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Thermoregulatory and metabolic responses of Japanese quail to hypoxia

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Abstract

Common responses to hypoxia include decreased body temperature (T_b) and decreased energy metabolism. In this study, the effects of hypoxia and hypercapnia on T_b and metabolic oxygen consumption ($\dot{V}_{O_2}$) were investigated in Japanese quail (*Coturnix japonica*). When exposed to hypoxia (15, 13, 11 and 9% O_2), T_b decreased only at 11% and 9% O_2 compared to normoxia; quail were better able to maintain T_b during acute hypoxia after a one-week acclimation to 10% O_2. $\dot{V}_{O_2}$ also decreased during hypoxia, but at 9% O_2 this was partially offset by increased anaerobic metabolism. T_b and $\dot{V}_{O_2}$ responses to 9% O_2 were exaggerated at lower ambient temperature (T_a), reflecting a decreased lower critical temperature during hypoxia. Conversely, hypoxia had little effect on T_b or $\dot{V}_{O_2}$ at higher T_a (36°C). We conclude that Japanese quail respond to hypoxia in much the same way as mammals, by reducing both T_b and $\dot{V}_{O_2}$. No relationship was found between the magnitudes of decreases in T_b and $\dot{V}_{O_2}$ during 9% O_2, however. Since metabolism is the source of heat generation, this suggests that Japanese quail increase thermolysis to reduce T_b. During hypercapnia (3, 6 and 9% CO_2), T_b was reduced only at 9% CO_2 while $\dot{V}_{O_2}$ was unchanged.

1. Introduction

A common response to hypoxia exposure among vertebrates and invertebrates is to reduce O_2 demand. Reduced body temperature (T_b) and the associated metabolic depression protect against hypoxia by reducing metabolic O_2 consumption and, in many species, lowering T_b also enhances O_2 loading at gas exchangers by increasing the O_2 affinity of respiratory pigments (Steiner and Branco, 2002; Bicego et al., 2007). Both decreased T_b and metabolic depression have been shown to increase survival in hypoxia (Steiner and Branco, 2002).

Thermoregulatory and metabolic responses to hypoxia have been extensively studied in mammalian and ectothermic models (Hill, 1959; Dupre et al., 1988; Wood, 1991; Gautier, 1996). Frappell et al. (1992) observed decreases in oxygen consumption ($\dot{V}_{O_2}$) in 26 of 27 mammalian species studied, with most species showing a concomitant decrease in T_b. Reduced T_b via behavioral thermoregulation has even been recorded in the protozoan *Paramecium caudatum* during hypoxia (Malvin and Wood, 1992). In mammals, the response to hypoxia is most dramatic in newborn and small species (Hill, 1959; Frappell et al., 1992; Mortola, 1996, 1999).

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Despite overwhelming evidence for each response, the relationship between changes in T_b and metabolic rate during hypoxia remains unclear. In mammals, it is frequently reported that the decrease in T_b is a direct result of a decrease in metabolic rate (i.e., reduced heat production), with the decrease in metabolic rate always observed first (Hill, 1959; Gautier and Bonora, 1994; Gautier, 1996). On the other hand, there have been several studies in which the decrease in T_b reportedly precedes the decrease in metabolic rate. For example, the little brown bat (*Phyllostomus discolor*) decreases T_b at 12% O_2 despite no measurable reduction in metabolic rate at this level of hypoxia (Walsh et al., 1996). Similarly, the golden-mantled ground squirrel (*Spermophilus lateralis)* exhibits an immediate decrease in T_b followed by a delayed suppression of metabolic rate upon exposure to hypoxia (Barros et al., 2001). This suggests that increased heat loss (thermolysis) may also be an important mechanism for the decrease in T_b in some mammals. The decrease in T_b could, in turn, depress metabolic rate directly (i.e., Q_{10} effect), although active suppression of metabolism may also contribute to metabolic depression in hypoxia (Barros et al., 2001).

It is generally accepted that the decrease in T_b during hypoxia is a regulated process (i.e., anapyrexia; Steiner and Branco, 2002; Bicego et al., 2007). In behaviorally thermoregulating ectotherms, this is reflected in a change in preferred T_b (Wood, 1991; Tattersall and Boutilier, 1997), although there is also evidence for a shift in the temperature at which lizards begin evaporative cooling during hypoxia (Dupre et al., 1986). In mammals, the regulated decrease in T_b is evidenced by a shift in the thermoneutral zone to lower T_a during hypoxia (Dupre et al., 1988; Barros et al., 2001). This shift reduces metabolically expensive processes such as shivering and non-shivering thermogenesis at low T_a, which also explains why metabolic depression is exaggerated at low T_a (Gautier, 1996). The mechanism by which hypoxia initiates this process is largely unknown, but reduced oxidative metabolism in the central nervous system (CNS) (Steiner and Branco, 2002) and/or changes in the thermal sensitivity of preoptic neurons during hypoxia (Tamaki and Nakayama, 1987) have been implicated. Indeed, nitric oxide, serotonin and dopamine all appear to be important modulators of hypoxic anapyrexia within the preoptic area (Branco et al., 2006).

The effects of hypoxia on T_b and metabolism have been studied far less in birds than mammals, and many of the previous reports have yielded conflicting results. Body temperature has been reported to decrease during hypoxia in most avian species studied to date, including the Japanese quail (studied at T_a = 5°C; Weathers and Snyder, 1974), bobwhite quail (Boggs and Kilgore, 1983), greylag goose (Scott et al., 2008), bar-headed goose (Scott et al., 2008), burrowing owl (Boggs and Kilgore, 1983; Kilgore et al., 2008), house sparrow (Tucker, 1968) and rufous-collared sparrow (Novoa et al., 1991), although a study on the rosy finch and house finch reported no change in T_b during hypoxia (Clemens, 1988). The pekin duck also appears to decrease T_b in hypoxia (Faraci et al., 1984; Scott et al., 2008), but this has not been consistently observed (Kiley et al., 1985, Bouverot and Hildwein, 1978). In contrast, $\dot{V}_{O_2}$ has only been shown to decrease during hypoxia in the Japanese quail (studied at T_a = 5°C; Weathers and Snyder, 1974) and, at least in one study, the rufous-collared sparrow (Castro et al., 1985; but see Novoa et al., 1991). Rather, many avian species do not change $\dot{V}_{O_2}$ during hypoxia, including the bobwhite quail (Boggs and Kilgore, 1983), burrowing owl (Boggs and Kilgore, 1983; Kilgore et al., 2008) and several small passerines (Novoa et al., 1991) or actually *increase* $\dot{V}_{O_2}$ during hypoxia, including the greylag goose (Scott et al., 2008), bar-headed goose (Black and Tenney, 1980; Scott et al., 2008), house sparrow (Tucker, 1968), and the rosy and house finches (Clemens, 1988). Pigeons and pekin ducks have variously been shown to have either no change (duck: Kiley et al., 1985, Bouverot and Hildwein, 1978; pigeon: Bouverot et al., 1976) or an increase in $\dot{V}_{O_2}$ during hypoxia (duck: Black and Tenney, 1980; Scott et al., 2008; pigeon: Barnas et al., 1986). Since factors such as the level of hypoxia, T_a and duration of the hypoxic exposure have been shown to influence the magnitude of the changes in T_b and metabolic rate in mammals (Gautier, 1996; Barros et al., 2001), some of this intraspecific and

interspecific variation could reflect differences in experimental design. However, it is difficult to address these questions because of the limited data available for any one species.

The purpose of this study was to investigate the effects of hypoxia on T_b and metabolic rate in Japanese quail (*Coturnix japonica*). Several levels of hypoxia were investigated to determine the severity of hypoxia required to elicit a response, and a week-long chronic hypoxia exposure was used to determine whether these responses exhibit plasticity. Quail were also exposed to hypoxia at several T_a to test the hypothesis that there would be a shift in the thermoregulatory set point similar to that observed in mammals. Finally, several levels of hypercapnia were studied to determine whether another respiratory stimulant elicits similar changes in T_b and/or metabolic rate in this species.

2. Materials and methods

2.1 Experimental animals

Fertile Japanese quail (*Coturnix japonica*) eggs were purchased from a commercial supplier (Boyd's Birds, Pullman, WA, USA) and were hatched and raised to adulthood in-house. Adult birds were maintained on a 12:12 light cycle and provided food and water *ad libitum*. A total of 26 adult males, between 11 and 24 weeks of age, were used in these experiments; individuals were generally used in more than one experimental protocol (see below) with a minimum of one week between protocols. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Bates College.

2.2 Surgical preparation

At least two weeks prior to experimentation, a temperature transponder (E-mitter G2; Respironics, Bend, OR) was surgically implanted into the abdominal cavity of each quail. Quail were anesthetized with isoflurane, first in a closed box and then maintained via nose cone (2.5% isoflurane, balance O_2). The transponder was then placed into the abdominal cavity through a ventral midline incision. Carprofen (30 mg kg^{-1} i.m., Rimadyl, Pfizer, Exton, PA, USA) was administered post-operatively as an analgesic (Hocking et al., 2005).

2.3 Body temperature and metabolism

Metabolic rate was measured using open-system respirometry. A cylindrical, opaque respirometer chamber (15.2 cm diameter, 14.6 cm height) was housed in an incubator (Model 818, Precision, Winchester, VA, USA) to maintain constant ambient temperature. Mixtures of N_2, O_2, and/or CO_2 were forced through the chambers at a flow rate of 1 liter/min using precision rotameters (7300 and 7400 series, Matheson, Montgomeryville, PA); flow rate (STPD) was continuously monitored with a mass flowmeter (G265, Qubit Systems, Kingston, ON, Canada) and recorded to a computer. At this flow rate, it took less than ten min to switch from 21% O_2 to 9% O_2.

The fractional concentrations of O_2 and CO_2 entering (F_{IO2} and F_{ICO2}) and exiting (F_{EO2} and F_{ECO2}) the chamber were measured using a gas analyzer (PowerLab ML206, ADinstruments, Colorado Springs, CO, USA); air was dried (Drierite, W.A. Hammond Drierite Co., Xenia, OH, USA) prior to analysis. Ambient temperature (T_a) was measured via a T-type thermocouple situated within the respirometer chamber, just below the lid; unless otherwise noted, T_a was 23°C for all measurements. T_a, gas flow rate, and O_2 and CO_2 concentrations were recorded to computer during each experiment (PowerLab/8sp, Chart 5.2 ADinstruments). Body temperature (T_b) was simultaneously monitored by telemetry (ER-4000 energizer receiver and VitalView 4.1 software; Respironics, Bend, OR, USA).

2.4 Lactate measurements

To measure blood lactate, the toe nail (claw) was visualized to determine the location of the blood vessel. The claw was then clipped so as to release a few drops of blood. Blood was collected directly onto test strips and analyzed for lactate concentrations using a blood lactate analyzer (Lactate Pro, Arkray, Kyoto, Japan).

2.5 Experimental protocols

Metabolic rate, body temperature, and/or lactate were measured during five different experimental protocols. Each quail was exposed to the respirometry chamber on at least two occasions prior to the study (~60 min per exposure). Animals were not fasted prior to experimentation. When a protocol required the same individual to be studied on multiple days (see below), the individual was studied at approximately the same time each day; all measurements were made during the light period of the light cycle. Barometric pressure averaged 754 ± 5 (S.D.) mmHg across all protocols

2.5.1 Protocol 1: Graded hypoxia—On the day of study, each quail was weighed and placed in the respirometer chamber (T_a = 23°C). Quail remained at normoxia (21% O_2, 0% CO_2) for 100 min to establish a baseline and were then switched to one of five test gases for 60 min: 21% (time control), 15%, 13%, 11%, and 9% O_2. Only one test gas was used each day, so each individual was studied on five consecutive days; the order in which each quail was exposed to the five test gases was randomly assigned. A total of 16 quail were used in this protocol.

2.5.2 Protocol 2: Blood lactate—Quail were placed in a respirometer chamber and exposed to normoxia for 90 min, then exposed to normoxia (21% O_2) or hypoxia (11% or 9% O_2) for 30 additional min. At the end of this exposure, quail were quickly removed from the chamber and sampled for blood lactate concentrations. Each quail was exposed to only one test gas per day, and the order of the test gases was assigned randomly. Six quail were used in this protocol.

2.5.3 Protocol 3: Chronic hypoxia—As in protocol 1 (see section 2.5.1), quail were placed in a respirometer chamber to measure T_b and metabolic rate. After 100 min of normoxia (21% O_2), the test gases were switched to 9% O_2 for 60 min. Quail were then placed in large acrylic chambers for one week at 10% O_2; quail were individually caged and provided food and water *ad libitum*. Chambers were opened briefly (<10 min) each day to clean cages and replenish food and water. After seven days, T_b and metabolic rate measurements were repeated as described above. Ten quail were used in this protocol.

2.5.4 Protocol 4: Graded T_a—On the day of study, each quail was placed in the respirometer chamber at one of eight ambient temperatures: all sixteen quail used in this protocol were studied at 13°, 18°, 23°, 28°, and 33°C, and six of these were also studied at 30° and 36°C to better estimate the thermoneutral zone. As described in protocol 1 (see section 2.5.1), quail remained at normoxia (21% O_2) for 100 min to establish a baseline before being switched to 9% O_2 for 60 min. Only one T_a was used each day, so each individual was studied on 5–7 consecutive days; the order of exposure to each T_a was randomly assigned. To verify that the observed changes in T_b and metabolic rate reflected the effect of hypoxia versus the duration of time spent in the respirometer chamber, six quail were also studied for 160 min in normoxia at 13°C and 33°C (i.e., time controls).

2.5.5 Protocol 5: Graded hypercapnia—As in protocol 1 (see section 2.5.1), quail were placed in a respirometer chamber to measure T_b and metabolic rate. Quail remained in normoxic normocapnia (21% O_2, 0% CO_2) for 100 min and were then switched to one of four

test gases for 60 min: 0%, 3%, 6% and 9% CO_2. Only one test gas was used each day, so each individual was studied on four consecutive days; the order in which each quail was exposed to the four test gases was randomly assigned. Ten quail were used in this experiment.

2.6 Data analysis

Body temperature was recorded every five min for the final 30 min of baseline and throughout the 60 min of the test gas exposure. Metabolic O_2 consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$) were calculated based on inspired and expired gas levels averaged over 30 s and sampled every 10 min during the final 30 min of baseline and during exposure to the test gas; reported $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ are corrected to standard temperature and pressure (STPD). At the most severe levels of hypoxia, $\dot{V}_{CO_2}$ could be affected by lactate production (see section 3.3). Preliminary analysis showed that $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ yielded similar results for all experimental conditions, so only $\dot{V}_{O_2}$ is reported in the figures. T_b and $\dot{V}_{O_2}$ are reported both as the minimum value achieved during the 60 min of test gas exposure ("minimum T_b" or "minimum $\dot{V}_{O_2}$") and as the corresponding change from baseline, where baseline represents the average T_b or $\dot{V}_{O_2}$ for the final 30 min of the 100-min baseline (i.e., normoxic and normocapnic) exposure. Normalization to baseline reduces the effects of day-to-day variation that might result from factors such as acclimation to the chamber over repeated measurements or time since the last meal.

The effects of graded hypoxia and hypercapnia on T_b, $\dot{V}_{O_2}$ and/or lactate were analyzed using one-way repeated measures ANOVA followed by Newman-Keuls post hoc tests. The effects of chronic hypoxia on T_b and $\dot{V}_{O_2}$ responses to hypoxia were analyzed by paired t-tests, and by two-way repeated measures ANOVA with Bonferroni post hoc tests (comparing all time points to baseline) to further analyze the time course of changes in T_b and $\dot{V}_{O_2}$ during acute hypoxia. Two-way repeated measures ANOVA followed by Student-Newman-Keuls post hoc tests were used test the effect of hypoxia on T_b and $\dot{V}_{O_2}$ at different T_a; only a subset of quail were studied at two additional T_a (30 and 36°C), so separate paired t-tests were used to test the effects of hypoxia on T_b and $\dot{V}_{O_2}$ at these T_a. Where appropriate, one-way repeated measures ANOVA and/or one-sample t-tests were used to determine whether there was a significant change in T_b or $\dot{V}_{O_2}$ with time in 21% O_2 trials (i.e., time controls). Linear regression was used to determine whether hypoxia-induced changes in T_b were related to changes in $\dot{V}_{O_2}$. Statistical tests were run using GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, CA, USA) or SigmaStat 3.11 (Systat Software, Inc., San Jose, CA, USA). The threshold of significance was set at $P < 0.05$ for all tests. Values in text are means ± SEM, unless otherwise noted.

3. Results

3.1 Normoxic T_b and metabolic rate

The resting T_b and metabolic rate for adult quail breathing 21% O_2 are reported in Table 1. Since individuals were studied multiple times, the average value for each individual was computed prior to calculating the group mean. Although T_b was fairly consistent across days within an individual (coefficient of variation (CV) = 0.005±0.002), individuals showed considerable day-to-day variation in $\dot{V}_{O_2}$ (CV = 0.22±0.09). The average T_b (41.2°C) was similar to previous reports for this species (e.g., 40.8°C; Bavis and Kilgore, 2001), whereas average $\dot{V}_{O_2}$ (4.3 mL O_2 min^{-1} $100g^{-1}$) tended to be greater than previously reported (e.g., 3.1 mL O_2 min^{-1} $100g^{-1}$;Bavis and Kilgore, 2001). Ten individuals were also studied at least twice after an additional 60 min in the respirometer chamber (i.e., total exposure 160 min) (Table 1). For these individuals, there were no significant differences between 100 min and 160 min measurements for T_b (P=0.38), $\dot{V}_{O_2}$ (P=0.10), $\dot{V}_{CO_2}$ (P=0.08) or respiratory quotient (P=0.64).

3.2 Protocol 1: Effect of graded hypoxia

Body temperature was analyzed both as the minimum value achieved during the 60 min of test gas exposure ("minimum T_b") and as the change from baseline. In both analyses, T_b consistently decreased during moderate/severe hypoxia. When compared to the minimum T_b observed during an equivalent duration at 21% O_2 (T_b=41.0±0.1°C), T_b was reduced during hypoxia (P<0.001), but only at 11% (T_b=40.7±0.1°C; P<0.05) and 9% (T_b=40.3±0.2°C; P<0.05) O_2 (Fig. 1A); T_b at 9% was significantly lower than at 11% (P<0.001). Although the minimum T_b of quail maintained at 21% was also slightly lower than baseline in this group of quail (ΔT_b=0.2°C, P<0.001), the decrease was greater at 11% (T_b=0.5°C, P<0.05) and 9% (ΔT_b=0.9°C, P<0.05) (Fig. 1B). In contrast, no significant reduction in T_b was observed at 15% or 13% O_2 compared to 21% O_2 (Figs. 1A, B; both P>0.05).

The rate of oxygen consumption tended to decrease during hypoxia as well. Although minimum $\dot{V}_{O_2}$ did not vary with inspired O_2 (P=0.07, Fig. 1C), there was a trend for $\dot{V}_{O_2}$ to be lower in hypoxia. Indeed, there was a significant overall effect of hypoxia on $\dot{V}_{O_2}$ when normalized to baseline (P=0.03, Fig. 1D). Post hoc tests were unable to distinguish which groups varied (all P>0.05), but $\dot{V}_{O_2}$ appeared to be reduced at 11% and 9% O_2 compared to at 21% O_2. Indeed, $\dot{V}_{O_2}$ was significantly reduced during 9% O_2 in subsequent experiments (e.g., Fig. 4 and Fig 5, see below). Similar to T_b, minimum $\dot{V}_{O_2}$ at 21% O_2 during the 60-minute test gas exposure was lower than during baseline (P<0.001).

3.3 Protocol 2: Blood lactate during hypoxia

A decrease in $\dot{V}_{O_2}$ during hypoxia could reflect an overall decrease in energy metabolism. Alternatively, the decrease in aerobic metabolism could be offset by an increase in anaerobic metabolism. To test this possibility, blood lactate levels were measured during 21%, 11% and 9% O_2. Overall there was an increase in blood lactate compared to normoxia (P=0.003, Fig. 2). Post hoc analysis revealed that blood lactate levels in quail exposed to 9% O_2 increased by 186% over the normoxic values (P<0.05), but no increase in lactate concentration was observed at 11% O_2 (P>0.05).

3.4 Protocol 3: Effect of chronic hypoxia

To determine whether chronic hypoxia alters the T_b or metabolic responses to hypoxia in quail, T_b and $\dot{V}_{O_2}$ responses to 9% O_2 were measured in a group of ten quail before and after 7 days at 10% O_2; T_b data were unavailable for three of the quail following chronic hypoxia because of technical problems, so n=7 for T_b and n=10 for $\dot{V}_{O_2}$. T_b decreased compared to baseline during acute hypoxia both before and after acclimation, but the average decrease after chronic hypoxia was 51% less (P=0.01, Fig. 3A). To determine whether this difference was an artifact of the rate at which T_b decreased versus the actual T_b achieved, we examined the time course for the change in T_b during hypoxia (Fig. 4). There was a significant statistical interaction between treatment and time (P<0.001). Normoxic T_b was not altered by acclimation (41.4±0.1° C before and after acclimation) and the decrease in T_b was noticeable within 15–20 min of switching to hypoxia in each group. However, T_b began to level off within 40–45 min in acclimated quail (i.e., after acclimation) while it continued to decrease until 50–55 min in the unacclimated condition (i.e., before acclimation). Since T_b stabilized in the recording period for both groups, higher hypoxic T_b in acclimated quail was not simply due to a slower decrease in T_b.

There was no change in baseline $\dot{V}_{O_2}$ (4.2±0.3 and 3.7±0.3 mL O_2 min^{-1} $100g^{-1}$ for before and after acclimation, respectively; P=0.12), or in the $\dot{V}_{O_2}$ response to acute hypoxia following acclimation (P=0.52; Fig. 3B). $\dot{V}_{O_2}$ decreased within 20 min of the onset of acute hypoxia and remained fairly constant (on average) between min 20 and 60 of the exposure (Fig. 4B).

3.5 Protocol 4: Effect of graded T_a

The effect of hypoxia on T_b varied with ambient temperature ($F_{IO2} \times T_a$, $P< 0.001$). Body temperature was lower during 9% O_2 compared to 21% O_2 at the five T_a (13°, 18°, 23°, 28°, and 33°C) at which all quail were studied (all $P<0.001$; Fig. 5A), but this difference tended to be greater at low T_a. T_b during normoxia was slightly higher at 33°C than at lower T_a, but remained consistent for all other T_a studied. In contrast, T_b tended to decrease as T_a decreased during hypoxia, which equates to a larger decrease in T_b (versus normoxia) at lower T_a. Conversely, the effect of hypoxia on T_b appeared quite small at higher T_a. Six quail were studied at ambient temperatures of 30° and 36°C (Fig. 5A). Although these data were not included in the overall statistical analysis discussed above, the effects of hypoxia on T_b were tested by separate paired t-tests at 30° and 36°C for this subset of quail. Hypoxia had no effect on T_b at 36°C ($P=0.73$), the highest T_a studied; in contrast, hypoxia significantly reduced T_b at 30°C ($P=0.02$).

The effect of inspired O_2 on $\dot{V}_{O2}$ also varied with T_a ($F_{IO2} \times T_a$, $P<0.001$). $\dot{V}_{O2}$ decreased during 9% O_2 compared to 21% O_2 at the five T_a (13°, 18°, 23°, 28°, and 33°C) at which all quail were studied (all $P\leq0.02$, Fig. 5B). During normoxia, $\dot{V}_{O2}$ increased as T_a decreased. A similar trend was seen during hypoxia, but the increase was less dramatic with $\dot{V}_{O2}$ only increasing at $T_a<23$°C. When additional T_a (30° and 36°C) are considered, the threshold at which $\dot{V}_{O2}$ begins to increase (i.e. lower critical temperature) occurs somewhere between 30 and 33°C in normoxia whereas it appears to occur at approximately 23°C in 9% O_2. As with T_b, hypoxia had no apparent effect on $\dot{V}_{O2}$ in the subset of quail studied at 36°C (paired t-test, $P=0.32$), the highest T_a studied (Fig. 5B); $\dot{V}_{O2}$ was reduced by hypoxia at 30°C in this same subset of quail ($P=0.01$).

As reported above, there was a decrease in minimum $\dot{V}_{O2}$ compared to baseline in quail maintained at 21% O_2 for an additional 60 min post-baseline in protocol 1 (see section 2.5.1). To ensure that the decrease in metabolic rate observed during hypoxia was not due to a time-dependent decrease in baseline $\dot{V}_{O2}$, several quail (n=6) were maintained in normoxia at 13°C and 33°C for 160 min (i.e., the equivalent of the entire protocol). No change in $\dot{V}_{O2}$ from baseline was observed at either T_a (both $P>0.9$; Fig. 6). As expected, $\dot{V}_{O2}$ was greater at 13°C than at 33°C. No change in T_b was observed from baseline at 33°C ($P=0.08$), but a gradual decrease was observed at 13°C ($P<0.001$). However, the greatest decrease in T_b during this time control experiment was 0.2°C compared to the average 0.7°C decrease observed at 9% O_2 (Fig. 5A).

Given that T_b decreased as T_a decreased in hypoxia (Fig. 5A), the relationship between T_a and $\dot{V}_{O2}$ during hypoxia is influenced by the direct effects of T_b depression on $\dot{V}_{O2}$ (i.e., Q_{10} effect). To look at the T_b-independent effects of T_a and hypoxia on $\dot{V}_{O2}$, we corrected $\dot{V}_{O2}$ for the change in T_b by assuming a constant Q_{10} of 3 (Fig. 5C); a Q_{10} of 2–3 is typical of biological reactions and has been used by others to investigate the temperature-independent effects of hypoxia on metabolic rate in ground squirrels (Barros et al., 2001). Specifically, we corrected the hypoxic $\dot{V}_{O2}$ for each quail to the normoxic T_b of the same individual at the corresponding T_a. Analyzed this way, the effect of inspired O_2 on $\dot{V}_{O2}$ still varied with T_a ($F_{IO2} \times T_a$, $P=0.002$). However, $\dot{V}_{O2}$ was only reduced by hypoxia at 13°, 18°, 23 and 28°C (and in the subset of quail studied at 30°C; paired t-test, $P=0.03$), and not at 33°C (nor in the subset of quail studied at 36°C; paired t-test, $P=0.33$). Temperature-corrected $\dot{V}_{O2}$ increased as T_a decreased at $T_a<28$°C but was not affected by changes in T_a between 28 and 33°C. Thus, after controlling for the direct effects of T_b depression on $\dot{V}_{O2}$, the lower critical temperature occurred somewhere between 23 and 28°C in hypoxia, which is lower than observed in normoxia (i.e., between 30 and 33°C).

3.6 Relationship between T_b and metabolic responses to hypoxia

To further investigate the relationship between the T_b and metabolic responses to hypoxia, we combined data for all 23 quail studied at T_a = 23°C without prior exposure to chronic hypoxia into a single regression analysis. There was no relationship between the decrease in $\dot{V}_{O_2}$ and the decrease in T_b during exposure to 9% O_2 (r^2=0.01, P=0.65). Indeed, individuals with the least (ΔT_b=0.1°C) and greatest (ΔT_b=2.6°C) decreases in T_b had similar decreases in $\dot{V}_{O_2}$ relative to baseline ($\Delta\dot{V}_{O_2} \sim -60\%$).

3.7 Protocol 5: Effect of graded hypercapnia

Body temperature decreased during hypercapnia (P<0.001). Post hoc analysis revealed that minimum T_b decreased significantly at 9% CO_2 compared to 0% CO_2 (P<0.05, Fig. 7A); T_b was unaffected by 3% or 6% CO_2. Similar results were observed for the change in T_b from baseline, with a significantly larger decrease in T_b at 9% CO_2 than at any other level of inspired CO_2 (Fig. 7B). $\dot{V}_{O_2}$ was unaffected by the level of inspired CO_2 (all P>0.05; Figs 7C, D).

4. Discussion

Mammals generally reduce O_2 demand in hypoxia by decreasing both their body temperature and their energy metabolism (Wood, 1991; Frappell et al., 1992; Gautier, 1996; Mortola, 1996, 1999; Steiner and Branco, 2002; Bicego et al., 2007), but equivalent responses have not been consistently reported in birds. The present study demonstrated both reduced T_b and reduced metabolic rates in adult Japanese quail acutely exposed to moderate-severe hypoxia ($F_{IO_2} \leq 11\%$). These responses were relatively small compared to those observed in small mammals and were most evident at low T_a. In addition, our results indicate that the T_b response is modifiable by chronic exposure to hypoxia (i.e., exhibits phenotypic plasticity). These data confirm and extend an earlier report by Weathers and Snyder (1974) who observed decreases in T_b and $\dot{V}_{O_2}$ during hypoxia in Japanese quail when studied at low ambient temperatures (5° C).

4.1 Methodological considerations

Quail were allowed 100 min to adjust to the respirometry chambers before switching to the test gas condition. Despite this, T_b and $\dot{V}_{O_2}$ tended to decrease during the subsequent 60 min even when kept at 21% O_2 (e.g., Fig. 1B,D and Fig 7B,D); this gradual decrease was not immediately obvious during data collection. The apparent time-dependent decrease in T_b and $\dot{V}_{O_2}$ may be overestimated here since the minimum value recorded over the 60-min test gas exposure is being compared against baseline values that represent an average of multiple time points. Indeed, $\dot{V}_{O_2}$ did not decrease during normoxia in the time control experiments presented in Figure 6. However, the quail in Figure 6 had been studied up to ten previous times (i.e., these experiments were completed after those presented in Fig. 1 and Fig 7), and these repeated measurements may have caused the quail to settle more quickly after being handled and placed into the respirometer chamber. Moreover, T_b did decrease slightly over time in these time control experiments (Fig. 6A). Thus, quail may require more than 160 min to adjust to the chamber after handling in order to obtain stable measurements, or may require multiple chances to become accustomed to handling and the experimental apparatus. As such, the effects of hypoxia and/or hypercapnia on T_b and $\dot{V}_{O_2}$ are most appropriately evaluated with reference to quail maintained under normoxic and normocapnic conditions (i.e., time controls).

We sampled gases exiting the respirometer chamber and calculated $\dot{V}_{O_2}$ at ten minute intervals during the test gas exposure. Although it was not possible to observe the activity level of the birds directly, it was obvious based on fluctuations in F_{EO_2} and F_{ECO_2} that individuals were active at some time points. Moreover, T_b and/or $\dot{V}_{O_2}$ are expected to change gradually after the onset of hypoxia or hypercapnia until a new steady-state is established (e.g., Fig. 4), and the specific

timing of these changes may differ among individuals. Therefore, with the exception of the time course data in Figure 4, we used the minimum T_b and $\dot{V}_{O_2}$ observed for each quail during the 60-min test gas exposure for statistical analyses; this typically occurred between the 40 and 60 minute time points. This approach reduced confounding variation in T_b and $\dot{V}_{O_2}$ contributed by periodic activity and thus improved our ability to detect differences between experimental conditions.

4.2 Effect of ambient temperature

The effects of hypoxia on T_b and $\dot{V}_{O_2}$ were most apparent at lower T_a in Japanese quail, as previously reported in mammals (Gautier, 1996). In mammals, this reflects reduced shivering and nonshivering thermogenesis associated with a shift in the thermoneutral zone to lower T_a (Dupre et al., 1988; Barros et al., 2001). Previous work demonstrated that shivering thermogenesis is also attenuated by hypoxia in the pigeon (Gleeson et al., 1986a,b; Barnas and Rautenberg, 1990), suggesting that the thermoneutral zone may be altered by hypoxia in birds as well. The present study supports this hypothesis. In normoxia, the threshold T_a at which $\dot{V}_{O_2}$ began to increase (i.e., lower critical temperature, LCT) occurred between 30° and 33°C, similar to a previous report of 29°C for this species (Marjoniemi, 2000). When exposed to 9% O_2, however, the LCT occurred closer to 23°C. In mammals, the shift in the LCT is less apparent after correcting for the direct effects of depressed T_b on $\dot{V}_{O_2}$ (i.e., Q_{10} effect) (Barros et al., 2001), but the LCT appears to be reduced somewhat (perhaps by 2–10°C) in quail exposed to 9% O_2 even after temperature correction. The upper critical temperature was not determined in the present study, so it is unclear whether this represents a widening of the thermoneutral zone or a shift of the entire thermoneutral zone to lower T_a.

Interestingly, quail exhibited a decrease in metabolic rate during hypoxia at T_a within lower end of the normoxic thermoneutral zone (e.g., 33°C). Thus, inhibition of shivering and nonshivering thermogenesis below the LCT is not the only mechanism for reducing metabolic expenditure in this species (see section 4.3, below). It should be noted, however, that the effects of hypoxia on T_b and $\dot{V}_{O_2}$ are diminished at higher T_a and that no effect was observed in quail studied at 36°C, a temperature also within the normoxic thermoneutral zone for this species. To the extent that other species have been studied within or above their thermoneutral zones, this observation may help to explain some of the discrepancies in T_b and $\dot{V}_{O_2}$ responses to hypoxia (or lack thereof) reported for birds.

In mammals, shifts in the lower critical temperature (Dupre et al., 1988; Barros et al., 2001) and preferred T_a (Gordon and Fogelson, 1991) have been interpreted as a change in the thermoregulatory set point during hypoxia. Thus, the decreased T_b during hypoxia is a regulated event (i.e., anapyrexia) rather than hypothermia. Taken together, the results of the present study indicate that quail, and perhaps all birds, exhibit hypoxia-induced anapyrexia as well. It is somewhat surprising, however, that T_b continued to fall in hypoxic quail as T_a decreased below the LCT (e.g., 13 vs. 18°C, Fig. 5A); this also appears to be the case in the rat (Dupre et al., 1988) and the golden-mantled ground squirrel (Barros et al., 2001). In hypoxia, therefore, it appears that increased heat production below the LCT is not adequate to maintain a constant T_b. This is different than what is generally observed in torpid birds and mammals, for example, where T_b stabilizes once the new LCT is reached even as T_a continues to fall (e.g., Wolf and Hainsworth, 1972; Buck and Barnes, 2000). $\dot{V}_{O_2}$ did not increase significantly at T_a between 18 and 13°C in the Japanese quail at 9% O_2, so the ability to increase $\dot{V}_{O_2}$ may have been compromised by inadequate O_2 delivery or, alternatively, this may reflect the direct effects of falling T_b on $\dot{V}_{O_2}$ (i.e., Q_{10} effect). Our data support the latter hypothesis. Specifically, $\dot{V}_{O_2}$ increased progressively as T_a decreased below the LCT after correcting for the effects of hypoxia-induced T_b depression, and the slope of this response approximates that observed in normoxia (Fig. 5C). A similar pattern was observed in ground squirrels exposed to hypoxia,

with little apparent change in $\dot{V}_{O_2}$ or $\dot{V}_{CO_2}$ as T_a decreased unless these values were corrected for the falling T_b (Barros et al., 2001). Thus, the further decrease in T_b as T_a falls may serve to reduce energetic expenditure (and conserve O_2) at very low T_a.

4.3 Relationship between decreases in T_b and metabolic rate during hypoxia

Changes in T_b and energy metabolism clearly influence one another in endotherms. Indeed, T_b depression during hypoxia will decrease metabolic rate through the Q_{10} effect, all else being equal. As previously discussed, we temperature-corrected the $\dot{V}_{O_2}$ data for hypoxic quail to their normoxic T_b to estimate the T_b-independent effect of hypoxia on $\dot{V}_{O_2}$ at various ambient temperatures. The actual Q_{10} for metabolic rate is not known for Japanese quail, so we assumed a constant Q_{10} of 3 (cf. Barros et al., 2001). Because of the relatively small T_b depression exhibited by quail at 9% O_2, this operation had only a modest effect on $\dot{V}_{O_2}$ (e.g., +11% at 13° C and +5% at 33°C). Even so, corrected $\dot{V}_{O_2}$ no longer differed between normoxia and hypoxia at 33°C (Fig. 5C); there was also no effect of hypoxia on either the corrected or uncorrected $\dot{V}_{O_2}$ at 36°C, which is noteworthy since T_b was not depressed at this T_a. These results must be interpreted cautiously since we used an assumed value for Q_{10}, but they suggest that the passive effects of lower T_b on energy metabolism can completely explain the hypoxia-induced decreases in $\dot{V}_{O_2}$ observed within the normoxic thermoneutral zone. This may also be the case in mammals: correction of $\dot{V}_{O_2}$ to normoxic T_b (Q_{10}=3) appears to explain most (if not all) of the hypoxia-induced reduction in $\dot{V}_{O_2}$ observed in ground squirrels at thermoneutrality (Barros et al., 2001). Corrected $\dot{V}_{O_2}$ was still significantly reduced by hypoxia at T_a below the normoxic LCT in quail; however, it is likely that metabolic depression below the nomoxic LCT reflects the combined effects of T_b depression and the downward shift of the LCT (and associated decrease in shivering/nonshivering thermogenesis at a given T_a). Importantly, these results do not preclude an important role for active metabolic suppression in the thermoregulatory and metabolic responses of Japanese quail to hypoxia. In ground squirrels, for example, it has been suggested that metabolic suppression facilitates the decline in T_b soon after the onset of hypoxia (Barros et al., 2001).

Despite the obvious interaction between T_b and $\dot{V}_{O_2}$, there was no apparent relationship between the magnitudes of the decreases in T_b and $\dot{V}_{O_2}$ during hypoxia in Japanese quail (section 3.6, above). This is consistent with several other studies on birds which note decreases in T_b with no decrease, and perhaps even an increase, in $\dot{V}_{O_2}$ during hypoxia (Boggs and Kilgore, 1983; Kilgore et al., 2008; Scott et al., 2008). Thus, the decrease in either T_b or $\dot{V}_{O_2}$ during hypoxia is not solely dependent on a decrease in the other. Moreover, metabolic heat production during hypoxia may be underestimated by measuring $\dot{V}_{O_2}$ – anaerobic mechanisms may maintain metabolic heat production (at least temporarily) even as $\dot{V}_{O_2}$ falls. Although direct calorimetry is needed to fully address this issue, the observed increase in blood lactate levels during exposure to 9% O_2 supports this hypothesis. Blood lactate levels also increase in rats during acute hypoxia (7% O_2) (Bicego et al., 2002); interestingly, there was no correlation between blood lactate levels and T_b depression in the rat, suggesting that there is no direct relationship between anaerobic heat production and T_b depression in that species. These data suggest that increased thermolysis is principally responsible for the reduced T_b during hypoxia in Japanese quail, and perhaps in other birds.

Although not specifically investigated here, two major routes of thermolysis are likely involved in hypoxia-induced reduction of T_b in quail: (1) peripheral vasodilation and (2) convective and evaporative cooling associated with increased ventilation. First, a recent study by Scott et al. (2008) demonstrated increased bill surface temperature during hypoxia in the pekin duck, greylag goose and bar-headed goose, suggesting that birds increase blood flow to poorly insulated regions of the body (*i.e.*, thermal windows) during hypoxia to promote heat loss. A similar strategy has been proposed for the golden-mantled ground squirrel (Tattersall and

Milsom, 2003). In the latter species, surface temperatures of various body regions (e.g., feet, ears, nose) increase during the onset of hypoxia and then return toward normal, presumably once the new T_b set point is achieved (Tattersall and Milsom, 2003). Together, these data indicate that birds can regulate peripheral blood flow to reduce T_b during hypoxia independent of changes in metabolic rate.

Second, the hypoxic ventilatory threshold (*i.e.*, where ventilation increases by at least 10%) occurs at an inspired partial pressure of O_2 (P_{O_2}) of 90–100 Torr in adult, male Japanese quail (R.W. Bavis & D.L. Kilgore, Jr., unpublished data). This threshold is equivalent to approximately 13% O_2, with ventilation expected to increase by ~30% and ~50% at 11% O_2 and 9% O_2, respectively, almost exclusively through an increase in respiratory frequency (R.W. Bavis & D.L. Kilgore, Jr., unpublished data). Thus, respiratory heat loss could contribute to the progressive decrease in quail T_b observed at FI_{O_2}<13% O_2. Consistent with this hypothesis, T_b also decreased during exposure to hypercapnia, a powerful respiratory stimulant that is not consistently reported to reduce T_b or $\dot{V}_{O_2}$ in mammals (Mortola and Lanthier, 1996). Interestingly, we observed moisture inside the respirometry chamber after exposure to moderate-severe hypercapnia, implying increased respiratory water loss. However, the present study cannot rule out the possibility that hypercapnia itself induces an anapyrexic response (Bicego et al., 2007).

On the other hand, increased ventilation will also increase heat production by the respiratory muscles due to their increased metabolic demands. The additional metabolic costs of the respiratory muscles could partially offset, or perhaps even exceed, temperature-dependent decreases in $\dot{V}_{O_2}$ and/or active metabolic depression elsewhere in the body. Indeed, variation in the energetic costs of the cardiorespiratory responses to hypoxia, and the associated heat production, may contribute to variation in the T_b and metabolic responses to hypoxia within and among bird species, as well as to differences between birds and mammals. Given that most endothermic vertebrates exhibit decreased T_b during acute hypoxia, however, it appears that hypoxia-induced thermolysis generally exceeds the increased heat production associated with cardiorespiratory responses to hypoxia.

4.4 Acclimation to hypoxia

Japanese quail were better able to maintain T_b during acute hypoxia following acclimation to chronic hypoxia. This result is similar to that of Weathers and Snyder (1974) for quail acclimated to simulated high altitude (6100 m) for six weeks and studied at 5°C. The present study, however, demonstrates that this plasticity is observable in as little as one week, and that this effect is evident at relatively mild T_a. Contrary to the earlier study, acclimation had no effect on $\dot{V}_{O_2}$ in normoxic or hypoxic conditions; Weathers and Snyder (1974) observed a considerably greater $\dot{V}_{O_2}$ in acclimated quail under normoxic conditions, although this difference tended to decrease as inspired P_{O_2} decreased. It is possible that longer durations of hypoxia have greater effects on metabolic rates.

The ability to maintain T_b better following acclimation likely reflects increased capacity to deliver O_2 to the tissues responsible for anapyrexia. In birds, similar to mammals, chronic hypoxia tends to increase parabronchial ventilation (i.e., increased ventilatory chemosensitivity; Powell et al., 2000), although the time course of these changes are not known for the Japanese quail. Blood O_2 carrying capacity also increases, both due to progressive increases in hematocrit and hemoglobin concentration in Japanese quail (Jaeger and McGrath, 1974; Weathers and Snyder, 1974). Importantly, these changes begin within the first week of chronic hypoxia (Jaeger and McGrath, 1974) and, therefore, may contribute to changes in the thermoregulatory response to hypoxia observed in the present study. It is also possible that chronic hypoxia alters the hypoxic sensitivity of CNS structures mediating anapyrexia, but this hypothesis awaits further study.

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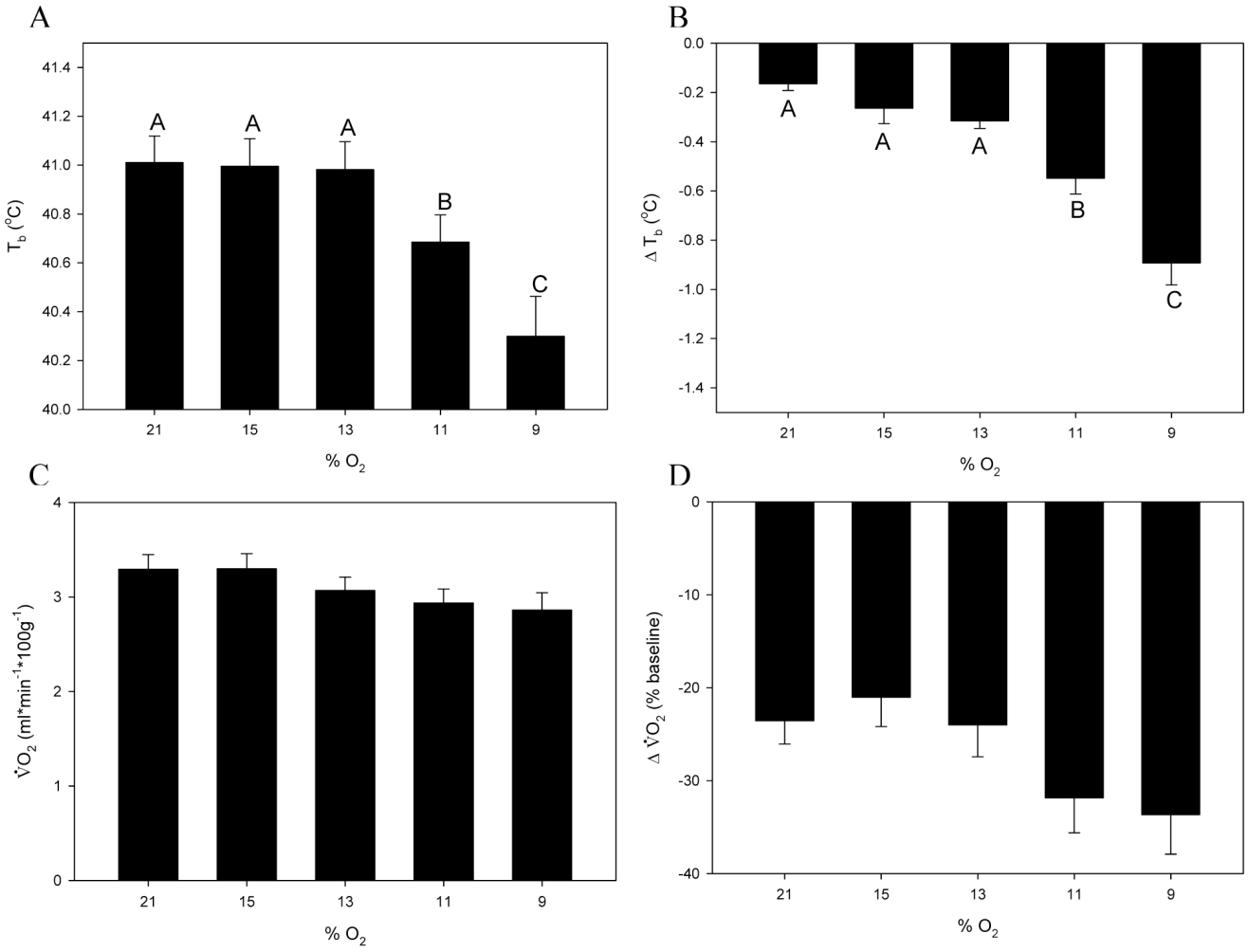

Fig. 1.

Body temperature (T_b) and O_2 consumption ($\dot{V}_{O_2}$) of quail during normoxia (21% O_2) and during exposure to four levels of hypoxia (15, 13, 11, and 9% O_2) (T_a = 23°C). T_b and $\dot{V}_{O_2}$ are presented both as the minimum value achieved during the 60-min test gas exposure (A, C) and as the corresponding change from baseline (B, D); see text for details. Values are mean ± SEM; n = 16. Letters denote statistical comparisons: groups with different letters are significantly different (P<0.05). In panel D, one-way repeated measures ANOVA detected a significant effect of hypoxia (P=0.03), but post-hoc tests were unable to determine which groups differed.

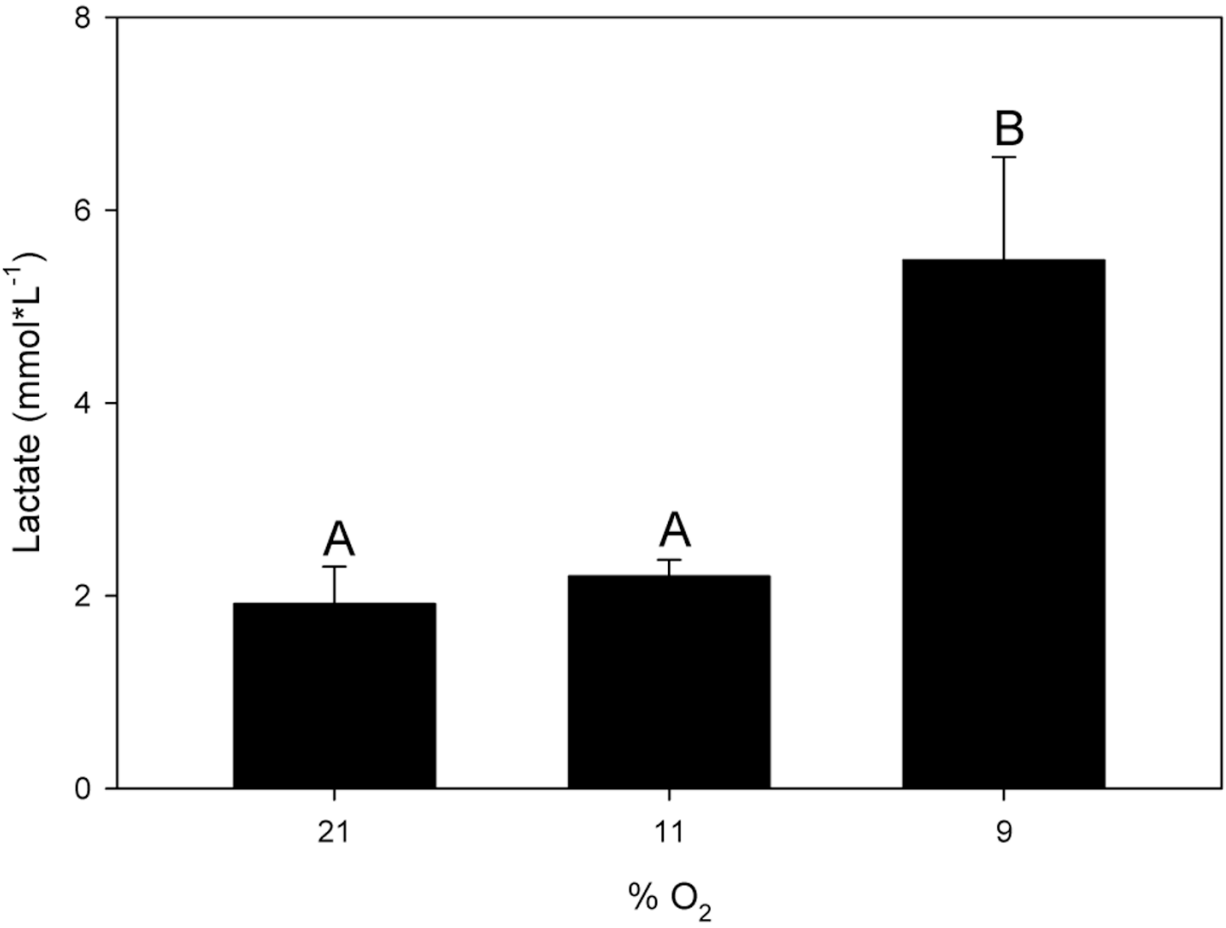

Fig. 2.

Blood lactate concentrations for quail exposed to normoxia (21% O_2) and two levels of hypoxia (11% and 9% O_2) (T_a = 23°C). Values are mean ± SEM; n=6. Groups with different letters are significantly different (P<0.05).

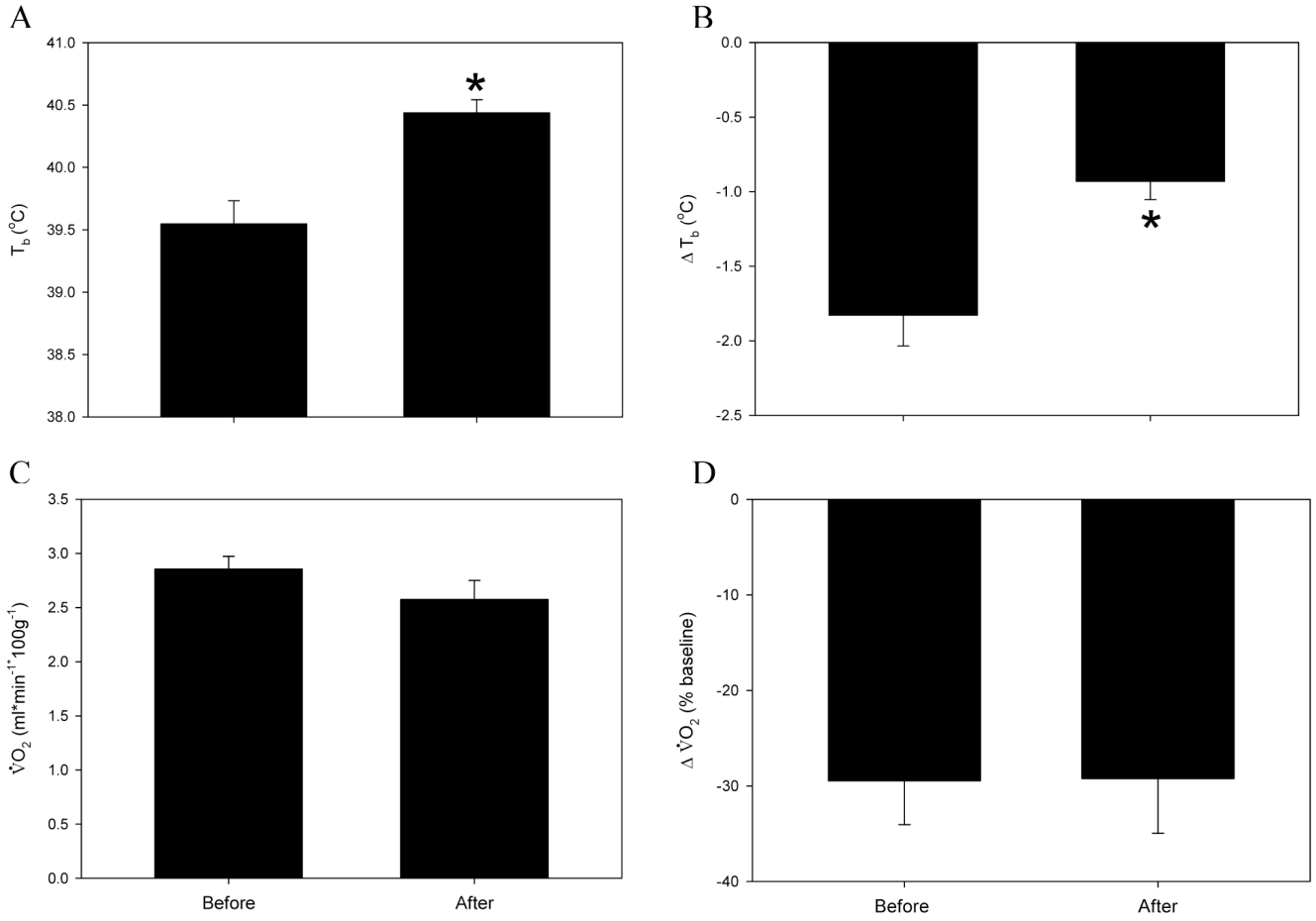

Fig. 3.

Effect of acclimation to chronic hypoxia on the body temperature (T_b) and metabolic ($\dot{V}_{O_2}$) responses to acute hypoxia (9% O_2). Quail were studied before and immediately after a 7-day exposure to 10% O_2. T_b and $\dot{V}_{O_2}$ are presented both as the minimum value achieved during the 60-min acute exposure to 9% O_2 (A, C) and as the change from baseline (B, D) (T_a = 23°C). Values are mean ± SEM; n=7 for T_b, n=10 for $\dot{V}_{O_2}$. * P<0.05 vs. before acclimation values.

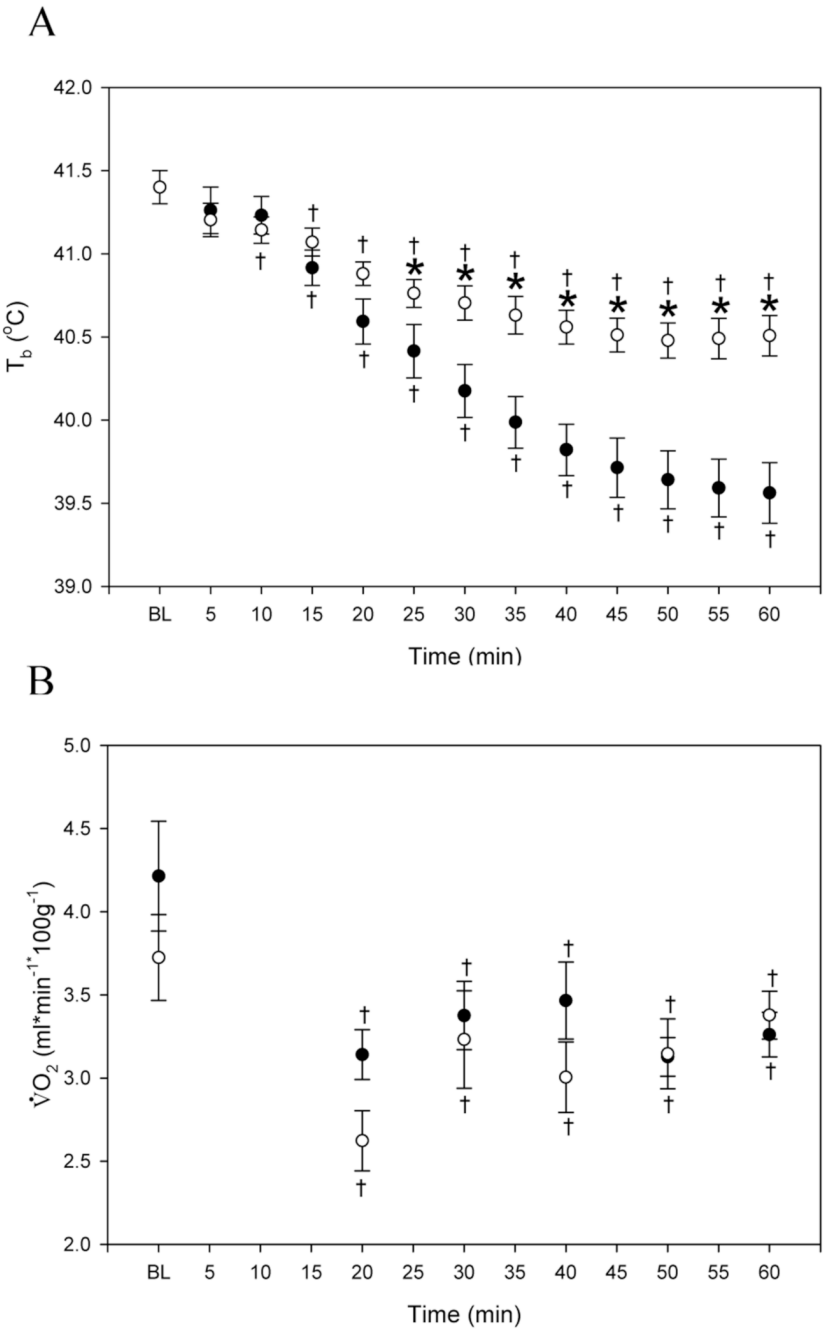

Fig. 4.

Time course for changes in T_b and $\dot{V}_{O_2}$ during acute hypoxia (9% O_2) in quail before (●) and after (○) acclimation to chronic hypoxia (7 days, 10% O_2) (T_a = 23°C). Baseline (BL) represents the average value during the preceding 30 min of normoxia. T_b and $\dot{V}_{O_2}$ are reported at 5- and 10-min intervals after the switch to hypoxia, respectively; to ensure that O_2 levels reached a steady state within the respirometer after the switch to 9% O_2 prior to calculating $\dot{V}_{O_2}$, $\dot{V}_{O_2}$ is not reported for the 10 min time point. Values are mean ± SEM; n=7. * P<0.05 vs. before acclimation values at the same time point; † P<0.05 vs. BL.

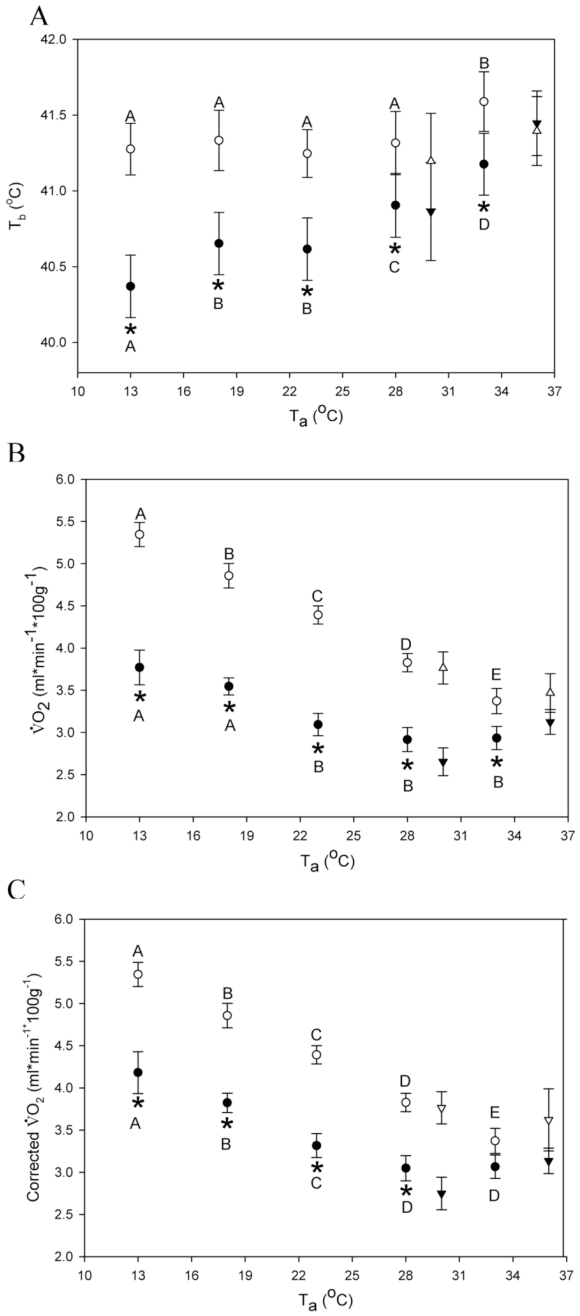

Fig. 5.

Effect of ambient temperature on T_b (A) and $\dot{V}_{O2}$ (B) for quail during normoxia (○; 21% O_2) or hypoxia (●; 9% O_2). Values are mean ± SEM; n=16. * P<0.05 vs. normoxia at the same T_a. For the effect of T_a within each treatment (i.e., within normoxia or within hypoxia), groups with different letters differ from one another (P<0.05). Triangles (△ normoxia, ▲ hypoxia) represent a subset of quail (n=6) studied at 30 and 36°C; these data were not included in the overall statistical analysis. In panel C, $\dot{V}_{O2}$ during hypoxia has been temperature corrected to the normoxic T_b at the corresponding T_a, assuming a Q_{10} of 3; see text for details.

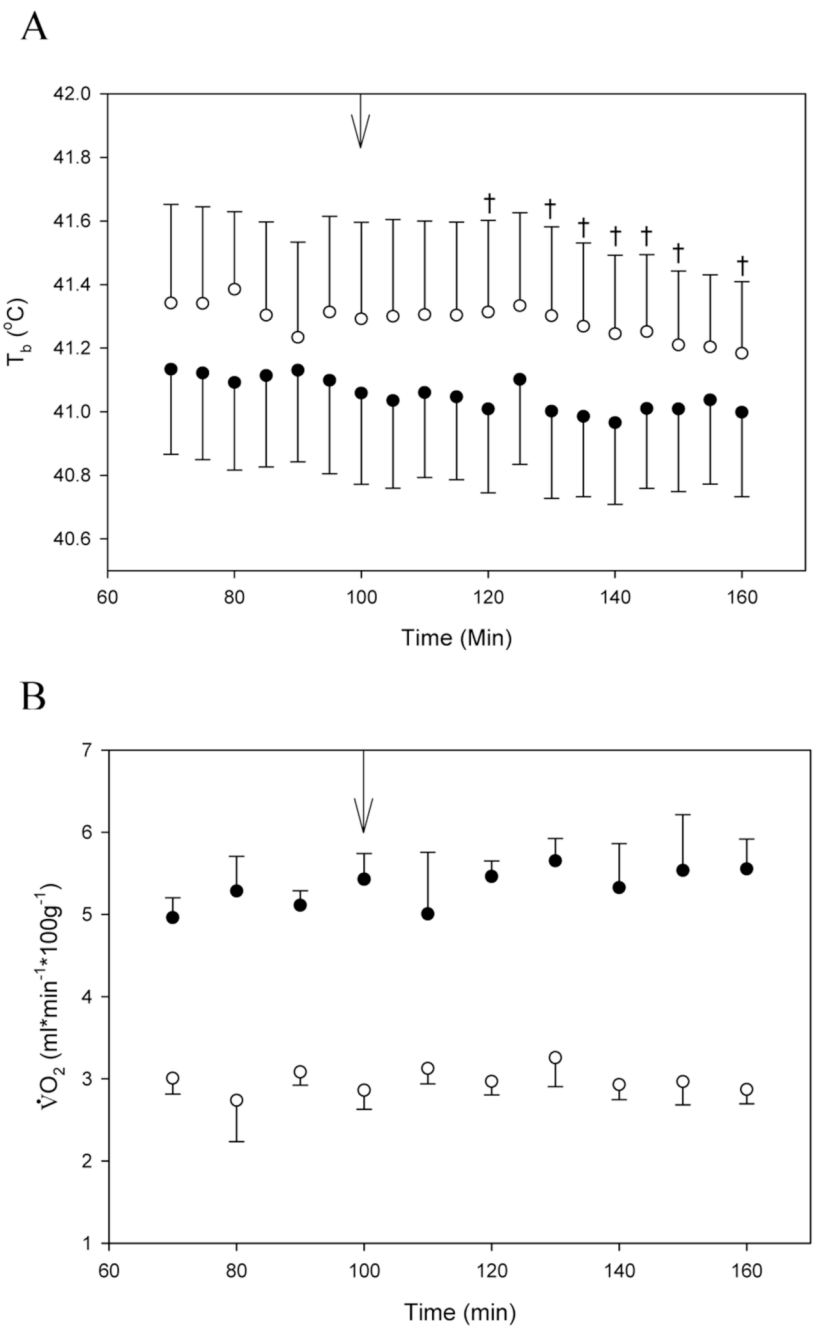

Fig. 6.

Time-dependent changes in T_b (A) and $\dot{V}_{O2}$ (B) of quail maintained in normoxia (21% O_2) for 160 min. Quail were studied at ambient temperatures of either 33°C (○) or 13°C (●). Although quail were exposed to normoxia for the entire 160 min, the arrow at minute 100 indicates the time at which quail would have been switched to hypoxia in a typical experiment. Values are mean ± SEM; n = 6. † $P<0.05$ vs. baseline (i.e., the average value for min 70–100).

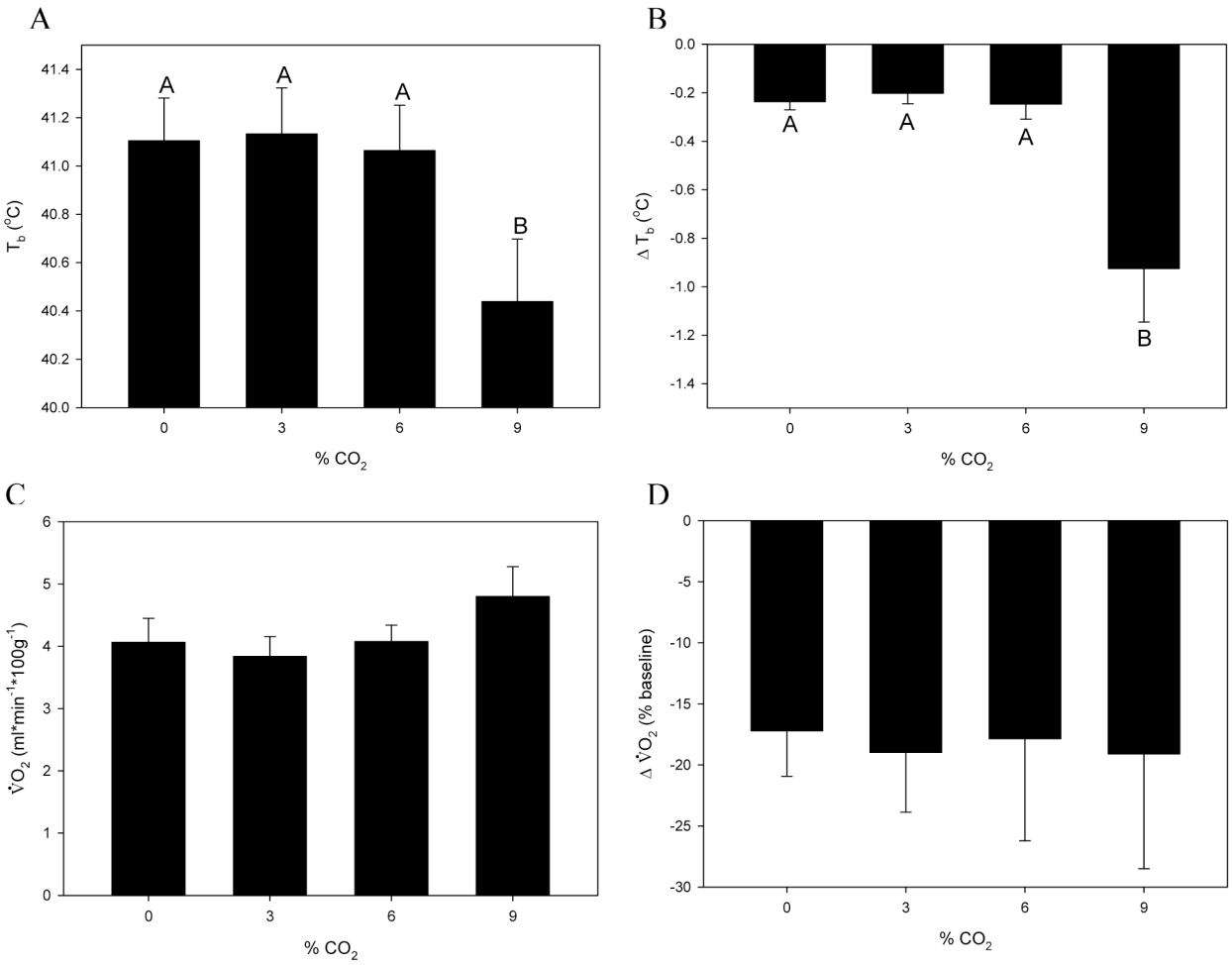

Fig. 7.

Effect of acute hypercapnia on body temperature (T_b) and O_2 consumption ($\dot{V}_{O2}$) of adult quail. Quail were exposed to normocapnia (0% CO_2) and three levels of hypercapnia (3, 6, and 9% CO_2) (T_a = 23°C). T_b and $\dot{V}_{O2}$ are presented both as the minimum value achieved during the 60-min test gas exposure (A, C) and as a change from baseline (B, D). Values are mean ± SEM; n = 10. Groups with different letters are significantly different (P<0.05).

Table 1

Normoxic T_b and metabolic rate of adult quail after 100 min and after 160 min in the respirometer chamber (T_a = 23°C).

	100 min	160 min
n	22 (10)	10
Mass (g)	117±1 (119±2)	
T_b (°C)	41.2±0.1 (41.3±0.2)	41.2±0.1
$\dot{V}O_2$ (mL O_2 min^{-1} $100g^{-1}$)	4.3±0.2 (4.8±0.4)	4.3±0.3
$\dot{V}CO_2$ (mL CO_2 min^{-1} $100g^{-1}$)	3.2±0.1 (3.6±0.4)	3.2±0.2
RQ	0.77±0.03 (0.77±0.03)	0.75±0.02

Values are mean ± S.E.M. For the 100 min time point, values in parentheses are for the subset of quail also studied at 160 min.