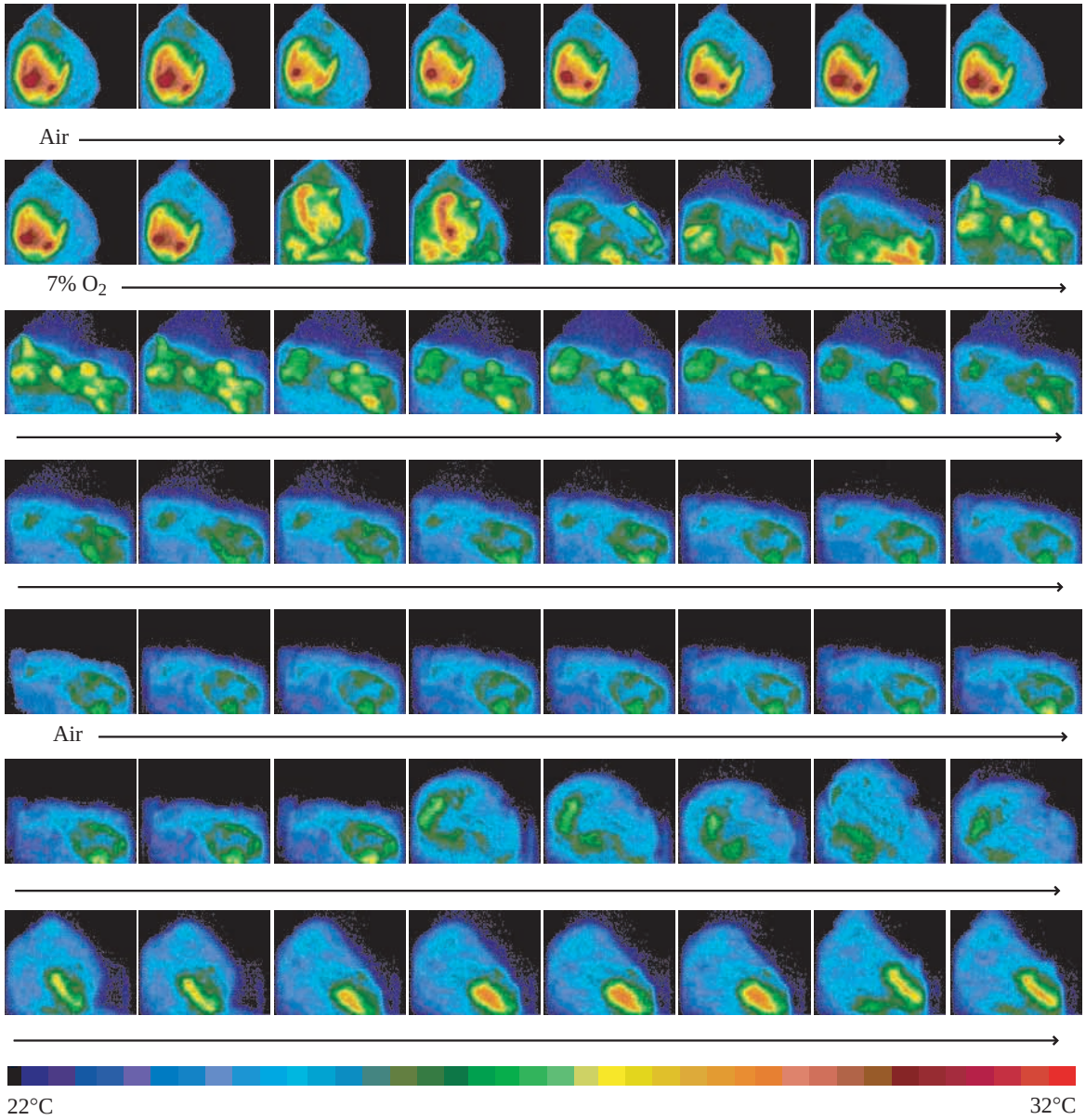


Fig. 5. Time series of infrared thermograms from one squirrel ($T_a$=22°C) during normoxia (30 min), hypoxia (60 min) and normoxic recovery (30 min). Images represent pictures taken at approximately 2 min intervals (eight images correspond to 30 min duration). Note the feet and ears warming up during the early period of hypoxic exposure, and the lack of peripheral warming during the recovery period of intense metabolic activity.

of a significant thermogenesis, a larger fall in $T_b$ and the subsequent $Q_{10}$ effects on metabolic rates (cf. Barros et al., 2001).

The kinetics of the post-hypoxic recovery of metabolic rate also support this view. At the lowest ambient temperature, the post-hypoxic metabolic rate showed a large overshoot before returning to pre-hypoxic levels. This probably reflects both the greater metabolic suppression in hypoxia and a more pronounced decline in $T_b$. Upon return to normoxia, the $T_b$ set point immediately shifts back to normothermic levels and, given increased input from peripheral thermosensors associated with the lower ambient temperature, and increased input from deep body (hypothalamic and spinal) thermosensors due to the greater fall in $T_b$, provokes a large increase in thermogenesis (shivering, and possibly non-shivering), bringing $T_b$ back to 38°C. In the squirrels held at 22°C, the post-hypoxic overshoot in metabolic rate was smaller although still significant, probably reflecting lower feedback from peripheral and deep body thermosensors, and less drive to increase thermogenesis.

At 30°C ambient temperature, not only was there no overshoot in metabolic rate post-hypoxia, but $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were reduced for 10–20 min. Frappell et al. (1991) also found that a post-hypoxic metabolic suppression could be transiently

present in kittens. This result is somewhat perplexing but may reflect the shift of the TNZ to lower temperatures during hypoxia that has been described by others (Barros et al., 2001; Dupré et al., 1988). If the TNZ was lowered during hypoxia, 30°C may have been above the upper critical temperature of the new TNZ. As a result, the steady state metabolic rate at 30°C during hypoxia may have included costs associated with maintaining a $T_b$ below $T_a$. Upon switching the inspired gas back to normoxia, these costs would disappear and metabolic rate would be lowered transiently until the TNZ and $T_b$ returned to pre-hypoxic levels.

*Hypoxia induced changes in* $T_b$ via *regulated heat loss*

During the early stages of hypoxia, the surface temperature of particular body surfaces with minimal insulation warmed in a fashion that was ambient temperature specific (Fig. 3B). At 10°C and 22°C ambient temperature, the $T_s$ of the nose, ears and feet increased for the first 20–30 min in hypoxia, while at 30°C, the increases in $T_s$ were small and confined to the nose and feet (Table 2; Figs 4, 6). These increases in surface temperature occurred simultaneously with reductions in metabolic heat production and at a time when $T_b$ was known to be falling (*cf.* Barros et al., 2001). This suggests that core heat was being shifted to the periphery, and is consistent with the hypothesis that this facilitates a regulated drop in body temperature.

The kinetics and magnitude of the changes in $T_s$ of the feet, ears and nose are also consistent with this hypothesis. Changes in $T_s$ were of shorter duration at 10°C than at 22°C and smaller in magnitude at 30°C than at either of the other temperatures. Thermoregulatory control of $T_b$ within the TNZ is predominantly mediated through changes in peripheral vasomotor tone. At or above the upper critical temperature, the periphery is already maximally vasodilated, and there is little room for more vasomotor thermoregulatory adjustments. At temperatures well below the lower critical temperature, peripheral changes in $T_s$ occur rapidly as the greater temperature gradient from the animal to the environment allows for more rapid body cooling.

Although not specifically measured in this study, total heat loss in hypoxia is predicted to increase in parallel with changes in surface temperature. Previous studies have shown this in other species. Gordon (1997) measured heat loss in hypoxic rats with direct calorimetry, and demonstrated that heat loss increased and remained elevated above heat production for nearly an hour in hypoxia. Similar results were observed in a small primate breathing 10% $O_2$ (Tattersall et al., 2002), although the duration of elevated heat loss was shorter, reflecting the more

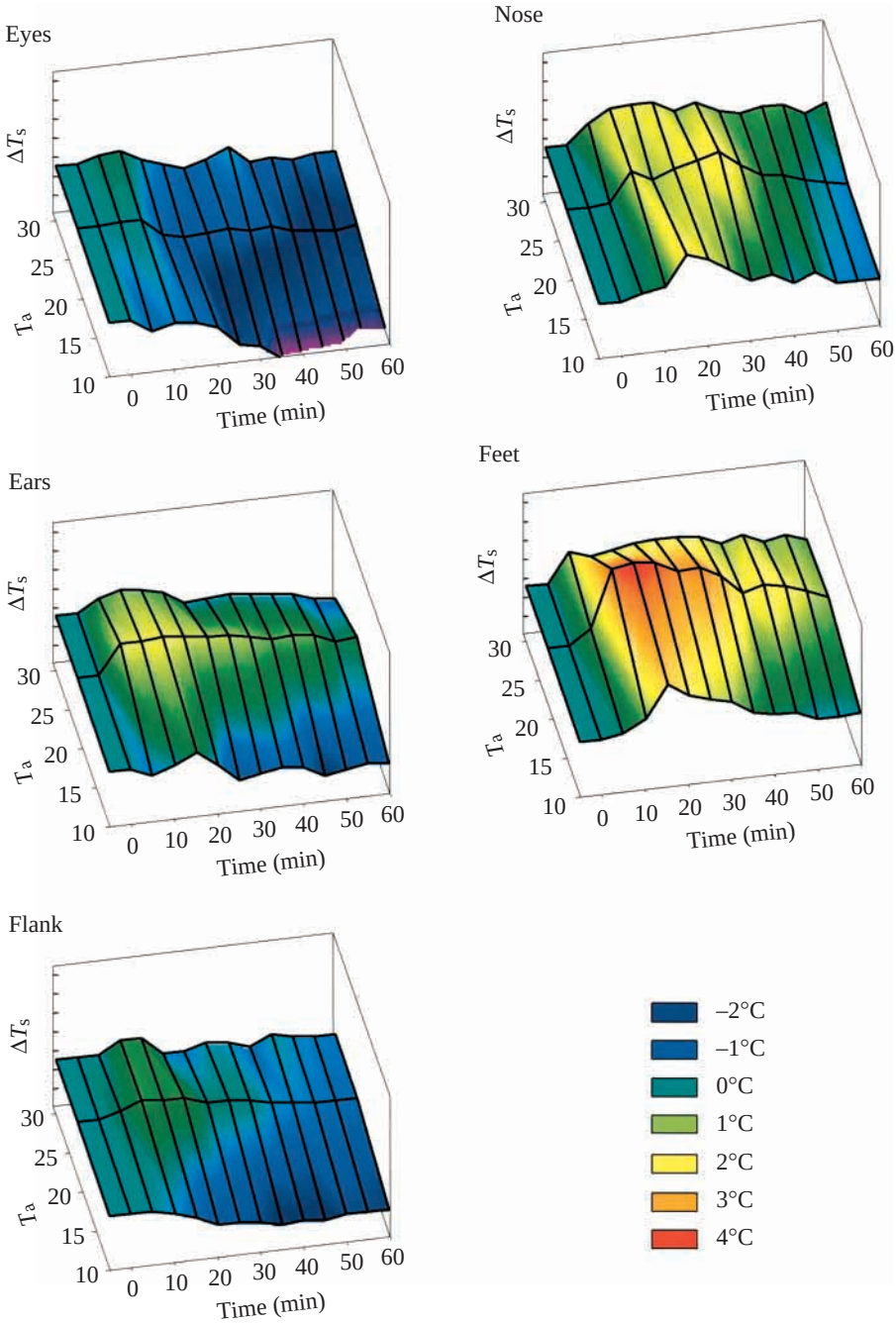


Fig. 6. Change in surface temperature $T_s$ during hypoxia ($\Delta T_s$) as a function of time and ambient temperature $T_a$ on the different body surfaces. Colours indicate the $\Delta T_s$, as shown in the key.

modest decrease in $T_b$ in the primate (only 2°C *versus* a 4–5°C drop in the rat). While it would be possible for $T_b$ to decline without an increase in heat loss, provided metabolic rate (i.e. heat production) decreased, the decrease in $T_b$ would be a passive process and the speed with which it fell would be much slower than that which would be observed if heat loss were also increased.

There is also evidence from studies on other mammals to suggest that levels of hypoxia similar to those used in the present study lead to neurally mediated changes in cutaneous vascular resistance that can account for the changes in peripheral blood flow and heat loss. Systemic hypoxia evoked a reflex dilation of the saphenous (leg) vein in the dogs, a mechanism thought to result from the withdrawal of sympathetic tone (Britton, 1984). Similar results were found in the ear of the rabbit (Iriki and Kozawa, 1976). While neither study inferred a thermoregulatory role for this decreased cutaneous sympathetic tone in hypoxia, these decreases in cutaneous sympathetic tone and vascular resistance were accompanied by increases in sympathetic tonus and resistance to other regions of the body (splenic, muscle), suggesting that the opening up of cutaneous vascular beds is preferentially regulated in hypoxia.

There is further indirect evidence to suggest that $T_s$ was being regulated for thermoregulatory purposes during hypoxia, including: (1) some squirrels were observed to adopt apparent heat-loss postures, lying with their feet above their bodies during hypoxia (Fig. 5); and (2) the lack of peripheral warming during the post-hypoxic period, despite the increased metabolic rate (and hence, heat production) at 10 and 22°C. At this stage, the squirrels were shivering intensely, and $\dot{V}_{O_2}$ was 2–3 times higher than pre-hypoxic levels, yet there was no apparent change in $T_s$ in any part of the body, suggesting that the heat was being maintained within the core. In fact, $T_s$ passively warmed or did not change at all as the animals' body temperature and metabolism returned to normal. This suggests that during normoxic recovery, $T_s$ was being controlled to conserve the heat being produced by shivering muscles and brown adipose tissue to warm the squirrel back to its normoxic $T_b$.

### *Conclusions and perspectives*

There now exists a considerable amount of data to indirectly support the hypothesis that moderate to severe levels of hypoxia lead to a controlled decline in $T_b$. This hypothesis has been difficult to test directly. The current study, however, adds to the indirect evidence by demonstrating that changes in $T_s$, as a possible reflection of changes in peripheral blood flow and heat loss, proceed in a coordinated fashion that is consistent with a carefully regulated, hypoxia-induced decline in $T_b$. While it remains to be shown quantitatively that these changes in $T_s$ do contribute to total heat loss, and that this is indeed a reflexly mediated process, the changes in $T_s$ do appear to be differentially controlled in a manner that is dependent upon ambient temperature, and consistent with the general hypothesis.

### List of symbols and abbreviations

| | |
|---|---|
| IR | infrared radiation |
| RER | respiratory exchange ratio |
| $T_a$ | ambient temperature |
| $T_b$ | body temperature |
| $T_s$ | surface temperature |
| $\Delta T_{s-a}$ | difference between surface and ambient temperatures |
| $\Delta T_s$ | change in $T_s$ from normoxic control |
| TNZ | thermoneutral zone |
| $\dot{V}_{CO_2}$ | rate of $CO_2$ production |
| $\dot{V}_{O_2}$ | rate of oxygen consumption |

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