HUMMINGBIRD HOVERING PERFORMANCE IN HYPEROXIC HELIOX: EFFECTS OF BODY MASS AND SEX

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Summary

Owing to their small size and hovering locomotion, hummingbirds are the most aerobically active vertebrate endotherms. Can hyperoxia enhance the flight performance of this highly oxygen-dependent group? Hovering performance of ruby-throated hummingbirds (*Archilochus colubris*) was manipulated non-invasively using hyperoxic but hypodense gas mixtures of sea-level air combined with heliox containing 35 % O_2. This manipulation sheds light on the interplay among metabolic power input, mechanical power output and aerodynamic force production in limiting flight performance. No significant differences in flight mechanics and oxygen consumption were identified between hyperoxic and normoxic conditions. Thus, at least in the present experimental context, hyperoxia did not change the major metabolic and mechanical parameters; O_2 diffusive capacities of the respiratory system were probably not limiting to a significant extent. Compared with hummingbirds in our previous studies, the present experimental birds were heavier, had resultant shorter hover-feeding durations and experienced aerodynamic failure at higher air densities. Because hummingbirds have relatively stable wingbeat frequencies, modulation of power output was attained primarily through variation in stroke amplitude up to near 180 °. This result indicates that maximum hovering performance was constrained geometrically and that heavier birds with greater fat loads had less margin for enhancement of power production. Sexual dimorphism in flight adaptation also played a role, with males showing more limited hovering capacities, presumably as a trade-off for increased maneuverability.

Key words: air density, body mass, heliox, hovering flight, hummingbird, hyperoxia, muscle power, oxygen consumption, sexual dimorphism, *Archilochus colubris*.

Introduction

The mass-specific oxygen consumption of flying animals is markedly higher than that of running animals and is inversely proportional to body mass (Lindstedt *et al.* 1991; Wells and Ellington, 1994). Small hovering hummingbirds exhibit the highest mass-specific rates of aerobic metabolism measured in vertebrates (Lasiewski, 1963; Wolf and Hainsworth, 1971; Berger and Hart, 1972; Epting, 1980; Bartholomew and Lighton, 1986; Suarez *et al.* 1990; Wells, 1993*a*,*b*; Chai and Dudley, 1996). Consequently, hummingbirds possess numerous morphometric, physiological and biochemical adaptations which enhance oxygen uptake and delivery by the respiratory/cardiovascular systems and oxygen utilization by the mitochondria in flight muscles (Dubach, 1981; Johansen *et al.* 1987; Suarez *et al.* 1988, 1991; Mathieu-Costello *et al.* 1992; Hochachka, 1994).

Much progress has been made in understanding the limiting factors in exercise performance and in the oxidative metabolism of running mammals (reviewed by Jones and Lindstedt, 1993). But studies on the limits of aerobic performance in flying birds, the other endothermic vertebrate class, seem disproportionately few presumably because of enormous technical difficulties (Berger and Hart, 1974; Butler, 1991; Saunders and Fedde, 1994). Air density is a major determinant of aerodynamic power requirements (Norberg, 1990), and the flight mechanics of birds can be conveniently varied through density manipulation of normoxic gas mixtures. Chai and Dudley (1995) determined the limits to flight performance of hummingbirds hovering in normoxic but hypodense mixtures of air and heliox. This manipulation is analogous to increasing the treadmill speed for runners in that hummingbirds must increase their mechanical power output to generate sufficient lift force to stay airborne. Ruby-throated hummingbirds (*Archilochus colubris*) demonstrate considerable power reserves under these conditions. Maximum aerobic capacity at low air densities is unequivocally indicated by aerodynamic failure when the birds dramatically descend to the chamber floor.

In low-density gas mixtures, ruby-throated hummingbirds

modulate their power output primarily through variation in wingstroke amplitude. If hyperoxia does enhance oxidative capacities and mechanical power output, this enhancement could be manifested by an increased wingbeat frequency once maximum stroke amplitude (near 180°) has been attained. Studies on running mammals have demonstrated that increasing stride frequency is increasingly energetically more costly and oxygen-demanding (Taylor, 1987; Heglund and Taylor, 1988). It is thus interesting to determine whether hovering hummingbirds in hyperoxic gas mixtures can increase their wingbeat frequency and hence the rate of cross-bridge cycling. However, hovering hummingbirds are also mechanically constrained by their wing morphology and kinematics, and it is conceivable that increases in mechanical power output and lift force generation do not occur in tandem at lower air densities (Ellington, 1991). If performance enhancement under hyperoxia is not observed and aerodynamic failure occurs at air densities comparable with those observed in normoxic heliox, two conclusions are possible: (1) when mechanical power output remains the same, diffusive limitations in oxygen uptake by the lungs do not constrain hovering, or (2) when mechanical power output is increased, maximum hovering performance cannot be enhanced because an asymptotic limit exists for the production of lift force.

In the present study, the hovering performance of ruby-throated hummingbirds was investigated in hyperoxic but hypodense gas mixtures of air and heliox containing 35 % O_2. To our knowledge, this is the first study investigating the effects of hyperoxic gas mixtures on the flight performance of birds. We observed no significant differences in flight mechanics and oxygen consumption between hyperoxic and normoxic conditions. We thus conclude that hyperoxia, in our experimental context, enhances neither metabolic power input nor mechanical power output to a significant extent during hovering flight. Seven birds (four males and three females) were studied to allow an evaluation of sexual dimorphism in flight performance and to facilitate comparisons with previous studies (Chai and Dudley, 1995, 1996).

Materials and methods

Ruby-throated hummingbirds (*Archilochus colubris* L.) were mist-netted in the vicinity of Austin, Texas, during the autumn migratory season of 1995 and were housed in screen cages of dimensions equal to those of the experimental cube (90 cm×90 cm×90 cm). Hummingbird care was in accordance with federal and state guidelines. Two or three birds were housed together within one cage.

The experimental procedures and equipment have been described previously (Chai and Dudley, 1995, 1996), and only a brief account will be given here. Birds were trained to feed through a cylindrical mask attached to a hanging syringe. Seven individual hummingbirds with intact flight feathers (three adult males, one juvenile male and three females) were used in experiments. Each bird was subjected to two trials:

(1) the experimental hyperoxic heliox containing 35 % O_2 (density 0.57 kg m^{-3}), and (2) the control normoxic heliox containing 21 % O_2 (density 0.40 kg m^{-3}) conducted over two consecutive days (sea-level atmospheric air has a density of 1.20 kg m^{-3}). The sequence of gas manipulation was arbitrarily decided, and three birds began with the hyperoxic treatment on the first day (see Table 1). Both treatments were conducted only once because previous studies with normoxic and hypoxic treatments found no significant trial effect. Hyperoxic heliox (35 % O_2/65 % He) provides the highest O_2 concentration that is still likely to induce aerodynamic failure in these birds, given the average failure density of 0.54 kg m^{-3} measured in our previous study (see Chai and Dudley, 1995).

Flight experiments were carried out within an airtight acrylic cube (90 cm×90 cm×90 cm). Data were collected initially from birds hover-feeding in unmanipulated sea-level air. Air within the cube was then gradually replaced by filling with hyperoxic or normoxic heliox while allowing the cube contents to escape from an additional port. Hover-feeding flight was then video-recorded (at 60 fields s^{-1}) approximately every 15–20 min when the bird was hungry and came to feed. The duration of hover-feeding flight t was timed from video recordings. Heliox filling was terminated after the bird showed aerodynamic failure while hover-feeding, and reverse pumping of ambient air was then initiated. The experiment was generally stopped when the bird could hover longer and feed normally. During the hyperoxic experiments, O_2 concentration never exceeded 34 %. Oxygen concentration in the chamber increased asymptotically towards 35 % as the oxygen difference between air and the heliox within the cube was greatest at the outset of filling and then rose more slowly. We were unable to induce aerodynamic failure in two female birds using heliox of 35 % O_2 (see Table 1). After more than 3 h of filling with heliox, the rise in O_2 tension became too slow and too wasteful to continue. To identify the failure density, we then switched to filling with heliox containing 21 % O_2. This treatment eventually led to failure of hover-feeding at lower air densities, but also at lower O_2 tension.

Density reduction associated with replacement of normal air by heliox was monitored acoustically (Dudley, 1995). Metabolic power input (P_{input}) during hovering was obtained from measurements of rates of oxygen consumption ($\dot{V}_{O_2}$) using an open-flow feeder-mask respirometry system. Oxygen consumption was expressed at STP. Horizontal projections of wingbeat kinematics of each hover-feeding sequence were video-recorded through a mirror oriented above the bird at 45° to horizontal. Wingbeat kinematics recorded for each hover-feeding sequence, together with morphological parameters for individual birds, were used to estimate the mechanical power requirements of flight using a detailed aerodynamic model of hovering flight (Ellington, 1984*a–f*). Wingbeat kinematics measured for each hovering sequence included wingbeat frequency n and stroke amplitude Φ. Morphological parameters used in aerodynamic calculations included body mass m, relative wing mass $\hat{m}_w$ for both wing pairs and expressed as a fraction of body mass, wing length R, total wing

area S (the area of both wing pairs), wing loading p_w (mg/S, where g is gravitational acceleration) and aspect ratio $\mathcal{R}$ ($4R^2/S$). For humane reasons, none of the experimental birds was killed for *post mortem* analysis. Wing mass and its spanwise distribution were estimated from results of previous studies using the same species (Chai and Dudley, 1995, 1996). For each hovering flight sequence, the mechanical power requirements of flight were estimated by evaluating the individual components of profile (P_{pro}), induced (P_{ind}) and inertial power during the first half of a half-stroke (P_{acc}). Total power expenditure for a flight sequence was calculated for the two cases of zero (P_{zero}) and perfect (P_{per}) elastic storage of wing inertial energy, representing maximum and minimum estimates of required mechanical power respectively (Ellington, 1984*f*). Thus, $P_{zero}=(P_{pro}+P_{ind}+P_{acc})/2$, assuming zero elastic energy storage, and $P_{per}=P_{pro}+P_{ind}$, assuming perfect elastic energy storage. P_{zero} and P_{per} are expressed in muscle mass-specific form, assuming that flight muscle equals 25 % of the body mass (Chai and Dudley, 1995). Muscle mechanical efficiency η_m was estimated as $P_{per}/(0.9P_{input})$, assuming 90 % direct energy expenditure by flight muscle. P_{per} was used because hummingbirds can probably store kinetic energy elastically during the deceleration phase of the wing stroke (Wells, 1993*a*).

The effects of hyperoxic density reduction and normoxic density reduction treatments were evaluated for each kinematic, metabolic and aerodynamic variable using repeated-measures analysis of variance (ANOVA) (SAS Institute, 1989). Hover-feeding events were first grouped by rounding air densities at which feeding events occurred to the nearest $0.1\,kg\,m^{-3}$. To achieve a paired statistical design, data points from each bird were derived as the difference between hyperoxic experimental values and normoxic control values (i.e. the value in normoxia minus the value in hyperoxia; the value was the mean of each treatment × density level). No difference in such values should appear if hyperoxia and normoxia treatments yielded similar effects; conversely, a trend towards greater differences across the density levels

should emerge if the two treatments differed consistently in effect. Two explanatory variables were tested: the density-reduction effect was modelled as a within-subject source of variation, whereas the sex of birds was modelled as a between-subject variation. Ruby-throated hummingbirds are sexually dimorphic: males are smaller with shorter and more pointed wings (Johnsgard, 1983). With four males (including one juvenile male) and three females, the effect of sex is incorporated into the statistical analysis. However, the males showed aerodynamic failure at higher densities, and consequently only four density levels over the range that both sexes overlapped (1.2–$0.8\,kg\,m^{-3}$) were used in statistical analysis. Since the three females failed at lower densities, repeated-measures ANOVA was also conducted for females using six density levels (1.2–$0.6\,kg\,m^{-3}$). For $\dot{V}_{O_2}$ and η_m (P_{per}), the final density level of $0.6\,kg\,m^{-3}$ was dropped from the analysis because oxygen consumption rates near failure could not be reliably obtained given the short duration of hover-feeding.

Results

Because of sexual dimorphism, males and females differed in major morphological variables (Table 1). Males showed much higher wing loading; their smaller wing area relative to body mass will increase flight costs and decrease lift production (Rayner, 1988; Norberg, 1990). This is probably the primary reason that males showed aerodynamic failure at much higher air densities than did the females (Table 1) as well as shorter hover-feeding durations at the lower air densities (Fig. 1).

Although intersexual differences in flight energetics were obvious (see Figs 2–5), the effects of hyperoxia relative to normoxic controls did not suggest a significant effect of sexual dimorphism on any of the variables (Table 2). For neither sex did hyperoxia produce statistically significant effects on any of the variables across air density levels. The four marginally significant results presented in Table 2 did not indicate overall significance after sequential Bonferroni adjustment for
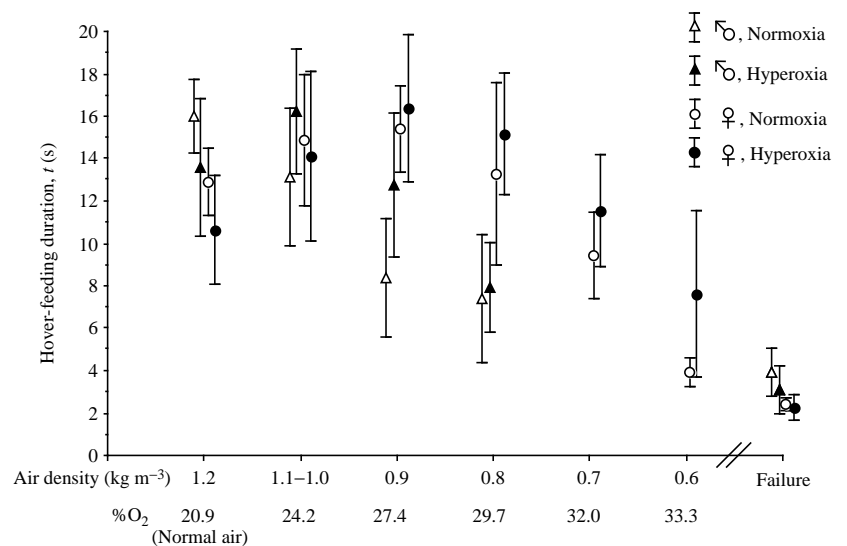

Fig. 1. Hover-feeding duration (mean ± 1 S.E.M.) of male ($N=4$) and female ($N=3$) hummingbirds during hyperoxic (35 % O_2) or normoxic (21 % O_2) heliox replacement of normoxic sea-level air. Mean values of means from individual birds at each density level are shown; %O_2 is the oxygen concentration at each density level under hyperoxia. A separate category (Failure) shows measurements at maximum hovering performance prior to aerodynamic failure.

Table 1. *Morphological variables and the air density and oxygen concentration at aerodynamic failure for seven ruby-throated hummingbirds during hyperoxic (35 % O_2) or normoxic (21 % O_2) heliox replacement of sea-level air on two consecutive days*

Bird, sex and treatment sequence	m (g)	R (mm)	$A\!R$	P_w (N m^{-2})	S (cm^2)	$\hat{m}_w$ (%)	Failure ρ (kg m^{-3})	Failure O_2 (%) in hyperoxia
1, M, **35 %**/21 %	**3.83**/3.58	41	7.34	**41.0**/38.4	9.2	3.6	**0.76**/0.69	31.4
2, M, 21 %/**35 %**	3.67/**3.54**	41	6.96	37.3/**35.9**	9.7	3.7	0.79/**0.70**	32.1
3, M, 21 %/**35 %**	4.01/**4.13**	40	7.17	44.1/**45.3**	8.9	3.3	0.79/**0.77**	29.9
4, M, 21 %/**35 %**	4.16/**4.09**	43	8.13	44.9/**44.1**	9.1	3.2	0.76/**0.69**	32.0
5, F, **35 %**/21 %	**4.61**/4.36	49	7.55	**35.6**/33.6	12.7	3.3	**0.56**/0.52	28.3 (33.6)*
6, F, 21 %/**35 %**	4.36/**4.17**	48	7.18	33.3/**31.9**	12.8	3.5	0.53/**0.48**	25.4 (33.8)*
7, F, **35 %**/21 %	**4.51**/4.18	49	8.00	**36.9**/34.2	12.0	3.4	**0.64**/0.58	33.6

Values for hyperoxic (35 % O_2) are given in bold type.

Bird 4 was a juvenile male.

*Numbers in parentheses represent the highest attained oxygen concentration; filling the experimental cube with hyperoxic heliox (35 % O_2) did not induce aerodynamic failure, and filling was switched to normoxic heliox to reduce air density further at the expense of a decreased oxygen concentration.

Body mass m (on treatment day by treatment sequence), wing length R, aspect ratio $A\!R$, wing loading P_w (by treatment sequence due to mass change), total wing area S, relative wing mass $\hat{m}_w$, air densities at failure ρ by treatment sequence in hyperoxic and normoxic gas mixtures, and oxygen level at failure in hyperoxic gas mixture O_2.

multiple statistical tests (Holm, 1979). Thus, the mechanical and metabolic variables remained similar in hypodense gas mixtures under either hyperoxic or normoxic treatment.

The overall patterns of changes in feeding duration (Fig. 1), kinematic (Fig. 2), aerodynamic (Fig. 3), mechanical (Fig. 4) and metabolic (Fig. 5) parameters during density reduction in air and heliox mixtures were very similar to previous studies (Chai and Dudley, 1995, 1996). The duration of feeding bouts declined more sharply at lower air densities (Fig. 1). Density reduction beyond 0.8 kg m^{-3} for males and 0.6 kg m^{-3} for females resulted in aerodynamic failure to sustain hovering during a feeding bout lasting 2–4 s. Density reduction altered wingbeat kinematics (Fig. 2), with increases in both wingbeat frequency and stroke amplitude at lower air densities. The change in wingbeat frequency was relatively small, and modulation of stroke amplitude up to near 180 ° was the more important means of raising lift force and power output. As a result of alterations in wingbeat kinematics, mean lift coefficients actually increased, despite a reduction in the mean Reynolds number at low air densities (Fig. 3). This lift production incurred a cost in increased mechanical power requirements (Fig. 4) as well as in aerobic metabolism (Fig. 5). The muscle mechanical efficiency generally remained stable except for females at a density of 0.7 kg m^{-3} (Fig. 5); efficiency increased marginally at this density.

Among-individual mass and sex variation

Of six birds captured during the autumn migratory season of 1994, Chai and Dudley (1995) studied four birds (one adult male, two juvenile males and one female) in the autumn of 1994. Chai and Dudley (1996) studied five of these birds (three adult males and two females) in the spring of 1995. Two males (juveniles in the autumn of 1994) and one female were each studied twice. Because they completed their spring moult with a new set of flight feathers and were older, they are treated here

Table 2. *Results of repeated-measures ANOVA for kinematic, mechanical and metabolic variables comparing effects due to hyperoxic (35 % O_2) and normoxic (21 % O_2) heliox replacement of normal air*

Variable	P value (d.f.)		
	Density	Sex	Density × Sex
All birds (4 M/3 F):			
Δt	0.163 (3,15)	0.787 (1,5)	0.549 (3,15)
Δn	0.892 (3,15)	0.825 (1,5)	0.574 (3,15)
$\Delta\Phi$	0.034 (3,15)*	0.616 (1,5)	0.012 (3,15)*
$\Delta\overline{Re}$	0.161 (3,15)	0.769 (1,5)	0.324 (3,15)
$\Delta\overline{C_L}$	0.228 (3,15)	0.560 (1,5)	0.212 (3,15)
ΔP_{zero}	0.340 (3,15)	0.818 (1,5)	0.579 (3,15)
ΔP_{per}	0.657 (3,15)	0.761 (1,5)	0.727 (3,15)
$\Delta\dot{V}_{O_2}$	0.682 (3,15)	0.478 (1,5)	0.054 (3,15)
$\Delta\eta_m$ (P_{per})	0.400 (3,15)	0.416 (1,5)	0.047 (3,15)*
Females only:			
Δt	0.666 (5,10)		
Δn	0.889 (5,10)		
$\Delta\Phi$	0.360 (5,10)		
$\Delta\overline{Re}$	0.147 (5,10)		
$\Delta\overline{C_L}$	0.141 (5,10)		
ΔP_{zero}	0.047 (5,10)*		
ΔP_{per}	0.079 (5,10)		
$\Delta\dot{V}_{O_2}$	0.157 (4,8)		
$\Delta\eta_m$ (P_{per})	0.163 (4,8)		

Derived variables represent differences (Δ) between hyperoxic experimental measurements and normoxic controls.

P values are from F-test; d.f., degrees of freedom.

Hover-feeding duration Δt, wingbeat frequency Δn, stroke amplitude $\Delta\Phi$, Reynolds number $\Delta\overline{Re}$, mean lift coefficient $\Delta\overline{C_L}$, mechanical power output per unit flight muscle mass assuming zero (ΔP_{zero}) and perfect (ΔP_{per}) elastic energy storage, rate of oxygen consumption $\Delta\dot{V}_{O_2}$, and muscle mechanical efficiency $\Delta\eta_m$ for P_{per}.
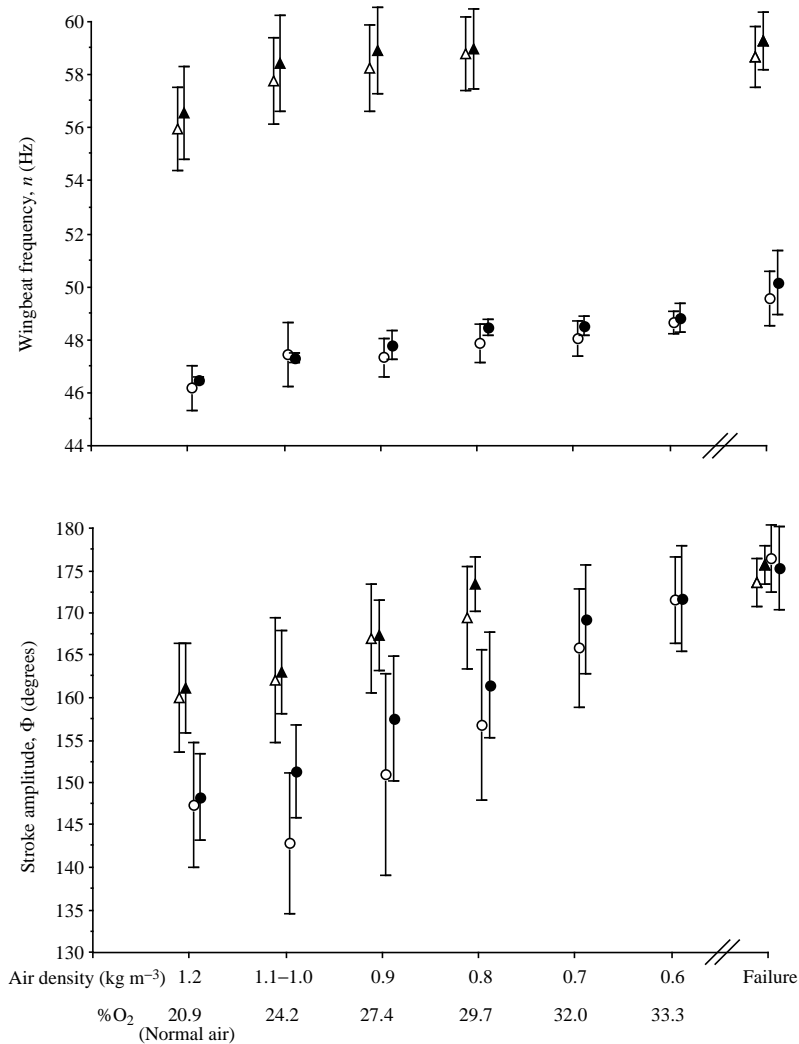
*$P<0.05$.

Fig. 2. Wingbeat frequency and stroke amplitude during hyperoxic (35 % O₂) and normoxic (21 % O₂) heliox replacement of normal air. Other details and symbols are as in Fig. 1.

as different birds (juvenile males gain their adult plumage after their first moult; wing area and shape in juvenile males are more similar to those of females). Thus, together with the birds in the present study, a total of 16 samples from 13 birds offers a reasonably large set to examine within-species variation in hovering performance using wing loading as the predictive variable (Fig. 6).

Compared with the birds in our previous studies, the experimental birds of the present study were heavier. For adult males, the body mass and wing loading of the three birds of this study averaged $3.79\pm0.2\,\mathrm{g}$ (1 s.d.) and $40.3\pm4.1\,\mathrm{N\,m^{-2}}$, respectively, compared with $3.28\pm0.2\,\mathrm{g}$ and $34.1\pm2.6\,\mathrm{N\,m^{-2}}$ for the four birds in the previous studies. For adult females, the three birds in this study averaged $4.37\pm0.1\,\mathrm{g}$ body mass and $34.2\pm1.5\,\mathrm{N\,m^{-2}}$ wing loading, compared with $3.52\pm0.1\,\mathrm{g}$ and $31.8\pm1.5\,\mathrm{N\,m^{-2}}$, respectively, for the three females in the previous studies. Johnsgard (1983) listed the mean mass of males of this species as 3.03 g (range 2.5–4.1 g) and that of females as 3.34 g (range 2.7–4.8 g) on the basis of several hundred specimens. During the present study period, we observed many fewer attacks and much less fighting between cagemates. This allowed uninterrupted feeding and resulted in fattening under *ad libitum*

food provision (see also Wells, 1993*a*). More subdued aggression relative to previous studies may reflect ontogenetic variation; earlier studies used two juvenile males and one feather-damaged adult female (out of six captive birds), whereas in the present study there was only one juvenile male and no birds with badly damaged flight feathers (out of a total of eight birds). Juvenile males, moulting birds and birds with feather damage seem to be more aggressive. More studies are clearly needed to identify patterns of aggression in relation to age, sex, body mass and feather conditions of hummingbirds in the field and in captivity.

The generally heavier birds of the present study nevertheless provide an opportunity to evaluate the effect of body mass on flight performance. Ruby-throated hummingbirds begin their migration across the Gulf of Mexico with 40 % or more of their body mass occupied by fat (Odum *et al.* 1961). It is reasonable to assume that the added mass of the heavier birds of the present study (conducted in the autumn of 1995) was mostly fat (Carpenter *et al.* 1993). This fat load will increase wing loading and flight cost and reduce power reserves, as shown by shorter hover-feeding duration at or near normal air density $(1.2–1.0\,\mathrm{kg\,m^{-3}})$ and aerodynamic failure at air densities higher than those that characterize failure of less heavy birds (Fig. 6).
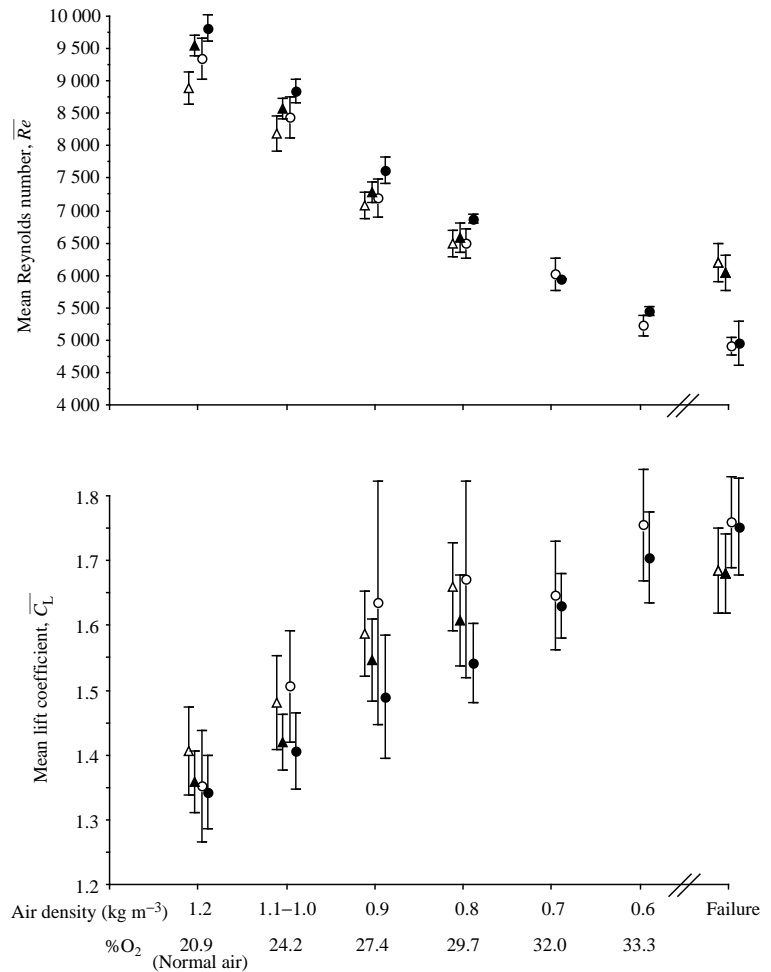
Fig. 3. Mean Reynolds number and mean lift coefficient during hyperoxic (35 % O$_2$) and normoxic (21 % O$_2$) heliox replacement of normal air. Other details and symbols are as in Fig. 1.

Moreover, sexual dimorphism also played a role. The seven adult males of this and previous studies had shorter wing lengths by 12 % than those of the six females (average 42 *versus* 48 mm), lower body mass by 11 % (3.5 *versus* 4.0 g) and higher wing loading by 11 % (37 *versus* 33 N m^{-2}).

Without immediate *post mortem* analysis, it is not possible accurately to determine the proportions of flight muscle and fat storage in relation to varying body mass (ruby-throated hummingbirds can undergo rapid body mass changes of up to 10 % in 1 day; P. Chai, unpublished observations). The reduced flight performance of males in the present study suggests considerable fat loads. This is also shown by their lower muscle mass-specific mechanical power output P_{per} at aerodynamic failure (Fig. 4). Muscle mass-specific power output in this and previous studies was derived assuming that the proportion of flight muscle equals 25 % of the body mass. Thus, muscle mass-specific power output will be underestimated if the flight muscle proportion is less than 25 % because of fat storage. To circumvent this limitation, the ratio of P_{per} at the two extreme density levels (i.e. in normal air and before aerodynamic failure) was used as an indicator of hovering capacity. In our two previous studies, this ratio ranged from 1.32 to 1.37 for the four adult males, from 1.32 to 1.39 for the two juvenile males and from 1.35 to 1.43 for

the three females. In the present study, the ratio ranged from 1.30 to 1.41 for the three females but only between 1.18 and 1.21 for the three adult males (1.22 for the juvenile male). Thus, males of the present study showed considerable less margin for power enhancement.

Discussion

For a hummingbird to hover, a continuous supply of muscle mechanical power fuelled by aerobic metabolism must be transduced into the appropriate wing kinematics to generate enough lift force. Wing morphology and air density also substantially influence lift production. Because no significant differences in oxygen consumption and flight mechanics are identified between hyperoxic and normoxic conditions, we conclude that, relative to the normoxic density reduction, exposure to hyperoxic but hypodense air does not change the major metabolic and mechanical parameters of flight. There is thus no interplay among metabolic power input, mechanical power output and aerodynamic lift production in limiting flight in hypodense air. Two earlier studies on the ventilation pattern of running fowl (Brackenbury *et al.* 1982) and on the behaviour and heart rate of diving ducks (Butler and Stephenson, 1988) also showed hyperoxia-insensitivity in these birds. In contrast,
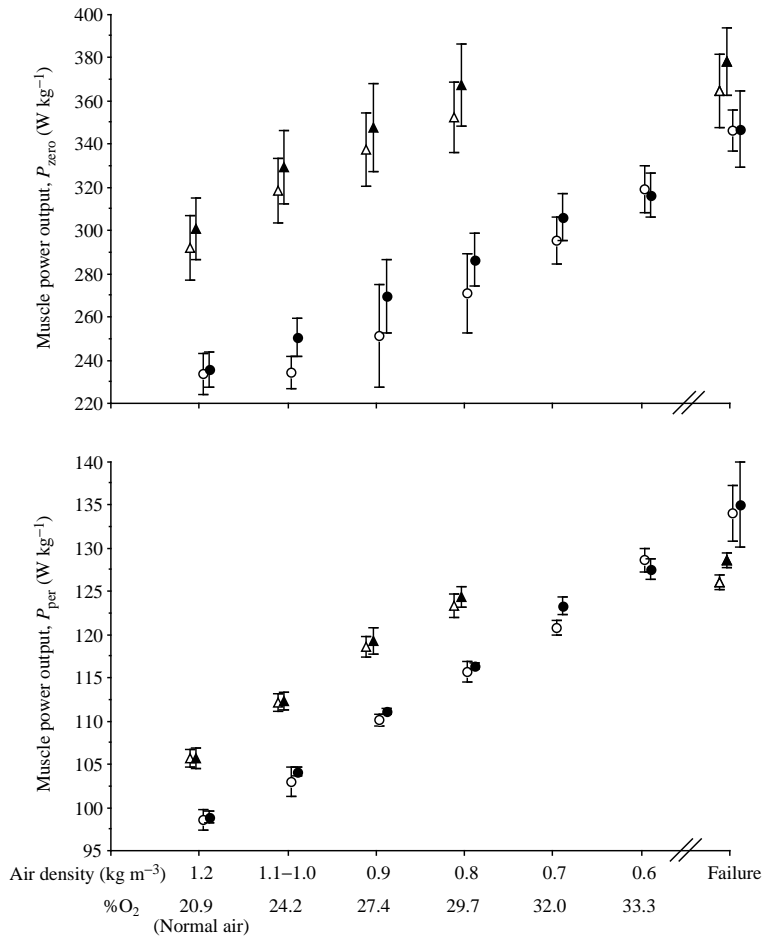
Fig. 4. Muscle mass-specific mechanical power output assuming zero or perfect elastic energy storage during hyperoxic (35 % O_2) and normoxic (21 % O_2) heliox replacement of normal air. Other details and symbols are as in Fig. 1.

oxygen supply is more limiting than are flight mechanics in hypoxic and hypodense air (Berger, 1974; Chai and Dudley, 1996). Thus, increasing O_2 tension in hyperoxic air and heliox mixtures does not enhance hovering performance, whereas decreasing O_2 tension of hypoxic air and helium mixtures ultimately reduces hovering performance.

Helium is characterized by low density and high thermal conductivity (Reid *et al.* 1987). Heliox is likely to improve the physiological performance of hovering hummingbirds in two ways: low density can increase internal air circulation and reduce ventilatory costs (Brackenbury, 1991), while high thermal conductivity can alleviate thermal stress when metabolic rate is high (López-Calleja and Bozinovic, 1995). Thus, hypodense air and heliox mixtures may improve gas exchange even during normoxia. However, it is unclear exactly how heliox affects the gas exchange of exercising birds because of the unique design of bird lungs. Cross-current and unidirectional airflow patterns coupled with aerodynamic valving depend on both respiratory gas velocity and density (Banzett *et al.* 1987; Wang *et al.* 1988). Hummingbirds hovering in gas mixtures of air and heliox do not demonstrate reduced flight performance at failure when simultaneously breathing normal air pumped into the same feeder mask used previously in respirometry (P. Chai and R. Dudley, unpublished data). Diffusive limitation at the level of

pulmonary oxygen loading thus appears not to limit the aerobic performance of hummingbirds. With the present experimental design using hyperoxic and hypodense heliox, the use of 35 % O_2 represents an upper experimental limit because a higher oxygen concentration results in a gas density too high to induce maximum performance and aerodynamic failure. Hyperoxic effects on flight performance at even higher oxygen tensions are thus unclear.

In two mammalian taxa (horses and humans), aerobic performance can be limited by the oxygen transport system and by pulmonary gas exchange (reviewed by Jones *et al.* 1993; Jones and Lindstedt, 1993). Exercise performance is consequently improved under hyperoxia, although the physiological effects are multiple and complex (Welch, 1982). Jones and Lindstedt (1993) suggested that, for highly aerobic athletic mammalian runners, maximal performance may eventually be limited by the diffusive capacity of the lung. Among vertebrates, birds possess a unique lung structure and pattern of air circulation and are thought to implement more effective gas exchange (Brackenbury, 1991; Faraci, 1991; Saunders and Fedde, 1994). In contrast, rates of oxygen consumption by flying bats do not differ from those of flying birds (Thomas, 1975; Carpenter, 1985, 1986; Butler, 1991). Further studies are needed to compare the limiting factors on flight performance for avian and mammalian fliers.
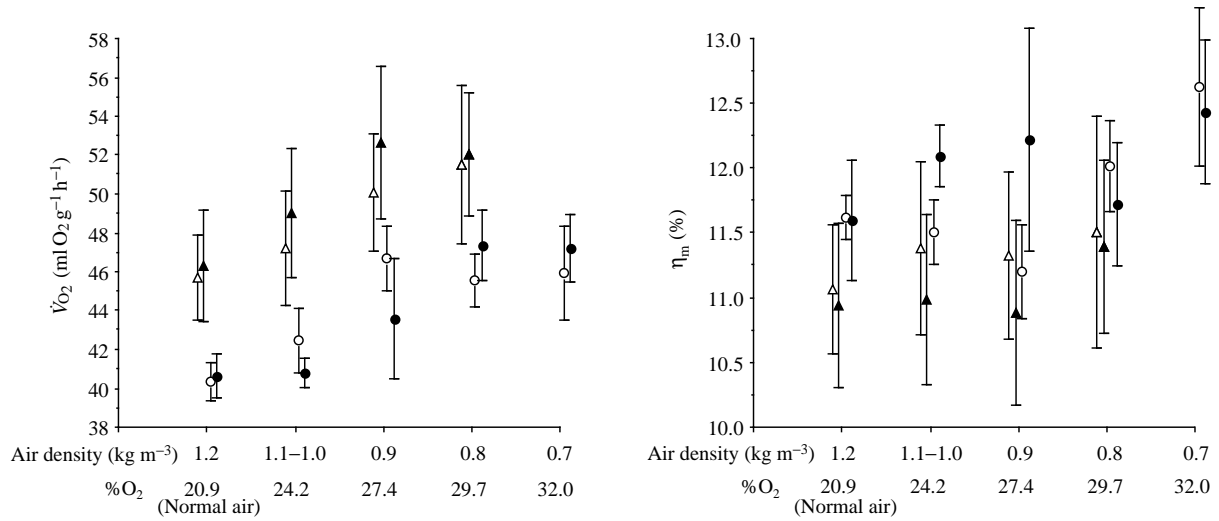
Fig. 5. Rate of oxygen consumption ($\dot{V}_{O_2}$) and muscle mechanical efficiency (η_m) assuming perfect elastic energy storage during hyperoxic (35 % O_2) and normoxic (21 % O_2) heliox replacement of normal air. Other details and symbols are as in Fig. 1.

Hummingbirds, and birds in general, have only a limited capacity for modulation of wingbeat frequency (Greenewalt, 1962, 1975; Hagiwara *et al.* 1968; Rayner, 1985; Pennycuick, 1990, 1992). The stable wingbeat frequency reflects physical constraints because a mechanically efficient wingbeat should operate at its natural frequency determined by the wing mass, shape and area (Greenewalt, 1975; Pennycuick, 1990, 1992). The shortening velocity of the flight muscle should also be tuned to match this natural frequency (Rayner, 1985; Pennycuick, 1992). However, some hummingbird individuals
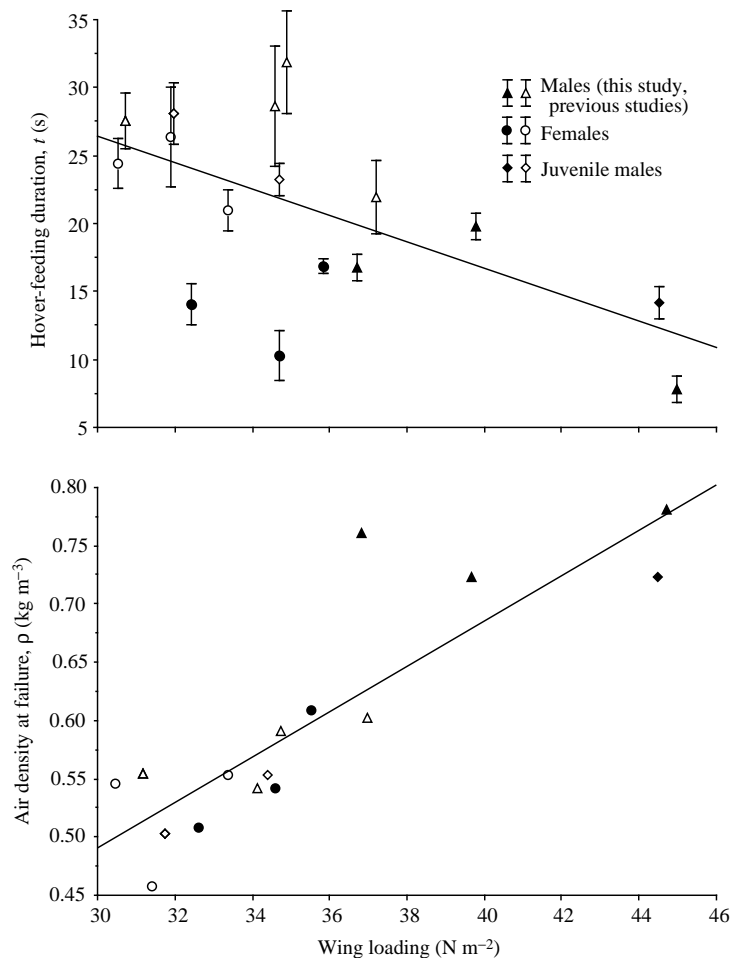

Fig. 6. Hover-feeding duration (mean ± 1 s.e.m., sample size ranging from 6 to 17 per bird) at or near normal air density (1.2–1.0 kg m^{-3}) and mean air density at aerodynamic failure (sample size 1–3 trials per bird) as a function of wing loading for 16 hummingbird individuals. Mean values for individual birds were used in the regression analysis (hover-feeding duration: $y=-0.97x+55.3$, $r=-0.60$, $P=0.014$; air density at failure: $y=0.02x-0.10$, $r=0.87$, $P<0.001$)

are capable of increasing their wingbeat frequency by more than 10 % (Wells, 1993*b*; Chai and Dudley, 1995). For ruby-throated hummingbirds, similar energetic limits to performance under hyperoxic and normoxic treatments with heliox indicate that hovering flight is not constrained by oxygen availability. Rather, the wingbeat frequency is fairly stable and is presumably tuned to a naturally resonant frequency, whereas stroke amplitude is limited geometrically.

For mammalian runners, higher rates of cyclic locomotor events (stride frequency) are energetically more expensive than lower rates (Taylor *et al.* 1980; Heglund and Taylor, 1988). This may be the reason why ruby-throated hummingbirds, and probably birds in general, use wingstroke amplitude modulation to vary mechanical power output rather than frequency modulation (Rayner, 1985). The wingbeat geometry of hovering animals may thus constrain their maximum power output, as indicated by aerodynamic failure occurring near stroke amplitudes of 180 °. Alternatively, wings could be lengthened to augment lift force and circumvent geometrical limitations, but at the expense of maneuverability, acceleration and wing moment of inertia (Rayner, 1988; Norberg, 1990). Wing morphology thus represents a compromise between energetic efficiency and acrobatic performance, as reflected in differences in hovering capacity between male and female ruby-throated hummingbirds.

For smaller species of North American hummingbirds, male fitness is closely linked to aerial display and agility in pursuit (Feinsinger and Chaplin, 1975; Hixon and Carpenter, 1988; Calder *et al.* 1990). Male birds are characterized by shorter, more pointed wings and by lower body mass (Johnsgard, 1983). This trade-off for acrobatic performance in males presumably increases flight costs and decreases hovering capacity in low air densities. The reduced power margin in flight may contribute to a lower survivorship of male ruby-throated hummingbirds in the wild (Mulvihill *et al.* 1992). Although wing shape and area reflect ontogenetic and phylogenetic controls, the individual can still exert morphological control of flight performance through varying body mass and accompanying wing loading. Breeding male broad-tailed hummingbirds refrain from feeding, presumably in order to reduce their body mass and to enhance their flight performance (Calder *et al.* 1990). Among-individual variation shown in the present study indicates the flexible nature of flight performance within the limits set by wing morphology and kinematics. This flexibility presumably reflects different priorities of individual birds in the wild, e.g. courtship and reproduction *versus* migration and survival.

In progressively hypodense gas mixtures, we have consistently observed a decline in hover-feeding duration which eventually leads to the 2–4 s of hovering at maximum performance, followed by aerodynamic failure. In the present study, heavier birds with higher wing loading generally exhibited shorter hover-feeding durations. This result indicates that increasing costs of flight due to lower air density or higher fat load will reduce hover-feeding duration, whereas the occurrence of aerodynamic failure suggests physiological and/or mechanical limitations. Although hummingbirds rely exclusively on aerobic metabolism, their flight muscles nevertheless contain high levels of creatine phosphokinase (Suarez *et al.* 1986), indicating transient use of creatine phosphate for high power output. Phosphagen-based burst performance is only possible for a short time and leads to a power reduction after depletion of the auxiliary creatine phosphate (Hochachka, 1994). This may explain why maximum hovering performance is not sustainable. During the less oxygen-dependent burst phase, aerodynamic lift force production from the wings may be limiting, or nature may select the hummingbird flight machinery in such a way that the amount of flight muscle is adjusted in accordance with the wing morphology so that power and lift production reach maxima simultaneously. That moulting hummingbirds with reduced wing area typically lose weight lends support to the latter possibility (Wells, 1990; Hiebert, 1993; P. Chai, unpublished data).

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