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Hypoxia reduces the hypothalamic thermogenic threshold and thermosensitivity

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Hypoxia is well known to reduce the body temperature (T_b) of mammals, although the neural origins of this response remain uncertain. Short-term hypoxic exposure causes a reduction in the lower critical temperature of the thermal neutral zone and a reduction in whole body thermal conductance of rodents, providing indirect support that hypoxia lowers T_b in a regulated manner. In this study, we examined directly the potential for changes in central thermosensitivity to evoke the hypoxic metabolic response by heating and cooling the preoptic area of the hypothalamus (the area which integrates thermoreceptor input and regulates thermoeffector outputs) using chronic, indwelling thermodes in ground squirrels during normoxia and hypoxia (7, 10 and 12% O_2). We found that the threshold hypothalamic temperature for the metabolic response to cooling (T_{th}) of ∼38°C in normoxia was proportionately reduced in hypoxia (down to 28–31°C at 7% O_2) and that the metabolic thermosensitivity (α; the change in metabolic rate for any given change in hypothalamic temperature below the lower critical temperature) was comparatively reduced by 5 to 9 times. This provides strong support for the hypothesis that the fall in temperature that occurs during hypoxia is the result of a reduction in the activation of thermogenic mechanisms. The decrease in the central thermosensitivity in hypoxia, however, appears to be a critical factor in the alteration of mammalian T_b. We suggest, therefore, that an altered central thermosensitivity may provide a proximate explanation of how low oxygen and similar stressors reduce normal fluctuations in T_b (i.e. circadian), in addition to the depression in regulated T_b.

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Abbreviations α, metabolic thermosensitivity during hypothalamic cooling; CGS, Columbian ground squirrel; CNS, central nervous system; EBZ, ear-bar zero (stereotactic terminology); f_H, heart rate; f_R, breathing frequency; GMGS, golden-mantled ground squirrel; NTS, nucleus tractus solitarii; RER, respiratory exchange ratio (whole animal respiratory quotient); RTN, reticular thalamic nucleus; T_b, body temperature; T_h, hypothalamic temperature; $\overline{T}_h$, steady state T_h, measured without thermal manipulation; T_{th}, threshold T_h below which metabolic heat production is activated above basal levels during hypothalamic cooling; $\dot{V}_E$, rate of ventilatory air exchange (minute ventilation); $\dot{V}_{CO_2}$, rate of carbon dioxide production; $\dot{V}_{O_2}$, rate of oxygen consumption; V_{TI}, tidal volume of air exchanged with each breath.

Introduction

Hypoxia can manifest as changes in environmental oxygen, which occur naturally in some burrow-dwelling animals (Birchard *et al.* 1984; Kuhnen, 1986), or as hypoxaemia associated with circulatory disorders, particularly those related to septicemic or endotoxic shock (Kozak, 1997; Romanovsky *et al.* 2005; Kozak *et al.* 2006). Both forms of oxygen limitation reduce metabolism and body temperature (T_b) in many small mammals (Frappell *et al.* 1992; Mortola, 1993), primates (Tattersall *et al.* 2002) including humans (Kottke *et al.* 1948), and a host of ectothermic vertebrates (Wood, 1991; Wood & Gonzales, 1996). This reversible metabolic suppression, however, is also accompanied by a behavioural selection of lower temperatures (Gordon & Fogelson, 1991; Brown *et al.* 2005), an increase in behavioural responses that facilitate heat loss (e.g. adoption of postures conducive to heat loss; Mortola & Feher, 1998) and physiological responses that preferentially 'dump' core heat to the periphery (Tattersall & Milsom, 2003). Extensive evidence suggests that the reduction in T_b results from

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neurophysiological control rather than a failure of thermoeffectors. For example, non-shivering thermogenesis in rats is inhibited by hypoxic stimulation of arterial chemoreceptors, mediated via brainstem mechanisms involving the NTS, and possibly the reticular thalamic nucleus (RTN) (Madden & Morrison, 2005). Shivering has also been demonstrated to be rapidly and reversibly reduced in hypoxic mammals (Barros *et al.* 2001). These thermoregulatory adjustments, however, occur without evidence of anaerobic metabolism or oxygen limitation in the tissues, *per se* (Frappell *et al.* 1991; Rohlicek *et al.* 1998). Combined, this evidence points to neural mechanisms being strongly involved in temperature regulation during hypoxic conditions.

Thus, the lowering of T_b in hypoxia appears to result from a suite of mechanisms that cause a decrease in thermoeffector activity and lead to a decline in T_b. This has not, however, been definitively proven or tested centrally. Indeed, as a result of the diverse and indirect approaches to this question, the hypoxic thermoregulatory response has been referred to by assorted names: regulated hypothermia (Gordon, 1983), hypoxia-induced torpor (Hayden & Lindberg, 1970), hypoxic hypometabolism (Mortola, 1993; Rohlicek *et al.* 1998), hypoxia-induced hypothermia (Wood & Gonzales, 1996; Branco *et al.* 1997; Fabris *et al.* 1999), hypoxia-induced anapyrexia (Steiner & Branco, 2002; Steiner *et al.* 2002), and the hypoxic metabolic response (Barros *et al.* 2001; Tattersall & Milsom, 2003). Since evidence to date suggests functional thermoregulatory control in hypoxia, any use of the prefix 'hypo' to describe this decline in T_b is misleading since it implies that T_b falls uncontrollably (IUPS Thermal Commission, 2001).

Since the central nervous system (CNS) T_b regulator has been observed to exhibit similar characteristics in euthermic and hibernatory states, hibernation may offer insight into the question of whether central mechanisms are responsible for the hypoxic thermoregulatory response. The preoptic area of the anterior hypothalamus of mammals contains thermosensitive neurons that respond to cooling and warming of hypothalamus temperature (T_h), effecting physiological changes that act in a feedback fashion to reverse the change in T_h (Heller & Hammel, 1972; Heller & Colliver, 1974; Heller & Glotzbach, 1977). Cooling this region results in a proportional increase in metabolic heat production and a peripheral vasoconstriction. Warming this region produces the opposite response (decreased heat production and increased heat loss). In fact, the threshold for activation of thermogenic defences (T_{th}) can be explored at the same time as hypothalamic thermosensitivity (i.e. sensitivity of the CNS T_b regulator), where the latter value should be an estimate of the intrinsic temperature-related output of thermosensitive hypothalamic neurons. To examine the nature of the central control of T_b, T_h can be manipulated, producing responses that adhere to the following control equation (Hammel, 1968):

$$R - R_0 = \alpha(T_h - T_{th}) \quad (1)$$

where R is heat production, R_0 is basal heat production, and the incremental change in heat production above basal levels ($[R - R_0] \geq 0$) is proportional to the product of thermosensitivity (α) and the difference between hypothalamic temperature (T_h) and a threshold temperature (T_{th}). This product is the effective error signal with which the CNS regulator operates, producing heat production responses that are related to α (provided $T_h < T_{th}$). The farther that T_{th} is from the actual T_h, the larger the error signal, and thus, the larger corrective heat production required.

The usefulness of this technique in assessing thermoregulatory responses is its ability to examine the central control of T_b in a closed loop system by 'opening the feedback loop' that influences T_b (Hammel, 1968). By manipulating only T_h, numerous processes and stimuli have been established that alter T_{th} and α (Heller & Hammel, 1972; Hammel *et al.* 1973; Glotzbach & Heller, 1975; Florant & Heller, 1977; Florant *et al.* 1978; Sakaguchi *et al.* 1979). Low ambient temperatures raise T_{th} by stimulating cutaneous cold thermoreceptors which relay information to the preoptic area (Glotzbach & Heller, 1975); thus, the threshold T_h for activation of thermogenesis is higher at low ambient temperatures, ensuring that any deviation in brain temperature is met with compensatory heat production. Neural output from the preoptic area is also state dependent (Sakaguchi *et al.* 1979; Glotzbach & Heller, 1984); the normal facilitation (high α and T_{th}) that occurs during wakefulness is reduced during slow-wave sleep (SWS), leading to a reduction in T_{th} and α. In REM sleep, no facilitation occurs, and no discernible hypothalamic thermosensitivity can be measured; thus, there are no thresholds that elicit either thermogenesis during cooling, or respiratory panting during warming (Parmeggiani *et al.* 1973; Glotzbach & Heller, 1976, 1984). Finally, exercise and infectious agents that cause fever raise T_{th} for both heat production and heat loss pathways (Hammel *et al.* 1973). Whether alterations in blood or cerebral fluid oxygen levels alter the integration of thermal signals by the CNS regulator of T_b is unknown; however, the idea of an adjustable T_{th} with respect to hypoxia is compatible with the models for the CNS regulation of T_b (Kozak, 1997; Cabanac, 2006; Kozak *et al.* 2006). The question of whether T_{th} is reduced by hypoxia, however, has only ever been indirectly assessed from whole animal cooling experiments; squirrels and other rodents actively recruit thermogenesis in hypoxia, but at reduced levels, and T_b is not as tightly defended, varying, instead with ambient temperature (Dupré *et al.* 1988; Barros

et al. 2001). Thus, from these kinds of experiments, it is uncertain whether hypoxia alters the capacity to generate body heat under low temperature conditions, alters heat loss and heat production pathways differently, or reflects an underlying central mechanism.

Ground squirrels and other hibernating mammals have served as model animals for elucidating underlying thermoregulatory mechanisms common to mammalian T_b control (Hammel *et al.* 1973; Heller *et al.* 1974; Florant & Heller, 1977; Larkin & Heller, 1996). Not only do they exhibit a profound reduction in T_b and metabolism in hypoxia (Barros *et al.* 2001) and a high degree of hypoxia tolerance (Drew *et al.* 2007; Levesque & Tattersall, 2009), they also possess a very high α (Heller, 1978, 1979), a profound, cold-activated thermogenesis from brown adipose tissue (Milner *et al.* 1989), and have been shown to progressively lower T_{th} as they enter into hibernation (Heller *et al.* 1977). Thus, ground squirrels are a powerful model for exploring questions concerning the hypoxic response and thermoregulatory control. We describe, in this study, experiments designed to probe the changes in central T_{th}, the intrinsic thermosensitivity (α) of heat production, and associated cardiorespiratory indicators during the hypoxia-induced periods of reduced T_b. The specific objectives were: (i) to assess the hypothalamic thermosensitivity and threshold temperature (T_{th}) for metabolic heat production; (ii) to assess the integration of relevant ventilatory and cardiovascular parameters with thermoregulatory adjustments; and (iii) to determine the characteristics of the CNS temperature regulator operating under hypoxic conditions. By fulfilling these objectives, we anticipate addressing whether the threshold for activation of hypothalamic heat production defences (T_{th}) decreases in hypoxia and whether the thermosensitivity of the central regulator of T_b is reduced by hypoxia, and thereby determine how the CNS exerts regulatory influences on T_b in hypoxia.

Methods

Ethical approval

All experiments were approved by the University of British Columbia Animal Care and Use Committee and conformed to Canadian Council for Animal Care guidelines, and to the principles and policies outlined by Drummond (2009).

Animals

Golden-mantled ground squirrels ($N = 7$; average mass 250 ± 18 g) and Columbian ground squirrels ($N = 7$; average mass 496 ± 62 g) were caught from wild populations in Redding, CA, USA and Manning Park, British Columbia, Canada. All squirrels were housed at 20°C and studied during the months of July and August, when they were normothermic. No squirrel underwent torpor during the period of experimentation, as verified by daily observations. Animals were supplied *ad libitum* with standard rat chow, supplemented with sunflower seeds and fruit. Both male and female golden-mantled squirrels (GMGS) were examined, and only female Columbian ground squirrels (CGS) were studied. In GMGS, sex did not exert a significant effect and, thus, data were pooled for both sexes.

Surgeries and thermode implantation

Squirrels were anaesthetized with 65 mg kg^{-1} sodium pentobarbital (I.P.), and throughout surgeries maintained within an appropriate surgical plane using supplemental injections as required. Water-perfused metal thermodes were implanted bilaterally (2 mm from midline) in the medial preoptic area of the hypothalamus as described in Heller & Hammel (1972), allowing for the manipulation of T_h. T_h was measured using a 30 gauge thermocouple wire that was placed down a one-ended re-entrant tube (26 gauge needle with a welded end) located half-way between the two thermodes and 1.5 mm caudal. The placement of the thermodes and re-entrant tube was determined using established skull suture line morphologies (Heller & Hammel, 1972; Heller & Colliver, 1974; Heller *et al.* 1974). Using the nose bar of the stereotaxic apparatus set +5 mm provided the appropriate correction factor to ensure that skull morphologies, lambda and bregma were within the same horizontal plane (Harris & Milsom, 2001), allowing the use of rat brain coordinates (Paxinos & Watson, 1997). The stereotaxic coordinates used for GMGS were 9.5 mm rostral to EBZ and 1.5 mm lateral to midline, and those for CGS were 11 mm rostral to EBZ and 1.5 mm lateral to midline. In both species, the thermodes were implanted to a depth of 9 mm. As Heller & Hammel (1972) found, depth rather than rostral–caudal or lateral orientation had the greatest influence on evoking metabolic changes. Placing thermodes only 8 mm deep did not evoke significant changes in metabolic rate with hypothalamic cooling, whereas thermodes placed 1 mm rostral or caudally (in separate instances) evoked similar increments in metabolic rate with cooling. Since the total distance from the top of the cranium to the base was between 9 and 10 mm, 9 mm depth was chosen to minimise damage to the neural structures of interest.

After placing the thermode assembly, four bone screws were fixed bilaterally into the occipital and parietal bones. Dental cement and dental acrylic were used to fuse the plexiglass thermode assembly to the bone screws. Proper placement of the thermodes and re-entrant

tube was verified initially by perfusing the thermodes with cool water which evoked strong and rapid shivering in awake animals, and later in animals post mortem, through brain dissections. Thermode placement was verified to be directly above the optic chiasm through visual examination of the brain of animals that were humanely killed with an overdose of sodium pentobarbital. Electroencephalogram (EEG) leads were attached to two of the bone screws, allowing for contralateral cortical EEG recordings (in the GMGS only). Due to limited input channels on the data acquisition system and electrical interference that resulted from multiple temperature probes, EEG measurements were only conducted in the GMGS, where T_b was not measured. Electrocardiogram (ECG) and electromyogram (EMG) leads were implanted subcutaneously in the pectoral and neck muscles, respectively, for the assessment of heart rate and shivering as described previously (Barros *et al.* 2001; Zimmer & Milsom, 2001). A thermocouple wire housed within a small, sealed cannula was implanted into the peritoneal cavity and was passed subcutaneously back to the thermode assembly where the copper and constantan poles were left available as contact points for recording core T_b. Unfortunately, the core temperature thermocouples were prone to malfunction not long after implantation, providing limited data on core temperature. T_h was necessarily the best estimate of core temperature during periods when water was not perfusing through the thermode assemblies. Following surgeries, squirrels were allowed to recover for 12 h in a warm environment (24–28°C) while being monitored for vital signs. Following recovery, squirrels were returned to their cages, and not studied until fully healed (approximately 3 weeks following surgery). Immediately following surgery, squirrels were provided with an antibiotic (Baytril, 5–10 mg kg^{-1}, I.M.), in addition to daily applications of a topical xylocaine/clove oil mixture to the wound site. By the time of experimentation, wounds were fully healed, and no animal exhibited any signs of fever.

Data acquisition

All parameters (O_2, CO_2, differential pressure, T_b, T_h, EMG, ECG and EEG) were recorded at a sampling rate of 200 Hz using an 8-channel Windaq data acquisition system (Data Instruments, Akron, OH, USA). Due to a limitation in number of input channels, EEG was recorded only in GMGS in place of T_b, whereas T_b was recorded in CGS in place of EEG. Temperatures were recorded using thermocouple meters (Model BAT-12, Physitemp Instruments). ECG and EMG voltages were first passed through an amplifier (Model 7P511L, Grass Instruments). ECG values were low-pass (LP) and high-pass (HP) filtered (50% filters: LP, 30 Hz; HP, 0.1 kHz), providing appropriate resolution of the QRS complex for peak determination. EMG voltages were low-pass filtered (50% filter LP, 300 Hz) prior to integration and analysis. EMG data were integrated over 5 s ($\int$EMG), and the average $\int$EMG values during periods of interest obtained and expressed as percentages relative to resting, non-disturbed values. EEG voltages were low-pass and high-pass filtered (50% filters: LP, 1 Hz; HP, 30 kHz), prior to being processed. EEG values were only examined during stable periods of normoxia and hypoxia. Multiple (approximately 10 per squirrel per O_2 level) 2 min epochs of EEG voltages were fourier transformed, producing power spectra where total power (P_{EEG}; area under the power spectrum, units: V^2 s^{-1}) was compared between normoxia and hypoxia, as well as the ratio of power within the 2–4 Hz band *versus* the 13.5 to 20 Hz band ($P_{2-4\ Hz}$:$P_{13.5-20Hz}$), as an indicator of slow:fast EEG activity. Due to electrical interference, accurate EEG measures during T_h manipulation were not practical. In addition to the 2 h transition periods into hypoxia (see experimental protocol below for details), the periods during which T_h was manipulated were exported to custom spreadsheets for peak detection (f_R and f_H), as well as $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, V_{TI} and $\int$EMG. Average values during the stable portion of the manipulation period were obtained.

Metabolic rate determination

Metabolic rates ($\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$) were determined using flow-through respirometry as described in Tattersall & Milsom (2003). Briefly, squirrels were placed in 0.5 l plexiglass chambers with incurrent gas flow set to 1000–1500 ml min^{-1} (depending on the squirrel size). Gases (7, 10, 12 or 21% O_2) were mixed and flow controlled using a gas mixing flow meter (Cameron GF-3/MP). A subsample (200 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 (200 ml min^{-1}) 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 reported at STPD (ml kg^{-1} h^{-1}). The respiratory exchange ratio (RER) was determined from $\dot{V}_{CO_2}$: $\dot{V}_{O_2}$.

Cardiovascular and ventilatory parameters

Heart rate (f_H), tidal volume (V_{TI}; ml kg^{-1}), breathing frequency (f_R), minute ventilation ($\dot{V}_E$; ml kg^{-1} h^{-1}) and air convection requirement ($\dot{V}_E$: $\dot{V}_{O_2}$) were determined as outlined in Barros *et al.* (2001). Peak detection for the determination of interbeat interval (IBI) and f_H (60 × IBI^{-1}; min^{-1}) was determined offline using custom

peak detection spreadsheets. Ventilation was assessed with whole body plethysmography using a flow-through system (Tattersall *et al.* 2002), consisting of two identical 0.5 l chambers. One chamber served as the respirometer for the animal (see above), while the other as the pressure reference chamber. Gas flows to each chamber were set to be balanced at 1500 ml min^{-1} each by producing a negligible pressure-differential between them (total flow of mixed gas was 3000 ml min^{-1}). The pressure signal was measured using a differential pressure transducer (Model DP103-8, Validyne Engineering, Northridge, CA, USA), amplified (Model 7P122E, low-level direct current amplifier, Grass Instruments), and recorded to the computer data acquisition system. The plethysmograph was calibrated using pulse injections of air (3 ml), as well as confirmed with dynamic pressure signals generated as described by McArthur & Milsom (1991). The time constant of the pressure signal was >4 s, producing negligible filtering of the pressure amplitude. All ventilatory variables (V_{TI}, $\dot{V}_E$) are reported at BTPS (ml kg^{-1} and ml kg^{-1} h^{-1}, respectively).

Experimental protocol

GMGS were studied at 21 and 7% O_2, whereas CGS were studied at 21, 12, 10 and 7% O_2; therefore, most experimental results presented are from CGS, with reference to GMGS made where appropriate. Protocols for both species were identical, except for the additional oxygen levels in CGS. At the beginning of the experimental period, an individual squirrel was placed into the respirometer, which was housed within a temperature-controlled environmental chamber held at 20–22°C throughout all experiments. Squirrels were unrestrained and allowed 30–60 min to habituate to the respirometer before altering oxygen levels or manipulating T_h (the last 10 min of this initial period served as the control, non-manipulated values). T_h was altered above and below resting (non-manipulated) values for 10 min intervals. Inbetween manipulations, squirrels were given 10 min to recover before a change in T_h was induced, long enough for values to return to resting levels. In normoxia, T_h was manipulated from a resting value of ~38°C down to ~33°C and up to ~43°C (exact values were measured for each manipulation). Squirrels were left in normoxia (21% O_2) for 2 h, during which time T_h was lowered or raised. After completing changes to T_h, squirrels were exposed to hypoxic gases for at least 150 min to allow T_h and physiological parameters to reach a new, lowered value. Only one level of hypoxia was examined per day. After steady state values had been reached (2 h was sufficient; see Fig. 1), T_h was manipulated at temperatures above and below the hypoxic T_h value, as in normoxia. For 12% O_2, T_h was manipulated from ~31 to ~41°C, for 10% O_2, from ~29 to ~41°C, and at 7% O_2, from ~26 to ~40°C; the lower extreme values were chosen because they generated maximum heat production responses. Since each animal was examined over a period of 3 days, multiple periods of normoxia were possible during the start of each experiment, allowing for completion of T_h manipulations in normoxia. During all periods of T_h manipulation, the following parameters were measured (average of the values between the 2nd and 8th minute of manipulation): O_2 consumption ($\dot{V}_{O_2}$), CO_2 production ($\dot{V}_{CO_2}$), tidal volume (V_{TI}), breathing frequency (f_R), total ventilation ($\dot{V}_E$), heart rate (f_H) and integrated neck muscle electromyographic activity ($\int$EMG). Due to variability in electrode placement and quality of the electrical signal, $\int$EMG was expressed as percentage of the steady state values. During T_h manipulations, squirrels were periodically observed to ensure that recording occurred during waking states.

Data analysis

Steady state values for all physiological parameters were determined after 2 h at each O_2 level. For the T_h manipulation results, all physiological parameters were measured as the average values during the 2nd to 8th minute of all periods of T_h manipulation to ensure suitable time for physiological equilibration. At least six, and usually up to nine separate 10 min manipulations at different T_h values were obtained at each oxygen level. Subsequently, slopes (analogous to α) of the various physiological parameters *versus* manipulated T_h (e.g. ml O_2 g^{-1} h^{-1} °C^{-1} for $\dot{V}_{O_2}$) were determined from each animal both below (cool slope) and above (warm slope) the steady state T_h, to explore the thermosensitivity of physiological parameters above and below the steady state T_h. Slopes were chosen not necessarily because a linear relationship was expected in all cases, but to simplify the analysis for comparison to previous research (Heller *et al.* 1974; Florant *et al.* 1978) and to readily incorporate the slopes into subsequent statistics. Cases where the slopes were zero at one level of oxygen, but positive or negative in another, would still reveal evoked physiological changes associated with T_h changes. After calculating the slopes, T_{th} for metabolic heat production was determined using eqn (1) as described by Heller *et al.* (1974) for each animal and at each level of oxygen. One additional parameter was examined: the 'heat gain ratio' (Cormareche-Leydier *et al.* 1985). Immediately following every 10 min T_h manipulation (ΔT_h; $T_{h,before} - T_{h,duringmanipulation}$), a change in T_b ($\Delta T_b = T_{b,before} - T_{b,aftermanipulation}$) manifested from the altered balance between metabolic heat production and heat loss. Thus, we examined the ratio of ΔT_b:ΔT_h as an indicator of how much T_b changed for a given T_h

stimulus, across the different oxygen treatments, using a one-way repeated measures (rm) ANOVA. This ratio provided an indication of the effectiveness of hypothalamic manipulation to manifest a change in heat load, as well as information on extrahypothalamic feedback. Steady state physiological parameters, cooling and warming slopes from T_h manipulations, and T_{th} were examined statistically using a one-way rmANOVA, with O_2 level as the factor. *Post hoc* multiple comparisons were made using a Holm–Sidak test compared against the normoxic value. All statistical tests were considered significant at an α of 0.05. Data are presented as mean ± S.D., except for graphical clarity where S.E.M. is utilised.

Results

Throughout, results are presented primarily for CGS, with data from GMGS provided in accompanying tables and figures for comparative purposes. Results where species differences occur or where data from only one species was collected are highlighted below.

Metabolic and cardiorespiratory responses to hypoxia

All physiological parameters reached steady state values within 1–2 h of exposure to hypoxia (Fig. 1). T_b and T_h were strongly dependent on oxygen level (12, 10 and 7% significantly lower than 21% O_2); however, only 7% O_2 led to significant reductions in $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ (Tables 1 and 2).

Table 1. Cardio-respiratory parameters in normoxic (21% O_2) and hypoxic (7% O_2) golden-mantled ground squirrels during steady state conditions[1]

Variable	21% O_2	7% O_2
T_h (°C)	38.0 ± 0.6	30.8 ± 1.6‡
$\dot{V}_{O_2}$ (ml O_2 kg^{-1} min^{-1})	27.5 ± 7.2	19.8 ± 2.1‡
$\dot{V}_{CO_2}$ (ml CO_2 kg^{-1} min^{-1})	23.5 ± 5.7	17.4 ± 4.7†
RER (ml CO_2 (ml O_2)$^{-1}$)	0.86 ± 0.08	0.87 ± 0.11
f_H (beats min^{-1})	342 ± 28	284 ± 35†
V_{TI} (ml kg^{-1})	8.71 ± 3.62	16.4 ± 2.4†
f_R (beats min^{-1})	150 ± 53	121 ± 22
$\dot{V}_E$ (ml kg^{-1} min^{-1})	1150 ± 260	1960 ± 330‡
$\dot{V}_E$: $\dot{V}_{O_2}$ (ml O_2 ml^{-1})	43.9 ± 12.6	101 ± 24‡
P_{EEG} (V^2 s^{-1})	11581 ± 6064	13728 ± 12443
P_{2-4Hz}:$P_{13.5-20Hz}$	2.44 ± 1.44	4.75 ± 2.53*

Values are mean ± S.D. [1]Steady state refers to measurements obtained after 2 h. RER, respiratory exchange ratio. *Significantly different from normoxic values ($P < 0.05$). †Significantly different from normoxic values ($P < 0.01$). ‡Significantly different from normoxic values ($P < 0.001$).

Steady state RER values were not influenced by inspired oxygen. Compared to normoxia, steady state f_H values exhibited a stimulation at 12 and 10% O_2, which did not persist at 7% O_2 (Table 2). Steady state V_{TI} values were not affected by oxygen, but in combination with an increase in f_R at all levels of hypoxia, $\dot{V}_E$ was significantly higher at 12 and 10% O_2. $\dot{V}_E$: $\dot{V}_{O_2}$ was progressively higher with increasing degree of hypoxia.

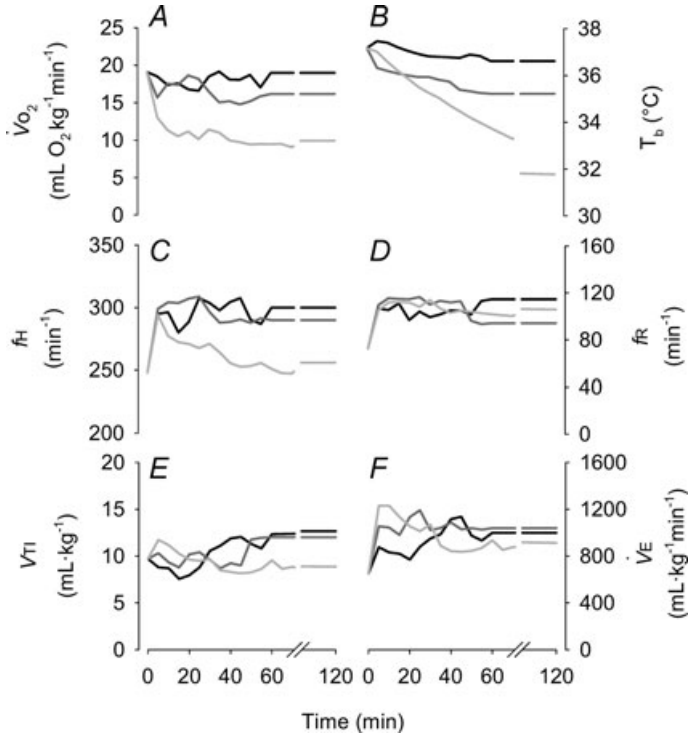

Figure 1. Time course (average values; error bars not shown for visual clarity) of the pertinent physiological factors under exposure to different levels of oxygen in the Columbian ground squirrel, demonstrating steady state values were achieved by the second hour of exposure
Black lines indicate 12% O_2, dark grey indicate 10% O_2 and light grey indicate 7% O_2. Shown are $\dot{V}_{O_2}$ (*A*), T_b (*B*), heart rate (*C*), breathing frequency (*D*), tidal volume (*E*) and minute ventilation (*F*).

Table 2. Cardio-respiratory parameters in normoxic (21% O_2) and hypoxic (12, 10 and 7% O_2) Columbian ground squirrels during steady state conditions[1]

Variable	21% O_2	12% O_2	10% O_2	7% O_2
T_h (°C)	37.7 ± 0.4	36.8 ± 0.4†	35.4 ± 0.9‡	31.9 ± 0.6‡
T_b (°C)	37.5 ± 0.6	36.7 ± 0.3*	35.1 ± 1.0‡	31.4 ± 0.6‡
$\dot{V}_{O_2}$ (ml O_2 kg^{-1} min^{-1})	19.1 ± 2.6	17.7 ± 2.0	16.0 ± 1.6	8.80 ± 2.60‡
$\dot{V}_{CO_2}$ (ml CO_2 kg^{-1} min^{-1})	17.2 ± 2.4	17.5 ± 2.8	15.7 ± 1.9	7.91 ± 2.46‡
RER (ml CO_2 (ml O_2)$^{-1}$)	0.90 ± 0.04	0.99 ± 0.06	0.99 ± 0.13	0.93 ± 0.15
f_H (beats min^{-1})	248 ± 28	300 ± 12†	295 ± 31†	251 ± 14
V_{TI} (ml kg^{-1})	9.70 ± 3.53	11.8 ± 4.2	12.5 ± 5.6	8.87 ± 3.0
f_R (beats min^{-1})	72.5 ± 15.1	103 ± 30*	99.3 ± 37.9*	103 ± 7*
$\dot{V}_E$ (ml kg^{-1} min^{-1})	650 ± 168	1130 ± 384†	1080 ± 298†	897 ± 270
$\dot{V}_E$: $\dot{V}_{O_2}$ (ml O_2 ml^{-1})	34.0 ± 7.4	62.2 ± 16.1†	67.4 ± 17.3‡	103 ± 14‡

Values are mean ± S.D. [1]Steady state refers to measurements obtained after 2 h. *Significantly different from normoxic values ($P < 0.05$). †Significantly different from normoxic values ($P < 0.01$). ‡Significantly different from normoxic values ($P < 0.001$).

Temperatures during normoxic–hypoxic transitions

In CGS, we were able to record both T_h and T_b simultaneously during entry and recovery from 7% O_2. The rate of change for T_b was 0.058°C min^{-1} during hypoxia, and 0.2°C min^{-1} during re-warming from hypoxia. Typically, T_h was higher than T_b; however, this differential diminished during hypoxia, until such a time as T_h reached a steady state value after 2 h (Fig. 2). During re-warming, T_h rose prior to T_b, creating an initial, large differential between T_h and T_b until T_h approached 36°C, after which the rate of T_h increase diminished.

EEG responses to hypoxia in golden-mantled ground squirrels

Cortical EEG measurements were made in GMGS during their transition to 7% O_2. Power spectra of EEG traces revealed that high frequency components declined in hypoxia, while low frequency components rose compared to normoxia (the ratio of low to high frequency components was significantly affected by oxygen levels; Table 1). Overall power, however, did not change between normoxia and hypoxia.

Responses to T_h manipulation

Only data where proportionality constants were significantly different from zero in normoxia were reported and analysed. Warming the hypothalamus did not produce proportionality constants significantly different from zero.

Metabolic responses to induced T_h changes

Brief, 10 min manipulations of T_h led to reversible changes in metabolic parameters in squirrels under normoxic and hypoxic conditions (Fig. 3). Hypothalamic cooling led to a proportional change in $\dot{V}_{O_2}$ in both CGS and GMGS at all levels of oxygen tested (Table 3; Fig. 4). Hypoxia had strong influences on the proportionality constants for $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ (Table 3), with all levels of hypoxia (7, 10 and 12%) showing a significant attenuation of the metabolic response to hypothalamic manipulation (Fig. 4). Cooling the hypothalamus was accompanied by activation of shivering thermogenesis (Fig. 5). In accord with changes in $\dot{V}_{O_2}$, hypothalamic cooling led to proportional changes in the activation of nuchal muscle EMG. These proportional changes (Table 3) were significantly attenuated by hypoxic exposure compared to normoxia (Fig. 4, Table 3). Return

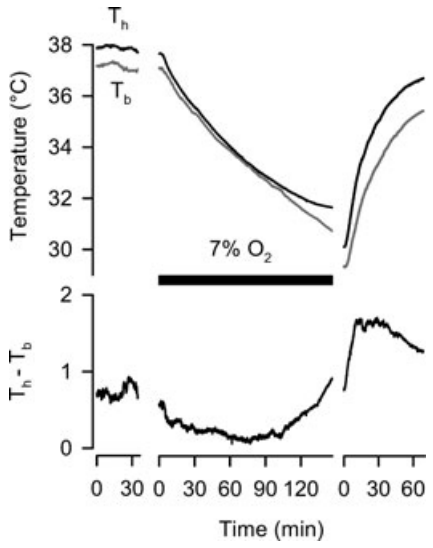

Figure 2. Time course (average values, top trace) of hypothalamic (T_h) and T_b from Columbian ground squirrels during entry and recovery from 7% O_2 (black bar)
Normally, T_h is elevated slightly above T_b, although this difference (bottom trace) undergoes dynamic changes, decreasing during the initial exposure to hypoxia, and subsequently increasing during re-oxygenation.

Table 3. Slopes resulting from T_h cooling trials in normoxic (21% O_2) and hypoxic (12,10 and 7% O_2) golden-mantled ground squirrels (GMGS) and Columbian ground squirrels (CGS)

	GMGS		CGS			
	21% O_2	7% O_2	21% O_2	12% O_2	10% O_2	7% O_2
$\dot{V}_{O_2}$	−12.1 ± 1.9	−1.58 ± 0.71‡	−6.01 ± 1.51	−2.70 ± 0.78‡	−2.03 ± 0.42‡	−1.10 ± 0.52‡
$\dot{V}_{CO_2}$	−9.64 ± 1.86	−0.954 ± 0.331‡	−4.62 ± 0.84	−2.46 ± 0.86‡	−1.79 ± 0.31‡	−0.986 ± 0.40‡
f_H	−21.6 ± 15.3	−1.38 ± 1.99*	−20.4 ± 2.80	−9.90 ± 3.96‡	−7.70 ± 4.46‡	−0.932 ± 3.15‡
$\int EMG$	−81.0 ± 45.7	−19.0 ± 22.0*	−32.4 ± 23.2	−12.0 ± 12.0†	−11.9 ± 9.3†	−4.12 ± 5.70†
V_{TI}	−1.62 ± 0.76	−0.132 ± 0.342†	−0.566 ± 0.604	−0.148 ± 0.303	0.126 ± 0.694	−0.504 ± 0.259
f_R	1.47 ± 9.62	−3.70 ± 1.60	−5.48 ± 4.76	−4.58 ± 3.99	−5.29 ± 1.41	−1.64 ± 1.67*
$\dot{V}_E$	−331 ± 85	−90.0 ± 56.3‡	−111 ± 61.2	−69.9 ± 78.9	−63.2 ± 56.9	−64.2 ± 39.4

All values are mean ± S.D., expressed in units $^{\circ}C^{-1}$ (i.e. per change in T_h) – negative values indicate that cooling T_h caused that parameter to rise. *Significantly different from normoxic values ($P < 0.05$). †Significantly different from normoxic values ($P < 0.01$). ‡Significantly different from normoxic values ($P < 0.001$).

to normoxic conditions was met with rapid initiation of shivering and increments in metabolic rate (Fig. 6), suggesting a rapid return to normoxic thermoregulatory patterns. In one case, however, we transiently suppressed this post-hypoxic shivering by warming the hypothalamus to 40°C (Fig. 6).

T_b responses to induced T_h changes

In addition to metabolic changes, manipulation of T_h also led to changes in T_b itself (Fig. 7*A–D*). Indeed, in normoxic squirrels, warming the hypothalamus led to a decline in T_b, whereas cooling raised T_b. The relationship between changes in T_h and T_b manifested in a linear 'heat gain ratio' in normoxic squirrels of approximately 0.1 (Fig. 7*E*). Exposure to hypoxia, however, abolished the heat gain ratio, such that warming or cooling T_h produced little change in T_b (Fig. 7*E* and *F*). The effect of oxygen on the heat gain ratio was significant ($F_{3,11} = 17.0$; $P = 0.00019$), with 7 and 10% O_2 values higher than normoxia, but not significantly different from zero ($t_5 = 1.8$ and 0.44; $P = 0.07$ and 0.31, respectively). Heat gain ratios for 21 and 12% O_2 were significantly different from zero ($t_5 = 4.7$ and 4.2; $P = 0.003$ and 0.004, respectively).

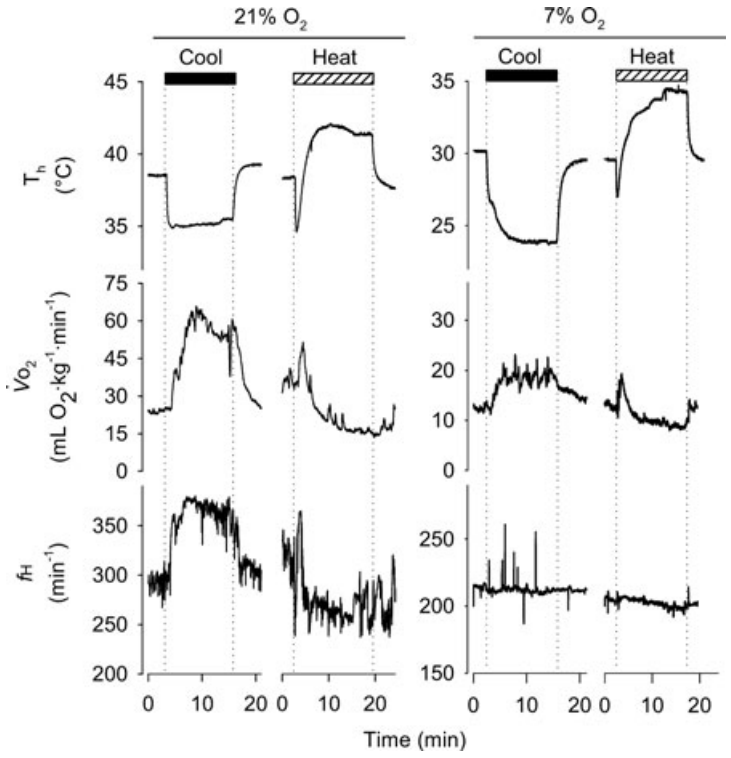

Figure 3. Sample traces of the influence of T_h manipulation on metabolic heat production ($\dot{V}_{O_2}$) and heart rate (f_H) under normoxia (left traces) and hypoxia (7% O_2; right traces) in Columbian ground squirrels

Traces depict raw data from one squirrel before, during and after 10 min periods of artificial manipulations of T_h below (black bars) and above (hatched bars) normal values. Different scales for normoxic and hypoxic values are shown to demonstrate the influence of T_h manipulation under both oxygen conditions.

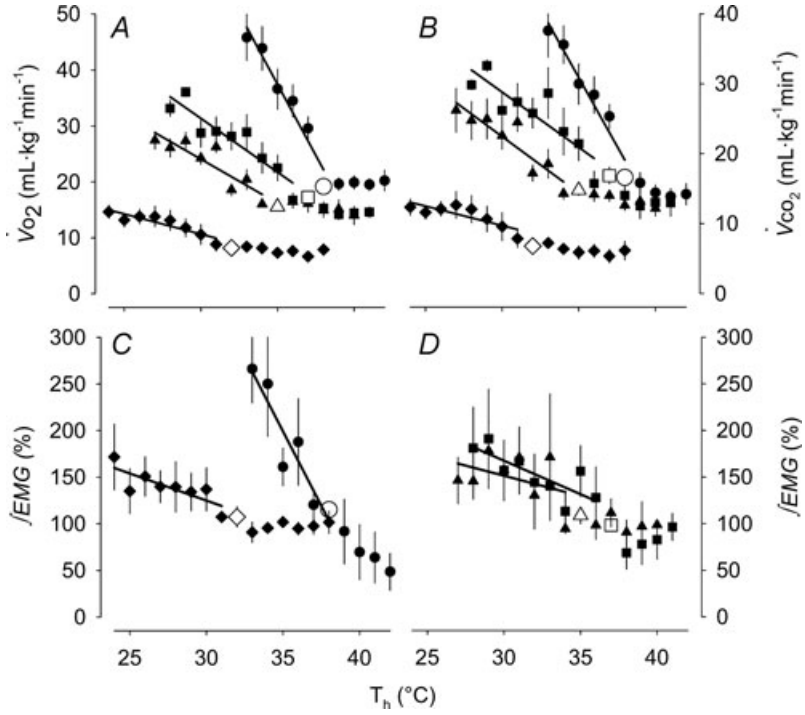

Figure 4. Summary (mean ± S.E.M.) metabolic heat production values from the T_h manipulation experiments in Columbian ground squirrels
$\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ are shown in *A* and *B*, respectively, for all levels of inspired O_2, whereas EMG responses are split into *C* (21 and 7% O_2) and *D* (12 and 10% O_2) for visual clarity. Open symbols represent the steady state, non-manipulated values, whereas filled symbols represent values measured during the 10 min T_h manipulation trials. For all graphs (*A–D*), circles refer to 21%, squares refer to 12, triangles refer to 10 and diamonds refer to 7% inspired O_2. Regression lines are plotted to demonstrate the degree of proportionality resulting from hypothalamic cooling.

Cardiorespiratory responses to T_h cooling and warming

Cardiovascular changes in response to hypothalamic manipulations closely mirrored metabolic changes (Fig. 8). Heart rate rose with hypothalamic cooling in normoxic squirrels. Furthermore, the proportionality constants of f_H for hypothalamic cooling were significantly influenced by hypoxia (Table 3; Fig. 8*A* and *B*); all levels of hypoxia significantly attenuated the influence of cooling on the f_H response.

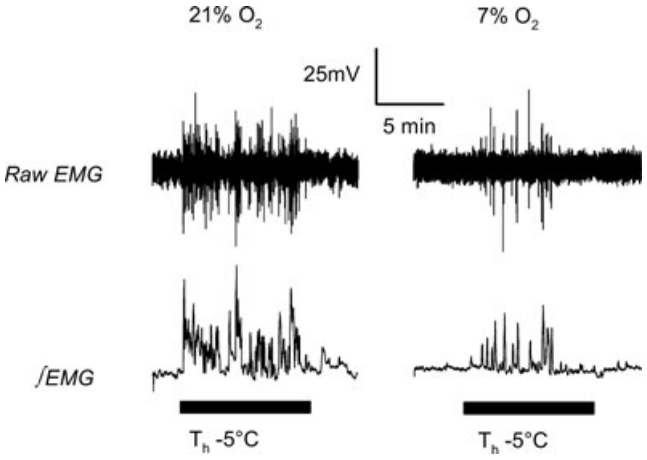

Figure 5
Sample traces of raw EMG voltage (top) and integrated EMG voltage (∫EMG; bottom) during 10 min periods (black bars) of T_h manipulation 5°C below steady state values in normoxia (left) and 7% O_2 (right) in a Columbian ground squirrel.

Ventilatory responses during hypothalamic manipulations were more complex than metabolic and cardiovascular responses. Generally, hypothalamic cooling induced increases in V_{TI}; however, the proportionality constants were not affected by hypoxia (Table 3), except in GMGS where 7% O_2 attenuated

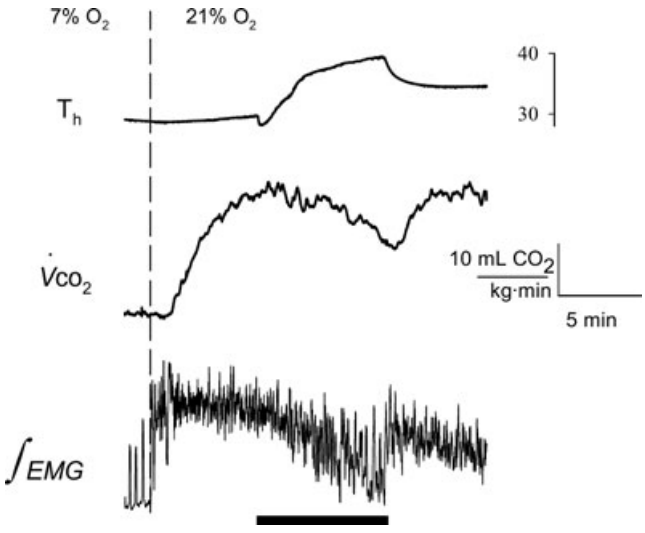

Figure 6. Sample trace of a Columbian ground squirrel re-warming following exposure to 7% O_2, demonstrating a rapid onset of shivering thermogenesis with normoxia
During re-warming, T_h (top trace) was transiently warmed to 40°C (black bar), which was the minimum temperature required to offset the post-hypoxic rise in metabolism ($\dot{V}_{CO_2}$, middle trace), which can be readily observed by the rapid, transient changes in neck muscle electromyography (bottom trace).

the influence of T_h cooling. f_R, on the other hand, only manifested a significant influence on the proportionality constant for T_h cooling in CGS at 7% O_2, where the change in f_R with T_h was significantly diminished compared to normoxia. $\dot{V}_E$ was affected by hypothalamic cooling, exhibiting proportionality constants different from zero in all cases (Fig. 9); however, oxygen did not significantly influence the proportionality constants in CGS. In GMGS, on the other hand, 7% O_2 significantly attenuated the ventilatory changes associated with T_h cooling (Table 3).

α and T_{th}

α values for metabolic heat production (calculated from $\dot{V}_{O_2}$ values) are reported in Table 4. It was evident that α was linearly related to the level of inspired oxygen in CGS (Fig. 10*A*; $r = 0.91$; $F_{1,20} = 91$; $P < 0.00001$), whereas T_{th} demonstrated a more non-linear response, with larger declines in T_{th} occurring at the lowest levels of oxygen (Table 4). However, the difference between T_{th} and steady state T_h ($T_{th} - \overline{T}_h$; where $\overline{T}_h$ is the T_h in the absence of thermal manipulation) decreased linearly with hypoxia, becoming statistically significant from normoxic values at 10 and 7% O_2 (Table 4, Fig. 10*B*; $r = 0.51$; $F_{1,20} = 7.1$; $P = 0.014$). Correlations between α and T_{th} were evident from slopes derived from Arrhenius plots of ln (α) *vs.* T_{th}^{-1} (Fig. 11), yielding estimates of the activation energy, which were subsequently converted into Q_{10} values. In CGS, the Q_{10} for the relationship between α and T_{th} was 12.0, and in GMGS the Q_{10} was 9.78.

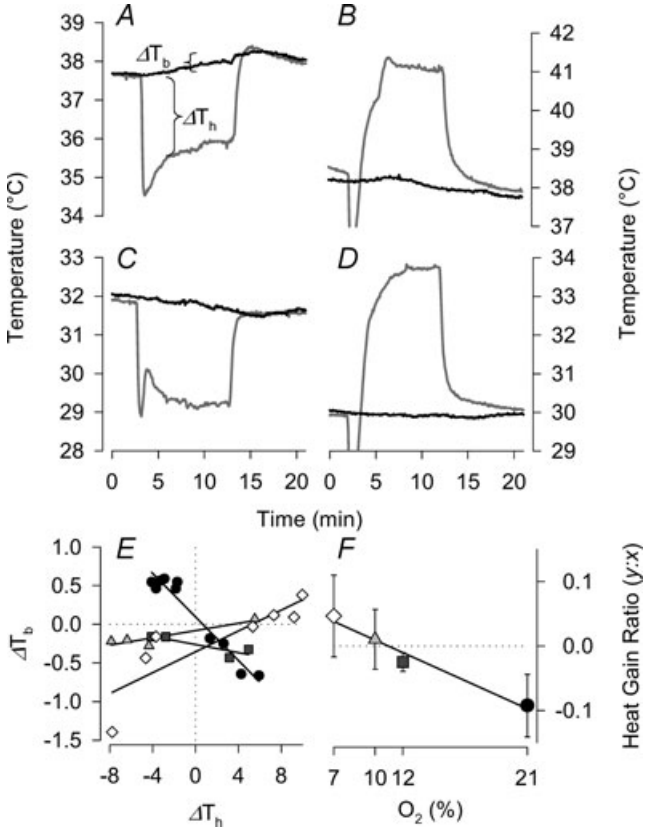

Figure 7. Change in T_h (ΔT_h is the difference between manipulated and unmanipulated T_h) over 10 min periods of manipulations induce changes in T_b ($\Delta T_b = T_b$ after manipulation $- T_b$ before manipulation) in Columbian ground squirrels

Sample traces of manipulations from normoxic (*A* and *B*) and hypoxic (7% O_2; *C* and *D*) ground squirrels during T_h cooling (*A* and *C*) and T_h warming (*B* and *D*). Sample results of multiple T_h manipulations from one squirrel are shown in *E*. Average slope (ratio of ΔT_b:ΔT_h) values from all Columbian ground squirrels combined are shown in *F*. In both *E* and *F*, the symbol fills refer to inspired oxygen level: 21% (black circles), 12% (dark grey squares), 10% (light grey triangles) and 7% O_2 (white circles).

Discussion

This study examined the properties of the CNS T_b regulator that change in hypoxia. Central regulation of T_b remains operational in hypoxia, however, low oxygen progressively reduces T_{th} as well as suppressing metabolic thermosensitivity. Combined, this suggests that the hypoxic thermoregulatory response results from a regulated decline in body temperature, similar to that observed during sleep states, torpor, and hibernation.

Steady state responses to hypoxia

Acute, but prolonged (i.e. > 2 h) exposure to hypoxia leads to major adjustments in mammalian cardiorespiratory, metabolic and thermoregulatory homeostasis (Powell *et al.* 1998; Mortola & Frappell, 2000). The results of the present study are no exception (Fig. 1). The overall response to hypoxia that occurred in these two ground squirrel species was: (1) a progressive reduction in T_b which is accompanied by a reduction in metabolic heat production, (2) a ventilatory stimulation (increased $\dot{V}_E$: $\dot{V}_{O_2}$), driven initially by an increase in heart rate, and later on by changes in tidal volume, and (3) a hypoxic tachycardia in mild hypoxia, that converted to a bradycardia as the metabolic requirements dropped in extreme hypoxia. The overall responses support the idea that hypoxia initiates a chemoreceptor-driven ventilatory stimulation and a decline in metabolic heat production accompanied by a fall in T_b. Ultimately, this cascade of events is geared toward the balance of oxygen supply and demand through changes in delivery (cardiorespiratory) and consumption (metabolic) pathways. This physiological response, often termed hypoxic-anapyrexia (Steiner & Branco, 2002; Steiner *et al.* 2002), was relatively stable throughout the experimental period, allowing for the manipulation of T_h. In order to ascertain whether the hypoxic decline in T_b resulted from a regulated decrease, it was necessary to

Table 4. Parameters (mean ± S.D.) related to metabolic heat production resulting from hypothalamic thermal manipulations in ground squirrels

	GMGS		CGS			
	21% O_2	7% O_2	21% O_2	12% O_2	10% O_2	7% O_2
α (W kg^{-1} °C^{-1})	−4.41 ± 1.14	−0.506 ± 0.110‡	−2.00 ± 0.50	−0.879 ± 0.31‡	−0.594 ± 0.204‡	−0.367 ± 0.182‡
T_{th} (°C)	37.9 ± 0.6	28.4 ± 0.8‡	38.1 ± 0.2	36.3 ± 0.5‡	34.9 ± 0.5‡	31.3 ± 0.3‡
$T_{th} - \overline{T}_h$	−0.080 ± 0.56	−2.38 ± 0.893‡	0.070 ± 0.38	−0.38 ± 0.52	−0.50 ± 0.71†	−0.78 ± 0.68‡

†Significantly different from normoxic values ($P < 0.01$). ‡Significantly different from normoxic values ($P < 0.001$).

manipulate T_h under these steady state conditions and observe whether concomitant changes in physiological responses reflected attempts to defend homeostatic states.

Hypothalamic control of physiological responses in hypoxia

The most profound results from the hypothalamic manipulation trials were manifested in the metabolic changes. Cooling the hypothalamus led to activation of thermogenic mechanisms proportional to the degree of cooling. This has been observed in prior work on a range of mammals from mice, rats, cats, dogs and seals (Simon *et al.* 1986). The proportionality of the central mechanisms for heat production are thought to reflect the presence of cold-responsive neurons (or, more specifically, from inhibitory warm-sensitive neurons) within the CNS regulator, whose output is coupled to thermogenic activation in the periphery (Bligh, 1998). The metabolic thermosensitivity is reduced, but not fully eliminated by hypoxic exposure; α values decrease 5.4- and 8.7-fold, respectively in CGS and GMGS comparing 21 to 7% O_2. Shivering can still be recruited in hypoxia by hypothalamic cooling, albeit at reduced levels (Fig. 5). This alone demonstrates that the hypoxic decline in T_b is supported by thermogenic mechanisms acting with reduced amplitude (Barros *et al.* 2001). For the most part, the proportionality constants for cooling in other physiological parameters (e.g. f_H, f_R) also exhibited similar declines in hypoxia. We did not record major physiological changes that reflected regulatory responses to hypothalamic warming (in normoxia or hypoxia); ideally, however, these experiments need to be conducted in a species that exhibits profound thermolytic responses to hypothalamic heating (Parmeggiani *et al.* 1973) to resolve this question. An important caveat from this study, however, is that other thermoeffectors may not

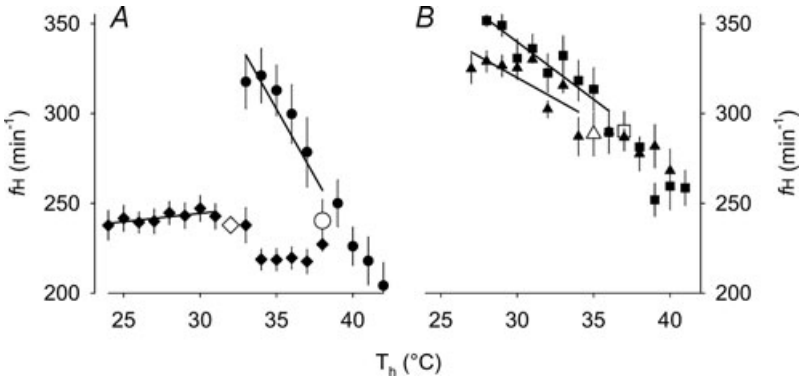

Figure 8. Summary (mean ± S.E.M.) cardiovascular values from the T_h manipulation experiments in Columbian ground squirrels
f_H is shown in *A* (21 and 7% O_2) and *B* (12 and 10% O_2). Open symbols represent the steady state, non-manipulated values, whereas filled symbols represent values measured during the 10 min T_h manipulation trials. Circles refer to 21%, squares refer to 12%, triangles refer to 10% and diamonds refer to 7% inspired O_2. Regression lines are plotted to demonstrate the degree of proportionality resulting from hypothalamic cooling.

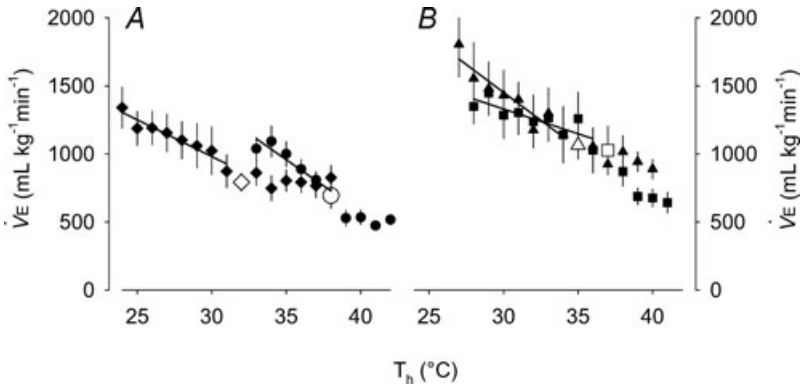

Figure 9. Summary (mean ± S.E.M.) minute ventilation ($\dot{V}_E$) values from the T_h manipulation experiments in Columbian ground squirrels
$\dot{V}_E$ is shown in *A* (21 and 7% O_2) and *B* (12 and 10% O_2), for visual clarity. Open symbols represent the steady state, non-manipulated values, whereas filled symbols represent values measured during the 10 min T_h manipulation trials. Circles refer to 21%, squares refer to 12%, triangles refer to 10% and diamonds refer to 7% inspired O_2. Regression lines are plotted to demonstrate the degree of proportionality resulting from hypothalamic cooling.

exhibit similar thresholds for activation in hypoxia, since recent evidence points toward multiple, independent temperature sensors and pathways within the brain responsible for activating heat loss, heat production and temperature-seeking behaviour (Nagashima *et al.* 2000).

Hypoxic thermoregulatory threshold and thermosensitivity

The ultimate goal of this study was to shed light on whether hypoxia induces a decrease in hypothalamic 'set-point' for T_b regulation, as hypothesised by Wood (1991) . The term 'set-point', however, has produced much debate, confusion and controversy, and recently inspired abandonment (Romanovsky, 2004; Cabanac, 2006). Part of the debate stems from different uses of the term (Cabanac, 2006), as well as from an expectation that a stable 'reference' neuron must exist that maintains a constant firing rate across a range of brain temperatures. Hammel's early neuron network model (Hammel, 1968, 1972), however, did not require constancy in a reference neuron, but simply a network of interacting neurons with differing thermal sensitivities (Bligh, 1998; Cabanac, 2006). In fact, simple changes in the pattern of neuronal firing (see Hori *et al.* 1987, 1988; Koga *et al.* 1987) would help explain how the hypothalamic threshold actually varies; changes in physiological state, such as fever, exercise, sleep and hibernation, may alter the inherent thermosensitivities of the neurons within the network, and thereby alter the balance point (Romanovsky, 2004, 2007) that represents the regulated T_b. We have used the term T_{th} similarly to Heller & Hammel (1972), as a threshold temperature that activates/deactivates the intrinsic thermosensitivity of the central T_b regulator.

Indeed, one of the major results from the current study is that the T_{th} is reset progressively to lower levels by hypoxic exposure. The notion that T_b is down-regulated has been a prevailing hypothesis in the literature on hypoxic anapyrexia (Kozak, 1997; Barros *et al.* 2001; Tattersall & Milsom, 2003). However, to date, the most compelling evidence for this assertion has come from work done on ectotherms, which actively select lower environmental temperatures in hypoxia (Dupré & Wood, 1988; Wood & Malvin, 1993; Tattersall & Boutilier, 1997; Bicego *et al.* 2006; Cadena & Tattersall, 2009). An active preference for lower T_b requires neurophysiological coordination, and thus strongly implies that the central thermoregulatory mechanisms are reset, since effectors for T_b control include behavioural as well as physiological mechanisms. Lowered temperature preferences have also been observed in

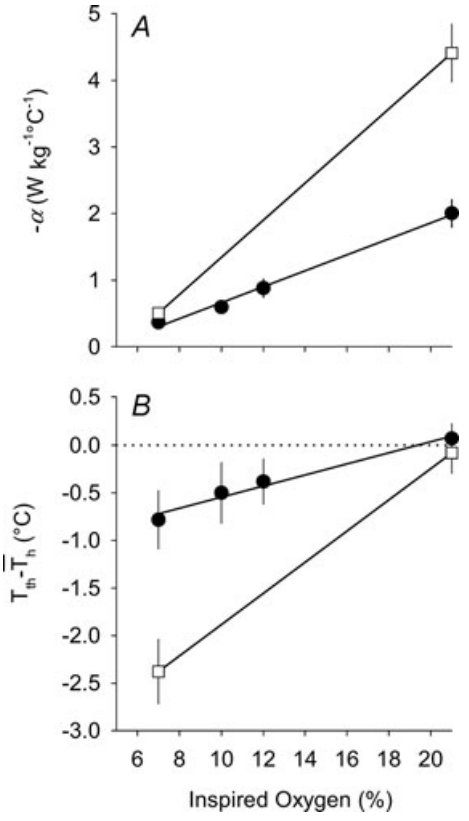

Figure 10. Mean (± S.E.M.) values for *A*, hypothalamic thermosensitivity (α) and *B*, $T_{th} - \overline{T}_h$ (difference between T_{th} and steady state T_h; $\overline{T}_h$) during normoxic and hypoxic exposure in Columbian ground squirrels (filled circles) and golden-mantled ground squirrels (open squares)
α in CGS was strongly correlated ($r = 0.91$; $F_{1,20} = 91$; $P < 0.00001$) with inspired oxygen, whereas $T_{th} - \overline{T}_h$ demonstrated a weaker, albeit significant correlation ($r = 0.51$; $F_{1,20} = 7.1$; $P = 0.014$).

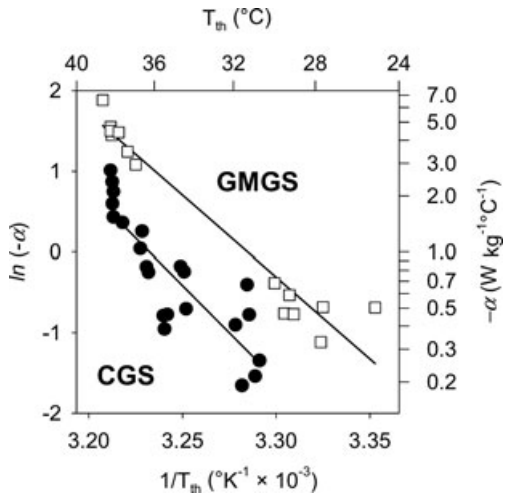

Figure 11. Correlation of hypothalamic thermosensitivity (α) and the threshold hypothalamic temperature (T_{th}) from individual animals required to elicit a rise in metabolic heat production in Columbian ground squirrels (CGS, filled circles) and golden-mantled ground squirrels (GMGS, open squares)
Arrhenius plots revealed Q_{10} values of 12 for CGS and 10 for GMGS, much higher than that predicted from simple temperature effects (Q_{10} 2–3).

endotherms (Gordon & Fogelson, 1991; Wood, 1995; Gordon, 1997) as well as a wide range of animals (Wood & Gonzales, 1996), suggesting an adaptive value to lowering T_b during hypoxic stress. Therefore, if hypoxia down-regulated T_b, then it should follow that correlates of energy metabolism, thermoregulation and heat loss would operate in a coordinated fashion to facilitate this drop in T_b. Further indirect evidence for hypoxia eliciting apparent declines in regulated T_b are found in cats and rabbits where artificially raising T_b back to normothermic temperatures elicits a heat stress or relative hyperthermic responses (Rohlicek *et al.* 1996; Seifert *et al.* 2006). Furthermore, changes to the thermosensitivity of hypothalamic neurons (both warm- and cold-responsive neurons) do occur (Koga *et al.* 1987; Tamaki & Nakayama, 1987) in hypoxia, suggesting that the hypoxic effects on α may be a direct effect on neuronal activity in the hypothalamus, as has been implied for osmotic and cardiovascular influences on T_b (Hori *et al.* 1987, 1988).

It is also possible, however, that the decline in α in hypoxia is a passive consequence of the drop in T_b itself, due to inherent thermal effects on neuronal firing rates (Heller & Colliver, 1974; Heller *et al.* 1974). We can address this by examining the temperature sensitivity for α; the relationship between α and T_{th} yielded Q_{10} values ranging from 10 to 12, substantially higher than that expected from simple temperature effects, as well as higher than that observed in hibernation (Florant & Heller, 1977; Florant *et al.* 1978), where T_{th} has been well established to be co-ordinately lowered. Indeed, the likely explanation is that the central thermosensitivity changes in hypoxia result from a direct suppressive effect on thermosensitive neuronal activity (or an alteration in the balance of excitatory and inhibitory inputs), rather than α values passively following T_b. One consequence of a suppression of neuronal activity is that the hypoxic decline in T_b produces a more variable thermoregulatory pattern due to the reduced gain or sensitivity of the CNS T_b regulator. Precedence for this is observed in ectothermic studies showing a decreased precision of regulated T_b in hypoxia (Cadena & Tattersall, 2009), as well as studies in mammals that demonstrate that the hypoxic decline in T_b is more variable and also strongly dependent on ambient temperature (Barros *et al.* 2001; Bishop *et al.* 2001; Levesque & Tattersall, 2009). Indeed, one interesting result from the present study is the remarkable similarity between the changes in α and T_{th} and those observed during different sleep states in rodents. During slow wave sleep (SWS), α and T_{th} are partially reduced, whereas during REM sleep, T_{th} and α are entirely absent (Glotzbach & Heller, 1975, 1976; Sakaguchi *et al.* 1979), which has led to the notion that REM sleep is essentially a period where thermoregulatory defences are abandoned. Hypoxia appears to produce a qualitatively similar state to SWS; coincidentally, hypoxia also produces alterations in EEG patterns, which show a relative increase in slow wave activity. These similar patterns suggest parallel mechanisms between SWS and hypoxia, although whether hypoxia influences the hypothalamic regulator via similar processes is unknown.

Under resting, normoxic conditions, feedback from extrahypothalamic warming would appear to be minor (Heller *et al.* 1974) since T_b continues to rise linearly during T_h cooling without abatement. Indeed, ground squirrels exhibit little inhibitory extra-hypothalamic feedback during euthermia and hibernation (Heller & Hammel, 1972). In hypoxia, the relative amount of extrahypothalamic sensitivity (assessed by the heat gain ratio) trended toward zero, since cooling T_h, which augments heat production, does not evoke the same rise in T_b as it did in normoxia. This may actually be the result of a sustained peripheral vasodilatation in hypoxia (Tattersall & Milsom, 2003), allowing the centrally derived heat to be driven from the animals, or from the involvement of peripheral chemoreceptor activation acting via medullary pathways producing a sustained reduction in sympathetic nervous activation of thermogenesis (Madden & Morrison, 2005). Combined with the low hypothalamic thermosensitivity, this may explain why hypoxia also produces an ambient-temperature-dependent T_b in addition to lowering T_{th} (Barros *et al.* 2001). Similarly, we observed that $T_{th} - \overline{T}_h$ was directly related to oxygen level, indicating that the threshold for heat activation is actually below the apparent steady state $\overline{T}_h$ value in hypoxia. Thus, despite an achievement of steady state physiological responses to hypoxia, T_{th} drops below and in advance of T_h, similar to what is observed during entrance into hibernation (Heller *et al.* 1977). Furthermore, we also observed instances in hypoxic squirrels where T_h did not return to previous values after completion of T_h manipulation (Fig. 7), reinforcing that the threshold to activate thermogenesis in hypoxia is very often well below that of T_h.

Interestingly, upon initial exposure to hypoxia, $T_h - T_b$, which is normally positive, decreases, implying that the stimulus to regulate an elevated T_h is diminished. After 2 h of exposure to hypoxia, however, this difference begins to rise, driven by a stabilisation of T_h (Fig. 2). This suggests that T_h is being regulated in hypoxia; upon re-oxygenation, the $T_h - T_b$ difference rapidly rises, for, perhaps two reasons. Firstly, T_{th} for thermogenesis would instantly return to normal values (as witnessed by the near-instantaneous activation of shivering upon returning oxygen levels to 21%; Fig. 6), and given the small size of the brain relative to its high blood flow, maximal thermogenesis would raise T_h prior to T_b. It is also possible that the greater difference between T_h and T_b at this point is due to selective brain warming, which is consistent with how many hibernators re-warm

from torpor; an elevated thoracic and head temperature is achieved prior to re-warming the rest of the body, achieved via sympathetically mediated constriction in the periphery (Osborne *et al.* 2005).

Universality of the hypoxic thermoregulatory response?

Although the present study demonstrated a robust decline in T_b, interestingly, acute hypoxia does not exert profound changes in T_b or $\dot{V}_{O_2}$ in larger mammals (>2 kg; Frappell *et al.* 1992), which may be due to a size-limited inability to cool significantly, akin to the size constraints of deep hibernation observed in small mammals (Geiser, 1998). Given, however, that the lowering of T_b is a nearly ubiquitous response to hypoxia in the animal kingdom, large mammals may still exhibit the appropriate control mechanisms for reducing T_b in hypoxia, but are simply incapable of doing so due to size restrictions. Equally, however, the slight decline in T_b that is observed in large mammals in hypoxia may be due to scaling influences on hypothalamic thermosensitivity. Mammalian hypothalamic thermosensitivity scales negatively (exponent $= -0.37$) with body mass (Heller, 1978), suggesting a diminishing importance of central neuronal thermosensitivity in maintaining T_b in large animals. If hypoxia reduces primarily central thermosensitivity, with minimal effects on peripheral thermosensation, this may explain the profound reduction in T_b of small mammals. Further examination of central and peripheral thermosensitivities in hypoxic states is warranted to test this hypothesis.

Conclusions and perspectives

The present study illustrates that informative features of how the CNS T_b regulator operates can be elucidated from simple changes in inspired oxygen. This approach has shed light on a fundamental homeostatic mechanism (temperature regulation), demonstrating that a change in oxygen, which could be manifested in a number of ways (e.g. alteration in brain blood flow, airway occlusion/asphyxia, sleep apnoea, as well as naturally hypoxic states), produces a rheostatic adjustment in homeostasis (Mrosovsky, 1990). Indeed, T_b regulation is lowered from ∼38°C in normoxia to ∼30°C in hypoxia. Central thermoregulatory control, however, is still intact in hypoxia, although the thresholds for changes in metabolism and shivering are reduced in magnitude. The degree of central thermosensitivity is drastically reduced, in a manner similar, but more profound, to that observed during SWS. Thus, although hypoxia produces a decrease in the regulated T_b, this begs the question about whether the term 'hypoxia-induced anapyrexia' (Steiner & Branco, 2002; Steiner *et al.* 2002) is entirely appropriate to apply to a state that reduces both the regulated temperature and the sensitivity (α) of the regulator itself. Equally, however, hypoxia does not produce a state where thermoregulatory responses are completely abandoned, arguing against 'hypothermia' being an appropriate description of the hypoxic thermoregulatory response.

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Conception and design: G.J.T. and W.K.M. Data collection: G.J.T. Analysis and interpretation: G.J.T. Article drafting, revisions and final approval: G.J.T. and W.K.M.

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