

The metabolic power requirements of flight and estimations of flight muscle efficiency in the cockatiel (*Nymphicus hollandicus*)

Charlotte R. Morris, Frank E. Nelson* and Graham N. Askew[†]

Institute of Integrative and Comparative Biology, University of Leeds, Leeds, LS2 9JT, UK

*Present address: Department of Radiology, University of Washington, 1959 NE Pacific St. Seattle, WA98195-7115, USA

[†]Author for correspondence (g.n.askew@leeds.ac.uk)

Accepted 6 April 2010

SUMMARY

Little is known about how *in vivo* muscle efficiency, that is the ratio of mechanical and metabolic power, is affected by changes in locomotory tasks. One of the main problems with determining *in vivo* muscle efficiency is the large number of muscles generally used to produce mechanical power. Animal flight provides a unique model for determining muscle efficiency because only one muscle, the pectoralis muscle, produces nearly all of the mechanical power required for flight. In order to estimate *in vivo* flight muscle efficiency, we measured the metabolic cost of flight across a range of flight speeds (6–13 ms⁻¹) using masked respirometry in the cockatiel (*Nymphicus hollandicus*) and compared it with measurements of mechanical power determined in the same wind tunnel. Similar to measurements of the mechanical power–speed relationship, the metabolic power–speed relationship had a U-shape, with a minimum at 10 ms⁻¹. Although the mechanical and metabolic power–speed relationships had similar minimum power speeds, the metabolic power requirements are not a simple multiple of the mechanical power requirements across a range of flight speeds. The pectoralis muscle efficiency (estimated from mechanical and metabolic power, basal metabolism and an assumed value for the ‘postural costs’ of flight) increased with flight speed and ranged from 6.9% to 11.2%. However, it is probable that previous estimates of the postural costs of flight have been too low and that the pectoralis muscle efficiency is higher.

Key words: efficiency, metabolic, power.

INTRODUCTION

Flight is one of the most energetically demanding forms of locomotion (Schmidt-Nielsen, 1972), consuming energy at high rates. Consequently many aspects of a bird’s physiology, ecology and behaviour are affected by the demands of flight. The mechanical power requirements of flight give us insight into the demands of flight in relation to flight speed; however, the generation of mechanical power by the pectoralis muscles does not represent the sole use of metabolic energy during flight. The ‘postural costs’ of flight encompass the energy expenditure above basal metabolism of all non-muscular physiological systems and muscles other than the pectoralis muscles. Thus, the relationship between the mechanical and metabolic power requirements of flight may vary with flight speed because of changes in the postural costs of flight and changes in muscle efficiency. The actual postural cost of flight is unknown. Others have assumed that the additional energy expenditure includes basal metabolism and an additional 10% of the metabolic cost of flight, based on estimates of the energy consumed by the heart and respiratory system in flight (Pennycuik, 1975; Pennycuik, 1989; Tucker, 1973; Ward et al., 2001). However, this may not be an accurate assumption (in particular given that the energy expenditure of physiological systems other than the heart and respiratory system, and muscles other than the pectoralis muscles have not been considered) and may vary with bird size and flight speed. Ward et al. (Ward et al., 2001) found that flight muscle efficiency ranges from 13% to 23% with flight speed, and flight muscle efficiency may also increase with bird body size (Ward et al., 2001) as has been shown in flying insects (Casey and Ellington, 1989). Variation in both the postural cost of flight and muscle

efficiency with flight speed would result in a non-linear relationship between mechanical and metabolic power.

Comparisons have been made between mechanical and metabolic power requirements of flight across a range of flight speeds, assuming that the postural costs consist of 10% of the metabolic cost, in order to examine flight muscle efficiency (Chai et al., 1998; Chai and Dudley, 1995; Norberg et al., 1993; Ward et al., 2001). Flight muscle efficiency ranges from 13% to 23% in the European starling [*Sturnus vulgaris* (Ward et al., 2001)], from 11% (during hovering) to 15% (during forward flapping flight) in a nectar-feeding bat [*Glossophaga soricina* (Norberg et al., 1993)] and from 10% to 13% in the ruby-throated hummingbird (*Archilochus colubris*) during hovering (Chai et al., 1998; Chai and Dudley, 1995). All of the mechanical power estimates used in these calculations of flight muscle efficiency were calculated using aerodynamic models (Chai et al., 1998; Chai and Dudley, 1995; Norberg et al., 1993; Ward et al., 2001). As previously shown (Askew and Ellerby, 2007; Morris and Askew, 2010b), mechanical power calculated using aerodynamic models is highly sensitive to the values assumed for the aerodynamic coefficients used in the analysis. Thus, a comparison between mechanical and metabolic power requirements of flight, where the mechanical measurements have been made using a different approach, would be useful.

The mechanical and metabolic power requirements of flight have been determined in the European starling (Biewener et al., 1992; Ward et al., 2001; Ward et al., 2004), budgerigar [*Melopsittacus undulatus* (Tucker, 1968; Bundle et al., 2007; Ellerby and Askew, 2007)] and cockatiel [*Nymphicus hollandicus* (Hedrick et al., 2003; Bundle et al., 2007)]. However, these power measurements may be

affected by different experimental protocols and wind tunnels. For example, the birds may have been subjected to differing amounts of ground effect which would alter the mechanical power requirements of flight (see Morris and Askew, 2010b). In the budgerigar and cockatiel the power–speed relationships have been examined (Bundle et al., 2007; Ellerby and Askew, 2007; Hedrick et al., 2003; Tucker, 1968): these studies all measured U-shaped power–speed relationships. In the budgerigar, the minimum power speed for the mechanical and metabolic power measurements is approximately 10 m s^{-1} (Bundle et al., 2007; Ellerby and Askew, 2007; Tucker, 1968). However, the minimum power speeds were extremely different for mechanical and metabolic power measurements in the cockatiel; 5 and 10 m s^{-1} , respectively (Bundle et al., 2007; Hedrick et al., 2003). Considering the similar mechanical and metabolic power speeds measured in three separate studies for the budgerigar, this twofold variation in the minimum power speed is unlikely to be a real feature of the power requirements of flight in the cockatiel. In the accompanying papers (Morris and Askew, 2010a; Morris and Askew, 2010b), we estimated a minimum mechanical power speed of 8 m s^{-1} which is similar to the minimum metabolic power speed of 10 m s^{-1} measured by Bundle et al. (Bundle et al., 2007). This suggests the minimum mechanical and metabolic power speeds may also correspond in the cockatiel.

In order to clarify the minimum power speed in the cockatiel, the metabolic and mechanical power–speed relationships were compared. We also calculated the flight efficiency and the effects of respirometry equipment on flight performance.

MATERIALS AND METHODS

Animals and flight training

Cockatiels (*Nymphicus hollandicus* Kerr 1792; $N=5$, $92.0\pm 8.6\text{ g}$, mean body mass \pm s.d.) were purchased from local bird suppliers and housed in an indoor aviary in a temperature ($18\text{--}24^\circ\text{C}$)-and humidity ($45\text{--}65\%$)-controlled room with a 12h:12h light:dark cycle. Food and water were available *ad libitum*.

The birds were trained to fly in a variable speed Eifel design low-turbulence wind tunnel as previously described (Morris and Askew, 2010a). Birds were flown five times a week for a minimum of 8 weeks before the introduction of respirometry training masks. When the birds would fly continuously for 8 min at 12 m s^{-1} in the training mask they were introduced to a range of flight speeds from 6 to 14 m s^{-1} . The birds became accustomed to flight in the training mask for 8 weeks before the respirometry experiments.

Respirometry

Oxygen consumption is proportional to the metabolic energy consumed during aerobic exercise, thus the rate of oxygen consumption can represent the metabolic power input (Tucker, 1968). We used an open-flow respirometry system to measure the bird's oxygen consumption and carbon dioxide production by withdrawing exhaled and ambient air from a loose fitting mask worn by the birds.

Mask design

Light-weight respirometry and training masks were made from acetate sheet and secured onto the birds head by two strips of rubber. Initially the birds were trained to fly in training masks, which allowed the birds to become accustomed to flying with something on their head without the added weight and drag of the full respirometry equipment.

The full respirometry mask (Fig. 1) was connected to the respirometry system by vinyl tubing (internal diameter 2 mm, outer



Fig. 1. Cockatiel wearing a respirometry mask.

diameter 3 mm; Portex Limited, Kent, UK). The tubing was 90 cm long from the mask to where it exited the working section of the wind tunnel. The mass of the mask and tubing supported by the bird was 4.0 g.

Respirometry setup

Air was drawn through the respirometry mask by a vacuum pump (model DOA-P725-BN, GAST Manufacturing Inc., MI, USA). The air removed from the respirometry mask initially passed through a Drierite column to remove water and then entered the mass flow control valve (840 Side-Trak Mass Flow Controller; Sierra Instruments Inc., CA, USA) before the vacuum pump. The flow control valve was controlled by a mass flow controller (MFC-2 Mass Flow Valve Controller; Sable Systems International Inc., NV, USA), which allowed the rate of air flow through the flow control valve to be set and determined accurately. The output of the pump entered an open-end manifold, from which air was sub-sampled. The sub-sample passed through a magnesium perchlorate column to remove any remaining water, before entering the carbon dioxide analyser (Foxbox O_2/CO_2 Analyser; Sable Systems International Inc., NV, USA). After the carbon dioxide (CO_2) analyser the sub-sample entered a column of ascarite to scrub the air of CO_2 , and magnesium perchlorate to remove the water created from CO_2 scrubbing before the sample entered the oxygen (O_2) analyser (Foxbox O_2/CO_2 Analyser).

Prior to each session of data collection the O_2 and CO_2 analysers were calibrated. A two point calibration was used for both the O_2 and CO_2 linear analysers (manufacturer's specification). Zero percent O_2 and CO_2 were calibrated by recording the voltage output when nitrogen was pumped in. Outside air was used to measure the voltage output at 20.95% O_2 , and a 5% CO_2 calibration gas (BOC Gases, Surrey, UK) was used for the second CO_2 calibration voltage. Two equations were then used to convert the voltage output of the two analysers into a percentage O_2 and CO_2 gas content.

In addition to measuring the percentage O_2 and CO_2 content of air, the Foxbox also measured temperature and pressure. These four parameters and the rate of air flow through the flow control valve

were output in volts to an analogue to digital converter (PowerLab, ADInstruments Inc., CO, USA) linked to a computer. The data were recorded on the computer in LabChart (Version 5.5.5, ADInstruments Inc., CO, USA). The O₂ and CO₂ content of the air was recorded in the program as a raw voltage, temperature and pressure measurements were converted to degrees Celsius and atmospheres, respectively, and recorded. The flow rate through the flow control valve was converted from percentage flow to millilitres per second and recorded. During all flights air was pumped from the respirometry mask at a rate of 108.3 ml s⁻¹. We confirmed that this flow rate was adequate to collect all of the exhaled gases by reducing the flow rate to 50% of this value. There was no effect on the fractional oxygen and carbon dioxide content of the air and hence no variation in the rate of oxygen and carbon dioxide uptake (\dot{V}_{O_2} and \dot{V}_{CO_2} , respectively), indicating that 108.3 ml s⁻¹ was an appropriate rate.

Data collection

Simultaneous measurements of the bird's respiratory gases and mechanical power required for flight were recorded for all birds over the full range of speeds. At the beginning of each flight session each bird's mass was recorded. Prior to the respirometry mask being placed on the bird, the fractional oxygen and carbon dioxide content of the room air was measured. After being fitted with the respirometry mask the bird was allowed to sit on the perch with the wind tunnel on low (~6 m s⁻¹), while the O₂ and CO₂ content of the exhaled air was measured. Once the fractional gas contents at rest had reached a plateau, the bird was flown at three of the five different flight speeds tested (6, 8, 10, 12 and 13 m s⁻¹). Each trial lasted no more than 20 min in total with flight durations at each speed of no more than 3 min. Between flights the bird was allowed to rest on the perch for at least 5 min, where their oxygen and carbon dioxide consumption returned to a resting level. Data were collected twice a week for a period of 4 weeks, until all the birds flew across the full range of speeds.

Flights were video recorded at 250 frames s⁻¹ (Troubleshooter, model TS500MS, Fastec Imaging, USA) with a shutter duration of 0.2 ms. The camera was positioned lateral to the working section of the wind tunnel and a mirror was placed on top of the working section at a 45 deg angle to simultaneously record a lateral and dorsal view of the bird.

Data analysis

Respirometry

Respirometry data analysis was done in IGOR Pro (version 5.0.5.7, WaveMetrics, USA). Initially the measurements of oxygen and carbon dioxide content of air were converted from volts to their percentage content of air using the calibration equations previously determined. The flow rate at which air was pumped through the mask (M_F , ml s⁻¹) was standardized to standard temperature and pressure (\dot{V}_E , ml s⁻¹):

$$\dot{V}_E = \frac{273 M_F p_r}{(273 + T_m)}, \quad (1)$$

where p_r is pressure in atmospheres and T_m is the temperature in degrees Celsius. The rate of carbon dioxide production (\dot{V}_{CO_2} , ml s⁻¹) was calculated using Eqn 2 (from Withers, 1977):

$$\dot{V}_{CO_2} = \frac{\dot{V}_E (F_{ECO_2} - F_{ICO_2})(1 - F_{IO_2}) - F_{ICO_2} (F_{IO_2} - F_{EO_2})}{(1 - F_{ICO_2} - F_{IO_2})}, \quad (2)$$

where F_{ICO_2} is the fractional carbon dioxide content of air entering the mask, and F_{ECO_2} is the fractional carbon dioxide content of air

leaving the mask. The rate of oxygen consumption (\dot{V}_{O_2} , ml s⁻¹) was calculated using Eqn 3 (Withers, 1977):

$$\dot{V}_{O_2} = \frac{\dot{V}_E (F_{IO_2} - F_{EO_2}) - \dot{V}_{CO_2} F_{IO_2}}{(1 - F_{IO_2})}, \quad (3)$$

where F_{IO_2} is the fractional oxygen content of air entering the mask, and F_{EO_2} is the fractional oxygen content of air leaving the mask. Both the rate of oxygen consumption and carbon dioxide production were calculated relative to body mass (ml s⁻¹ kg⁻¹). To give an indication of the metabolite, and more importantly, to assess whether aerobic or anaerobic metabolism was used, the respiratory exchange ratio (RER) was calculated:

$$RER = \frac{\dot{V}_{CO_2}}{\dot{V}_{O_2}}. \quad (4)$$

An RER less than or equal to one indicates that the flight is at least partially aerobic, whereas a RER greater than one indicates that flight is anaerobic.

For flights to be included in the data set a plateau had to be achieved for the oxygen consumption and carbon dioxide production, and the RER had to be less than or equal to one.

Metabolic power (P_{met} , W) was calculated using Eqn 5 (Romijn and Lokhorst, 1962):

$$P_{met} = 16.18 \dot{V}_{O_2} + 5.02 \dot{V}_{CO_2}, \quad (5)$$

where \dot{V}_{O_2} and \dot{V}_{CO_2} are expressed in ml s⁻¹ STPD.

Kinematic and aerodynamic analysis

Flight kinematic and aerodynamic analysis was performed as described previously (Morris and Askew, 2010b). Flight kinematics were measured for masked and un-masked flights, for each bird at each flight speed where there was respirometry data. Kinematics included wing beat frequency, relative shortening duration, relative flapping duration, stroke amplitude and stroke plane angle relative to the horizontal.

Aerodynamic analysis was done for all flights where P_{met} was measured. The mass and horizontal force of the respirometry equipment was accounted for in the aerodynamic analysis (see Morris and Askew, 2010b). The horizontal force resulting from the drag on the tubing was measured in the wind tunnel at speeds ranging from 0 to 16 ml s⁻¹ in 2 ml s⁻¹ intervals. The respirometry tube was attached to a force transducer (FORT100, WPI, FL, USA) in a position that reproduced its attachment to the bird during flight. A third order polynomial was fitted to this data and the force was calculated at 6, 8, 10, 12, and 13 ml s⁻¹. The aerodynamic coefficients used in the analysis were the coefficients that best fit the *in vitro* muscle power data ($k=1.2$, $C_{D,par}=0.13$, $C_{D,pro}=0.02$) (Morris and Askew, 2010b).

Flight muscle efficiency (η_{fm}) was calculated as:

$$\eta_{fm} = \frac{k_{pos}}{(P_{met} - P_b)} P_{mech}, \quad (6)$$

where k_{pos} is the postural cost (assumed to equal 1.1, as in previous studies), P_{mech} is the mechanical power requirements and P_b is basal metabolism (Chai and Dudley, 1995; Pennycuik, 1975; Pennycuik, 1989; Tucker, 1973). P_b was calculated from scaling equations for non-passerines (Lasiewski and Dawson, 1967).

Statistical analysis

To test for differences in the rate of oxygen consumption, carbon dioxide production and the RER in relation to flight speed, a mixed-

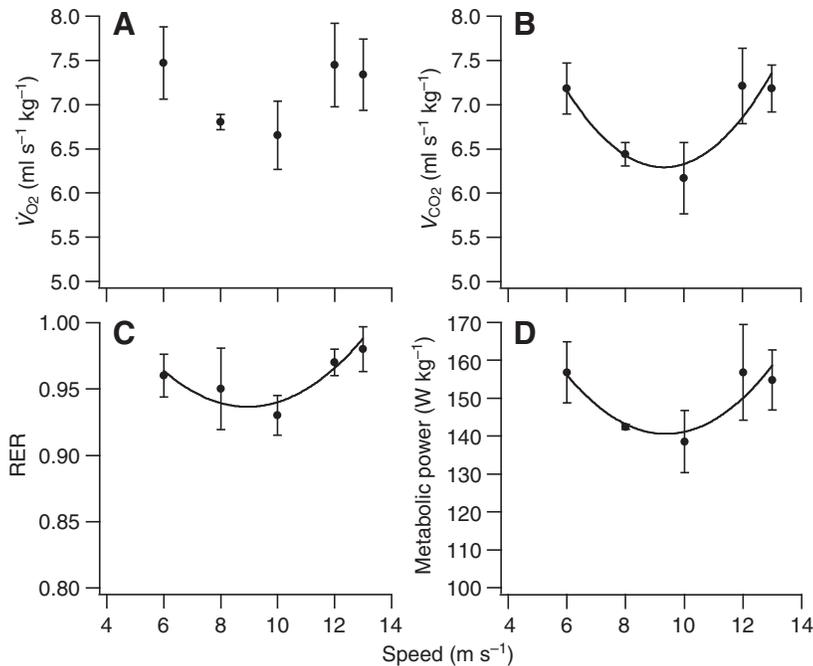


Fig. 2. The relationship between metabolic parameters and flight speed. (A) \dot{V}_{O_2} . (B) \dot{V}_{CO_2} . (C) RER. (D) Body mass-specific P_{met} . Data are shown as means \pm s.e.m. ($N=3, 3, 5, 3, 2$ for speeds 6, 8, 10, 12, 13 $m s^{-1}$, respectively). Third order polynomials are plotted for significant relationships.

effect model (type III) two-way general linear model was used. Individual birds were treated as a random factor and speed as a fixed factor. A fixed-effect model (type I) two-way general linear model was also used to test for difference in the aerodynamic power calculated during the masked flights, with *in vivo* muscle power measured from *in vitro* muscle performance (Morris and Askew, 2010a), and aerodynamic power calculated during respirometry data collection (Morris and Askew, 2010b), between 6 and 12 $ml s^{-1}$. Both flight speed and experimental condition were treated as fixed factors. Additionally paired *t*-tests were used to test for differences in flight kinematics between masked and un-masked flights. Kinematic parameters included wing beat frequency, relative shortening duration, relative flapping duration, stroke amplitude and stroke plane angle relative to the horizontal. All statistical analyses were performed in SPSS (version 14.0.2, SPSS Inc., USA).

RESULTS

Metabolic power

The relationship between the rate of oxygen consumption (\dot{V}_{O_2}) and flight speed had a shallow U-shape, where \dot{V}_{O_2} during flight was high at minimum and maximum flight speeds, and low at intermediate flight speeds with a minimum at 10 $m s^{-1}$ (Fig. 2A). \dot{V}_{O_2} ranged from $6.7 \pm 0.9 ml s^{-1} kg^{-1}$ at 10 $m s^{-1}$ to $7.5 \pm 0.7 ml s^{-1} kg^{-1}$ at 6 $m s^{-1}$. The variation in \dot{V}_{O_2} across flight speeds was not significant (GLM, $F_{4,22}=3.255$, $P=0.061$).

Both \dot{V}_{CO_2} (Fig. 2B) and RER (Fig. 2C) varied in a U-shaped relationship with flight speed, with maxima at 13 $m s^{-1}$; and similar to \dot{V}_{O_2} had minima at 10 $m s^{-1}$ (Fig. 2B,C). \dot{V}_{CO_2} ranged from $6.2 \pm 0.9 ml s^{-1} kg^{-1}$ at 10 $m s^{-1}$ to $7.2 \pm 1.0 ml s^{-1} kg^{-1}$ at 13 $m s^{-1}$. The variation in \dot{V}_{CO_2} across flight speeds was significant (GLM, $F_{4,22}=4.471$, $P=0.028$). The RER values measured in this study ranged from 0.93 ± 0.03 at 10 $m s^{-1}$ to 0.98 ± 0.02 at 13 $m s^{-1}$. The variation in RER across flight speeds was significant (GLM, $F_{4,22}=4.555$, $P=0.017$).

Metabolic power (P_{met}), as calculated using Eqn 5, varied in a U-shaped relationship with flight speed with a maximum at 6 $m s^{-1}$; and a minimum at 10 $m s^{-1}$. The variation in P_{met} with flight speeds

was significant (GLM, $F_{4,22}=3.609$, $P=0.048$). Body mass-specific P_{met} ranged from $138.6 \pm 8.2 W kg^{-1}$ at 10 $m s^{-1}$ to $156.9 \pm 8.0 W kg^{-1}$ at 6 $m s^{-1}$ (Fig. 2D).

Aerodynamic power

The aerodynamic power calculated for the masked flights was not significantly different from the previous measurements determined

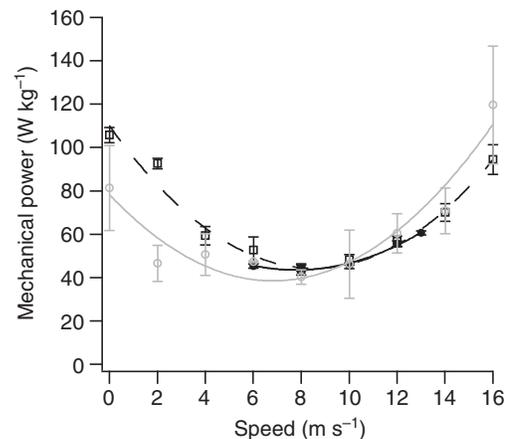


Fig. 3. The relationship between aerodynamic power and flight speed in comparison with *in vitro* muscle power. *In vitro* muscle power (open circle, solid grey line) (Morris and Askew, 2010a) is shown in relation to aerodynamic power, calculated using the best fit coefficients ($k=1.2$, $C_{D,par}=0.13$, $C_{D,pro}=0.02$) (Morris and Askew, 2010b), for the instrumented birds during *in vivo* sonomicrometry and EMG data collection (open square, broken black line) (Morris and Askew, 2010b) and masked flights (closed circles, solid black line). Data are shown as means \pm s.e.m. *In vitro* muscle power: $N=4, 4, 5, 3, 4, 3, 7, 3, 4$ for speeds 0, 2, 4, 6, 8, 10, 12, 14, 16 $m s^{-1}$, respectively; *in vitro* instrumented aerodynamic power: $N=4, 3, 5, 3, 4, 3, 7, 2, 4$ for speeds 0, 2, 4, 6, 8, 10, 12, 14, 16 $m s^{-1}$, respectively; masked aerodynamic power: $N=3, 3, 5, 4, 2$ for speeds 6, 8, 10, 12, 13 $m s^{-1}$, respectively.

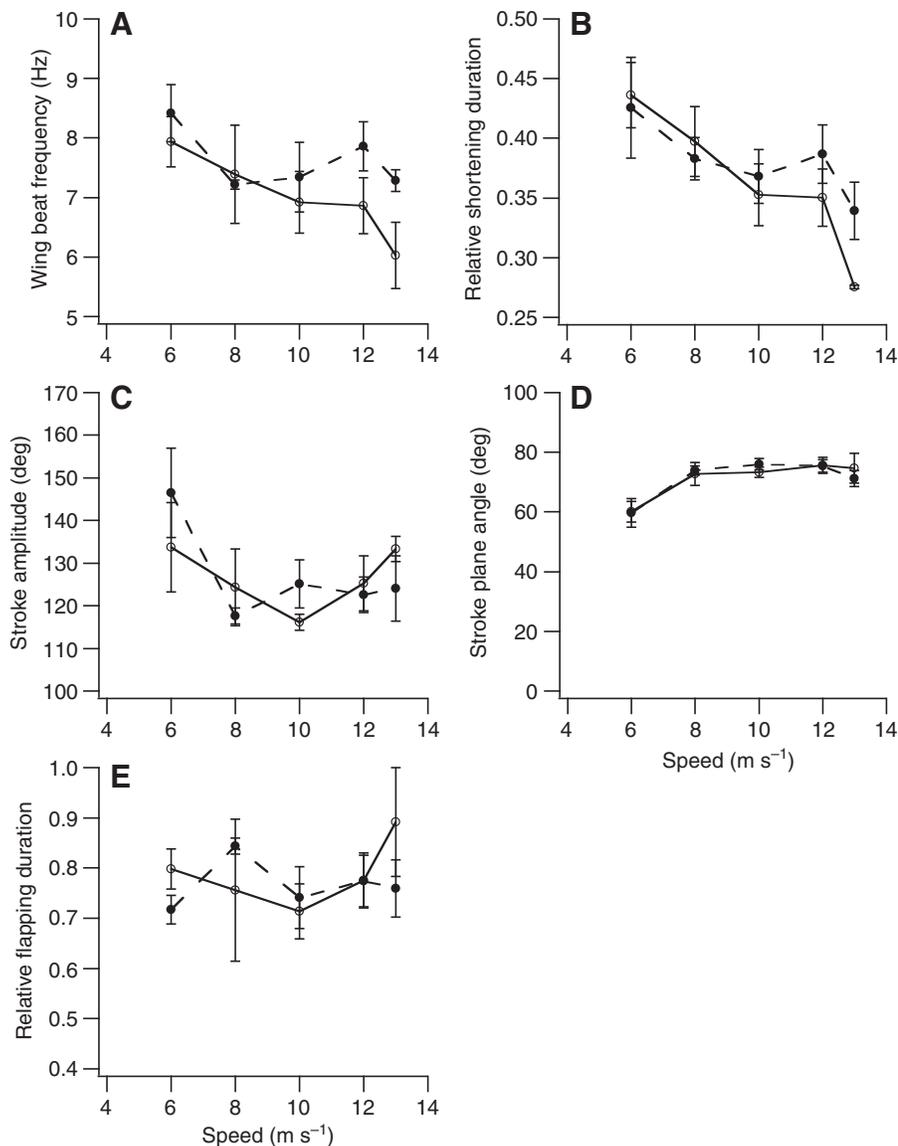


Fig. 4. Flight kinematics in relation to flight speed in masked (closed circles, dashed line) and un-masked (open circles, solid line) flights. (A) Wing beat frequency. (B) Relative shortening duration. (C) Stroke amplitude. (D) Stroke plane angle relative to the horizontal. (E) Relative flapping duration. Data are means \pm s.e.m. ($N=3, 3, 5, 4, 2$ for speeds 6, 8, 10, 12, 13 m s^{-1} , respectively).

using either *in vitro* muscle physiology (Morris and Askew, 2010a) or an aerodynamic model (Morris and Askew, 2010b) for the flight speeds between 6 and 12 m s^{-1} (GLM, $F_{2,48}=0.111$, $P=0.895$; Fig. 3).

Flight kinematics

No significant differences were detected in shortening duration (paired *t*-test, $t=-0.809$, $N=17$, $P=0.430$), relative shortening duration (paired *t*-test, $t=1.519$, $N=17$, $P=0.148$; Fig. 4B), relative flapping duration (paired *t*-test, $t=-0.210$, $N=17$, $P=0.836$; Fig. 4E), stroke amplitude (paired *t*-test, $t=0.516$, $N=17$, $P=0.613$; Fig. 4C) and stroke plane angle relative to the horizontal (paired *t*-test, $t=0.326$, $N=17$, $P=0.749$; Fig. 4D) between the masked and un-masked flights. However, a significant difference existed between masked and un-masked flights for wing beat frequency (paired *t*-test, $t=2.833$, $N=17$, $P=0.012$; Fig. 4A).

Flight muscle efficiency

Flight muscle efficiency also increased with flight speed, ranging from 6.9% at 8 m s^{-1} to 11.2% at 13 m s^{-1} (Fig. 5A).

DISCUSSION

In this study we made the first measurements of metabolic and mechanical power in cockatiels under similar flight conditions in the same wind tunnel. This allowed us to directly compare the metabolic and mechanical power speed relationships and to estimate flight muscle efficiency, without the uncertainty that the experimental conditions affected the results (see below).

Metabolic power requirements of flight

The metabolic power requirements of flight in cockatiels in this study ranged from 12.7 to 14.2 W. P_{met} for cockatiels is in the range previously measured in similarly sized birds [e.g. European starling *Sturnus vulgaris*: 8.1–14.9 W (Ward et al., 2001; Ward et al., 2004; Torre-Bueno and Larochelle, 1978); rose-coloured starling *Sturnus roseus*: 8.2 W (Engel et al., 2006); red knot *Calidris canutus*: 13.5 W (Kvist et al., 2001)], although a little higher than predicted from a scaling equation for bats and birds [10 W (Rayner, 1990)]. Metabolic power varied significantly in a U-shaped relationship with flight speed (Fig. 2D). Some studies (Bundle et al., 2007; Tucker, 1968) have also found a U-shaped metabolic power–speed relationship,

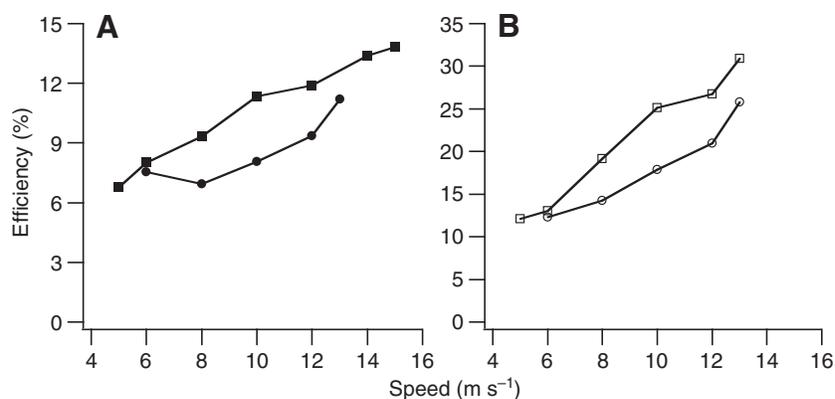


Fig. 5. The relationship between pectoralis muscle efficiency and flight speed. (A) Pectoralis muscle efficiency in relation to flight speed calculated from the mechanical power data presented in the accompanying paper (Morris and Askew, 2010a). Closed circles: metabolic power data presented in this study (mechanical power at 13 m s^{-1} was calculated from a third order polynomial fitted to the mechanical power–speed data). Closed squares: the metabolic power measurements by Bundle et al. (Bundle et al., 2007) (mechanical powers at 5 and 15 m s^{-1} were calculated from a third order polynomial fitted to the mechanical power–speed data). (B) Pectoralis muscle efficiency in relation to flight speed calculated from Hedrick et al.'s (Hedrick et al., 2003) mechanical power data. Open circles: metabolic power data presented in this study (mechanical power calculated from a third order polynomial fitted to the mechanical power–speed data; metabolic power at 13 m s^{-1} calculated from a third order polynomial fitted to the metabolic power–speed data). Open squares: metabolic power measurements by Bundle et al. (Bundle et al., 2007) (mechanical power was calculated from a third order polynomial fitted to the mechanical power–speed data; metabolic power at 13 m s^{-1} calculated from a third order polynomial fitted to the metabolic power–speed data).

whereas other studies have found a different shape to the metabolic power–speed relationship (Alexander, 1997; Bundle et al., 2007; Ellington, 1991).

\dot{V}_{O_2} and \dot{V}_{CO_2} varied in a U-shaped relationship with flight speed, with minima at 10 m s^{-1} (Fig. 2A,B). Bundle et al. (Bundle et al., 2007) also found a U-shaped relationship between \dot{V}_{O_2} and \dot{V}_{CO_2} and speed in cockatiels and the minimum power speed was the same as that reported here. Our \dot{V}_{O_2} values were similar to those previously reported for cockatiels; only the value at 8 m s^{-1} was significantly higher than previous measurements (Bundle et al., 2007). However, \dot{V}_{CO_2} was significantly higher than previously reported (Bundle et al., 2007). The difference in the \dot{V}_{CO_2} measurements between the two studies (this study) (Bundle et al., 2007) could result from one or more of the following reasons. First, there may be differences in the substrate utilized during flight. RER typically decreases during exercise in running mammals (Issekutz et al., 1967), flying mammals (Thomas, 1975) and flying birds (Butler et al., 1977; Polus, 1985; Rothe et al., 1987). This change is attributable to a switch in the substrate utilized, from primarily carbohydrate, at the start of exercise, to fat. In pigeons, \dot{V}_{O_2} reaches a steady level after approximately 10–15 min, whereas \dot{V}_{CO_2} takes approximately 45 min from the onset of flight (Rothe et al., 1987). In our study typical flight durations were shorter (1 to 3 min) than in Bundle et al.'s study (4 to >6 min) (Bundle et al., 2007) so, it is possible that the time course for the stabilization of \dot{V}_{CO_2} may have differed between the two studies. A second possibility is that anaerobic metabolism may have contributed to flight metabolism. This would increase the RER since less oxygen is required for a given amount of CO_2 during anaerobic metabolism. However, because in both studies RER values were less than 1, the contribution of anaerobic metabolism is presumed to have been relatively small (Fig. 2C) (Bundle et al., 2007). Other factors that may have contributed to the differences between the two studies are differences in the age of the birds, feeding conditions and season, all of which affected \dot{V}_{O_2} in pigeons (Rothe et al., 1987), and differences in the strain of cockatiels. Differences in the mass of the respirometry mask and tubing are likely to affect flight performance, although the mass of the

respirometry mask and tubing used in the Bundle et al. (Bundle et al., 2007) study (8.9 g or 11% of the birds' body mass) was higher than in our study (4.0 g or 4% of the birds' body mass; this study), and so is unlikely to explain the higher \dot{V}_{CO_2} reported here.

We found that the minimum mechanical power speed is close to the minimum metabolic power speed (10 m s^{-1} ; Fig. 2D, Fig. 3) (Morris and Askew, 2010b). Previous findings for the budgerigar also indicate that the minimum mechanical and metabolic power speeds correspond (Bundle et al., 2007; Ellerby and Askew, 2007; Tucker, 1973). Together the data for the cockatiel and budgerigar suggests that the minimum mechanical and metabolic power speeds correspond.

Flight muscle efficiency in cockatiels

Flight muscle efficiency in cockatiels can be estimated from the metabolic (this study) (Bundle et al., 2007) and mechanical (Morris and Askew, 2010a; Hedrick et al., 2003) power requirements of flight (Eqn 6). With these data, we can make a four-way comparison to determine a range of predicted efficiencies for each pairing (Table 1). For all four comparisons, flight muscle efficiency increased with increasing flight speed (Table 1; Fig. 5). The flight muscle efficiency calculated from our studies (this study and Morris and Askew, 2010a) is close to the values calculated from our mechanical power (Morris and Askew, 2010a) and Bundle et al.'s (Bundle et al., 2007) P_{met} . This is because our study and that of Bundle et al. (Bundle et al., 2007) had very similar values of P_{met} . However, the flight muscle efficiency is much higher when estimated using the higher mechanical power values of Hedrick et al. (Hedrick et al., 2003) [see Morris and Askew (Morris and Askew, 2010b) for discussion on the discrepancy in the two sets of mechanical power data].

Interference of air flow caused by the walls of the wind tunnel is expected to reduce the mechanical (and therefore metabolic) power requirements of flight, especially at low flight speeds (Rayner, 1994). These effects are likely to have been more significant in both metabolic studies, where smaller wind tunnels were used, compared with mechanical study of Hedrick et al. (Hedrick et al., 2003). At

flight speeds where ground effect is important, flight muscle efficiency may be over-estimated when comparing mechanical power determined in a relatively large wind tunnel with metabolic power measured in a relatively small wind tunnel. For this reason, caution must be applied when making comparisons between mechanical and metabolic power requirements determined in different studies: comparisons between studies made under similar experimental conditions are likely to be the most reliable (e.g. this study) (Morris and Askew, 2010a; Bundle et al., 2007).

Comparison with other efficiency estimates

Estimated flight muscle efficiency ranged from 6.9 to 11.2% (Table 1; Fig. 5). This range of efficiency values can be compared with direct measurements on isolated mammalian muscles and to estimates from other birds and mammals during flight. Our range of muscle efficiency is lower, but overlaps the range of measured muscle efficiencies in isolated mammalian skeletal muscle [10–19%; table 4.1 in Smith et al. (Smith et al., 2005)]. It is also slightly lower than previous muscle efficiency values calculated during flight [European starlings: 13–23% (Ward et al., 2001); bats: 15% (Norberg et al., 1993), and hummingbirds: 10–12% (Chai et al., 1998; Chai and Dudley, 1995)]. However, in making comparisons with these previously published estimates of flight muscle efficiency, the following assumptions must be taken into consideration.

The first is that mechanical power is accurately estimated. Estimation of mechanical power using an aerodynamic model is sensitive to the values assumed for a number of coefficients in the calculations (Askew and Ellerby, 2007; Morris and Askew, 2010b). For cockatiels a physiological approach was used to suggest a range of appropriate values for use in the calculation of mechanical power using an aerodynamic model (Morris and Askew, 2010a; Morris and Askew, 2010b). However, uncertainty remains on the appropriate values to be used in the equations in other species. In addition, the power requirements of flight in laboratory studies may be affected by the equipment used. For example, the mechanical costs of flight power determined within a wind tunnel may be affected by: (1) interference of the air flow resulting from the walls of the wind tunnel; and (2) increased drag and weight associated with recording equipment (e.g. respirometry mask and tubing, data recording cables, etc.). In our study, the wing span of cockatiels in relation to dimensions of the working section of the wind tunnel means that it is likely that ground effect does reduce the mechanical power requirements of flight (see also Morris and Askew, 2010b). In addition, wing beat kinematics and the use of intermittent flight were significantly different between the birds instrumented for respirometry and un-instrumented flights (Fig. 4) (see also Bundle et al., 2007). The changes in wing beat kinematics and the use of intermittent flight probably reflect the increase in the mechanical power requirements of instrumented flights associated with the additional mass and drag of the mask and tubing. There was no significant difference between the aerodynamic power estimated in birds instrumented for respirometry measurements and those instrumented for in vivo recording of muscle length change and activity patterns (Morris and Askew, 2010a) (Fig. 3). This suggests that it is valid to compare mechanical and metabolic measurements from this and our companion studies (Morris and Askew, 2010a). However, comparisons between separately collected mechanical and metabolic data in which it has not been established that the

Table 1. Summary of estimated muscle efficiencies

Efficiency (%)	Flight speed range (m s ⁻¹)	Metabolic study	Mechanical study
6.9–11.2	6–13	This study	Morris and Askew, 2010a
6.8–13.8	5–15	(Bundle et al., 2007)	Morris and Askew, 2010a
12.3–25.8	6–13	This study	(Hedrick et al., 2003)
12.1–30.9	5–13	(Bundle et al., 2007)	(Hedrick et al., 2003)

mechanical power requirements are similar under both conditions should be interpreted with caution.

Second, is the assumption that the metabolic power requirements are accurately measured. The metabolic power requirements of flight may also be affected by elevated stress resulting from flight in a restricted environment (Ward et al., 2001). We are unable to ascertain whether P_{met} during wind tunnel flight is higher than during free flight as a result of elevated stress levels. However, birds were flown on a regular basis in the wind tunnel, including flights with training masks, to accustom the birds to the experimental conditions. As noted above, the effects of instrumentation of birds on the mechanical power requirements is likely to also affect the metabolic power requirements of flight. It is therefore preferable to compare metabolic and mechanical power requirements of flight that have been determined under similar conditions. Comparisons where this is not the case must be interpreted cautiously.

Third, basal metabolic rate (P_b) is assumed to be constant across flight speed. We calculated P_b from previous published scaling equations (Lasiewski and Dawson, 1967), since neither our study nor that of Bundle et al. (Bundle et al., 2007) measured basal metabolic rate. However, it is not clear whether the amount of energy used to maintain life is constant during exercise. During running in birds, there is a doubling of blood flow to the brain (Ellerby et al., 2005). Without the use of an approach to determine regional energy expenditure during locomotion (e.g. Ellerby et al., 2005) we are unable to evaluate the validity of this assumption during flight. However, the error is likely to be relatively small since doubling our estimate of basal metabolic rate only increases the estimate of efficiency by approximately 6% of the values presented in Table 1.

The fourth assumption is that respiration and circulation each contribute 5% to P_{met} during flight (i.e. $k_{\text{pos}}=1.1$). Based on a previous study of bird running (Ellerby et al., 2005) we know the blood flow to the heart and muscles associated with respiration increase with the speed of locomotion. We do not know from this study exactly what percentage increase in total metabolic cost these increases represent, but the coronary circulation and respiratory muscles do appear to account for approximately 10% of the increase in cardiac output with exercise. However, we calculated that changing k_{pos} to 1.3 only slightly increased our estimate of muscle efficiency: 8.9% to 11.8%. Therefore, although the assumption that the heart and respiratory muscles always consume 10% of the total metabolic cost of locomotion regardless of the speed of locomotion is probably false, it should not dramatically affect estimations of muscle efficiency.

The fifth assumption is that no other physiological systems consume metabolic energy. This assumption is likely to be false. Approximately one-third of the flight muscle mass in cockatiels is muscles (K. M. C. Tjørve and G.N.A., unpublished data) that are unlikely to contribute to the mechanical power required to impart momentum to the air to provide lift, and instead serve to change wing shape, vary the angle of the stroke plane and stabilize joints. Clearly their importance as contributors to the total energy costs of flight cannot be ignored. These 'accessory muscles' probably

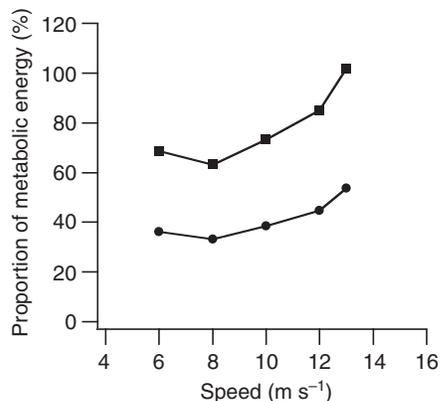


Fig. 6. The proportion of metabolic energy consumed by the pectoralis muscle in relation to flight speed. Calculated from the metabolic data presented in this study and the mechanical power data from Morris and Askew, 2010a when the pectoralis muscle operates at an assumed 10% (closed squares) and 19% (closed circles) efficiency. Mechanical power measurement at 13 m s⁻¹ was calculated from a third order polynomial fitted to the mechanical power–speed data.

contribute a significant proportion of the energy costs of flight. Furthermore, changes in wing kinematics that occur with speed as a result of the changing aerodynamic function of the upstroke (Spedding et al., 2003) probably result in a change in the relative contribution of these muscles to the energy budget. There are currently no data on the energy contribution of these accessory muscles; however, estimates of the mechanical power output of the supracoracoideus muscle in pigeons (Tobalske and Biewener, 2008) and the inertial power of the wing in cockatiels (Hedrick et al., 2004) both indicate that the energy contribution of the accessory muscles could be relatively high. The proportion of the total flight metabolic rate accounted for by pectoralis muscle metabolism, from which the energy use by other physiological processes, including the accessory muscles, can be estimated (see below).

Allocating increased metabolic cost to various muscles

The proportion of energy use by the pectoralis muscles can be estimated as follows:

$$\%P_{\text{met,pec}} = \frac{P_{\text{mech}}}{\eta_{\text{fm}}(P_{\text{met}} - P_{\text{b}})} \times 100, \quad (7)$$

where % $P_{\text{met,pec}}$ is the percentage of the total metabolic cost of flight consumed by the pectoralis muscles and assuming η_{fm} ranges between 10% and 19% (Smith et al., 2005).

When operating with an assumed efficiency of 19% the cost of pectoralis muscle mechanical power production (measured by Morris and Askew, 2010a) represents 36–54% of the increase in metabolic cost above rest, at flight speeds between 6 and 13 m s⁻¹ (measured in this study; Fig. 6). Therefore, other physiological systems (including the circulatory and respiratory systems, P_{b} and accessory muscles) contribute 46–64% of P_{met} . If the pectoralis muscle operated with an efficiency of 10%, the metabolic cost of the pectoralis muscle is 69–102% of the increase in metabolic cost above rest, at flight speeds from 6 to 13 m s⁻¹ (Fig. 6). Other physiological processes contribute 0–31% of P_{met} . Without better estimates of the pectoralis muscle efficiency or a means of measuring the metabolic energy expenditure of the pectoralis muscle, it is not currently possible to improve the estimate of how P_{met} is partitioned between different physiological systems.

Concluding remarks

The metabolic power requirements of flight varied in a U-shaped relationship with flight speed with a minimum at 10 m s⁻¹. Although this is similar to the minimum mechanical power speed (Morris and Askew, 2010a), the metabolic power requirements are not simply a multiple of the mechanical power requirements because of the possible variations in muscle efficiency and in the energy distribution between physiological systems, and the accessory and main flight muscles with flight speed. In order to obtain more accurate measures of *in vivo* muscle efficiency the metabolic cost of the flight muscles would need to be separately determined.

ACKNOWLEDGEMENTS

This research was supported by a Biotechnology and Biological Sciences Research Council (BBSRC) research grant (BB/C503703/1) and a BBSRC strategic research studentship (BBS/S/H/2005/11890). The work complied with The Animals (Scientific Procedures) Act 1986 and was carried out under license from the British Home Office with approval from the local ethical review committee.

REFERENCES

- Alexander, R. M. (1997). The U, J and L of bird flight. *Nature* **390**, 13.
- Askew, G. N. and Ellerby, D. J. (2007). The mechanical power requirements of avian flight. *Biol. Lett.* **3**, 445–448.
- Biewener, A. A., Dial, K. P. and Goslow, G. E. (1992). Pectoralis-muscle force and power output during flight in the starling. *J. Exp. Biol.* **164**, 1–18.
- Bundle, M. W., Hansen, K. S. and Dial, K. P. (2007). Does the metabolic rate-flight speed relationship vary among geometrically similar birds of different mass? *J. Exp. Biol.* **210**, 1075–1083.
- Butler, P. J., West, N. H. and Jones, D. R. (1977). Respiratory and cardiovascular responses of the pigeon to sustained level flight in a wind tunnel. *J. Exp. Biol.* **71**, 7–26.
- Casey, T. M. and Ellington, C. P. (1989). Energetics of insect flight. In *Energy Transformation in Cells and Organisms* (ed. W. Wieser and E. Gnaiger), pp. 200–210. Stuttgart: Georg Thieme Verlag.
- Chai, P. and Dudley, R. (1995). Limits to vertebrate locomotor energetics suggested by hummingbirds hovering in heliox. *Nature* **377**, 722–725.
- Chai, P., Chang, A. C. and Dudley, R. (1998). Flight thermogenesis and energy conservation in hovering hummingbirds. *J. Exp. Biol.* **201**, 963–968.
- Ellerby, D. J. and Askew, G. N. (2007). Modulation of flight muscle power output in budgerigars *Melopsittacus undulatus* and zebra finches *Taeniopygia guttata*: in vitro muscle performance. *J. Exp. Biol.* **210**, 3780–3788.
- Ellerby, D. J., Henry, H. T., Carr, J. A., Buchanan, C. I. and Marsh, R. L. (2005). Blood flow in guinea fowl *Numida meleagris* as an indicator of energy expenditure by individual muscles during walking and running. *J. Physiol.* **564**, 631–648.
- Ellington, C. P. (1991). Limitations on animal flight performance. *J. Exp. Biol.* **160**, 71–91.
- Engel, S., Biebach, H. and Visser, G. H. (2006). Metabolic costs of avian flight in relation to flight velocity: a study in rose coloured starlings (*Sturnus roseus*, Linnaeus). *J. Comp. Physiol. B* **176**, 415–27.
- Hedrick, T. L., Tobalske, B. W. and Biewener, A. A. (2003). How cockatiels (*Nymphicus hollandicus*) modulate pectoralis power output across flight speeds. *J. Exp. Biol.* **206**, 1363–1378.
- Hedrick, T. L., Usherwood, J. R. and Biewener, A. A. (2004). Wing inertia and whole-body acceleration: an analysis of instantaneous aerodynamic force production in cockatiels (*Nymphicus hollandicus*) flying across a range of speeds. *J. Exp. Biol.* **207**, 1689–1702.
- Issekutz, B., Paul, P. and Miller, H. I. (1967). Metabolism in normal and pancreatized dogs during steady-state exercise. *Am. J. Physiol.* **213**, 857–862.
- Kvist, A., Lindström, Å., Green, M., Piersma, T. and Visser, G. H. (2001). Carrying large fuel loads during sustained bird flight is cheaper than expected. *Nature* **413**, 730–732.
- Lasiewski, R. C. and Dawson, W. R. (1967). A re-examination of relation between standard metabolic rate and body weight in birds. *Condor* **69**, 13–23.
- Morris, C. R. and Askew, G. N. (2010a). The mechanical power output of the pectoralis muscle of cockatiel (*Nymphicus hollandicus*): the *in vivo* muscle length trajectory and activity patterns and their implications for power modulation. *J. Exp. Biol.* **213**, 2770–2780.
- Morris, C. R. and Askew, G. N. (2010b). Comparison between mechanical power requirements of flight estimated using an aerodynamic model and *in vitro* muscle performance in the cockatiel (*Nymphicus hollandicus*). *J. Exp. Biol.* **213**, 2781–2787.
- Norberg, U. M., Kunz, T. H., Steffensen, J. F., Winter, Y. and Vonhelversen, O. (1993). The cost of hovering and forward flight in a nectar-feeding bat, *Glossophaga soricina*, estimated from aerodynamic theory. *J. Exp. Biol.* **182**, 207–227.
- Pennycuik, C. J. (1975). Mechanics of flight. In *Avian Biology*, vol. 5 (ed. D. S. Farner and J. R. King), pp. 1–75. London, UK: Academic Press.
- Pennycuik, C. J. (1989). *Bird Flight Performance: a Practical Calculation Manual*. Oxford: Oxford University Press.
- Polus, M. (1985). Quantitative and qualitative respiratory measurements on unrestrained free flying pigeons by AMACS (Airborne Measuring And Control Systems). In *BIONA Report 3* (ed. W. Nachtigall), pp. 293–301. Stuttgart: G. Fischer.
- Rayner, J. M. V. (1990). The mechanics of flight and bird migration performance. In *Bird Migration*, (ed. E. Gwinner), pp. 283–299. Heidelberg: Springer Verlag.
- Rayner, J. M. V. (1994). Aerodynamic corrections for the flight of birds and bats in wind tunnels. *J. Zool.* **234**, 537–563.

- Romijn, C. and Lokhorst, W.** (1962). Some aspects of energy metabolism in birds. In *Symposium on Energy Metabolism 2. Methods and Results* (ed. E. Brouwer and A. J. H. van Es), pp. 49-59. Wageningen: E.A.A.P.
- Rothe, H.-J., Biesel, W. and Nachtigall, W.** (1987). Pigeon flight in a wind tunnel. II. Gas exchange and power requirements. *J. Comp. Physiol. B* **157**, 99-109.
- Schmidt-Nielsen, K.** (1972). Locomotion-energy cost of swimming, flying, and running. *Science* **177**, 222-228.
- Smith, N. P., Barclay, C. J. and Loiselle, D. S.** (2005). The efficiency of muscle contraction. *Progr. Biophys. Mol. Biol.* **88**, 1-58.
- Spedding, G. R., Rosén, M. and Hedenström, A.** (2003). A family of vortex wakes generated by a thrush nightingale in free flight in a wind tunnel over its entire natural range of flight speeds. *J. Exp. Biol.* **206**, 2313-2344.
- Thomas, S. P.** (1975). Metabolism during flight in two species of bats, *Phyllostomus hastatus* and *Pteropus gouldii*. *J. Exp. Biol.* **63**, 273-293.
- Tobalske, B. W. and Biewener, A. A.** (2008). Contractile properties of the pigeon supracoracoideus during different modes of flight. *J. Exp. Biol.* **211**, 170-179.
- Torre-Bueno, J. R. and Larochele, J.** (1978). The metabolic cost of flight in unrestrained birds. *J. Exp. Biol.* **75**, 223-229.
- Tucker, V. A.** (1968). Respiratory exchange and evaporative water loss in flying budgerigar. *J. Exp. Biol.* **48**, 67-87.
- Tucker, V. A.** (1973). Bird metabolism during flight-evaluation of a theory. *J. Exp. Biol.* **58**, 689-709.
- Ward, S., Moller, U., Rayner, J. M. V., Jackson, D. M., Bilo, D., Nachtigall, W. and Speakman, J. R.** (2001). Metabolic power, mechanical power and efficiency during wind tunnel flight by the European starling *Sturnus vulgaris*. *J. Exp. Biol.* **204**, 3311-3322.
- Ward, S., Moller, U., Rayner, J. M. V., Jackson, D. M., Nachtigall, W. and Speakman, J. R.** (2004). Metabolic power of European starlings *Sturnus vulgaris* during flight in a wind tunnel, estimated from heat transfer modelling, doubly labelled water and mask respirometry. *J. Exp. Biol.* **207**, 4291-4298.
- Withers, P. C.** (1977). Measurement of VO_2 , VCO_2 , and evaporative water-loss with a flow-through mask. *J. Appl. Physiol.* **42**, 120-123.