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HOVERING PERFORMANCE OF HUMMINGBIRDS IN HYPEROXIC GAS MIXTURES

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Summary

Hummingbirds evolved during a period of decline in atmospheric oxygen concentration and currently encounter varying levels of oxygen availability along their elevational distribution. We tested the hypothesis that inspiration of hyperoxic gas increases hummingbird hovering performance when birds are simultaneously challenged aerodynamically. We measured the maximum duration of hovering flight while simultaneously monitoring the rate of oxygen consumption of ruby-throated hummingbirds (*Archilochus colubris*) in low-density heliox that was either normoxic (21 % O_2) or hyperoxic (35 % O_2). As air density decreased below 0.85 kg m^{-3}, hummingbirds hovered significantly longer in hyperoxia than in normoxia, but the air density at which the birds could no longer sustain hovering flight was independent of oxygen concentration. At low air densities in hyperoxia flight trials, hummingbirds appeared to increase their rate of oxygen consumption relative to flight sequences at equivalent densities in normoxia trials, but these differences were not significant. We tested the hypothesis that hummingbirds can discriminate between environments that differ in oxygen concentration. In another density-reduction experiment, hummingbirds were allowed to choose between artificial feeders infused with either normoxic or hyperoxic gases. The hypothesis was not supported because birds failed to associate oxygen concentration with a particular feeder independently of air density. Supplemental oxygen thus yields increased hovering duration at intermediate air densities, but the minimum density at which birds can fly is limited exclusively by aerodynamic considerations.

Key words: *Archilochus colubris*, hovering, flight performance, hyperoxia, ruby-throated hummingbird.

Introduction

Flying hummingbirds exhibit the highest mass-specific rates of aerobic metabolism among vertebrates, twice as high as those of exercising mammals (Suarez, 1992; Suarez et al., 1991; Wells, 1993). However, *in vitro* measurements of respiratory rates per unit mitochondrial volume reveal no significant differences between hummingbirds and mammals (Suarez et al., 1991). The elevated metabolic rate of hummingbirds *in vivo* is probably attained through a suite of physiological adaptations including high mitochondrial density, high pulmonary diffusing capacity, high cardiac output, a high ratio of capillary surface area to muscle fiber surface area, increased cristae density and high concentrations of enzymes involved in energy metabolism (Suarez, 1998). Thus, it seems likely that the increased rates of mitrochondrial respiration of hummingbirds result from a higher rate of oxygen delivery compared with other vertebrates (Suarez et al., 1991). If this is true, then maximum hovering performance in hummingbirds should increase with increased oxygen availability.

Previous studies of hummingbird flight performance in hypodense, normoxic heliox revealed that hovering flight performance is limited by the maximum stroke amplitude of the wings. As air density decreased, hummingbirds increased their stroke amplitude to values near 180 °, at which point they could no longer sustain hovering flight and exhibited aerodynamic failure (Chai and Dudley, 1995). The effects of oxygen availability on hovering ability were also studied for hummingbirds hovering in low-density heliox that was either hyperoxic or normoxic (Chai et al., 1996). The hypotheses tested were that hummingbirds breathing hyperoxic heliox (35 % O_2) would increase their rates of oxygen consumption and exhibit aerodynamic failure at a lower air density than birds breathing normoxic heliox (21 % O_2). These hypotheses were not supported, although there was some indication of increased hovering duration at intermediate air densities. Thus, elevated oxygen levels might enhance hovering flight performance at intermediate air densities, whereas the density at which aerodynamic failure occurs is ultimately limited by the geometry of the wing motions.

Here, we address three questions. (i) Do hummingbirds hover longer when breathing 35 % oxygen at intermediate air densities compared with birds breathing normoxic air at equivalent densities? (ii) Do hummingbirds breathing 35 % oxygen consume more oxygen at intermediate air densities than hummingbirds breathing 21 % oxygen? (iii) Do hummingbirds discriminate between micro-habitats that differ

in oxygen availability as air density decreases? These questions were addressed through a combination of three experiments. The first experiment was performed in 1995 (Chai et al., 1996), and here we present a re-analysis of these data focusing on the intermediate air densities (hereafter referred to as the aerodynamic failure experiment). The second experiment determined the hovering durations and rates of oxygen consumption of hummingbirds in gases with equivalent air densities (the density experiment). The third experiment tested the hummingbirds' ability to discriminate between hyperoxic and normoxic gas (the choice experiment). The first question was addressed in all three studies. The second question was first addressed using the aerodynamic failure experiment and then re-tested in the density experiment. The third question was addressed through the choice experiment.

Materials and methods

Animals

All experiments were conducted with captive ruby-throated hummingbirds (*Archilochus colubris* L.) mist-netted in Travis County, Texas, USA, during the autumn migratory seasons (September) of 1995 and 1996. Because the birds would normally have over-wintered in Mexico and then returned to the USA for the summer breeding season, we maintained a 12 h:12 h light:dark cycle during the autumn and winter, and then used natural light cycles after April 1. One to three birds were housed together in nylon mesh cages (90 cm×90 cm×90 cm), and birds were fed daily on a sugar solution that also included low concentrations of proteins and lipids (Roudybush). Hummingbird care was in accordance with federal, state and university animal care guidelines.

Aerodynamic failure experiment

In 1995, three adult females (mean mass 4.365±0.175 g, mean wing loading 34.25±1.772 N m^{-2}), three adult males (mean mass 3.793±0.239 g, mean wing loading 40.33±3.792 N m^{-2}) and one juvenile male (mass 4.125 g, wing loading 44.5 N m^{-2}) (means ± S.D.) were used to test the aerodynamic failure hypothesis. Hovering flight was studied in a Plexiglas chamber with the same dimensions as the nylon cages. The chamber contained a perch for resting and a feeder covered by an artificial tubular corolla and a trap door. Every 20 min, the trap door was opened, and hovering birds could feed by inserting their entire head into the corolla. The trap door was kept open only while the birds were hovering at the feeder. Hummingbirds were exposed to the feeding protocol for 2 days prior to the experiment to ensure that they would feed through the corolla and would remain feeding until satisfied or until they could no longer sustain hovering flight at the feeder.

Hovering flight performance was studied in two separate density-reduction trials that differed in oxygen concentration: infusion of hyperoxic heliox (35 % O_2, balance He; density 0.57 kg m^{-3}) and infusion of normoxic heliox (21 % O_2, balance He; density 0.40 kg m^{-3}). The gases were infused into the chamber at a constant rate and gradually replaced normoxic and normodense air. The trials progressed until the bird reached aerodynamic failure (see Chai and Dudley, 1995). Immediately following failure, normal air (21 % O_2, 79 % N_2; density 1.2 kg m^{-3}) was pumped into the chamber. Trials were conducted on separate days, and the treatment order (hyperoxia *versus* normoxia) was determined arbitrarily.

All trials began by allowing hummingbirds several feeding bouts in normal air. The birds were allowed to feed once every 15–20 min. Air density was determined acoustically (see Dudley, 1995), and the oxygen concentration of the corolla's air volume was measured using an Applied Electrochemistry S-3A/I oxygen analyzer. Four variables were measured or calculated for each feeding bout: (i) oxygen consumption was monitored within the artificial corolla during feeding; (ii) the oxygen concentration of the flight chamber was measured immediately before and after a feeding bout; (iii) hover-feeding duration was timed from video recordings (60 frames s^{-1}); and (iv) muscle mechanical power output was estimated using a detailed model of hovering aerodynamics (Ellington, 1984) incorporating kinematic and morphological data obtained from individual hummingbirds as well as the physical properties of the gases.

The effects of chamber air density and oxygen treatment on hover-feeding duration and the rate of oxygen consumption were tested through repeated-measures analysis of variance (ANOVA). We compared only the two feeding bouts just prior to the bout in which the birds exhibited aerodynamic failure.

Density experiment

In 1996, we captured three adult female (mean mass 4.452±0.526 g, mean wing loading 36.782±4.650 N m^{-2}) and four juvenile male (mean mass 4.340±0.489 g, mean wing loading 42.159±3.504 N m^{-2}; means ± S.D.) hummingbirds to test hovering performance at intermediate air densities and to determine whether hummingbirds responded to differences between normoxic and hyperoxic gas mixtures. Because the external morphology of juvenile males is similar to that of females (Chai and Dudley, 1999), we avoided the confounding effects of gender-specific body morphology on hovering flight performance.

In addition to the confounding effects of gender-specific morphology, three other aspects of the aerodynamic failure experiment may have confounded the results. First, the experiment was performed on two separate days, between which slight differences in mass and behavior within individuals could have significant effects. Second, the birds were allowed access to the feeders every 20 min, but the air density and oxygen concentration were not identical within and among trials. Third, the densities of normoxic and hyperoxic heliox differ. To control for these effects, we performed a second experiment that was a modified version of the aerodynamic failure experiment. The second experiment used a smaller chamber (60 cm×60 cm×60 cm) so that aerodynamic failure could be reached more quickly and both hyperoxia and normoxia trials could be performed on the same day. At the

start of a trial, the chamber contained only normodense and normoxic air. This ambient air was then gradually replaced *via* gas infusion at a constant rate (5 l min^{-1}). Gas infusion continued during hover-feeding trials to ensure that feeding bouts occurred at equivalent air densities among trials. We used the same hyperoxic heliox (35 % oxygen, balance He; density 0.57 kg m^{-3}) for the hyperoxia trials but a denser normoxic mixture (21 % O_2, 16 % N_2, balance He; density 0.57 kg m^{-3}) for the normoxia trials.

As in the aerodynamic failure experiment, the hover-feeding duration and wingbeat kinematics were measured from video recordings. Oxygen concentration was measured from the corolla volume, and power output was calculated using the aerodynamic model of hovering flight (Ellington, 1984). Each bird was tested in only one trial of each of the two gas treatments.

Repeated-measures ANOVA was used to test for the effects of gas treatment and air density on hover-feeding duration and rates of oxygen consumption. Only the three hovering flights prior to aerodynamic failure were analyzed for differences in hover-feeding duration because previous studies had indicated no difference in hover-feeding duration at the density of aerodynamic failure. Oxygen consumption was analyzed for the two feeding bouts prior to aerodynamic failure.

Choice experiment

The birds from 1996 were also tested for their ability to discriminate between hyperoxia and normoxia as the larger Plexiglas chamber was gradually infused with normoxic heliox to lower air density. Trials were made up of individual feeding sessions at each air density until the birds reached the density of aerodynamic failure. A feeding session consisted of three feeding bouts. During the first bout, the bird had access to only one of two feeders that had either normoxic heliox or hyperoxic heliox infused into the corolla. Because the bird's head was completely within the corolla during feeding, the inspired gas mixture during hovering was controlled. In the second feeding bout, the bird had access to the feeder infused with the alternative gas mixture. In the third feeding bout, both trap doors were removed, and the hummingbird was allowed to choose between the two gas-infused feeders. The order of exposure to the two gases alternated between the first two feeding bouts, but both feeders were always accessible in the third feeding bout. The positions of the feeders were consistent throughout the trial, so that hummingbirds could associate feeder position with gas mixture. The entire trial was performed twice on each bird to control for a positional bias; the relative locations (left *versus* right) of the normoxia-infused feeder and hyperoxia-infused feeder were reversed between the two trials.

Hover-feeding duration during each feeding bout was measured with a stopwatch. During the third bout of each feeding session, we also recorded which feeder was chosen. We analyzed the effects of density and gas treatment on hovering duration using repeated-measures ANOVA. We examined the feeder preference in three ways: (i) analysis of the two feeding sessions just prior to failure, (ii) analysis of the complete trial, and (iii) analysis of the feeding session at failure.

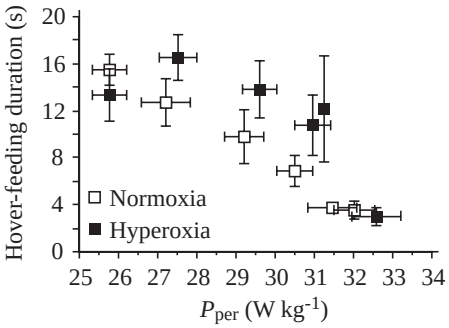

Fig. 1. Estimated body mass-specific power output (W kg^{-1}) assuming perfect elastic energy storage (P_{per}) *versus* the measured hover-feeding duration of ruby-throated hummingbirds (means ±1 S.E.M., N=6) hovering in either hyperoxic (filled squares) or normoxic (open squares) heliox. Data are from the hummingbirds used in the aerodynamic failure experiment.

Results

Aerodynamic failure experiment

In both normoxia and hyperoxia, hummingbird hovering duration decreased as the calculated muscle mechanical power output increased (Fig. 1). The initial analysis of the hover-feeding duration data from 1995 was performed using a repeated-measures ANOVA that included all flight sequences and failed to detect any difference (all P>0.15) in hovering duration or oxygen consumption between gas treatments (Chai et al., 1996). Our present analysis of hovering performance at intermediate densities used a restricted subset of the same data. Hovering duration and rates of oxygen consumption at these densities tended to increase in hyperoxia, but these trends were not statistically significant (Table 1). However, the restricted analysis did exhibit stronger effects of gas treatment on both independent variables.

Table 1. *Results of a repeated-measures ANOVA investigating the effects of gas treatment and density on hover-feeding duration and rate of oxygen consumption for the birds tested in the aerodynamic failure experiment*

	d.f.	MSE	F	P
Hover-feeding duration				
Treatment	1,5	102.67	5.83	0.061
Density	1,5	87.17	6.19	0.055
Treatment × density	1,5	1.25	0.35	0.579
Rate of oxygen consumption				
Treatment	1,5	14.20	3.84	0.107
Density	1,5	2.37	0.42	0.547
Treatment × density	1,5	0.71	0.14	0.725

MSE, mean squared error.

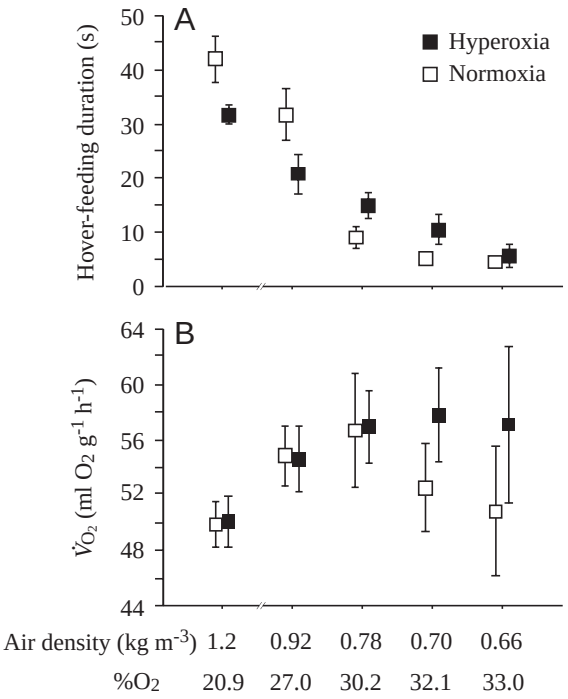

Fig. 2. Hover-feeding duration (A) and rates of oxygen consumption ($\dot{V}_{O_2}$) (B) across decreasing densities as normal air was gradually replaced with either hyperoxic (filled squares) or normoxic (open squares) heliox. Values are means ±1 S.E.M. (N=6) from individual birds at each air density level. %O_2 is the oxygen concentration at each density under hyperoxia. Data are from the hummingbirds used in the density experiment.

Density experiment

The density experiment tested hummingbirds at equivalent air densities between the two gas treatments. Each bird was tested in both low-density hyperoxic gas and low-density normoxic gas at five air densities from 1.2 kg m^{-3} (normal air) to 0.66 kg m^{-3}. Hover-feeding events were grouped by rounding air densities at which feeding occurred. Some birds reached aerodynamic failure at densities of 0.7 kg m^{-3} or above. These birds were forced to reduce mass by limiting their diet and were then tested again to provide flight sequences for all birds at all densities.

As in earlier experiments, the hover-feeding duration of ruby-throated hummingbirds decreased with decreasing air density (Fig. 2A). The effects of gas treatment and air density on hover-feeding duration were tested using the three hovering sequences prior to failure. Both variables significantly affected hover-feeding duration (Table 2).

Rates of oxygen consumption increased during hovering with decreasing density in the hyperoxic gas treatment (Fig. 2B). However, no effects of either gas treatment or air density for the two flight sequences prior to failure for each bird were detected (Table 2). Similarly, body mass-specific power output increased with decreasing density (Fig. 3), but the estimated muscle power output was the same for each density irrespective of gas treatment.

Table 2. *Results of a repeated-measures ANOVA investigating the effects of gas treatment and density on hover-feeding duration and rate of oxygen consumption for the birds tested in the density experiment*

	d.f.	MSE	F	P
Hover-feeding duration				
Treatment	1,5	149.74	10.12	0.025
Density	2,10	148.24	11.64	0.002
Treatment × density	2,10	20.40	1.31	0.313
Rate of oxygen consumption				
Treatment	1,5	45.98	2.85	0.152
Density	1,5	0.48	0.03	0.874
Treatment × density	1,5	26.04	0.71	0.439

MSE, mean squared error.

Choice experiment

During the choice experiment, hummingbirds also decreased their hover-feeding duration with decreasing air density. When feeding from the hyperoxia-infused corollas at intermediate densities (below 0.85 kg m^{-3} but above the density of aerodynamic failure), the hummingbirds hovered for longer than when feeding through normoxia-infused corollas at the same density. Furthermore, these differences between gas treatments became more pronounced with decreasing air density (Fig. 4). Analysis of only the last two bouts prior to failure revealed a significant effect of gas treatment on hover-feeding duration, but the mean hover-feeding duration during the bout just prior to failure was not statistically different from that of the preceding bout (Table 3). Despite increased hovering performance when feeding from masks infused with hyperoxia, the hummingbirds did not discriminate between masks infused with hyperoxic and normoxic mixtures (Fig. 5).

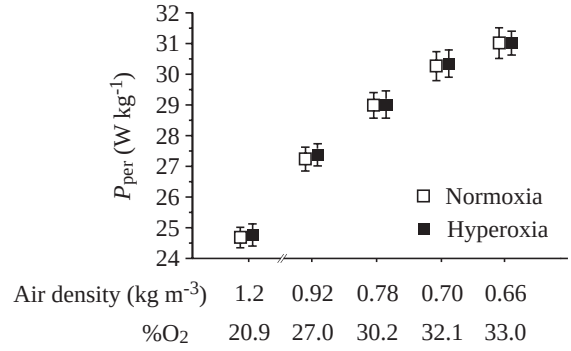

Fig. 3. Estimated body mass-specific mechanical muscle power output (W kg^{-1}) assuming perfect elastic energy storage (P_{per}) during hyperoxic (filled squares) and normoxic (open squares) heliox replacement of normal air. Values are means ±1 S.E.M. (N=6) from individual birds at each air density level. %O_2 is the oxygen concentration at each density under hyperoxia. Data are from the hummingbirds used in the density experiment.

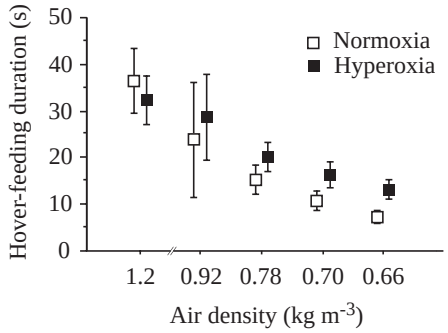

Fig. 4. Hover-feeding duration (means ±1 S.E.M., *N*=7) of ruby-throated hummingbirds across decreasing air densities as normal air was gradually replaced with either hyperoxic (filled squares) or normoxic (open squares) heliox. Values are means ±1 S.E.M. from individual birds at each density level. $\%O_2$ is the oxygen concentration at each density under hyperoxia. Data are from the hummingbirds used in the choice experiment.

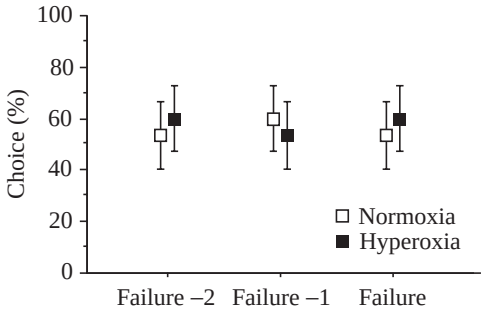

Fig. 5. Discrimination between feeders with hyperoxia-filled (filled squares) or normoxia-filled (open squares) corollas during failure and the two feeding bouts just prior to failure of hovering. Values are the mean incidence ±1 S.E.M. of choice for hummingbirds at that density (*N*=7). Birds that sampled both feeders during one feeding bout received a score of 1 for both feeders. Data are from the hummingbirds used in the choice experiment.

Discussion

Ruby-throated hummingbirds decreased their hovering duration as the estimated whole-bird power output increased. This relationship suggests that birds are limited in their ability to produce power as requirements for hovering flight increase. Accordingly, power output also increased as air density decreased (Fig. 3). What is particularly notable about this relationship is that power output did not differ between gas treatments at equivalent densities, indicating that the power requirements are identical between normoxia and hyperoxia at any given air density.

In general, birds are extremely tolerant of variations in oxygen and carbon dioxide concentrations. In deep hypoxia, however, respiration is ultimately limited by blood perfusion (Fedde et al., 1989; Shams and Scheid, 1989). Birds can tolerate hypoxia because avian hemoglobins have a high oxygen affinity; one of the highest is the oxygen affinity of the hemoglobin of bar-headed geese (*Anas indicus*), which breed in the Tibetan Plateau and have been recorded migrating at elevations greater than 9000 m (Black and Tenney, 1980). Other features of avian respiration physiology that aid in oxygen delivery include: (i) multiple exchanges of air in the lung per individual inspiration; (ii) an enormous increase in the exchange surface as a result of the very small diameters of the air capillaries; (iii) maximal thinning of the air/blood tissue barrier; and (iv) a large relative lung-to-blood volume ratio with a marked increase in the capillary volume (Dubach, 1981).

Hummingbirds possess three additional features of their muscular system that act to enhance oxygen delivery: (i) a small muscle fiber size, (ii) a large capillary length per fiber volume, and (iii) a high mitochondrial volume density per volume of muscle fiber. The ratio of capillary supply to mitochondria volume gives an index termed the ‘capillary-to-fiber interface’, which is the capillary surface area per fiber surface area. The value of this index is particularly high in hummingbird flight muscles and may allow for a greater oxygen flux rate from the capillaries to the muscle fiber mitochondria (Mathieu-Costello et al., 1992). In addition, hummingbirds have very high rates of breathing ranging from 180 to 600 breaths min^{-1} (Lasiewski, 1964) and rates of oxygen consumption ranging between 40 and 50 $ml\,O_2\,g^{-1}\,h^{-1}$ for hummingbirds weighing between 3 and 10 g (Bartholomew and Lighton, 1986). However, the rate of oxygen consumption does not correlate with body mass, but rather with wing disc loading (the ratio of the body mass to the area swept by the wings) or other measures that incorporate the costs of hovering flight (Bartholomew and Lighton, 1986).

The hummingbirds in our hyperoxic treatments exhibited a slight, although not statistically significant, increase in the rate of oxygen consumption with decreasing air density. This result is consistent with other measures of avian locomotor performance in hyperoxia. Diving behavior in the tufted duck (*Aythya fuligula*) was studied in individuals with denervated carotid bodies (CB; a center for breathing control). CB-denervated ducks significantly increased dive duration and significantly decreased pre-dive heart rate compared with control ducks (Butler and Stephenson, 1988). Similarly, ducks running in heliox had a slightly, but not significantly, higher respiratory frequency (Brackenbury et al., 1982). Thus, one possibility is that changes in rates of O_2 consumption of birds

Table 3. *Results of a repeated-measures ANOVA investigating the effects of gas treatment and density on hover-feeding duration for the birds tested in the choice experiment*

	d.f.	MSE	*F*	*P*
Hover-feeding duration				
Treatment	1,6	199.15	31.14	0.001
Density	1,6	136.26	2.74	0.149
Treatment × density	1,6	2.09	0.32	0.594

Only the two feeding bouts just prior to aerodynamic failure were included in the analysis.

MSE, mean squared error.

in hyperoxia are minor, indicating that the aerobic limit to avian skeletal muscle performance occurs during oxygen transport or some other process after air is inspired. Another possibility is that the considerable measurement error in rates of oxygen consumption is larger than the gas treatment effects.

Given the slight increase in the rate of oxygen consumption in hyperoxia, it would be worthwhile to determine whether muscle performance is similarly enhanced. As noted above, however, the birds compared in the aerodynamic performance experiment included adult males, juvenile males and females. The considerably higher wing-loading of adult males relative to adult females added a confounding variable that decreased the statistical power to detect differences based only on gas treatment. Our two new experiments showed a strongly significant increase in hover-feeding duration for birds supplied with increased oxygen relative to normal air and during the hovering bouts just prior to failure. Interestingly, a study of human exercise performance found no difference in either heart rate or blood lactate build-up (a signal of a shift towards anaerobic exercise) when subjects were supplied with hyperoxic air. However, the time to complete the tasks was significantly lower for subjects provided with elevated oxygen levels (Peterson et al., 2000). Other studies of human muscle performance have yielded similar results although, in general, adaptations in respiratory physiology have also been detected (Knight et al., 1993; Moore et al., 1992; Plet et al., 1992; Wilson and Welch, 1980). Taken together, these results suggest that elevated respiratory oxygen levels may produce increased skeletal muscle performance through a series of smaller physiological changes that interact either additively or multiplicatively.

Most experiments involving hyperoxia utilize oxygen concentrations ranging from 30 to 80 %. Although these high oxygen concentrations may elucidate the limits of different stages in a respiratory pathway, the earth's atmosphere has never reached the upper range of oxygen concentrations used in most of these studies. However, atmospheric oxygen concentration appears to have fluctuated between 15 % and 35 % since the mid-Devonian (380 million years ago), and two large pulses of oxygen occurred during the late Paleozoic and early Cenozoic (Berner, 1999; Berner and Canfield, 1989). Pulses in oxygen concentration above current levels would probably have influenced multiple features of organismal physiology (Graham et al., 1995). Furthermore, the increase in oxygen levels occurred without significant changes in nitrogen partial pressures in the atmosphere, resulting in an overall increase in atmospheric density, possibly facilitating the evolution of insect flight (Dudley, 2000). Previous exposure to increased oxygen levels in an evolutionary lineage can leave a physiological capacity to utilize increased oxygen in extant organisms (Gans et al., 1999). However, the extant hummingbird lineages appear to have originated in the early Miocene (15–20 million years ago) (Bleiweiss, 1998), an epoch predicted to have had an atmospheric oxygen concentration greater than 21 % but less than 25 %. We interpret our lack of discrimination between hyperoxia and normoxia in the choice experiment as reflecting an absence of previous exposure in the evolutionary lineage to the opportunity to discriminate among oxygen concentrations, at least none much greater than 21 %.

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