The Journal of Experimental Biology 213, 2770-2780

doi:10.1242/jeb.035691

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

Charlotte R. Morris and Graham N. Askew*

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

*Author for correspondence (g.n.askew@leeds.ac.uk)

Accepted 21 May 2010

SUMMARY

In order to meet the varying demands of flight, pectoralis muscle power output must be modulated. In birds with pectoralis muscles with a homogeneous fibre type composition, power output can be modulated at the level of the motor unit (*via* changes in muscle length trajectory and the pattern of activation), at the level of the muscle (*via* changes in the number of motor units recruited), and at the level of the whole animal (through the use of intermittent flight). Pectoralis muscle length trajectory and activity patterns were measured *in vivo* in the cockatiel (*Nymphicus hollandicus*) at a range of flight speeds (0–16 m s^{-1}) using sonomicrometry and electromyography. The work loop technique was used to measure the mechanical power output of a bundle of fascicles isolated from the pectoralis muscle during simulated *in vivo* length change and activity patterns. The mechanical power–speed relationship was U-shaped, with a 2.97-fold variation in power output (40–120 W kg^{-1}). In this species, modulation of neuromuscular activation is the primary strategy utilised to modulate pectoralis muscle power output. Maximum *in vivo* power output was 22% of the maximum isotonic power output (533 W kg^{-1}) and was generated at a lower relative shortening velocity (0.28 V_{max}) than the maximum power output during isotonic contractions (0.34 V_{max}). It seems probable that the large pectoralis muscle strains result in a shift in the optimal relative shortening velocity in comparison with the optimum during isotonic contractions as a result of length–force effects.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/213/16/2770/DC1

Key words: flight, modulation, power, recruitment.

INTRODUCTION

The need to modulate muscular power output is common to many activities. For example, during terrestrial locomotion the power output of the muscles driving the system is increased by 3.9- to 4.6-fold in order to meet the increased energy demands during incline running (Farley, 1997) and accelerations (Roberts and Scales, 2002) compared with steady speed locomotion. During avian flight mechanical power increases by 2.1- to 2.7-fold across a range of flight speeds (Hedrick et al., 2003; Tobalske et al., 2003; Ellerby and Askew, 2007a) compared with the minimum mechanical power requirements.

Animals use a range of strategies to modulate power (Hedrick et al., 2003; Ellerby and Askew, 2007a; Ellerby and Askew, 2007b). First, power can be modulated at the level of the motor unit *via* changes in the muscle length trajectory and activity pattern. Second, power can be modulated at the level of the muscle by changes in the numbers of motor units recruited within the muscle (including recruitment of motor units with different physiological properties). Third, power can be modulated at the level of the whole animal through the recruitment of different muscles: power can be increased within a group of muscle synergists by recruiting muscles with faster contracting fibres or by controlling the load on a muscle by changing the way in which a morphological structure (e.g. a limb) interacts with the environment (Marsh, 1999). Fourth, muscular power can be modulated behaviourally by the use of intermittent locomotion.

Bird flight provides an excellent model system for investigating power modulation mechanisms (Hedrick et al., 2003; Ellerby and Askew, 2007a; Ellerby and Askew, 2007b). First, the need to modulate flight muscle mechanical power output in birds has been demonstrated using a variety of approaches, including aerodynamic modelling (Pennycuick, 1975; Rayner, 1979; Askew and Ellerby, 2007), measurements of metabolic rate (Tucker, 1973; Ward et al., 1999; Bundle et al., 2007), measurements of bone strain as an index of muscle force (Biewener et al., 1992; Dial et al., 1997; Hedrick et al., 2003) or determined *in vitro* from the physiological properties of the flight muscles (Askew and Ellerby, 2007; Ellerby and Askew, 2007a). Although these approaches have produced power–speed relationships that vary both qualitatively and quantitatively, the general pattern that is observed is a U-shaped power–speed relationship. Therefore, mechanical power output must be modulated with flight speed in order to meet these changing power requirements. Second, the pectoralis muscles are the main source of mechanical power in birds, and birds are therefore unable to vary power by recruiting different muscle synergists.

Many large species of bird have flight muscles that have a heterogeneous fibre type composition (e.g. Rosser and George, 1986), providing the potential for differential fibre type recruitment and power modulation. In pigeons (*Columba livia*), several lines of evidence support the hypothesis that there is a bipartite division of labour between the fast glycolytic (FG) and fast oxidative glycolytic (FOG) muscle fibres types found within its pectoralis muscle. FG motor units are recruited during activities such as take-off and landing that demand a high power output, whereas FOG motor units are recruited during sustained level flight when power requirements

are lower (Sokoloff et al., 1998). By contrast, the pectoralis muscles in many small birds are homogeneous in their fibre type (Rosser and George, 1986), precluding the modulation of power by differential fibre type recruitment. For these species, power modulation can occur either at a motor unit or whole muscle level through changes in activity pattern, motor unit recruitment or strain trajectory, or at a behavioural level, by adopting an intermittent mode of flight.

Data exist on the power modulation strategies in small birds with homogenous pectoralis muscle fibre type composition. Both zebra finches and budgerigars modulate power using a combination of activation strategies, fascicle length trajectory and intermittent flight (Tobalske and Dial, 1994; Tobalske et al., 2005; Ellerby and Askew, 2007a). The relative importance of the strategy differs between these two species and with flight speed. In both species modulation in power occurs predominantly *via* changes in strain trajectory and neuromuscular activation (Ellerby and Askew, 2007a). However, at high flight speeds, intermittent flight is the dominant modulation mechanism in zebra finches. Overall, intermittent flight is a more important modulation mechanism in zebra finches than in budgerigars (Ellerby and Askew, 2007a). In cockatiels (*Nymphicus hollandicus*) power modulation occurs primarily through modulation of muscle force (Hedrick et al., 2003). However, as force modulation can result from both modulation of length trajectory and modulation of activation, distinguishing between modulation of power by strain trajectory and activation is not possible from this study (Ellerby and Askew, 2007a).

We hypothesised that the relative importance of each modulation strategy will vary with body mass for the following three reasons. First, twitch time decreases with increasing strain cycle frequency, scaling as frequency$^{-0.687}$ [figure 10 in Girgenrath and Marsh (Girgenrath and Marsh, 1999)], indicating that as strain cycle frequency increases muscles are relatively slower in relation to their strain cycle frequency. Therefore muscles operating at higher strain cycle frequencies may have less scope to modulate power by variation in activity duty cycle. For example, in hylid tree frogs the twitch rise time of the external oblique muscles represents a greater fraction of the cycle shortening duration in *Hyla chrysoscelis* (relative twitch duration 0.8; operating frequency 44 Hz) compared with *Hyla versicolor* (relative twitch duration 0.5; operating frequency 21 Hz). Second, in mammals, the number of motor units in a given muscle decreases with decreasing body mass (Buchthal and Schmalbruch, 1980; Ishihara et al., 2001). Assuming that the same relationship holds in birds and that there are relatively few motor units in small birds, smaller birds may be less well able to modulate power by the recruitment of different numbers of motor units. Third, the use of intermittent flight as a power modulation strategy appears to also be related to body mass with the use of intermittent flight decreasing with increasing body mass (Tobalske, 1996).

In contrast to the potential size-related differences in modulation of recruitment at the level of the motor unit and the use of intermittent flight, power modulation by variation in fascicle length trajectory appears to be a general feature of all muscles (Askew and Marsh, 1997; Girgenrath and Marsh, 1999) and the degree to which variation in length trajectory is used as a modulation strategy is unlikely to vary with body mass. Therefore the relative importance of different power modulation strategies in birds with homogeneous muscle composition are expected to vary with body mass.

In this study we measured the mechanical power–speed relationship of cockatiels (*Nymphicus hollandicus*) and determined the strategies utilised to modulate muscular power. We predicted that power modulation at the level of the motor unit *via* changes in the muscle length trajectory and neuromuscular activation will be more important than intermittent flight in comparison with smaller birds such as budgerigars and zebra finches (Tobalske and Dial, 1994; Tobalske et al., 2005; Askew and Ellerby, 2007; Ellerby and Askew, 2007a; Ellerby and Askew, 2007b; Tobalske, 2007). We used a physiological approach (Askew and Ellerby, 2007; Ellerby and Askew, 2007a; Ellerby and Askew, 2007b) to measure the mechanical power output of the pectoralis muscle in relation to flight speed. *In vivo* pectoralis muscle strain and activity patterns were measured at a range of flight speeds in a wind tunnel using sonomicrometry and electromyography (EMG). The *in vivo* muscle length change and activity patterns were then imposed onto an isolated bundle of pectoralis muscle fascicles *in vitro* and the mechanical power was measured using the work loop technique (Josephson, 1985a). This study, for the first time, imposes an individual bird's *in vivo* length change and activity pattern onto muscle fascicles isolated from the same individual. This ensures that mechanical power for an individual bird is estimated under the simulated *in vivo* conditions for the same bird.

MATERIALS AND METHODS

Animals and flight training

Cockatiels (*Nymphicus hollandicus* Kerr 1792) were purchased from local suppliers and housed in an indoor aviary in a temperature (20–22°C) and relative humidity (49–55%) controlled room with a 12 h:12 h light:dark cycle. Food and water was available *ad libitum*. Eight cockatiels (body mass 91.8±7.0 g; mean ± s.d.) were used for the *in vivo* data collection and *in vitro* muscle power measurements and seven cockatiels (body mass 97.2±6.6 g) were used for the *in vitro* isotonic shortening contractions.

The birds were trained to fly in a variable speed Eifel design low turbulence wind tunnel. The working section of the wind tunnel was 52 cm×52 cm×95 cm (width × height × length). Vertical nylon threads (0.4 mm diameter and spaced 15 mm apart upstream and 7.5 mm apart downstream) kept the bird in the working section. Prior to flight the birds were trained to sit on a perch. Flight was initiated by removing the perch and terminated by presenting the bird with the perch. Initially birds were flown at 10 m s^{-1} which was then increased to 12 m s^{-1} as they became accustomed to flying in the wind tunnel. Birds were flown five times a week for a minimum of 4 weeks. In the latter stages of training the birds were introduced to a range of flight speeds from 0–16 m s^{-1}. At the highest and lowest flight speeds the birds could not maintain steady flight for more than 30 s.

Sonomicrometry and electromyography

An implant was constructed for each bird, consisting of a female PCB socket (2 mm pitch), to which two 1.0 mm sonomicrometry transducers (Sonometrics Inc., London, Ontario, Canada), two silver bipolar EMG electrodes and a silver ground wire were soldered (Ellerby and Askew, 2007b). The bipolar EMG electrodes had bared tips of 1.5 mm with a 3 mm spacing between the two electrodes. The EMG electrodes and the ground wire were made from insulated silver wire (0.075 mm diameter; Goodfellow Cambridge Ltd, UK). To secure the sonomicrometry transducers in place and ensure correct crystal orientation when placed in the muscle, they were attached to metal holders. These holders were made from stainless steel insect pins bent into three arms, each of which was orientated at 90 deg to the other arms (Askew and Marsh, 2001). One arm was attached to the wire of the vertical sonomicrometry transducer using epoxy resin. The other two arms of the holder were used to secure

the crystal and holder to the surface of the muscle. The total mass of the implant was approximately 0.6 g.

The bird was connected to the data acquisition system by a light-weight cable (3.0 g). The cable entered the wind tunnel through a hole in the top and towards the rear of the working section. This cable consisted of two twisted pairs of insulated, stainless steel, 38 gauge wires and a seven core multi-stranded 0.07 mm enamelled copper wire. These wires were soldered to a male PCB socket. During data collection the male socket of the cable was attached to the female connector on the bird's back. The PCB socket was wired such that upon connection to the implant connector, the sonomicrometry transducers were connected to the twisted pairs of stainless steel wire and the EMG electrodes and ground wires were connected to the multi-stranded copper wire. The other end of the data cable was linked to the sonomicrometry system (TRX Series 8, Sonometrics, Ontario, Canada) and to two EMG pre-amplifiers (DAM50, WPI, USA).

Surgical procedures

The sonomicrometry transducers and EMG electrodes were implanted while the birds were under isoflurane-induced anaesthesia, which was administered at a concentration of 4% to induce and 1–2% to maintain the anaesthesia. The bird's temperature was maintained at 40°C using a heat pad placed under the bird throughout surgery. Feathers were removed from small areas of skin on the upper back, between the two scapulae, and from the skin overlying the left pectoralis muscle. These areas were sterilised with betadeine antiseptic solution. An approximately 10 mm cranio-caudal skin incision was made between the two scapulae and a 15 mm skin incision over the left pectoralis muscle, using a size 10 scalpel blade. Blunt dissection was then used to tunnel a path caudal to the wing between the two incision sites. The sonomicrometry transducers and EMG electrodes were threaded from the upper back incision site to the pectoralis muscle incision site.

The muscle fascia was pierced twice using a 23 gauge hypodermic needle parallel to a single muscle fascicle running between the intramuscular tendon and the sternum, and approximately 8–12 mm apart. The sonomicrometry transducers were inserted into these two holes and the holders were aligned and sutured to the muscle fascia with 6-0 silk to anchor the transducers in place. A 25 gauge hypodermic needle with a blunted internal edge was used to insert the EMG electrodes parallel to the fascicles between the two transducers. They were then sutured to the muscle fascia using 6-0 silk. The pectoralis muscle skin incision was sutured closed with 5-0 silk. The ground wire was placed subcutaneously.

The upper back skin incision was closed with 5-0 silk in a cranio-caudal direction and the external PCB socket was sutured to the caudal end of the incision. The bird was bandaged and allowed to recover for approximately 48 h before *in vivo* data collection.

In vivo data collection

Following recovery, the bird was placed in the wind tunnel and connected to the light-weight data cable. The bird was allowed to sit on the perch at an air speed of 6 m s^{-1} while the sonomicrometry system was adjusted to minimise noise and level shifts on the muscle length trace. The bird was then flown at a range of speeds in a random order from 0 to 16 m s^{-1} at 2 m s^{-1} intervals and sonomicrometry and EMG data was collected at a sampling rate of 2.7 kHz. Flights were recorded at 250 frames s^{-1} (Troubleshooter, model TS500MS, Fastec Imaging, USA) in a lateral view with a mirror placed on top of the working section at 45 deg in order to simultaneously record a dorsal view of the bird.

In vivo muscle length and activity data analysis

All data analysis was done in IGOR Pro (version 5.0.5.7, WaveMetrics, USA). EMG recordings were filtered with a digital high-band pass filter, designed with the IGOR Pro IFDL filter design package as in Ellerby and Askew (Ellerby and Askew, 2007b), to remove movement artefacts. Sonomicrometry recordings contained occasional point and level shifts in the data. Point shifts result from electrical noise and were corrected using a median filter macro implemented in Igor Pro (designed by R. L. Marsh). Any point lying more than 0.3 mm from the median of the preceding and following points was replaced with that median value. Level shifts in the length trace resulted from variations in the strength of the received signal caused by changes in crystal orientation and inter-crystal distance with muscle length change. Level shifts occur when, rather than triggering off the first peak of the received burst, triggering occurred off a subsequent voltage peak, resulting in a step change in length (i.e. a level shift) that was approximately proportional to a multiple of the ultrasound cycle duration. These artefacts were removed by moving the level shifted section of trace down to the pre-shifted level using a macro in IGOR Pro (designed by R. L. Marsh). All analyses were done on sonomicrometry recordings corrected for these two types of artefact.

At each flight speed three to five wing beat cycles were selected where the bird maintained a steady position in the wind tunnel (determined from the high-speed video recordings). Maximum and minimum inter-transducer distance for each of these wing beat cycles were measured along with the time at which they occurred. From these data the average muscle strain (relative to mid length during the cycles; L_{mid}), cycle frequency and relative shortening duration of the cycles were calculated. EMG onset relative to peak length, EMG duration and EMG intensity were also calculated for these cycles. In order to calculate EMG intensity, filtered EMG waves were rectified and integrated over the duration of the EMG burst (see Ellerby and Askew, 2007b). This integral was divided by burst duration in order to determine EMG intensity. To enable comparison between individuals and to give an index of relative motor unit activation, EMG intensity was scaled relative to the mean intensity at the highest flight speed, where the maximum intensity was typically recorded. All EMG data for each bird was calculated from the same EMG electrode, enabling comparison between flight speeds.

To obtain an average muscle length change pattern for each flight speed, which was subsequently used to measure the mechanical power output of the pectoralis muscle under simulated *in vivo* conditions, the muscle strain data of the selected cycles were smoothed by fitting a Fourier series to the data (see Askew and Marsh, 2001) with either three or four harmonics to the data to produce an average strain cycle (Fig. 1).

In vitro muscle physiology

The power output of the pectoralis muscle was determined *in vitro* using the work loop technique to simulate *in vivo* conditions (Josephson, 1985a) and during isotonic contractions. A bundle of pectoralis muscle fascicles was isolated under non-recovery isoflurane anaesthesia (administered at a concentration of approximately 4%). When the bird had reached a deep plane of anaesthesia a skin incision was made to expose the right pectoralis muscle. During the isolation of the bundle of muscle fascicles the muscle was continuously irrigated with oxygenated Krebs–Henseleit Ringer's solution chilled to 2°C. A suture was made around the intramuscular tendon with 4-0 silk, approximately 15 mm from its insertion on the deltopectoral crest of the humerus. An incision was

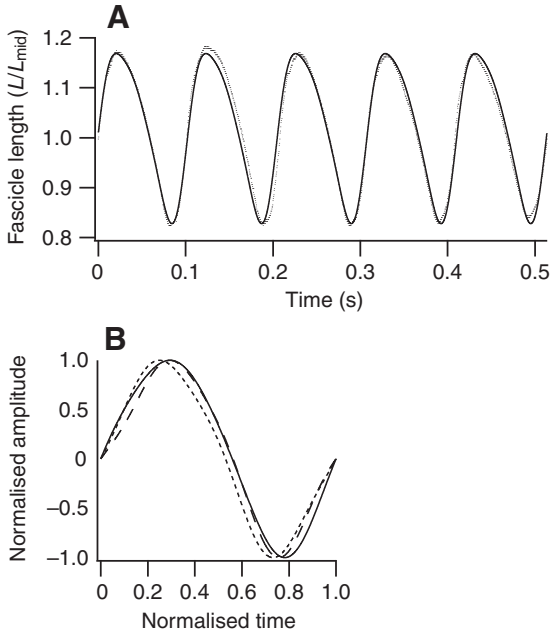


Fig. 1. Representative *in vivo* cockatiel pectoralis muscle length trajectory obtained at a flight speed of 10 m s^{-1}. (A) Data are muscle length relative to mid cycle length (L_{mid}) calculated from sonomicrometry recordings for five cycles (dots) and a Fourier smoothed wave calculated across all five cycles (solid line). Note that it is the Fourier smoothed wave that is imposed on the muscle fascicles *in vitro*. (B) Normalised Fourier smoothed muscle length waves for 0 m s^{-1} (solid line), 10 m s^{-1} (dotted line), 16 m s^{-1} (dashed line).

made above this suture parallel to the intramuscular tendon. A bundle of muscle fascicles was then removed from the pectoralis muscle by making two parallel incisions at approximately 30 deg relative to the surface of the muscle, 1–2 mm apart and parallel to the muscle fascicles, between the internal tendon and the muscle's origin on the sternum. A section of the sternum on either side of the bundle's origin was also removed. Note that the fascicles removed were from the same anatomical region of the contralateral pectoralis muscle from which the sonomicrometry and electromyography recordings were made.

Once isolated, the fascicle bundle was placed in a dish containing the chilled (2°C) oxygenated Krebs–Henseleit Ringer's solution while the free end of the suture was tied to a hook constructed from 00 insect pins, at the end of a silver chain (mass 45 mg). The isolated fascicles were then placed into a Perspex muscle chamber circulated with Krebs–Henseleit Ringer's solution, saturated with 95% oxygen, 5% carbon dioxide at a temperature of 2°C. The sternum adjacent to the fascicles was anchored to the base of the muscle chamber with stainless steel spring clips and the intramuscular tendon was attached to the arm of the muscle lever (model 300B-LR for isotonic contractions; model 305B-LR for *in vivo* simulations; Aurora Scientific, Aurora, Ontario, Canada) *via* the silver chain. A pair of parallel, platinum electrodes positioned on either side of the fascicle bundle were used to supramaximally activate the muscle fascicles. The temperature of the Krebs–Henseleit Ringer's solution was gradually increased from 2°C to an *in vivo* temperature of 40°C over a period of approximately 15 min.

The fascicle length at which the maximum force was produced during an isometric twitch (L_0), was found by performing a series of isometric twitch contractions using supramaximal 0.2-ms duration stimuli (applied using a Stimulus Isolation Unit, UISO model 236, Hugo Sachs Elektronik, March-Hugstetten, Germany) at different fascicle lengths. Fascicle length was altered by adjusting the height of the muscle lever relative to the base of the muscle chamber to which the muscle preparation was attached. Once the optimum length had been found, an isometric tetanic contraction was produced at L_0 by stimulating the muscle using a train of stimuli at a frequency of 200 Hz. Muscle fascicle bundles were used for either: (i) cyclical contractions simulating *in vivo* muscle length change and activity patterns; or (ii) isotonic shortening contractions.

Muscle power output during cyclical contractions simulating *in vivo* length change and activity patterns

Muscle fascicle bundles isolated from birds that had been previously used to determine *in vivo* muscle length change and activity patterns, were subjected to cyclical contractions *in vitro* that simulated the *in vivo* conditions. In these preparations, the muscle length was decreased from L_0 by the mean strain amplitude recorded across the different flight speeds such that the maximum length during cyclical contractions corresponded closely with that at which maximum isometric force was produced. The average *in vivo* muscle length and activation data for each flight speed that had previously been recorded were then imposed onto the fascicles using customised software (Testpoint, Version 3.4). Stimuli were delivered at the fusion frequency of the muscle (typically 200 Hz) *via* a stimulus isolation unit (UISO model 236, Hugo Sachs Elektronik, March-Hugstetten, Germany). For each simulated flight speed five continuous cyclical contractions were imposed on the fascicles. The resulting fascicle length change and force was measured at a rate of 1000 × cycle frequency. The instantaneous power output of the muscle was calculated from the product of muscle shortening velocity and force. The average shortening power was calculated for cycles three to five by averaging the instantaneous power during the shortening phase of the cycle over the cycle duration. The shortening power represents the rate at which useful work can be done on the environment over the wing stroke. Cyclical contractions simulating flight at 12 m s^{-1} were used as a control to assess the decline in muscle performance. Any decline in shortening power was corrected for by assuming linear decline in shortening power between controls (Stevenson and Josephson, 1990). Note that provided work loop shape is unaltered by preparation decline, as was the case in these experiments, power can be corrected by a simple multiplication factor. All mechanical power data recorded after a 35% decline in the muscle preparation's shortening power were excluded from the analysis.

After simulations at all flight speeds had been performed (or after control power had declined below 65% of the initial value) the muscle preparation was dissected leaving only fascicles that were intact between the sternum and intramuscular tendon. The mass of these fascicles was measured to enable the calculation of mass-specific power and to estimate muscle cross-sectional area in order to calculate muscle stress.

Calculation of *in vivo* mechanical power

The *in vitro* mechanical power measurements were obtained by supramaximally stimulating the muscle fascicles. However, power can be modulated by varying motor unit recruitment and/or firing rate (Farina et al., 2004). Power output measured in supramaximally activated muscle does not therefore indicate *in vivo* muscle power at all flight speeds. The mechanical power measurements for each flight speed were corrected for the relative volume of active muscle *in vivo* by multiplying the power by the relative EMG intensity (see Askew

and Ellerby, 2007; Ellerby and Askew, 2007a). In performing this correction it was assumed that there is a linear relationship between EMG intensity and work (see Bigland and Lippold, 1954; Adams et al., 1992; Del Valle and Thomas, 2005) and between motoneuron firing rate and force (Tansey et al., 1996). Intermittent flight also changes the power available during flight relative to that measured *in vitro*. During the non-flapping phase the mechanical power output of the pectoralis muscles is zero. To account for the use of intermittent flight, the *in vitro* mechanical power output was also multiplied by the proportion of time spent flapping which was determined for flights that were typically 5–6 s in duration (Fig. 4). The *in vitro* power corrected for both activation and intermittent flight give an estimate of the average mechanical power output available from the pectoralis muscles during flight in the wind tunnel. Estimating the total pectoralis muscle mechanical power output in this way assumes that the performance of our isolated bundle of muscle fascicles is representative of the whole pectoralis muscle. It is currently unknown whether there are regional differences in muscle performance within the pectoralis muscle of cockatiels. However, evidence from pigeons suggests that the relative timing of EMG activity within the majority of the pectoralis muscle is uniform (Boggs and Dial, 1993; Biewener et al., 1998; Soman et al., 2005) and, although there are some regional differences in strain, the majority of the muscle shows relatively uniform strain (Biewener et al., 1998; Soman et al., 2005). The error of extrapolating whole muscle performance from that of a bundle of muscle fascicles is likely to be small.

In vitro force–velocity measurements

The force–velocity characteristics of cockatiel pectoralis muscle were determined using a series of after-loaded isotonic shortening contractions. A separate group of birds were used for these experiments. Muscle length was increased to 5% above L_0 to ensure that during isotonic contractions the muscle would shorten through the plateau of the force–length relationship. The bundle of muscle fascicles was tetanically stimulated, and force was allowed to increase to a pre-set level, which was maintained by fascicle shortening. The resulting length and force traces were recorded at 5 kHz. Length was differentiated in order to calculate shortening velocity which was expressed relative to the optimum fascicle length (L_0) previously determined. The force was pre-set at relative forces in the range of approximately 0.95–0.05 of peak isometric tetanic stress (P_0). Isometric tetani were used as controls to measure any decline in muscle performance. This decline was assumed to be linear between controls. The shortening velocity was plotted against corrected relative force and the data were fitted with an exponential-linear equation (Marsh and Bennett, 1986). Maximal shortening velocity (V_{max}) was estimated by extrapolation to zero force. The power ratio, which is a measure of the curvature of the force–velocity relationship, was calculated as the ratio of the maximum isotonic power output to the product of P_0 and V_{max}.

After the *in vitro* force–velocity measurements were completed the fascicle bundle was dissected leaving only intact fascicles running between the sternum and intramuscular tendon, and their mass was measured. The cross-sectional area of the bundle was calculated by dividing its volume (calculated from fascicle mass assuming a muscle density of 1060 kg m^{-3}) by its length. Forces were expressed as a stress (relative to cross-sectional area, in kN m^{-2}).

Statistical analysis

To test for differences in the parameters associated with the muscle length trajectory, muscle activation and mechanical power output in relation to flight speed, a mixed-model (type III) two-way general linear model (GLM) was used. The parameters analysed included cycle frequency, strain (relative to cycle mid length; L_{mid}), shortening duration, relative shortening duration (proportion of the cycle spent shortening), shortening velocity, relative EMG intensity, onset of EMG activation relative to peak muscle length, EMG duty cycle (EMG duration relative to the cycle duration), relative flapping duration (proportion of time flapping relative to total flight duration), the mechanical power output prior to correction for recruitment and intermittent flight, mechanical power output after relative EMG intensity correction and mechanical power output after correction for recruitment and intermittent flight. All proportional data were arcsine-transformed prior to analysis and individual birds were treated as a random factor. A *post-hoc* Scheffé test was performed when a significant difference was identified. The GLM analysis was performed in SPSS (version 14.0.2, SPSS Inc., Chicago, IL, USA) and the *post-hoc* Scheffé tests were run by hand using formulae from Zar (Zar, 1999).

RESULTS

Muscle length trajectory

A significant difference was detected in pectoralis muscle strain with flight speed (F=14.08, P<0.001; Fig. 2A). Strain was relatively constant at low to intermediate flight speeds (8 m s^{-1} and slower), where strain ranged from 0.280 to 0.298. At speeds greater than 8 m s^{-1} strain increased, reaching a maximum of 0.365 at 16 m s^{-1}. There was a significant difference in the magnitude of strain (F=4.15, P=0.001) and how strain changed with speed between birds shown by the bird × speed interaction term (F=6.86, P<0.001).

Wing beat frequency varied significantly with flight speed (F=2.36, P=0.03; Fig. 2B). Wing beat frequency ranged from 8.23 to 9.78 Hz with a maximum at 0 m s^{-1}, which was significantly different from the minimum at 12 m s^{-1}. A significant difference was detected in relative shortening duration (F=2.28, P=0.036; Fig. 2C)

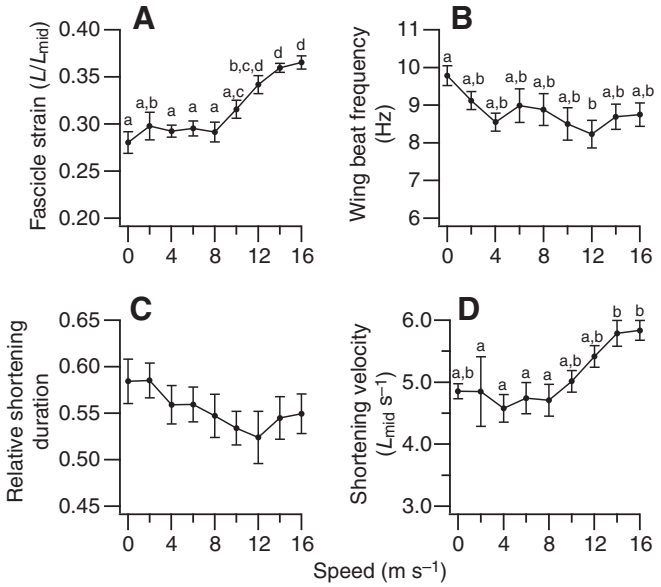


Fig. 2. The relationship between pectoralis muscle length trajectory and flight speed. (A) Fascicle strain. (B) Wing beat frequency. (C) Relative shortening duration. (D) Shortening velocity. The same letters above data points denote homogenous subsets between which no significant differences were detected using a Scheffé *post-hoc* test (P>0.05). Data are shown as means ± s.e.m. (N=6, 7, 8, 8, 8, 8, 8, 8, 6 for speeds 0, 2, 4, 6, 8, 10, 12, 14, 16 m s^{-1}, respectively).

with flight speed although the actual duration of a cycle spent shortening did not vary significantly with flight speed (F=2.11, P=0.051). The combined effect of modulating muscle strain, wing beat frequency and cycle shortening duration resulted in the muscle shortening velocity varying significantly with flight speed, being relatively constant between 0 and 8 m s^{-1} and increasing between 8 and 16 m s^{-1} (F=8.55, P<0.001; Fig. 2D) with flight speed, ranging from 4.58 to 5.83 L_{mid} s^{-1}.

Muscle activation

The EMG onset relative to peak muscle length did not vary significantly with flight speed (F=1.30, P=0.266). On average, muscle activation preceded peak muscle length by 6.3±5.7 ms (mean ± s.d.). EMG duration (F=5.07, P<0.001) and duty cycle (duration relative to cycle duration; F=4.55, P<0.001; Fig. 3B) varied significantly with flight speed. A significant difference was detected in the relative EMG intensity of muscle activation (F=15.03, P<0.001; Fig. 3A) with flight speed. The relative EMG intensity showed a U-shaped relationship with flight speed with a minimum at 4 m s^{-1} and a maximum at 16 m s^{-1}.

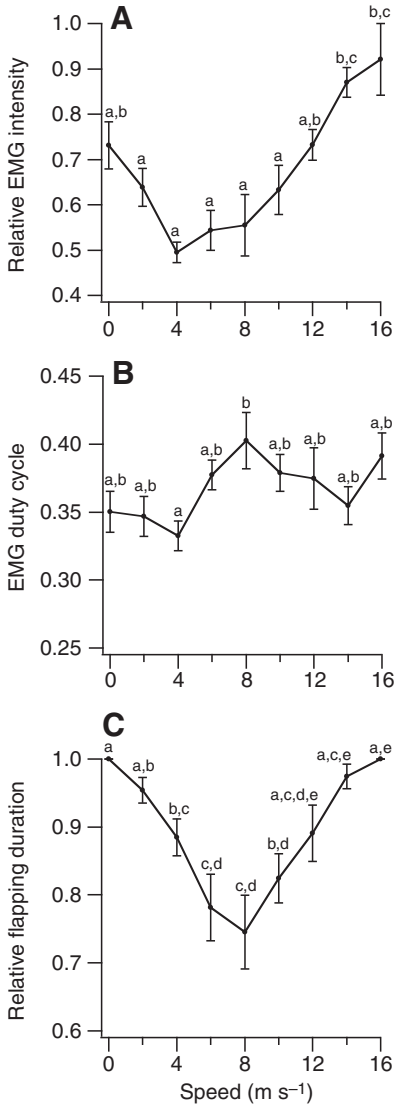


Fig. 3. The relationship between pectoralis muscle recruitment and flight speed. (A) Relative EMG intensity. (B) EMG duty cycle. (C) Relative flapping duration. The same letters above data points denote homogenous subsets between which no significant differences were detected using a Scheffé *post-hoc* test (P>0.05). Data are shown as means ± s.e.m. (A,B, N=6, 7, 7, 7, 7, 7, 7, 5 for speeds 0, 2, 4, 6, 8, 10, 12, 14, 16 m s^{-1}, respectively; C, N=6, 8, 8, 7, 8, 8, 7, 7, 6 for speeds 0, 2, 4, 6, 8, 10, 12, 14, 16 m s^{-1}, respectively.)

Intermittent flight

Cockatiels used intermittent flight where birds glided with the wings extended horizontally away from the body (Fig. 4). Not all birds utilised intermittent flight strategies and all birds flapped continuously at 0 and 16 m s^{-1}. Those that exhibited intermittent flight behaviour had longer periods of non flapping flight at intermediate flight speeds (Fig. 3C, Fig. 4). After accounting for the use of intermittent flight the relative flapping duration varied significantly, in a U-shaped relationship with flight speed (F=16.18, P<0.001; Fig. 3C).

Mean stress difference and mechanical power

The stress produced by the fascicles under an *in vivo* muscle length trajectory and activation pattern is shown in Fig. 5. Mean stress difference (corrected for intensity of activation) did not vary significantly with flight speed (F=1.11, P=0.39; Fig. 6) with an average of 28 kN m^2 across all flight speeds.

Pectoralis shortening power was calculated from the force produced during shortening and the velocity of fascicle shortening (Fig. 5A). The variation in the *in vitro* mechanical power across simulated flight speeds is modulated by variation in muscle length trajectory, timing and duration of muscle activation but not the intensity of activation, and was not significantly different among flight speeds being approximately 105 W kg^{-1} (F=1.62, P=0.176; Fig. 7). After correction for the intensity of muscle activation *in vivo*, the power varied significantly with flight speed (F=3.85, P=0.006; Fig. 7) as did the *in vivo* mechanical power required for flight (corrected for both activation and intermittent flight strategies; F=7.65, P<0.001; Fig. 7). Corrected *in vivo* shortening power had a minimum of approximately 40.3 W kg^{-1} at 8 m s^{-1} and a maximum of approximately 119.6 W kg^{-1} at 16 m s^{-1}.

In vitro contractile properties

The contractile properties of the pectoralis muscle fascicles during isometric and after-loaded isotonic contractions are shown in Table 1.

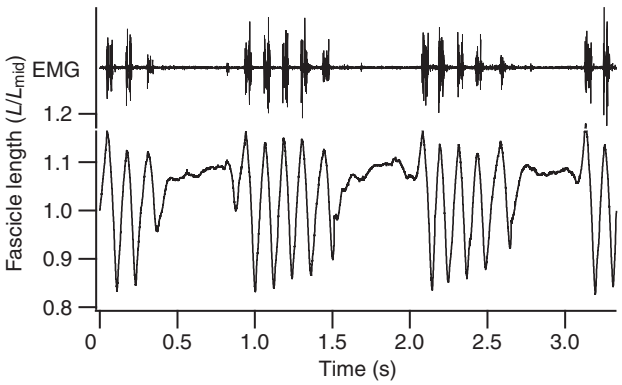


Fig. 4. *In vivo* pectoralis fascicle strain and EMG activity. Representative data from flight at 8 m s^{-1}. Fascicle length was measured *in vivo* by sonomicrometry and muscle activity was measured by electromyography. Fascicle length is relative to mid cycle length (L_{mid}).

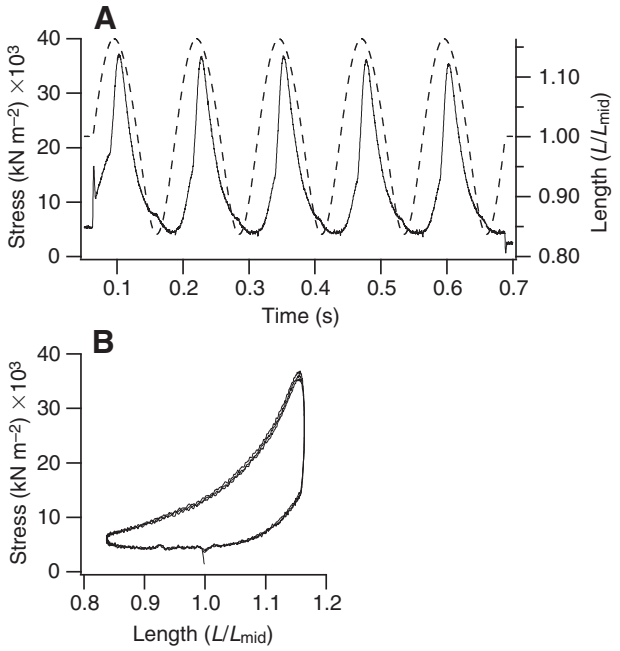


Fig. 5. Example of data obtained during *in vitro* work loop experiments at a simulated flight speed of 12 m s^{-1}. (A) The Fourier-smoothed wave represents the relative muscle length trajectory (dashed line) which was imposed on the muscle fascicles and the resultant stress (solid line) produced by the fascicles when simulated with the *in vivo* muscle length trajectory and activation pattern, are shown. (B) The corresponding work loop generated by plotting stress against relative muscle length for the last three cycles shown in A.

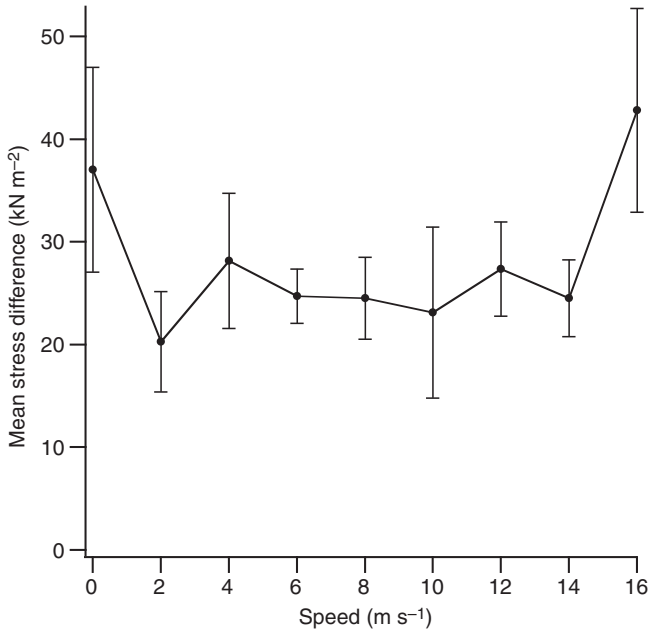


Fig. 6. The relationship between mean stress difference and flight speed. Data are presented as means ± s.e.m. (N=4, 4, 5, 3, 4, 3, 7, 3, 4 for speeds 0, 2, 4, 6, 8, 10, 12, 14, 16 m s^{-1}, respectively).

DISCUSSION

Cockatiel pectoralis muscle consists of fast oxidative glycolytic fibres (Rosser and George, 1986), therefore the available means of modulating power include varying muscle length trajectory and pattern of activation, the proportion of motor units recruited in the muscle, and the use of intermittent flight.

Variation in muscle length trajectory

Cockatiels modulated strain (1.30-fold, calculated as the ratio of the maximum and the minimum values; Fig. 2A) and cycle frequency (1.19-fold; Fig. 2B) with flight speed. The greater the shortening duration of a cycle, relative to the total cycle duration, the more positive work can be done (Askew and Marsh, 1997; Girgenrath and Marsh, 1999; Askew and Marsh, 2002). There was a 1.12-fold variation in the relative shortening duration (Fig. 2C) with flight speed. Variation in the relative shortening duration was due to an increase in the lengthening duration of a cycle as there was no significant difference in the actual duration of cycle shortening with flight speed as has been previously reported (Hedrick et al., 2003). Variation in strain trajectory, for example by changes in cycle frequency, strain and the proportion of time spent shortening, affects muscle shortening velocity. However shortening velocity remained relatively constant between flight speeds of 0 and 8 m s^{-1} (Fig. 2D) despite the variation in cycle frequency (Fig. 2B), and then increased 1.24-fold from 8 to 16 m s^{-1}.

The force–velocity characteristics of muscle dictate that increasing a muscle's shortening velocity will increase power up to an optimum with a subsequent decline in power thereafter (Josephson and Stokes, 1989; Josephson, 1993). Therefore increasing both strain and cycle frequency up to their optima increases power output (Josephson and Stokes, 1989; Askew and Marsh, 1997). The optimal strain and cycle frequency are unknown for cockatiel pectoralis muscle but it is probable that they are close to the conditions found *in vivo* where a large power output is required. The optimum shortening velocity under isotonic conditions may differ from that found to be optimal during cyclical contractions (Askew and Marsh, 1998). The shortening velocity measured during maximal power generation *in vivo* was 5.8 L_{mid} s^{-1}, which is lower than the optimal shortening velocity for maximum power output during and after loaded isotonic contractions (7.1 L_0 s^{-1}; Table 1). Similarly, in budgerigar, zebra finch and quail pectoralis muscles the peak isotonic power output occurs at higher relative shortening velocities than during *in vivo* contractions (Askew and Marsh, 2001; Ellerby and Askew, 2007a; Ellerby and Askew, 2007b). It has been shown that the optimal relative shortening duration during cyclical contractions, where the proportion of the cycle spent shortening exceeds that spent lengthening and where strain is relatively high, is lower than the optimal relative shortening velocity predicted from the force–velocity curve (Askew and Marsh, 1998). This is due to the reduction in force that occurs as a result of operating at lengths off the plateau of the length–force relationship. It seems probable that the large strains generally found in the avian pectoralis muscle during flight (Biewener et al., 1998; Askew and Marsh, 2001; Williamson et al., 2001; Hedrick et al., 2003; Ellerby and Askew, 2007b) result in a shift in the optimal relative shortening velocity in comparison with that which is optimal during isotonic shortening contractions due to length–force effects.

Considering the muscle length trajectory alone it appears that power is modulated at lower flight speeds by increasing cycle frequency, which increases the relative shortening duration of a cycle while maintaining a constant strain and shortening velocity. By contrast, at higher flight speeds power is modulated by increasing strain which increases muscle shortening velocity and by maintaining a constant cycle frequency and relative shortening duration.

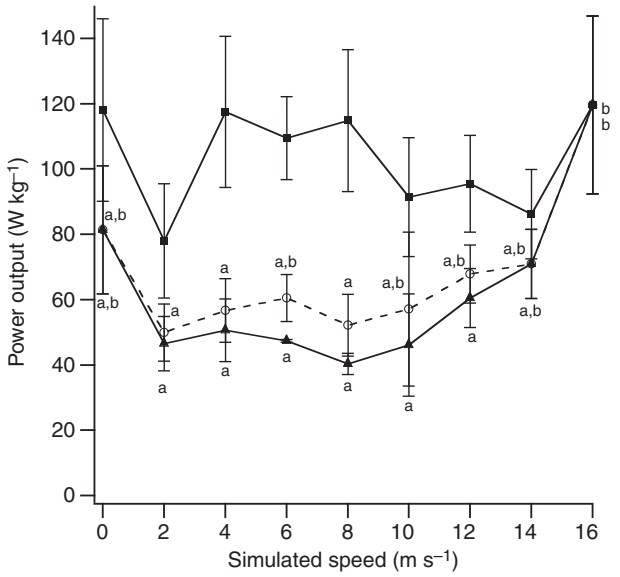


Fig. 7. The mechanical power–speed relationship for cockatiels. Traces show the power output of supramaximally stimulated fascicles (closed squares), power corrected for the variation of EMG intensity with flight speed (open circles) and power corrected for the variation of EMG intensity and the relative flapping duration (closed triangles) in relation to simulated flight speed. The same letters above the power data points corrected for the variation of EMG intensity and below the power data points corrected for the variation of EMG intensity and the relative flapping duration denote homogenous subsets between which no significant differences were detected using a Scheffé *post-hoc* test (P>0.05). Data are presented as means ± s.e.m. (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).

Table 1. Contractile properties of cockatiel pectoralis muscle fascicles

Parameter	Cockatiels
Peak isometric, tetanic stress, P_0 (kN m^{-2})	215±30
Twitch:tetanus ratio	0.65±0.02
Twitch-rise time (ms)	25.8±1
Twitch 50% relaxation time (ms)	34.2±1.7
Twitch 90% relaxation time (ms)	70.6±2.7
Maximum shortening velocity, V_{max} (L_0 s^{-1})	21.2±1.9
Velocity at maximum power (L_0 s^{-1})	7.1±0.3
Relative force at maximum power, P/P_0	0.35±0.03
Maximum isotonic power, $\dot{W}_{max}$ (W kg^{-1})	533±118
Power ratio, $\dot{W}_{max}$ ($V_{max}P_0$)	0.12±0.02

Values are means ± s.e.m. Isometric parameters, N=12; force–velocity parameters, N=3.

Muscle activation

There was no significant difference in the onset of muscle activation between flight speeds, with a mean of 6.3 ms prior to peak length; however, there was a significant difference in EMG duty cycle at 4 and 8 m s^{-1} (Fig. 3B). EMG duty cycle covered a similar range (0.33–0.40) to that reported in a variety of small birds (range 0.30–0.42 in zebra finch, budgerigar, blue-breasted quail, northern bobwhite and chukar) (Ellerby and Askew, 2007b; Askew and Marsh, 2001; Tobalske and Dial, 2000) but was lower than has been measured in the same species [0.41–0.46 (Hedrick et al., 2003)] and some larger species [0.42–0.51 in ring-necked pheasant, wild turkey and pigeon (Tobalske and Dial, 2000; Biewener et al., 1998)]. The general pattern of decreasing EMG duty cycle with increased cycle frequency is consistent with the increase in relative twitch duration with increasing cycle frequency (Girgenrath and Marsh, 1999).

There was a 1.95-fold variation in relative EMG intensity and this showed a U-shaped relationship with flight speed (Fig. 3A). As cockatiel pectoralis muscle is homogeneous in its muscle fibre type (Rosser and George, 1986) the relative EMG intensity indicates the level of muscle activation rather than the recruitment of different muscle fibre types. Our calculation of EMG intensity assumes, as did Ellerby and Askew (Ellerby and Askew, 2007a), the muscle is fully activated during at least one wing beat across all flight speeds for each bird where the EMG intensity is maximal. Recruiting more muscle fibres potentially increases muscle power output by increasing the force produced, however, the relationship between the level of activation and force cannot be established because work and power are both affected by the nature of the muscle length trajectory itself (Ellerby and Askew, 2007b).

Intermittent flight

Intermittent flight strategies were utilised by seven of the eight birds studied. Overall there was a 1.34-fold variation in the proportion of time spent flapping (Fig. 3C) and this showed a U-shape relationship with flight speed, similar to that previously reported in un-instrumented cockatiels (Bundle et al., 2007). The 'fixed gear hypothesis' suggests that these periods of flight where the power source is 'switched off', reducing the average power output, allows the active muscle to contract at the optimal shortening velocity for power generation (Rayner, 1985). However, in this study, shortening velocity was shown to vary from 4.58 to 5.83 L_{mid} s^{-1} with flight speed. This variation in shortening velocities demonstrates that the theory that an optimal shortening velocity is maintained across all flight speeds is over simplistic, as others have suggested (Tobalske et al., 2005; Ellerby and Askew, 2007b; Tobalske, 2007).

In vitro muscle performance

The isometric tetanic stress at L_0 was similar (215 kN m^{-2}) to other vertebrate striated muscles [e.g. 240–269 kN m^{-2} for mouse limb muscles (Askew and Marsh, 1997), 257–271 kN m^{-2} for turkey limb muscles (Nelson et al., 2004), 218 kN m^{-2} for lizard limb muscle at a similar temperature (Marsh and Bennett, 1985), 92–160 kN m^{-2} in frog vocalisation muscles (Girgenrath and Marsh, 1997; West et al., 2006)] and within the range reported for bird pectoralis muscles at optimal length [167–220 kN m^{-2} (Ellerby and Askew, 2007a; Askew and Ellerby, 2007)]. Therefore, our muscle preparations achieved a level of mechanical performance expected from a tissue of this type. However, although isometric stress can be useful in assessing muscle performance it is not particularly relevant to muscle performance during cyclical contractions, in which the muscle is not fully active and spends a large proportion of the cycle activating and deactivating. Mean stress difference is a measure of the mean stress generated during a cyclical contraction (Casey and Ellington, 1989; Askew and Marsh, 2002). In comparison with other aerobic, power generating muscles, the mean stress difference for cockatiel pectoralis muscle during power generation (calculated from the five highest power outputs measured) falls within the 95% confidence limits of the relationship between mean stress difference and shortening duration (supplementary material Fig. S1). The similarity between the data we have collected here and the scaling relationship between mean stress difference and shortening duration for other comparable muscles gives us confidence in the viability of our muscle preparations.

In order to maximise work, during a cyclical contraction a muscle must be alternately activated and deactivated such that force is

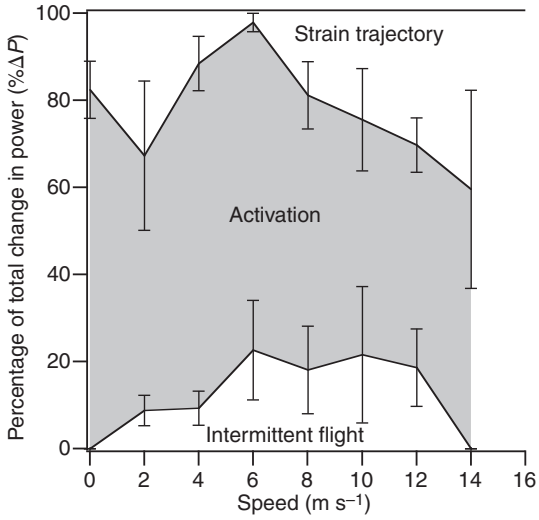


Fig. 8. Relative changes in pectoralis power output (%Δ*P*) resulting from modulation of strain trajectory, recruitment and intermittent flight (see Discussion for more detailed definitions) with flight speed. Data are presented as means ± s.e.m. (*N*=4, 4, 5, 3, 4, 3, 7, 3, 4 for speeds 0, 2, 4, 6, 8, 10, 12, 14, 16 m s^{-1}, respectively).

generated predominantly during shortening. The twitch-rise time is determined by the rate of deactivation of the muscle, since force only starts to decline once there is a net detachment of crossbridges due to Ca^{2+} removal (Marsh, 1990; Askew and Marsh, 2001). There is a strong correlation between twitch-rise time and muscle operating frequency (ESM; supplementary material Fig. S2) for a variety of muscles. The twitch-rise time measured in cockatiel pectoralis muscle was similar to that predicted from the scaling relationship between twitch-rise time and operating frequency and was longer (25.8 ms; Table 1) than has been previously measured in other avian pectoralis muscles (9.7 ms for zebra finch, 10.8 ms blue-breasted quail, 18.1 ms for budgerigar [(Askew and Marsh, 2001; Ellerby and Askew, 2007a)] which is consistent with the lower wing beat frequency of the cockatiel compared with the other species for which data are available (supplementary material Fig. S2).

The maximum shortening velocity (V_{max}) for cockatiel pectoralis muscle was 21.2 L_0 s^{-1} (Table 1) which is similar to that measured in zebra finch pectoralis muscle and faster than in budgerigar pectoralis muscle [21.3 L_0 s^{-1} and 14.7 L_0 s^{-1}, respectively (Ellerby and Askew, 2007a)]. The power ratio for cockatiel pectoralis muscle (0.12; Table 1) was much lower (indicating greater curvature of the force–velocity relationship) than has been previously reported for other avian flight muscles (0.22 in zebra finch, 0.17 in blue-breasted quail, and 0.17 in budgerigar). It has previously been argued that flattening of the force–velocity curve is a feature of power generating muscles that operate at high frequencies (Ellerby and Askew, 2007a). The reduction in power ratio with decreasing wing beat frequency in these species supports this hypothesis. Peak isotonic power output occurs at a lower relative shortening velocity with increased curvature of the force–velocity relationship: 0.34 V_{max} for cockatiel, 0.38 V_{max} for budgerigar, and 0.46 V_{max} for zebra finch pectoralis muscle (Ellerby and Askew, 2007a). Peak isotonic power occurs at a lower relative force with increased curvature of the force–velocity relationship: 0.35 P_0 for cockatiel, 0.44 P_0 for budgerigar, and 0.50 P_0 for zebra finch pectoralis muscle (Ellerby and Askew, 2007a). The lower relative force and lower relative shortening velocity in cockatiel pectoralis muscle resulted in the maximum instantaneous power output being lower (533 W kg^{-1}; Table 1) than that measured in zebra finch pectoralis muscle [730 W kg^{-1} (Ellerby and Askew, 2007a)], despite the relatively high V_{max} in cockatiels. By contrast, despite maximum isotonic power occurring at a higher relative force and velocity in budgerigars compared with cockatiels, the higher V_{max} in cockatiel pectoralis muscle resulted in the maximum instantaneous power output being similar in both species [522 W kg^{-1} in budgerigar (Ellerby and Askew, 2007a) compared with 533 W kg^{-1} in cockatiel (Table 1)].

Mechanical power output during simulated *in vivo* length and activity patterns

The mechanical power produced by the supramaximally stimulated muscle fascicles during simulated muscle length change and activity patterns was approximately 105 W kg^{-1} across all flight speeds. Relative EMG intensity (Fig. 3A) and the proportion of time spent flapping (Fig. 3C) both vary with flight speed. As a result, the mechanical power corrected for activation and intermittent flight did vary significantly with flight speed in an approximately U-shaped relationship with flight speed, varying 2.97-fold with a minimum power speed of 8 m s^{-1} (Fig. 7). This U-shaped mechanical power–speed relationship in the cockatiel agrees with that found in previous studies of the mechanical (Hedrick et al., 2003) and metabolic (Bundle et al., 2007) power–speed relationship for this species. There are differences between the minimum power speeds in that the minimum power speed estimated by Hedrick et al. [5 m s^{-1} (Hedrick et al., 2003)] is lower than has been measured using other approaches [8 m s^{-1} (this study); 10 m s^{-1} (Bundle et al., 2007); 9 m s^{-1} calculated using equation 1 in Pennycuick (Pennycuick, 2001)] in cockatiels. However, in several studies *post-hoc* tests did not identify significant differences in power across quite a broad range of flight speeds and therefore caution is warranted in making comparisons between the minimum power speed estimated from different studies (Fig. 7) (Bundle et al., 2007).

The minimum and maximum power required for flight was 40.3±6.6 W kg^{-1} (mean ± s.d.) and 119.6±54.4 W kg^{-1} (mean ± s.d.) of pectoralis muscle, respectively. These values are similar to the range in power measured in zebra finches (41–111 W kg^{-1}) and budgerigars (30–70 W kg^{-1}) (Ellerby and Askew, 2007a) but are lower than those measured in a previous study on cockatiels (Hedrick et al., 2003). Hedrick et al. (Hedrick et al., 2003) measured a minimum and a maximum power of 73.5±10.1 W kg^{-1} (mean ± s.d.) and 155.6±29.2 W kg^{-1} (mean ± s.d.), respectively, for pectoralis muscle.

Mechanisms of power modulation in comparison with other species

In cockatiels, the relative contribution of each modulation strategy to the variation in power with flight speed, was quantified by calculating the percentage of the change in power (%Δ*P*) resulting from (1) modulation of the muscle length trajectory and the timing and duration of activation ('strain trajectory' in Fig. 8); (2) modulation of neuromuscular activation ('activation' in Fig. 8); and (3) use of intermittent flight ('intermittent flight' in Fig. 8) for each speed in relation to the maximum power measured [following Ellerby and Askew (Ellerby and Askew, 2007a)]. At all flight speeds the primary mechanism by which power was modulated was variation in the volume of muscle activated, contributing 61–84% to the modulation (Fig. 8). At intermediate flight speeds (4–8 m s^{-1}) there was little modulation of power by

variation in muscle length trajectory. At speeds below 4 m s^{-1} and above 6 m s^{-1}, modulation of power by varying muscle length trajectory was more important, accounting for up to 39% of the modulation of power. The use of intermittent flight modulated power by 0–21%. The relative flapping duration varied in a U-shaped relationship with flight speed (Fig. 3C) and therefore modulation of power through the use of intermittent flight was greatest at intermediate flight speeds. Power modulation through the use of intermittent flight can be influenced by instrumentation. For example, cockatiels rarely if ever used intermittent flight while instrumented for respirometry, in contrast to the 1.6-fold variation in the proportion of time spent flapping in un-instrumented birds (Bundle et al., 2007). The similarity between the proportion of time spent flapping and flight speed in the birds in this study compared with data for un-instrumented birds (Bundle et al., 2007) gives us confidence that our estimation of the relative importance of intermittent flight as a power modulation strategy is realistic and is largely unaffected by the instrumentation for *in vivo* muscle length change and activity patterns. At the extreme flight speeds, muscle power output was maximal and not modulated.

We hypothesised that muscle activation would be the primary means by which power was modulated in cockatiels in comparison with smaller birds such as zebra finches and budgerigars. Our hypothesis was supported in that cockatiels were much less reliant on intermittent flight and length trajectory to modulate power. Unlike both zebra finches and budgerigars in which intermittent flight was increasingly important at high speeds [Fig. 5 in Ellerby and Askew (Ellerby and Askew, 2007a)], in cockatiels intermittent flight had the greatest effects on power modulation at intermediate flight speeds (Fig. 8). Intermittent flight in cockatiels accounted for a maximum of 21% of the power modulation, approximately half that measured in both budgerigars (38%) and zebra finches [41% (Ellerby and Askew, 2007a)]. As observed in budgerigars and zebra finches, there was a general decrease in the contribution that activation played in the modulation of power with increasing flight speed. The relative contribution to power modulation *via* changes in activation was greatest in cockatiels (61–84%) and decreased with decreasing body mass (budgerigars 29–81%; zebra finches 18–79%). The way in which power was modulated *via* changes in muscle length trajectory differed between the different species. In zebra finches, modulation by changes in length trajectory decreased with increasing speed; in budgerigars the contribution was highest at intermediate flight speeds; whereas in cockatiels the contribution was highest at the lower and higher flight speeds. We hypothesised that modulation of power by changes in muscle length trajectory is a general feature of muscle and that this strategy could be used by all species regardless of size. In support of this, it is found that modulation of wing beat frequency (1.1- to 1.2-fold variation) and strain (1.2- to 1.3-fold variation) are similar in a range of species (Fig. 2) (Hedrick et al., 2003; Tobalske et al., 2005; Ellerby and Askew, 2007b). However, the relative importance of variation of strain trajectory as a means of modulating power *does* vary with species. It seems likely that this reflects differences in the importance of other modulation strategies.

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. We would like to thank Frank Nelson for his helpful comments made during the writing of this manuscript and for assistance with the statistical analyses.

REFERENCES

Adams, G. R., Duvoisin, M. R. and Dudley, G. A. (1992). Magnetic-resonance-imaging and electromyography as indexes of muscle function. *J. Appl. Physiol.* **73**, 1578-1589.

Altringham, J. D. and Young, I. S. (1991). Power output and the frequency of oscillatory work in mammalian diaphragm muscle – the effects of animal size. *J. Exp. Biol.* **157**, 381-389.

Altringham, J. D., Wardle, C. S. and Smith, C. I. (1993). Myotomal muscle function at different locations in the body of a swimming fish. *J. Exp. Biol.* **182**, 191-206.

Askew, G. N. and Ellerby, D. J. (2007). The mechanical power requirements of avian flight. *Biol. Lett.* **3**, 445-448.

Askew, G. N. and Marsh, R. L. (1997). The effects of length trajectory on the mechanical power output of mouse skeletal muscles. *J. Exp. Biol.* **200**, 3119-3131.

Askew, G. N. and Marsh, R. L. (1998). Optimal shortening velocity (V/V_{max}) of skeletal muscle during cyclical contractions: length-force effects and velocity-dependent activation and deactivation. *J. Exp. Biol.* **201**, 1527-1540.

Askew, G. N. and Marsh, R. L. (2001). The mechanical power output of the pectoralis muscle of blue-breasted quail (*Coturnix chinensis*): the *in vivo* length cycle and its implications for muscle performance. *J. Exp. Biol.* **204**, 3587-3600.

Askew, G. N. and Marsh, R. L. (2002). Muscle designed for maximum short-term power output: quail flight muscle. *J. Exp. Biol.* **205**, 2153-2160.

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.

Biewener, A. A., Corning, W. R. and Tobalske, B. W. (1998). *In vivo* pectoralis muscle force-length behavior during level flight in pigeons (*Columba livia*). *J. Exp. Biol.* **201**, 3293-3307.

Bigland, B. and Lippold, O. C. J. (1954). The relation between force, velocity and integrated electrical activity in human muscles. *J. Physiol.* **123**, 214-224.

Boggs, D. F. and Dial, K. P. (1993). Neuromuscular organization and regional EMG activity of the pectoralis in the pigeon. *J. Morph.* **218**, 43-57.

Buchthal, F. and Schmalbruch, H. (1980). Motor unit of mammalian muscle. *Physiol. Rev.* **60**, 90-142.

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.

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.

Del Valle, A. and Thomas, C. K. (2005). Firing rates of motor units during strong dynamic contractions. *Muscle Nerve* **32**, 316-325.

Dial, K. P., Biewener, A. A., Tobalske, B. W. and Warrick, D. R. (1997). Mechanical power output of bird flight. *Nature* **390**, 67-70.

Ellerby, D. J. and Askew, G. N. (2007a). 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. and Askew, G. N. (2007b). Modulation of pectoralis muscle function in budgerigars *Melopsitaccus undulatus* and zebra finches *Taeniopygia guttata* in response to changing flight speed. *J. Exp. Biol.* **210**, 3789-3797.

Farina, D., Merletti, R. and Enoka, R. M. (2004). The extraction of neural strategies from the surface EMG. *J. Appl. Physiol.* **96**, 1486-1495.

Farley, C. T. (1997). Maximum speed and mechanical power output in lizards. *J. Exp. Biol.* **200**, 2189-2195.

Girgenrath, M. and Marsh, R. L. (1999). Power output of sound-producing muscles in the tree frogs *Hyla versicolor* and *Hyla chrysoscelis*. *J. Exp. Biol.* **202**, 3225-3237.

Goslow, G. E. and Dial, K. P. (1990). Active stretch-shorten contractions of the musculus pectoralis in the European starling (*Sturnus vulgaris*)-evidence from electromyography and contractile properties. *Neth. J. Zool.* **40**, 106-114.

Hagiwara, S., Chichibu, S. and Simpson, N. (1968). Neuromuscular mechanisms of wing beat in hummingbirds. *Z. Vgl. Physiol.* **60**, 209-218.

Hedrick, T. L., Tobalske, B. W. and Blewener, A. A. (2003). How cockatiels (*Nymphicus hollandicus*) modulate pectoralis power output across flight speeds. *J. Exp. Biol.* **206**, 1363-1378.

Ishihara, A., Ohira, Y., Tanaka, M., Nishikawa, W., Ishioka, N., Higashibata, A., Izumi, R., Shimazu, T. and Ibata, Y. (2001). Cell body size and succinate dehydrogenase activity of spinal motoneurons innervating the soleus muscle in mice, rats, and cats. *Neurochem. Res.* **26**, 1301-1304.

Josephson, R. K. (1985a). Mechanical power output from striated muscle during cyclic contraction. *J. Exp. Biol.* **114**, 493-512.

Josephson, R. K. (1985b). The mechanical power output of a Tettigoniid wing muscle during Singing and flight. *J. Exp. Biol.* **117**, 357-368.

Josephson, R. K. (1993). Contraction dynamics and power output of skeletal muscle. *Annu. Rev. Physiol.* **55**, 527-546.

Josephson, R. K. and Stevenson, R. D. (1991). The efficiency of a flight muscle from the locust *Schistocerca americana*. *J. Physiol. Lond.* **442**, 413-429.

Josephson, R. K. and Stokes, D. R. (1989). Strain, muscle length and work output in a crab muscle. *J. Exp. Biol.* **145**, 45-61.

Josephson, R. K. and Young, D. (1985). A synchronous insect muscle with an operating frequency greater than 500 Hz. *J. Exp. Biol.* **118**, 185-208.

Marsh, R. L. (1988). Ontogenesis of contractile properties of skeletal muscle and sprint performance in the lizard *Dipsosaurus dorsalis*. *J. Exp. Biol.* **137**, 119-139.

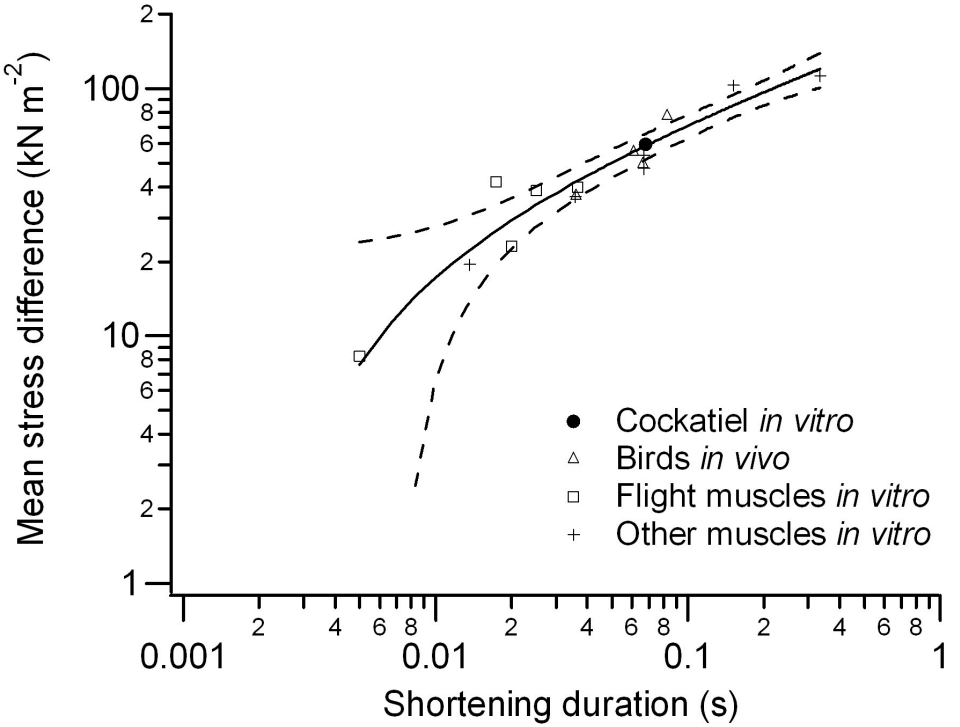
Marsh, R. L. (1999). How muscles deal with real-world loads: the influence of length trajectory on muscle performance. *J. Exp. Biol.* **202**, 3377-3385.

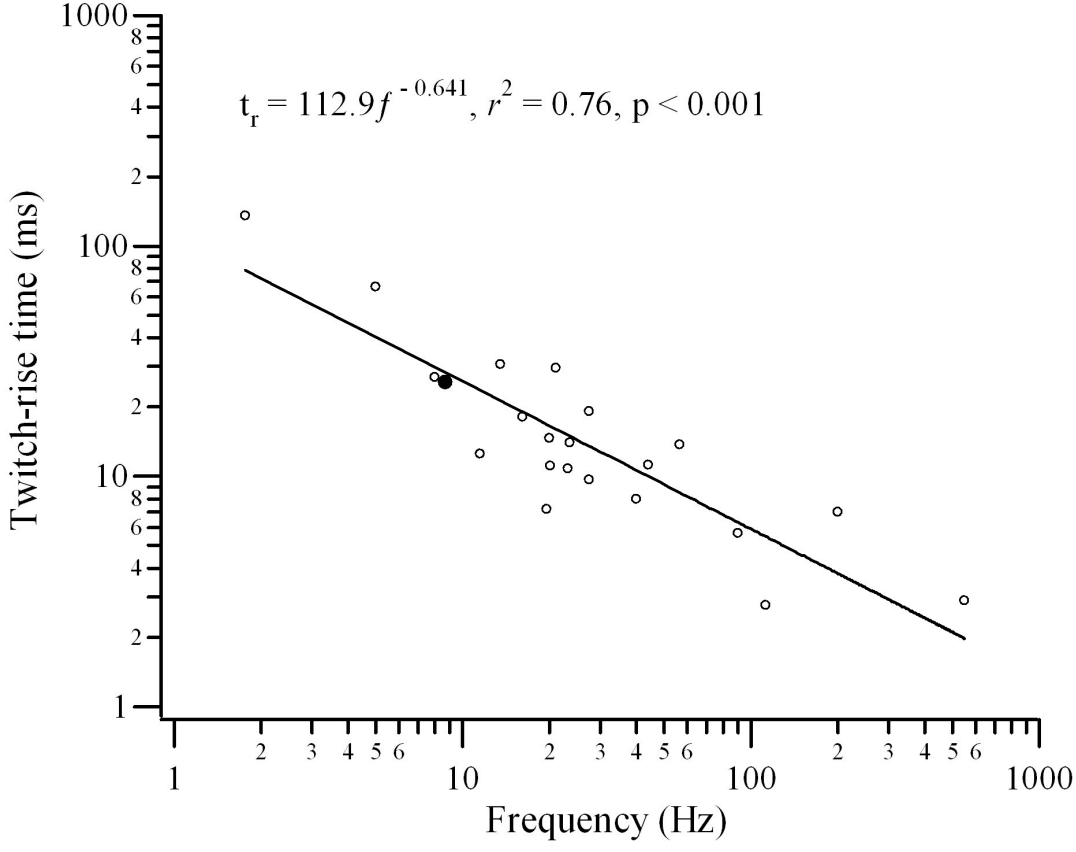
Marsh, R. L. and Bennett, A. F. (1986). Thermal dependence of contractile properties of skeletal muscle from the *lizard Sceloporus occidentalis* with comments on methods for fitting and comparing force-velocity curves. *J. Exp. Biol.* **126**, 63-77.

McLister, J. D., Stevens, E. D. and Bogart, J. P. (1995). Comparative contractile dynamics of calling and locomotor muscles in three Hylid frogs. *J. Exp. Biol.* **198**, 1527-1538.

Mendelso, M. (1969). Electrical and mechanical characteristics of a very fast lobster muscle. *J. Cell Biol.* **42**, 548-563.

Olson, J. M. and Marsh, R. L. (1993). Contractile properties of the striated adductor muscle in the bay scallop *Argopecten irradians* at several temperatures. *J. Exp. Biol.* **176**, 175-193.
Pennycuick, C. J. (1975). Mechanics of flight. In *Avian Biology*, vol. 5 (ed. D. S. Farner and J. R. King), pp. 1-75. London: Academic Press.
Pennycuick, C. J. (2001). Speeds and wingbeat frequencies of migrating birds compared with calculated benchmarks. *J. Exp. Biol.* **204**, 3283-3294.
Rayner, J. M. V. (1979). A new approach to animal flight mechanics. *J. Exp. Biol.* **80**, 17-54.
Rayner, J. M. V. (1985). Bounding and undulating flight in birds. *J. Theor. Biol.* **117**, 47-77.
Roberts, T. J. and Scales, J. A. (2002). Mechanical power output during running accelerations in wild turkeys. *J. Exp. Biol.* **205**, 1485-1494.
Rome, L. C., Syme, D. A., Hollingworth, S., Lindstedt, S. L. and Baylor, S. M. (1996). The whistle and the rattle: the design of sound producing muscles. *Proc. Natl. Acad. Sci. USA* **93**, 8095-8100.
Rosser, B. W. C. and George, J. C. (1986). The avian pectoralis-histochemical characterization and distribution of muscle fiber types. *Can. J. Zool.* **64**, 1174-1185.
Rosser, B. W. C., Wick, M., Waldbillig, D. M. and Bandman, E. (1996). Heterogeneity of myosin heavy-chain expression in fast twitch fiber types of mature avian pectoralis muscle. *Biochem. Cell Biol.* **74**, 715-728.
Sokoloff, A. J., Ryan, J. M., Valerie, E., Wilson, D. S. and Goslow, G. E. (1998). Neuromuscular organization of avian flight muscle: morphology and contractile properties of motor units in the pectoralis (pars thoracicus) of pigeon (*Columba livia*). *J. Morphol.* **236**, 179-208.
Soman, A., Hedrick, T. L. and Biewener, A. A. (2005). Regional patterns of pectoralis fascicle strain in the pigeon *Columba livia* during level flight. *J. Exp. Biol.* **208**, 771-786.
Stevenson, R. D. and Josephson, R. K. (1990). Effects of operating frequency and temperature on mechanical power output from moth flight muscle. *J. Exp. Biol.* **149**, 61-78.
Swoap, S. J., Johnson, T. P., Josephson, R. K. and Bennett, A. F. (1993). Temperature, muscle power output and limitations on burst locomotor performance of the lizard *Dipsosaurus dorsalis*. *J. Exp. Biol.* **174**, 185-197.
Swoap, S. J., Caiozzo, V. J. and Baldwin, K. M. (1997). Optimal shortening velocities for in situ power production of rat soleus and plantaris muscles. *Am. J. Physiol. Cell Physiol.* **42**, C1057-C1063.
Tansey, K. E., Yee, A. K. and Botterman, B. R. (1996). Activation of type-identified motor units during centrally evoked contractions in the cat medial gastrocnemius muscle. III. Muscle-unit force modulation. *J. Neurophysiol.* **75**, 51-59.
Tobalske, B. W. (1996). Scaling of muscle composition, wing morphology, and intermittent flight behavior in woodpeckers. *Auk* **113**, 151-177.
Tobalske, B. W. (2007). Biomechanics of bird flight. *J. Exp. Biol.* **210**, 3135-3146.
Tobalske, B. W. and Dial, K. P. (1994). Neuromuscular control and kinematics of intermittent flight in budgerigars (*Melopsittacus undulatus*). *J. Exp. Biol.* **187**, 1-18.
Tobalske, B. W. and Dial, K. P. (2000). Effects of body size on take-off flight performance in the Phasianidae (Aves). *J. Exp. Biol.* **203**, 3319-3332.
Tobalske, B. W., Hedrick, T. L., Dial, K. P. and Biewener, A. A. (2003). Comparative power curves in bird flight. *Nature* **421**, 363-366.
Tobalske, B. W., Puccinelli, L. A. and Sheridan, D. C. (2005). Contractile activity of the pectoralis in the zebra finch according to mode and velocity of flap-bounding flight. *J. Exp. Biol.* **208**, 2895-2901.
Tucker, V. A. (1973). Bird metabolism during flight-evaluation of a theory. *J. Exp. Biol.* **58**, 689-709.
Ward, S., Rayner, J. M., Moller, U., Jackson, D. M., Nachtigall, W. and Speakman, J. R. (1999). Heat transfer from starlings *Sturnus vulgaris* during flight. *J. Exp. Biol.* **202**, 1589-1602.
Williamson, M. R., Dial, K. P. and Biewener, A. A. (2001). Pectoralis muscle performance during ascending and slow level flight in mallards (*Anas platyrhynchos*). *J. Exp. Biol.* **204**, 495-507.
Zar, J. H. (1999). *Biostatistical Analysis.* New Jersey: Prentice Hall.


Mean stress difference (kN m^{-2})
2
100
8
6
4
2
10
8
6
4
2
1
2
4
6
8
2
4
6
8
2
4
6
8
0.001
0.01
0.1
1
Shortening duration (s)
Cockatiel *in vitro*
Birds *in vivo*
Flight muscles *in vitro*
Other muscles *in vitro*


$t_r = 112.9f^{-0.641}$, $r^2 = 0.76$, $p < 0.001$
Twitch-rise time (ms)
Frequency (Hz)
1000
100
10
1
1
10
100
1000