Ventilatory and metabolic responses to hypoxia in the smallest simian primate, the pygmy marmoset

GLENN J. TATTERSALL, JAMES L. BLANK, AND STEPHEN C. WOOD

Department of Biological Sciences, Kent State University, Kent, Ohio 44242

Received 24 May 2001; accepted in final form 17 September 2001

Tattersall, Glenn J., James L. Blank, and Stephen C. Wood. Ventilatory and metabolic responses to hypoxia in the smallest simian primate, the pygmy marmoset. J Appl Physiol 92: 202–210, 2002; 10.1152/japplphysiol.00500.2001.—The pygmy marmoset (Cebuella pygmaea) is the smallest New World Monkey (average body mass of 120–130 g). As such, it faces possible challenges to thermoregulation. Small mammals (e.g., rats) are well known to lower body temperature and metabolism in response to hypoxia; however, small primates have not been studied in this respect nor have, in general, the interactions between metabolism and ventilation. Because little is known about these responses in small primates, it seemed of great interest to assess the hypoxia-induced metabolic depression and drop in body temperature and the associated ventilatory requirements in this species under hypoxic conditions. Exposure to graded hypoxia (30 min at each of 18, 16, 14, 12, and 10% O2) caused body temperature to drop from the normoxic value of 39 to 37°C. This was accompanied by a marked metabolic depression (O2 consumption was ~68% of the normoxic value, implying a suppression of metabolism greater than that predicted from a typical value of the effect of 10°C change on metabolism of 2–3 times). Minute ventilation declined in parallel to metabolism, maintaining a constant air-convection requirement during hypoxia; thus this species did not show the typical mammalian hyperventilation. Acute exposure to 10% O2 led to a similar overall decline in metabolism and body temperature and qualitative differences in the timing of these changes. The pygmy marmoset shares some similarities in its hypoxic metabolic response with other mammals of similar size yet appears to be unique in its much diminished ventilatory response to hypoxia.

body temperature; thermoregulation; hypoxic ventilatory response; hypothermia; metabolic depression

MANY ANIMALS RESPOND TO HYPOXIA by reducing body temperature (Tb) and metabolic rate (46). Since the 1950s, this was known to occur in a broad range of small mammals (18) and in neonates of larger mammals, including humans (10, 25). This hypoxia-induced drop in Tb and metabolic depression serves a protective role by reducing O2 demand, eliminating costly thermogenesis, improving blood O2 affinity, and reducing the costs of ventilation (14, 27, 36, 38, 47, 48). Furthermore, numerous studies support the conclusion that hypoxia represses the hypothalamic thermoregulatory set point to a lower level (9, 15–17, 34), suggesting that this is a regulated process.

Metabolism and thermoregulation affect ventilatory control (13, 30). Pulmonary ventilation is controlled so that O2 delivery matches metabolic rate under varying states of metabolic demand (24). Thus breathing during hypoxic exposure is complicated by reduced metabolism and Tb in small mammals. Lowered Tb and metabolic rate decrease the metabolic drive to breathe (13), whereas hypoxia serves to increase the chemical drive to breathe. These drives for breathing conflict with one another during hypoxia, such that pulmonary ventilation is the net result of both drives and is best expressed as the air convection requirement [minute ventilation (V˙E)/O2 consumption (V˙O2)]. The response of most adult mammals is to hyperventilate (i.e., increase V˙E/V˙O2) during hypoxia, whether through an increased V˙E (hyperpnea), a decreased V˙O2 (metabolic depression), or both (12). Newborn mammals, on the other hand, tend to exhibit stronger decreases in V˙O2 while keeping V˙E constant or even slightly lower, however, which still results in a relative hyperventilation (28, 33). This hypoxic response is most important to newborn mammals, because this is a period during which hypoxic episodes can often occur when the thermoregulatory and respiratory control systems are still developing.

A large amount of work has been done on rodent models, with little certainty about how effectively these results can be extrapolated to other groups of mammals, particularly primates. Rodents, for the most part, have quite reduced chemosensitivities compared with primates and other mammal groups (5, 24). Their fossorial nature bestows them with a remarkable tolerance to hypoxia (49) and a reduced sensitivity to hypercapnic stimulation of breathing compared with many other mammals (5). Marmosets (family Callitrichidae), on the other hand, are small-bodied primates. They are heterothermic mammals (Tb fluctuates by 4°C daily) that inhabit lowland forests of the Amazon (40). Although never likely to encounter ambient hypoxia in the wild, marmosets provide a valuable comparison to rats due to their similar size and their status as a primate. The pygmy marmoset, cho-

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sen for this study, is the smallest simian primate (120–130 g) and, as such, serves as an interesting species in which to examine physiological and thermoregulatory function at the extremes of primate body size.

This study tested the following hypotheses: 1) that pygmy marmosets would exhibit the "typical" hypoxia-induced fall in metabolic depression and fall in \( T_b \), 2) that this lower \( T_b \) will be achieved in a coordinated fashion, where metabolic rate and heat loss act in concert, and 3) that the pygmy marmoset will exhibit a ventilatory response to hypoxia that is appropriate to the fall in metabolism and \( T_b \). Two experimental approaches were taken in this study: the first involved exposure to graded hypoxia at six different levels of \( O_2 \), and the second approach assessed the acute response to hypoxia (10% \( O_2 \)) and the subsequent recovery. The graded hypoxic exposure was used to assess the sensitivity of the marmoset to hypoxia. The subsequent acute exposure to hypoxia allowed for comparisons to studies performed on other mammals, as well as the examination of the hypoxic recovery mechanisms. We were also interested in examining the kinetics or temporal changes that occur during hypoxic exposure as a means of understanding the timing of the various coping mechanisms that marmosets may use during hypoxia.

**MATERIALS AND METHODS**

Animals

Pygmy marmosets (\( n = 10 \); average body mass of 156 g) belonged to a captive-bred colony residing at Kent State University. An equal number of males and females was used in this study. They were fed a mixture of marmoset chow (Zu-Preem) and various fruits ad libitum. Under normal housing conditions, animals are usually kept in same-sex cages and are removed from their cages for short periods of experimentation. Within their home cages, small nest boxes (≈2- to 4-liter plastic containers) are placed, into which more than one marmoset will enter to sleep at night. Animals are maintained on a 12:12-h light-dark schedule, with the ambient temperature (\( T_a \)) kept within thermoneutrality at 28°C and the relative humidity maintained between 40 and 60%. During experimentation, marmosets were transferred to a nest-box-like respirometer held inside a constant-temperature environmental chamber set to regulate temperature at 28 ± 0.05°C and equipped with a television camera. All experimental procedures were approved by the Kent State University’s Institutional Animal Care and Use Committee.

Ventilation Measurements

Ventilation in marmosets was determined using the barometric method (3, 11, 29) as adapted for flow-through respirometers by Jacky (22) and recently validated in rats by Seifert et al. (39). This method was chosen over the closed-chamber barometric method, because it facilitated long-term measurements on unrestrained, undisturbed animals and was found to be preferable in the marmosets, because they were observed to alter breathing patterns when gas flow was briefly stopped. Pressure inside the respirometer was measured with a Validyne differential pressure transducer (model DP45–16), sampled at 50 Hz, recorded on a data-acquisition system (Sable Systems), and later converted to ventilatory measurements. One-minute readings of pressure traces, representing 40–120 breaths, were recorded every 5 min for the duration of all experiments. All data were analyzed with a custom-written Excel spreadsheet, and tidal volume (\( V_t \)) was determined from the pressure trace deflections using the equation in Bartlett and Tenney (3). Additionally, an empirically determined adiabatic correction factor (1.3) was applied to the pressure deflection, as in Walker et al. (43), to account for temperature and pressure differences due to the rapid expansion of the calibration gas into the respirometer. \( T_a \), respirometer temperature (\( T_a \) measured with a thermocouple wire inserted through the lid of the respirometer), and relative humidity (determined with a Vaisala humidity probe placed in series with the excurrent gas) were also measured continuously and utilized in the estimation of \( V_t \). In the interest of minimizing distress and instrumentation of animals, \( V_t \) estimation from \( T_a \) was chosen over a nasal temperature measurement (23), which has recently been shown to overestimate \( V_t \) in certain circumstances (R. Stephenson, personal communication). Considerable efforts were made to validate this method before embarking on this study. First of all, we verified that \( V_t \) values from rats inside the same respirometers were comparable to literature values. Second, we assessed ventilation with the closed respirometer barometric method and found that the ventilatory response to hypoxia was similar to the response with the open, flow-through respirometers. Overnight recordings of ventilation with this method revealed that the air-convection requirement was maintained at constant values by keeping \( V_t \) constant, despite large fluctuations in breathing frequency (\( f \)) between sleep and wake (40–120 breaths/min), indicating that, at the same respiratory drive but different \( f \), \( V_t \) is virtually constant.

Ventilatory parameters measured included \( V_t \), inspiratory time (\( T_i \)), expiratory time (\( T_e \)), total breath duration (\( T_{breath} = T_i + T_e \)), \( f (f = 1/T_{breath}) \), \( V_e (V_e = V_t \times f) \), and mean inspiratory flow rate (\( V_t/T_i \)). All ventilatory volume measurements were expressed as BTPS.

Calibrations were performed at the beginning of an experiment using an artificial ventilator (Harvard Apparatus rodent ventilator model 683) set to deliver 5 ml of air at 90 breaths/min. The calibration was not found to alter >5% among animals or across the entire 6 mo of study and thus was a source of little error. Pilot studies showed that the flow-through respirometer attached to a similarly sized chamber through small aperture tubing produced a pressure leak with a time constant of ~3 s. This pressure leak did not significantly affect the \( V_t \) estimates, because, when verified with the ventilator, there was no attenuation of the calibration pressure signal between 30 and 180 breaths/min. The pressure head (20 mmHg) produced inside the chamber by the high-resistance tubing did not significantly affect calibration or marmoset \( V_t \) values.

Metabolic Rate Determination

The respirometer for measuring metabolic rate and ventilatory parameters was constructed from noncompressible, 3/8-in. Plexiglas (total volume, 4.5 liters). This size allowed for small changes in \( O_2 \) and \( CO_2 \) (0.1–0.5%) at a gas flow rate of 1,500 ml/min without confusing the marmosets unnecessarily. Gases were mixed using a gas-mixing flowmeter (model GF-3/MP, Cameron Instruments). Water vapor was completely eliminated using a Drierite column placed before the subsampled gas entering the \( CO_2 \) and \( O_2 \) analyzers.

J Appl Physiol • VOL 92 • JANUARY 2002 • www.jap.org
(Li-Cor and Sable Systems, respectively). CO₂ production (VCO₂) was calculated using the following equation

\[ V_{CO2} = \Delta CO2 \times Q/M_b \]

where \( \Delta CO2 \) is the difference between incident and excurrent percent CO₂, \( Q \) is the gas flow rate (1,500 ml/min) of gas through the respirometer, and \( M_b \) is marmoset body mass (kg). \( V_{O2} \) was calculated using Eq. 3b from Withers (45), taking into account the change in gas flow rate introduced from VCO₂. Both VCO₂ and VO₂ are expressed as milliliters per kilogram per minute, corrected to STPD. The respiratory exchange ratio (RER) was calculated as the ratio of VCO₂ to VO₂. This was determined empirically and the rate of change of the CO₂ and O₂ signals. Trial experiments of switching from 21 to 10% O₂ by Boutilier and Shelton (7), compensating for the washout effect of the respirometer using a time constant (~3 min) determined empirically and the rate of change of the CO₂ and O₂ signals. Trial experiments of switching from 21 to 10% O₂ without an animal in the respirometer resulted in a near-perfect transformation of the O₂ trace into a square-wave signal with this correction.

Before data were collected, marmosets were placed in the respirometer on numerous occasions to acclimatize them to the experimental conditions. VO₂, VCO₂, respirometer chamber temperature (Tc), flow rate, and chamber relative humidity were averaged and collected via the data-acquisition system every 0.5 min. Although the respirometer was housed in a temperature-controlled environmental chamber (set to 28.0 ± 0.05°C), when marmosets were placed inside the respirometer and gas flow was initiated, a small deflection in chamber temperature (0.3–0.8°C) was detectable. Thus we have recorded this deflection (ΔTc = Tc with marmoset − Tc without marmoset), which roughly approximates the heat lost by the marmoset to its environment. In long-term, overnight studies, this parameter undergoes a dramatic drop, coinciding with the daily changes in metabolic rate and Tb, and thus to some extent provides us with a useful approximation of heat lost to the respirometer by the animal (personal observations).

Tb Measurements

One month before any measurements were taken, batteryless telemeters (PDT-4000 E-Mitters, Mini-Mitter) were implanted in animals under halothane anesthesia (2%). Animals were first injected with 0.2 mg Butorphanol to serve as a muscle relaxant and analgesic. A midline incision was made along the abdomen large enough to allow the insertion of the 1-g telemeters, which had been sterilized with ethylene oxide. All surgeries were performed under sterile conditions, and care was taken to ensure that anesthesia was maintained and that suffering was minimal. No marmosets developed fevers after the surgeries, nor were any complications encountered in the months after implantations. Tb was measured every 0.5 min using Vitalview software from Mini-mitter.

Experimental Protocol

Graded hypoxia. To first determine the relative sensitivity of marmosets to reduced O₂, a graded exposure protocol was used. At 11:00 AM on the day of an experiment, the marmoset was coaxed to enter the respirometer, which was subsequently placed within the temperature-controlled environmental chamber. The lights inside the environmental chamber were kept at low levels, and no food was provided to the marmosets during the experiment. After 1 h of equilibration to the surroundings, the experiment was commenced. Animals were exposed to 30 min each of the following gas mixtures: 20.9, 18, 16, 14, 12, and 10% O₂. Throughout this period, Tb, Ta, CO₂, and ventilatory parameters were monitored and averaged over 5-min periods. Parallel measurements (not shown) in normoxia demonstrated that all of the above parameters did not significantly change over a 3-h period.

Acute hypoxia. Approximately 2 mo after the graded hypoxia exposures, the same animals were examined for their response to an acute change in inspired O₂ and their subsequent recovery. At 11:00 AM on the day of an experiment, the marmoset was taken from its home cage and allowed to enter the respirometer inside the environmental chamber. Lights were dimmed throughout the experiment, and food was not provided. One hour was allowed to elapse before the recording commenced to acclimatize the animal to its new surroundings. The marmosets were then exposed to 30 min of 20.9% O₂ (control period), 60 min of 10% O₂ (hypoxic period), and 60 min of 20.9% O₂ (recovery period).

Data Analysis

Unless otherwise stated, all data represent 5-min averages of data originally collected at 0.5-min intervals and are reported as means ± SE. (n = 10). All parameters were analyzed with repeated-measures ANOVA, and significant effects were tested using Tukey's post hoc comparisons. All results were deemed significantly different at a level of P < 0.05.

RESULTS

Graded Hypoxia

The overall summary of the effects of graded hypoxia is shown in Fig. 1 and Table 1. Marmosets exhibited a modest but significant decline in Tb, with Tb falling 1 and 2°C at 12 and 10% O₂, respectively (Figs. 1 and 2). However, no effect of graded hypoxia was observed on the heat loss estimate (ΔTb), which remained constant at all levels of O₂ (Fig. 1). VO₂ and VCO₂ were 25–30% lower at 12% O₂ and 30–35% lower at 10% O₂ compared with the normoxic values, whereas RER was not significantly affected by any level of hypoxia (Figs. 1 and 2; Table 1), indicating that VO₂ and VCO₂ changed in synchrony with hypoxia and that there was no significant hyperventilation.

VE was significantly lower than normoxic values at 12 and 10% O₂. This was accomplished exclusively by a lowering of respiratory frequency at these levels of hypoxia, as VT was constant at all levels of O₂. Both Ti and Tc increased significantly only at 12 and 10% O₂; however, the overall breathing pattern remained constant as VT/Tc did not change at any level of O₂ tested (Table 1). VT/Ti was also observed to decline at 12 and 10% O₂. As a consequence of the paralleled declines in VE and metabolic rate, the air convection requirement (VE/VO₂ or VE/VCO₂) remained constant at all levels of hypoxia.

The temporal changes in metabolic and ventilatory parameters to graded hypoxia revealed few trends, except that, in general, a new equilibrium is reached. By exposing marmosets to hypoxia in a graded fashion,
metabolic and ventilatory parameters adjust to lower levels more evenly without undergoing any apparent oscillations.

**Acute Hypoxia**

The overall effects of acute hypoxia are summarized in Fig. 3 and Table 2. After 60 min of exposure to 10% O₂, Tₜ fell significantly and recovered close to control values within 60 min of 20.9% O₂ exposure. In contrast to the graded hypoxic protocol, exposure to acute hypoxia resulted in a transient increase in the Dₜₐ during the first 15–25 min of hypoxia (Fig. 3). After this increase, Dₜₐ returned to control values and did not change during the recovery period. VᵪO₂ and VᵪCO₂ decreased.

Table 1. **Metabolic and respiratory parameters of pygmy marmosets exposed to graded hypoxia**

<table>
<thead>
<tr>
<th></th>
<th>20.9% O₂</th>
<th>18% O₂</th>
<th>16% O₂</th>
<th>14% O₂</th>
<th>12% O₂</th>
<th>10% O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>%V₀₂</td>
<td>100</td>
<td>91.9 ± 3.0</td>
<td>97.7 ± 5.7</td>
<td>86.1 ± 4.2</td>
<td>74.9 ± 2.8*</td>
<td>68.5 ± 5.5*</td>
</tr>
<tr>
<td>%V₀₂₀₂</td>
<td>100</td>
<td>90.6 ± 2.8</td>
<td>94.6 ± 4.5</td>
<td>82.4 ± 4.3</td>
<td>70.8 ± 2.7*</td>
<td>64.6 ± 5.6*</td>
</tr>
<tr>
<td>Vₜ, ml</td>
<td>1.57 ± 0.10</td>
<td>1.67 ± 0.11</td>
<td>1.60 ± 0.13</td>
<td>1.55 ± 0.09</td>
<td>1.43 ± 0.08</td>
<td>1.49 ± 0.12</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>86.1 ± 4.9</td>
<td>84.7 ± 6.5</td>
<td>84.1 ± 3.8</td>
<td>75.4 ± 6.3</td>
<td>64.8 ± 3.6*</td>
<td>61.4 ± 3.1*</td>
</tr>
<tr>
<td>Tᵢ, s</td>
<td>0.29 ± 0.02</td>
<td>0.30 ± 0.01</td>
<td>0.30 ± 0.01</td>
<td>0.34 ± 0.02</td>
<td>0.39 ± 0.02*</td>
<td>0.37 ± 0.03*</td>
</tr>
<tr>
<td>Tₑ, s</td>
<td>0.46 ± 0.05</td>
<td>0.48 ± 0.06</td>
<td>0.47 ± 0.04</td>
<td>0.54 ± 0.05</td>
<td>0.58 ± 0.04*</td>
<td>0.59 ± 0.06*</td>
</tr>
<tr>
<td>Tᵢ/Tₑ</td>
<td>0.75 ± 0.06</td>
<td>0.78 ± 0.07</td>
<td>0.77 ± 0.07</td>
<td>0.88 ± 0.07</td>
<td>0.98 ± 0.06*</td>
<td>0.96 ± 0.08*</td>
</tr>
<tr>
<td>Vₑ/Vᵪ₀₂</td>
<td>5.70 ± 0.54</td>
<td>5.94 ± 0.51</td>
<td>5.70 ± 0.55</td>
<td>4.98 ± 0.46</td>
<td>3.98 ± 0.43*</td>
<td>4.41 ± 0.61*</td>
</tr>
<tr>
<td>Vₑ/Vₑ₀₂</td>
<td>47.0 ± 3.7</td>
<td>54.5 ± 4.5</td>
<td>50.7 ± 5.1</td>
<td>49.6 ± 3.7</td>
<td>46.8 ± 4.4</td>
<td>54.0 ± 5.4</td>
</tr>
</tbody>
</table>

Values represent the 30th minute mean ± SE. V₀₂, O₂ consumption (percentage of normoxic value); V₀₂₀₂, CO₂ production (percentage of normoxic value); Vₜ, tidal volume; f, breathing frequency; Tᵢ, inspiratory time; Tₑ, expiratory time; Tᵢ/Tₑ, total breath duration; Tᵢ/Tᵢᵣ, duty cycle; Vₑ/Tᵢ, inspiratory flow; Vₑ, minute ventilation; Vₑ/Vₑ₀₂, air-convection requirement. *P < 0.05 vs. normoxia.
V̇E/V̇O₂ and V̇E/V̇CO₂ tended toward higher values during acute hypoxic exposure during recovery. The constant V̇T remained virtually unchanged throughout most of the hypoxic exposure, falling to ~70% of normoxic values after 20–25 min of hypoxic exposure (Fig. 3). However, there was a significant increase in RER during the initial 10–20 min of hypoxic exposure (Fig. 3). Nevertheless, once metabolic rates had stabilized, RER returned to normoxic levels. During recovery from hypoxia, both V̇O₂ and V̇CO₂ remained low for the first 5 min and then rose rapidly back to control levels within 10 min of normoxic exposure. RER underwent a transient decrease at the beginning of the recovery period in normoxia but was otherwise unaffected.

Vf underwent a similar decline to V̇O₂ during acute hypoxic exposure, falling to ~70% of normoxic values (Fig. 3; Table 2). Similar to graded hypoxic exposure, this was accomplished exclusively through a decline in f, as V̇T remained constant throughout hypoxic exposure (Table 2). This decline in f occurred through significant increases in both Tl and T_e, with no significant effect of hypoxia on Tl/T_t (Table 2). The constant V̇T and increased Tl during hypoxia translated into a decrease in V̇f/Tl during 10% O₂ exposure (Table 2). Both V̇e/V̇O₂ and V̇e/V̇CO₂ tended toward higher values during acute hypoxia, although neither was significant, implying that the air convection requirement remained virtually unchanged throughout most of the hypoxic exposure (Figs. 3 and 4; Table 2). All ventilatory parameters returned to control values within 10 min of normoxic exposure during recovery.

**DISCUSSION**

One of the challenges of working with small mammals during their normally active phase is an increased variability in the data. Most of the comparative work on the hypoxic decline in T_b and metabolic depression has been done with rodents, performed during their normal inactive period (i.e., during the day), whereas marmosets and other primates are diurnal. There are arguable advantages to studying the physiology of awake vs. sleeping animals, despite the increased variability. We found that awake pygmy marmosets control T_b and metabolism much like any other small mammal when encountering hypoxia. The pygmy marmoset exhibited a profound metabolic depression and a moderate decline in T_b, as expected. However, exceptionally for an adult mammal, the pygmy marmoset displayed no or little trend to hyperventilation in hypoxia even when accounting for the fall in metabolism.

**Thermoregulatory and Metabolic Responses to Hypoxia**

Both graded and acute hypoxia (<12% O₂) produced a profound and reversible metabolic suppression (32–40% below normoxic) that was accompanied by a drop in T_b. T_b fell by ~2°C, which is comparable to T_b changes observed in other small mammals, including primates over similar time courses (12, 19). Numerous previous studies suggest a drop in the thermoregulatory set point in hypoxia (2, 8, 9, 14, 16, 31, 36) rather than a substrate limitation-induced hypothermia. In fact, the pygmy marmoset demonstrated an almost dose response to hypoxia, with a greater drop in T_b occurring at lower O₂ levels and small drops in T_b occurring at rather high levels of O₂ (Fig. 2). However, the results from the acute hypoxic experiments support this thermoregulatory set-point change more strongly, especially when the total kinetics of metabolism, heat loss, and T_b are taken into account. Figure 5 shows a plot of T_b vs. V̇O₂ during normoxia, acute hypoxia, and subsequent normoxic recovery, demonstrating a hysteresis in the relationship. It is also clear that V̇O₂ reaches a new equilibrium long before T_b, and the same is seen during recovery; metabolic rate rises almost immediately back to prehypoxic values. Taking into account the lowered T_b and an effect of 10°C change on metabolism (Q₁₀), this translates most likely into a posthypoxic thermogenesis serving to fuel the return to normal T_b. Meanwhile, heat loss (∆T_a) during the first 15–25 min of hypoxic exposure undergoes an initial increase, after which it falls back to minimal levels, not changing during recovery (Fig. 3). This delay between V̇O₂ and T_b decline and the transient rise in ∆T_a all point to a coordinated, systemic, physiological response to hypoxia, serving primarily to reduce T_b as quickly as possible, first through a fall in metabolic heat production and then, presumably, through an increase in peripheral circulation. At this point, we cannot rule out the possibility that hypoxia induces peripheral and tissue vasodilation due to local hypoxia, accounting for the temporary rise in ∆T_a.

The thermoneutral zone for the pygmy marmoset has been established to extend from 27 to 34°C (26). Because the temperature used in this study was 28°C, the marmosets were within their thermoneutral zone. This could account for an augmented peripheral heat
loss in hypoxia, because a large degree of vasomotor control would still be available to allow for controlled heat loss through altered blood flow. In addition, the thermogenic processes are already minimal in normoxia at 28°C. Thus the decline in metabolic rate is not likely due to the shutting off of thermogenesis but instead results from the shutting down of other metabolic processes that appear to be extremely temperature sensitive. By virtue of the 2.7°C change in Tb and the 30–40% decline in V̇O₂, the temperature sensitivity of metabolic rate is roughly three times the typical Q₁₀ value of 2–3 at Q₁₀ > 6.7. This supports the hypothesis of an active suppression of metabolism. The precise makeup of this suppressed metabolism is unknown. Mortola and Gautier (32) proposed that nonmitochondrial V̇O₂ may be shut down during hypoxia. However, because these processes (e.g., cellular oxygenases) account for only 10–20% of total tissue V̇O₂ (37), a reduction of metabolic processes must be occurring in hypoxia.

Ventilatory Response to Hypoxia

The hypoxic ventilatory response of the pygmy marmoset was quite remarkable in that there was little or no increase in breathing during hypoxic exposure (Figs. 4 and 6). Typically, in mammals, the air-convection requirement doubles during exposure to 30 min of 10% O₂ (i.e., hyperpnea and hyperventilation; Fig. 6 and Ref. 12). This contrasts to the present study where V̇E/V̇O₂ does not significantly change (Figs. 1 and 3) from normoxia (~40) to acute (~50) or graded hypoxia (from 40–45). Much like metabolism and Tₘ, ventilation itself exhibited a marked decline during both

Fig. 3. Effects of acute hypoxia (30 min at 20.9% O₂, 60 min at 10% O₂, and 60 min at 20.9% O₂) on Tₘ, ΔTₘ, V̇O₂, V̇CO₂, respiratory quotient, V̇E, and V̇E/V̇O₂. Values are means ± SE. *Significant difference compared with the time 0 value, P < 0.05. No significant effects of graded hypoxia were seen in ΔTₘ, respiratory exchange ratio, and V̇E/V̇O₂.
graded and acute hypoxia, with the decline being facilitated by a drop in \( f \), whereas \( V_t \) remained constant (Tables 1 and 2). It is unlikely that we missed the window of any ventilatory increase during hypoxia, because most mammals exhibit up to 20 min of sustained ventilation before exhibiting any decline in \( V_E \). Thus there appears to be little or no ventilatory roll-off in the pygmy marmoset but rather a strong coupling of ventilation to metabolic rate with minimal chemosensory stimulation of breathing. The small ventilatory roll-off that occurs in acute hypoxia in marmosets (Fig. 3) can probably be attributed to the large drop in metabolic rate and thus the drop in metabolic drive to breathe; however, none of these changes in \( V_{E/VO_2} \) are statistically significant. It is also unlikely that the levels of \( O_2 \) examined were too high to produce a brisk ventilatory response for two reasons: 1) we observed that some marmosets appeared distressed at the lowest level of \( O_2 \) studied (10%) and that some exposed to 8% \( O_2 \) were unable to maintain their balance and equilibrium, appearing to be extremely sensitive to hypoxia; 2) all other studies of the hypoxic ventilatory response of mammals indicate that even poikilocapnic 10% \( O_2 \) is low enough to stimulate breathing.

### Table 2. Metabolic and respiratory parameters of pygmy marmosets exposed to acute hypoxia

<table>
<thead>
<tr>
<th>Condition</th>
<th>( %V_O_2 ) 100</th>
<th>( %V_CO_2 ) 100</th>
<th>( V_t ), ml</th>
<th>( f ), breaths/min</th>
<th>( T_I ), s</th>
<th>( T_E ), s</th>
<th>( T_{I/T} ), s</th>
<th>( V_{T/T} ), ml/s</th>
<th>( V_{E/VO_2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>1.58 ± 0.10</td>
<td>97.5 ± 5.6</td>
<td>0.26 ± 0.01</td>
<td>0.40 ± 0.03</td>
<td>0.40 ± 0.02</td>
<td>6.40 ± 0.40</td>
<td>56.3 ± 4.0</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>59.8 ± 5.5*</td>
<td>57.7 ± 4.3*</td>
<td>1.69 ± 0.10</td>
<td>65.2 ± 6.3*</td>
<td>0.43 ± 0.03a</td>
<td>0.59 ± 0.05a</td>
<td>0.42 ± 0.01</td>
<td>4.33 ± 0.41a</td>
<td>66.6 ± 4.2</td>
</tr>
<tr>
<td>Recovery</td>
<td>89.8 ± 2.2</td>
<td>82.6 ± 3.4</td>
<td>1.67 ± 0.09</td>
<td>81.3 ± 6.4</td>
<td>0.32 ± 0.02</td>
<td>0.49 ± 0.05</td>
<td>0.41 ± 0.02</td>
<td>5.58 ± 0.41</td>
<td>59.4 ± 3.7</td>
</tr>
</tbody>
</table>

Values represent the 30th, 60th, and 60th minute means ± SE of normoxic, hypoxic, and recovery time periods, respectively. *\( P < 0.05 \) vs. control.

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**Fig. 4.** Summary of \( V_t \) and \( V_{E/VO_2} \) in graded and acute hypoxia. \( V_t \), circles; \( V_{E/VO_2} \), squares; solid symbols, data from the graded hypoxic experiments; open symbols, data from acute hypoxic experiments. Values are means ± SE. *\( P < 0.05 \) vs. normoxia.

**Fig. 5.** Relationship between \( T_b \) and \( V_O_2 \) during normoxia (A), exposure to acute hypoxia (B), and subsequent recovery (C). Plot of \( T_b \) vs. \( V_O_2 \) was chosen to emphasize the temporal differences between these 2 parameters during hypoxic exposure. \( V_O_2 \) reaches a plateau before \( T_b \) during hypoxia (B). During normoxic recovery, a posthypoxic thermogenesis (C) serves to promote the slower recovery of \( T_b \) back to normoxic levels. Each point represents the 5-min averages shown in Fig. 3. Values are means ± SE.

**Fig. 6.** Plot of \( V_E \) vs. \( V_O_2 \) expressed as percentage of normoxic values, during graded and acute hypoxic exposure. Values are means ± SE. Circles, data from this study (solid symbols from the graded hypoxic experiments and open symbols from the acute hypoxic experiments); triangles, 10% \( O_2 \) values from 21 mammalian species (adults ranging in size from 8 g to 5 kg) from Frappell et al. (12). Different quadrants or sections refer to the qualitative change from normoxia. A, hyperventilation, hyperpnea, and metabolic depression; B, hypopnea, hyperventilation, and metabolic depression; C, hypopnea, hypoventilation, and metabolic depression. Numbers in figure refer to \( %O_2 \). Note that most adult mammals exhibit hyperventilation, hyperpnea, and metabolic depression during short-term hypoxia.
The cause of this low-ventilatory sensitivity is unknown. However, studies on rhesus monkeys have demonstrated that adults exhibit a robust ventilatory response to hypoxia (70% above normoxic levels; Refs. 20, 21) and even 20-day-old newborns increase \( V\dot{E} \) by at least 20%, even without accounting for the fall in metabolism (50). In contrast, the hypercapnic ventilatory response of the pygmy marmoset assessed with the same technique is quite strong: \( V_t \) triples and \( f \) doubles from air to 6% CO\(_2\) (unpublished observations). This is similar to the hypercapnic response of other nonrodent mammals (5). At the very least, these observations help back up the validity of the barometric method for assessing breathing in this study. Thus the low-hypoxic ventilatory sensitivity of the pygmy marmoset is unique in terms of it being both an adult and a primate.

Relevance of the Hypoxic Response

The combined metabolic and respiratory response to hypoxia is indicative of a coordinated response geared toward reducing O\(_2\) demand. In fact, the response of hypoxic pygmy marmosets is not unlike the physiological changes that occur during sleep, where metabolic rate, \( T_b \), and ventilation decline in a synchronous fashion, suggesting that similar control processes are in effect in hypoxia and sleep.

As mentioned earlier, the air-convection requirement was constant throughout hypoxic exposure. This implies a greater extraction efficiency (as defined in Ref. 41) in hypoxia. In graded hypoxia, O\(_2\) extraction efficiency \( \frac{V\dot{O}_2}{(expired O_2 fraction \times V\dot{E})} \) can reach 35%, as opposed to the normoxic values of 15%, whereas, in acute hypoxia, a value closer to 25% is possible. The reasons for this change in extraction efficiency must reflect the changes in the O\(_2\) uptake of the pulmonary system or changes in metabolic demand. A recent morphological study on common marmosets (a related species) calculated the pulmonary O\(_2\) diffusing capacity of the lungs to be more than double that of mammals of similar size (1). The authors attribute this increased capacity for gas exchange to the relatively large lung volumes of marmosets. An increased gas-exchange capacity would maintain adequate arterial oxygenation and thus serve to blunt the ventilatory response, because a high diffusing capacity could compensate for diffusion-limited gas exchange that can be present during severe hypoxia (44).

One hypothesis to explain the diminished ventilatory response to hypoxia could be a high hemoglobin affinity for O\(_2\). Walsh et al. (43a) found that the lesser spear-nosed bat had a low-hypoxic ventilatory response and attributed this to their relatively high hemoglobin affinity, given the small body size. It is possible that the pygmy marmoset has a similarly high affinity for O\(_2\) of heme proteins involved in O\(_2\) transport and sensing, accounting for the apparent low ventilatory response to hypoxia (6).

We also examined the \( V\dot{E}-V\dot{O}_2 \) relationship using the method of Frappell et al. (12), where hypoxic values of \( V\dot{E} \) and \( V\dot{O}_2 \) are expressed as a percentage of normoxic values (Fig. 6). Frappell et al. concluded that nearly all the mammals that they studied exhibited a hyperventilatory response, taking the form of either hypopnea combined with a greater metabolic depression or hyperpnea combined with metabolic depression. There was no systematic trend in these responses. In contrast, pygmy marmosets exhibit a relationship markedly different. Practically all values in hypoxia fall along a line where \%\( V\dot{E} \) and \%\( V\dot{O}_2 \) are directly proportional to one another. Thus the pygmy marmoset exhibits a blunted ventilatory response to hypoxia.

Conclusions

In summary, the pygmy marmoset exhibits metabolic depression combined with a lowering of \( T_b \) when faced with hypoxia, a response akin to that of other small mammals. This is achieved through a coordinated response, involving changes in heat production (\( V\dot{O}_2 \)) and heat loss proportional to the severity and abruptness of the hypoxia. Unlike other mammals, pygmy marmosets exhibit little to no ventilatory response to hypoxia (\( V\dot{E}/V\dot{O}_2 \)), even accounting for the decline in metabolism. Future research should address the reasons for this blunted ventilatory response: is it a metabolic drive overriding a chemical drive to breathe, or do adult marmosets have low chemosensitivity?

Many experimental models have demonstrated the benefits of a lowering in \( T_b \) on metabolism and ventilation. The body of work on mammals demonstrating a short-term decline in \( T_b \) in hypoxia emphasizes the solution that nature has arrived at for coping with short-term hypoxia. Interestingly, although hypoxic human infants spontaneously lower \( T_b \) metabolism, and ventilation if permitted to do so, current treatment dogma is to maintain a constant, normal \( T_b \). This practice may soon change, because clinical studies examining the feasibility and ethics of allowing a natural “hypothermia” in infants during asphyctic episodes are beginning (42). The issue of therapeutic hypothermia is complicated, and recent work on long-term hypoxic exposure shows that \( T_b \) returns to normal (4, 35) after 24 h of hypoxic exposure, emphasizing that the benefits offered through lower \( T_b \) may only be transient. This long-term response is not well understood and warrants further investigation.

We gratefully acknowledge the skilled assistance of E. Sheafor, W. Horne, D. Layne, and S. Tardif for discussions and help with the marmosets.

This study was funded through grants from Summa Health Systems, Akron, OH.

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