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Modulation of flight muscle power output in budgerigars *Melopsittacus undulatus* and zebra finches *Taeniopygia guttata*: *in vitro* muscle performance

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Summary

The pectoralis muscles are the main source of mechanical power for avian flight. The power output of these muscles must be modulated to meet the changing power requirements of flight across a range of speeds. This can be achieved at the muscle level by manipulation of strain trajectory and recruitment patterns, and/or by intermittent flight strategies. We have measured the *in vitro* power outputs of pectoralis muscle fascicles from budgerigars *Melopsittacus undulatus* and zebra finches *Taeniopygia guttata* under conditions replicating those previously measured *in vivo* during flight. This has allowed us to quantify the extent to which different power modulation mechanisms control flight muscle power output. Intermittent flight behaviour is a more important determinant of flight power in zebra finches than budgerigars. This behaviour accounts for 25–62% of power modulation relative to the maximum available mechanical power output in zebra finch, compared to 0–38% in budgerigars. Muscle level changes in fascicle strain trajectory and motor unit recruitment, rather than intermittent flight behaviours, are the main determinants of pectoralis muscle power output in budgerigars at all speeds, and in zebra finch at speeds below 14 m s^{-1}.

Key words: power, modulation, flight.

Introduction

The pectoralis muscles are the main power source during avian flight. They provide the mechanical power required to generate lift and thrust during the wing downstroke and to accelerate the wing. Aerodynamic calculations, measurements of metabolic rate and direct physiological measurements of flight muscle performance have shown that the power requirements of flight typically have a U-shaped relationship with speed (e.g. Tucker, 1973; Rayner, 1994; Biewener et al., 1992; Tobalske et al., 2003; Bundle et al., 2007; Askew and Ellerby, 2007). Pectoral muscle function must therefore be modulated across a range of speeds to meet these changing power requirements.

Power modulation can occur at the muscle level, by the manipulation of fascicle strain trajectory and motor unit recruitment (Tobalske et al., 2005; Ellerby and Askew, 2007), and behaviourally by intermittent flight (Rayner, 1985; Rayner et al., 2001). In the avian pectoralis muscle the highest levels of motor unit recruitment, fascicle strain and wingbeat frequency typically occur at the upper and lower extremes of the speed range (Tobalske and Dial, 1994; Hedrick et al., 2003; Tobalske et al., 2005; Ellerby and Askew, 2007). Respectively, these changes indicate increases in force production, the work done per wingbeat and work rate. Changes of this type are expected in order to conform to the U-shaped power–speed relationship.

Changes in muscle function occur in concert with intermittent flight, where bouts of flapping are interspersed with non-flapping phases. The simplest explanation for intermittent flight is as a means of power modulation, simply 'switching off' the power source periodically to control average power output. This would be consistent with the fixed gear hypothesis, where muscle function is constrained to optimise power production (Rayner, 1985; Rayner et al., 2001).

While indicative of changes in power output, strain and activation data alone are insufficient for determining any resulting changes in mechanical power output. The complexity of the interaction between the basic physiological properties of a given muscle, and its response to changing activation and length change patterns in terms of force and power output, defies a modelling approach (Curtin et al., 1998; Askew and Marsh, 1998). Accurate power measurements require direct simultaneous measurements of muscle force production and fascicle length. The absence of a free tendon makes *in situ* pectoral muscle force measurements impracticable. As a consequence the mechanical power requirements of flight have primarily been estimated from three indirect sources of information: metabolic power inputs (Tucker, 1973; Ward et al., 2001), bone strain as an index of muscle force (Biewener et al., 1992; Tobalske et al., 2003) and aerodynamic theory (Rayner, 1979; Pennycuick, 1975), rather than by measurement of mechanical performance at the muscle level.

In the absence of direct measurements for the power output of avian flight muscles, the relative importance of changes in

muscle function and intermittent flight as power modulation strategies remains unclear. In the companion paper (Ellerby and Askew, 2007) we quantified pectoralis muscle activity, strain trajectory and intermittent flight strategies in zebra finches *Taeniopygia guttata* and budgerigars *Melopsittacus undulatus* across a range of flight speeds. In the present study we applied these *in vivo* strain and activity data to pectoralis muscle fascicles *in vitro*, and measured their mechanical power output using the work loop technique (Josephson, 1985a). We hypothesised, based on the measured range of powers available from the pectoralis muscles in these species (Askew and Ellerby, 2007), that modulation of muscle activity and fascicle strain trajectory were the primary means of power modulation in these species, and that intermittent flight was of secondary importance as a power modulation strategy. *In vitro* measurements allow the effects of strain trajectory on power to be assessed separately from the effects of muscle recruitment intensity and intermittent flight. This approach will therefore reveal, for the first time, the relative importance of muscle level power modulation and intermittent flight as power control mechanisms.

Materials and methods

Experimental animals

Zebra finches *Taeniopygia guttata* Vieillot 1817 (mean body mass=13.1±0.8 g, *N*=7) and budgerigars *Melopsittacus undulatus* Shaw 1805 (mean body mass=42.0±1.1 g, *N*=11) were obtained from commercial suppliers. They were housed in wire cages with *ad libitum* access to a commercial seed mix and water under a 12 h:12 h light:dark cycle. Vitamin enriched, high-protein egg food was also provided once a day.

In vitro muscle power measurements

We used the work-loop technique (Josephson, 1985a) to measure the power output of fascicles from the pectoralis muscles of both species *in vitro*. The techniques used were the same in each case. Bundles of muscle fascicles were dissected out from a pectoralis muscle under non-recovery isoflurane anaesthesia. The pectoralis was exposed by a skin incision. A 6-0 silk suture was tied through the intramuscular tendon of the pectoralis approximately 1 cm from the insertion of the tendon on the humerus. This formed a distal attachment point for the fascicles. Two parallel scalpel incisions were made, running from the intramuscular tendon proximal to the suture to the sternum. The anterior incision started approximately 2 mm from the suture and reached the ventral margin of the pectoralis at approximately the mid point of the sternal keel. Throughout the dissection the fascicles were irrigated with chilled, oxygenated Krebs–Henseleit Ringer's solution at 5°C [composition in mmol l^{-1}: NaCl, 118.5; $NaHCO_3$, 25.0; KCl, 4.8; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 1.4; glucose, 11.0; pH 7.4 when saturated with 95% O_2, 5% CO_2 (Krebs and Henseleit, 1932)]. Care was taken to follow the margins of fascicles that were visible on the muscle surface. For each incision the blade was held at approximately a 30° angle to the muscle surface with the point of the blade under the fascicle bundle being freed from the main body of muscle. This freed a fascicle bundle with a triangular cross section approximately 3 mm wide and 2 mm thick at the apex of the triangle. The intramuscular tendon was cut proximal and distal to the suture and a section of sternum on which the fascicles originated excised by a series of scissor cuts. Finally, the bundle of fascicles was lifted clear from the pectoralis muscle and placed in a dish of chilled, oxygenated Ringer's solution at 5°C.

For connection to the muscle lever the silk suture attached to the intramuscular tendon was tied to a connector constructed from 00 insect pins and links from a silver chain (mass 45 mg). The fascicles were suspended vertically in a Perspex^{TM} tissue chamber perfused with Krebs–Henseleit solution saturated by bubbling with 95% O_2, 5% CO_2. The Ringer's solution was initially chilled to 5°C and allowed to warm to 40°C (the *in vivo* temperature of the pectoralis muscle during flight; D.J.E. and G.N.A., unpublished measurements) over approximately a 15 min period. The proximal end of the fascicle bundle was secured to the base of the muscle chamber using stainless steel spring clips to clamp the sternum on either side of the fascicle bundle. The lightweight connector linked the distal end of the fascicles to the muscle lever (model 300B, Aurora Scientific, Aurora, ON, Canada). The motor head of the muscle lever was attached to an adjustable mount, which allowed the starting length of fascicles to be changed. A series of isometric twitch contractions were used to set the operating length of the muscle for work loop measurements. The fascicles were activated by applying a supramaximal voltage *via* parallel, platinum electrodes, placed on opposite sides of the fascicles, and that ran the full length of the muscle preparation (stimulus pulse width 0.2 ms). The length for maximum isometric twitch force was determined. We then reduced fascicle length by approximately 7% so that when operating cyclically the muscle produced maximum force at the peak of the length cycle. This maximised power output by the muscle and approximated the *in situ* length of the fascicles measured before dissection with digital callipers.

The cyclical operating conditions imposed on the muscle fascicles replicated the *in vivo* strain trajectories and activity patterns measured during wind tunnel flight by sonomicrometry and electromyography (Ellerby and Askew, 2007). Length and activation were controlled using a virtual instrument designed in Testpoint (Version 3.4). This output a strain waveform appropriate to each simulated flight speed and triggered stimulation of the muscle by a stimulator (model S47, Grass-Telefactor, West Warwick RI, USA). The output of the stimulator was amplified *via* a Stimulus Isolation Unit (UISO model 236, Hugo Sachs Elektronik, March-Hugstetten, Germany), which provided a maximum stimulation current of 1 A. Stimuli were delivered at the fusion frequency of the muscle frequency (typically 275 Hz for zebra finch pectoralis and 200 Hz for budgerigar pectoralis). The resulting muscle force and length of the muscle were simultaneously recorded at 1000× cycle frequency on a personal computer *via* an A/D board (DAS1802AO, Keithley Instruments, Theale, UK). A composite strain waveform for each simulated flight speed was derived by Fourier smoothing *in vivo* strain trajectories and averaging the Fourier coefficients (Table 1). The strain, cycle frequencies and relative timings and durations of stimulation are given in Table 2. We used the 0 ms^{-1} and 4 ms^{-1} simulations as controls to monitor any decline in the performance of the zebra finch and budgerigar fascicles, respectively. Any decline in net power was corrected for by assuming a linear decrease in

Table 1. *Fourier coefficients of the strain waves applied to the pectoralis muscle fascicles in vitro*

Simulated speed (m s^{-1})	a_0	a_1	b_1	a_2	b_2	a_3	b_3	a_4	b_4
Zebra finch									
0	0.2860	0.2059	–0.8492	0.0168	0.2548	–0.0477	–0.1024	–0.0001	–0.0006
4	0.2013	0.1554	–0.9069	0.0109	0.2050	–0.0462	–0.0250	–0.0001	–0.0004
6	0.2627	0.2225	–0.8784	0.0312	0.2039	–0.0617	–0.0581	–0.0001	–0.0005
8	0.2530	0.1959	–0.8949	0.0246	0.2101	–0.0465	–0.0534	–0.0001	–0.0005
10	0.2251	0.1666	–0.8911	0.0108	0.2251	–0.0457	–0.0714	–0.00005	–0.0005
12	0.3062	0.2354	–0.8825	0.0353	0.2240	–0.0489	–0.0724	–0.0001	–0.0005
14	0.2926	0.2197	–0.8826	0.0312	0.2318	–0.0436	–0.0606	–0.0001	–0.0005
Budgerigar									
4	0.2944	0.1971	–0.8506	0.0053	0.2803	–0.0321	–0.0908	0.0178	0.0237
6	0.2734	0.2213	–0.9168	0.0484	0.1918	–0.0374	–0.0497	–0.00005	–0.0005
8	0.2368	0.2202	–0.9074	0.0574	0.1472	–0.0474	–0.0412	–0.0001	–0.0004
10	0.1699	0.1488	–0.7573	0.0669	0.0414	–0.0062	0.0123	0.0024	0.0098
12	0.1753	0.1947	–0.9259	0.0583	0.1096	–0.0399	–0.0265	0.0098	0.0149
14	0.1979	0.1506	–0.7678	0.0412	0.1256	–0.0239	–0.0338	–0.00003	–0.0004
16	0.1473	0.1331	–0.9508	0.0541	0.1196	–0.0078	–0.0181	–0.00002	–0.0004

The function $f(x)$ fitted to the raw sonomicrometry data from the accompanying paper (Ellerby and Askew, 2007) was in the form $f(x)=\frac{1}{2}a_0+\Sigma_{n=1}^{\infty}a_n\cos(nx)+\Sigma_{n=1}^{\infty}b_n\sin(nx)$. Fourth order series provided the best fits to the raw data.

Table 2. *Strain cycle parameters and relative timing and duration of stimulus applied to pectoralis muscle fascicles in vitro*

Simulated speed (m s^{-1})	Cycle frequency (Hz)	Strain (proportion L_0)	Stimulus duration (s)	Stimulus onset (s before peak length)
Zebra finch				
0	27	0.151	0.013	0.0056
4	26	0.133	0.016	0.0062
6	26	0.135	0.016	0.0062
8	28	0.138	0.014	0.0061
10	28	0.150	0.014	0.0055
12	30	0.155	0.014	0.0059
14	30	0.164	0.014	0.0060
Budgerigar				
4	16	0.154	0.019	0.0059
6	16	0.132	0.022	0.0105
8	16	0.124	0.022	0.0092
10	15	0.117	0.021	0.0105
12	16	0.128	0.020	0.0082
14	16	0.142	0.020	0.0089
16	16	0.149	0.019	0.0069

performance between consecutive controls. The positive power output during shortening (equivalent to the wing downstroke) was calculated from the force and strain trajectory data. Power output declined by 1–5% after each set of contractions. Any work-loop data that exhibited a greater than 30% decline in power output relative to the initial control were excluded from the final data set.

After completion of the power measurements the force–velocity characteristics of the fascicles were measured using a series of after-loaded isotonic tetanic contractions. The length of the fascicles was set to 10% above the starting length for the work-loop contractions so that when the muscle shortened it did so through the plateau of the force–length relationship. The fascicles were tetanically stimulated, and force allowed to rise to a pre-determined level, which was then maintained by fascicle shortening. The resulting length trace, recorded at 5 kHz, was differentiated to obtain shortening velocity. This was expressed relative to the muscle length at which velocity was measured. This procedure was carried out at relative forces from approximately 0.95–0.05 of the peak isometric tetanic stress (P_0). Isometric tetani were used as controls to monitor any decline in muscle performance. The decline was assumed to be linear between controls. Shortening velocity was plotted against corrected relative force. The data were fitted with an exponential-linear equation (Marsh and Bennett, 1986a), and maximal shortening velocity (V_{max}) was estimated by extrapolation to zero force. The power ratio was calculated as the ratio of the maximum isotonic power output to the product of P_0 and V_{max}, and is a measure of the curvature of the force–velocity relationship (note that a power ratio of 0.25 represents a linear force–velocity relationship).

After completion of the *in vitro* measurements the fascicle

Table 3. *Contractile properties of zebra finch and budgerigar pectoralis fascicles*

Parameter	Zebra finches	Budgerigars
Peak isometric, tetanic stress (kN m^{-2})	167±10	220±12
Twitch:tetanus ratio	0.35±0.07	0.51±0.07
Twitch rise time (ms)	9.7±0.7	18.1±0.5
Twitch 50% relaxation time (ms)	9.8±0.7	22.1±2.0
Twitch 90% relaxation time (ms)	20.2±2.5	48.2±4.0
Maximum shortening velocity (V_{max}, L_0 s^{-1})	21.3±1.0	14.7±0.7
Velocity at maximum power (L_0 s^{-1})	9.8±0.2	5.6±0.2
Relative force at maximum power (P/P_0)	0.50±0.01	0.44±0.01
Power ratio	0.22±0.01	0.17±0.003
Maximum isotonic power (W kg^{-1})	730±58	522±37

Values are means ± s.e.m.; *N*=7 zebra finches, isometric parameters, *N*=5 zebra finches, force–velocity parameters, *N*=9 budgerigars, all parameters.

bundle was dissected to remove any connective tissue and damaged fascicles. Power data are expressed relative to the mass of the remaining intact fascicles. The cross-sectional area of the fascicles was calculated by dividing their volume (calculated from fascicle mass assuming a muscle density of 1060 kg m^{-3}) by their length. Forces were expressed relative to this cross-sectional area.

Estimation of in vivo mechanical power

The *in vitro* work-loop measurements were obtained from supramaximally stimulated fascicles. Any measured change in fascicle power output across the range of simulated speeds was therefore due entirely to modulation of strain trajectory and the relative timing of activation. *In vivo* there are significant changes in pectoralis muscle EMG intensity in both zebra finches and budgerigars in relation to flight speed (Ellerby and Askew, 2007). Power outputs measured in supramaximally stimulated muscle are therefore not indicative of *in vivo* muscle power at all speeds. Where muscle force and EMG intensity (rectified burst area divided by burst duration) have been measured simultaneously, they are closely correlated (Adams et al., 1992; Hedrick et al., 2003). We therefore multiplied the measured *in vitro* powers by the relative EMG intensity at each simulated speed to give an estimate of *in vivo* muscle power output. Beyond modulation of muscle recruitment, intermittent flight further 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 this we multiplied the estimated muscle mechanical power output by the proportion of flight time spent flapping during instrumented flight. This gave an estimate of the average mechanical power available from the pectoralis muscles during flight.

Our estimates of the total power available from the pectoralis assume that the fascicles from which power measurements were made are representative of the whole pectoralis muscle. The only data concerning possible heterogeneity of avian pectoralis muscle function are from pigeons, where the timing of EMG activity is uniform in the majority of the muscle mass (Boggs and Dial, 1993; Biewener et al., 1998; Soman et al., 2005), the exceptions being the anterior portion of the sternobrachialis and part of the thoracobrachialis regions where the timing of activity is offset relative to the majority of the muscle. There is also some evidence for fascicle strain heterogeneity; higher strains were detected in the mid- relative to the anterior portion of the sternobrachialis (Biewener et al., 1998). This contrasts with other findings (Soman et al., 2005) where the authors measured uniform strains in the majority of the pectoral muscle mass, the exception being the posterior sternobrachialis region where strain was relatively lower. The extent of regional variations in pectoralis muscle function in smaller birds is unknown. However, given the relative uniformity of function in the majority of the pigeon pectoral muscle mass, the error in calculating whole pectoralis power output on the basis of measured powers from sternobrachialis fascicles is likely to be small.

Statistical analysis

A general linear model (GLM) was used to test for differences in mechanical power output in response to changing simulated speed and due to scaling of maximum *in vitro* powers to account for *in vivo* differences in EMG intensity and intermittent flight behaviour using the statistics package SPSS (Version 14, SPSS Inc., Chicago, IL, USA). An identifier for individual birds was included in the model as a random factor. Where significant differences were detected, a *post-hoc* Scheffé test was used to make a pairwise comparison of mean values.

Results

Isometric contractile properties

The basic contractile properties of the pectoralis fascicles in both species are shown in Table 3.

Isotonic contractile properties

Maximal shortening velocity (V_{max}), estimated by extrapolating a hyperbolic-linear fit to the force–velocity data to zero force (Marsh and Bennett, 1986a), was higher in zebra finch than in budgerigar muscle (Table 3, Fig. 1). This likely relates to the higher *in vivo* operating frequency of zebra finch pectoralis muscle (cycle frequency 26–30 Hz) relative to budgerigars [15–16 Hz (Ellerby and Askew, 2007)]. From the force–velocity relationship, the instantaneous power output was estimated to be 522 W kg^{-1} in budgerigar pectoralis muscle and

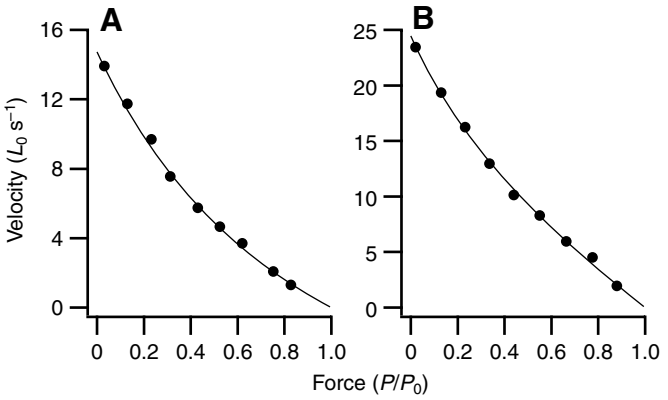

Fig. 1. Representative force–velocity relationships of zebra finch (B) and budgerigar (A) pectoralis muscle fascicles. Muscle fascicle velocity is expressed relative to resting muscle length, L_0. Muscle fascicle force is expressed relative to maximal isometric force, P_0.

730 W kg^{-1} in zebra finch pectoralis muscle. The velocity at which power was maximal was $0.46V_{max}$ in zebra finch pectoralis muscle and $0.38V_{max}$ in budgerigar pectoralis muscle. This is similar to the optimal relative shortening velocity in tree frog external oblique muscles (Girgenrath and Marsh, 1999; Marsh, 1999), but higher than the value reported in avian and mammalian hindlimb muscles [$0.22V_{max}$ to $0.26V_{max}$ (Askew and Marsh, 1997; Askew and Marsh, 1998; Nelson et al., 2004)]. At peak isotonic power the stress generated was 0.5× and 0.4× peak isometric tetanic force in zebra finch and budgerigar pectoralis muscles, respectively.

Muscle performance under in vivo length and activity patterns

Fig. 2 shows the mechanical performance of budgerigar pectoralis fascicles *in vitro*. Under *in vivo* operating conditions the mean stress difference, i.e. the mean difference between the stress developed during shortening and that developed during lengthening (Casey and Ellington, 1989), can be calculated to give a measure of force generation during locomotion. Significant changes in mean stress difference were found with flight speed (GLM; budgerigar, F=7.49, P<0.001; zebra finch, F=12.48, P<0.001; Fig. 3), ranging from 22 to 40 kN m^{-2} in budgerigar pectoralis muscle and 18 to 38 kN m^{-2} in zebra finch pectoralis muscle. Mean stress difference was lowest at 8 m s^{-1} in budgerigars and 10 m s^{-1} in zebra finches.

We detected significant changes in the positive power output of zebra finch pectoralis fascicles with simulated speed (Fig. 4). This was the case in supramaximally stimulated muscle (GLM, F=4.80, P=0.002), power values corrected for *in vivo* changes in EMG intensity (estimated *in vivo* pectoralis power output, GLM, F=11.36, P<0.001) and power values corrected for both *in vivo* changes in EMG intensity and flapping duration (estimated flight mechanical power, GLM, F=25.61, P<0.001). We also detected significant differences between maximal *in vitro* power, estimated *in vivo* pectoralis power, and estimated flight mechanical power (GLM, F=36.94, P<0.001). Zebra finch flight mechanical power ranged from 41 kg^{-1} at 6 m s^{-1} to 111 W kg^{-1} during hovering flight (Fig. 4).

We detected significant changes in the positive power output

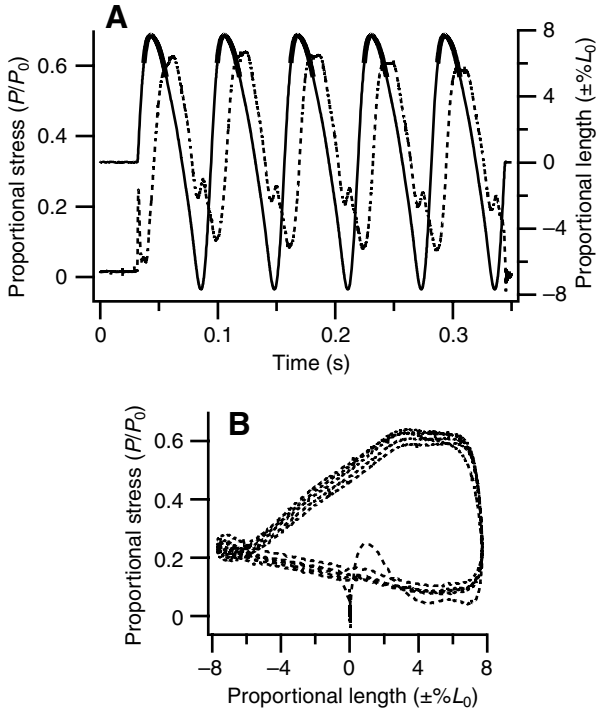

Fig. 2. Mechanical performance of a budgerigar pectoralis muscle fascicle *in vitro*. (A) Force production during strain and activation conditions measured *in vivo* at 4 m s^{-1} simulated flight speed. Length, unbroken lines; stress, broken lines. The bold line shows the timing of the stimulus relative to the strain cycle. (B) Work loops obtained by plotting fascicle stress against length for the data shown in A. Fascicle length is expressed relative to resting muscle length, L_0. Muscle stress is expressed relative to maximal isometric stress, P_0.

of budgerigar pectoralis muscles with simulated speed in supramaximally stimulated muscle (GLM, F=6.22, P<0.001), power values corrected for *in vivo* changes in EMG intensity (estimated *in vivo* pectoralis power output, GLM, F=10.58, P<0.001) and power values corrected for both *in vivo* changes in EMG intensity and flapping duration (estimated flight mechanical power, GLM, F=12.07, P<0.001). We also detected significant differences overall between maximal *in vitro* power, estimated *in vivo* pectoralis power, and estimated flight mechanical power (GLM, F=20.06, P<0.001). As a subset, however, we detected no significant difference between estimated *in vivo* pectoralis power, and estimated flight mechanical power. Budgerigar flight mechanical power ranged from approximately 30 W kg^{-1} at intermediate flight speeds (8–12 m s^{-1}) to approximately 70 W kg^{-1} during slow and fast flight (4 m s^{-1} and above 14 m s^{-1}; Fig. 4).

Discussion

Natural selection operates on the structural design of skeletal muscle to optimise functionality for the species (Hoppeler and Fluck, 2002). For the avian pectoralis muscle, the primary function is the generation of mechanical power to generate lift and overcome drag during flight. The mechanical power output must be modulated to meet speed-related changes in the power requirements of flight (Fig. 4) (Askew and Ellerby, 2007;

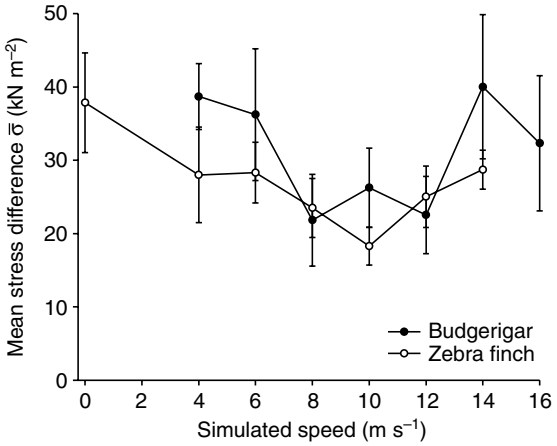

Fig. 3. Mean stress difference $\bar{\sigma}$ of zebra finch and budgerigar pectoralis muscle fascicles in relation to simulated flight speed. Values are means ± s.e.m. (budgerigars: N=11, 9, 9, 6, 9, 5, 7 for speeds 4, 6, 8, 10, 12, 14, 16 m s^{-1}, respectively; zebra finches: N=7, 6, 3, 5, 4, 4, 7 for speeds 0, 4, 6, 8, 10, 12, 14 m s^{-1}, respectively).

Ellerby and Askew, 2007). This could be achieved at the muscle level, and/or by changes in flight kinematics. The relative importance of these mechanisms for power modulation has remained unclear. Direct *in vitro* measurements have allowed us to quantify the effects of *in vivo* changes in strain trajectory, muscle recruitment and flight kinematics on pectoralis muscle power output in zebra finches and budgerigars.

Our *in vitro* power measurements were made in supramaximally stimulated muscle using strain trajectories and activation patterns measured *in vivo*. These data represent the maximum power outputs (Fig. 4) and mean stress differences (Fig. 3) achievable using the simulated *in vivo* conditions for each flight speed. Within this set of power measurements, any changes in power output across the speed range are due to changes in strain trajectory and the timing of activation. For each species we took the highest measured *in vitro* power output as a benchmark for comparison. At the other simulated flight speeds, any reduction in flight power below this maximum value is due to changes in activation timing and strain trajectory, activation intensity and intermittent flight. For a given simulated speed, the reduction in power output associated with each component of power modulation was expressed as a proportion of the total reduction in power output relative to the maximum value. The relative changes in power output (ΔP_{Rel}) for each power modulation mechanism are shown in Fig. 5. Estimating flight power in this way enables us to determine the relative contributions of changes in strain trajectory, motor unit recruitment and intermittent flight behaviour to modulating flight power output.

Muscle level changes in fascicle strain trajectory and motor unit recruitment, are the predominant means of modulating pectoralis power output in both species (Fig. 5). The exception is at the high end of the speed range in zebra finches where intermittent flapping is the main determinant of average flight power output. The major difference between the two species is the extent to which intermittent flight influences average power output. Intermittent flight is a more important power modulation mechanism in zebra finches than in budgerigars. This is a consequence of their lower relative flapping duration compared to budgerigars (Tobalske et al., 1999; Ellerby and Askew, 2007). Given the capacity for muscle level modulation of activation and strain trajectory and our data showing the extent to which this can change power output, it is clear that power modulation is not a primary explanation for intermittent flight behaviours in these species. During forward flight a role in energy economy has been identified (Rayner, 1985).

Muscle performance in relation to other power generating muscles

Our data show that the isometric force generated by the pectoralis muscle from budgerigars and zebra finches is not particularly impressive, being similar to that generated by many other vertebrate striated muscles [see Table 3; P_0=212–269 kN m^{-2} in mouse soleus muscle (Askew et al., 1997; Askew and Marsh, 1997); approximately 187 kN m^{-2} in lizard iliofibularis muscle (Marsh and Bennett, 1986b); 153 kN m^{-2} in bat biceps brachii muscle (Choi et al., 1998); and

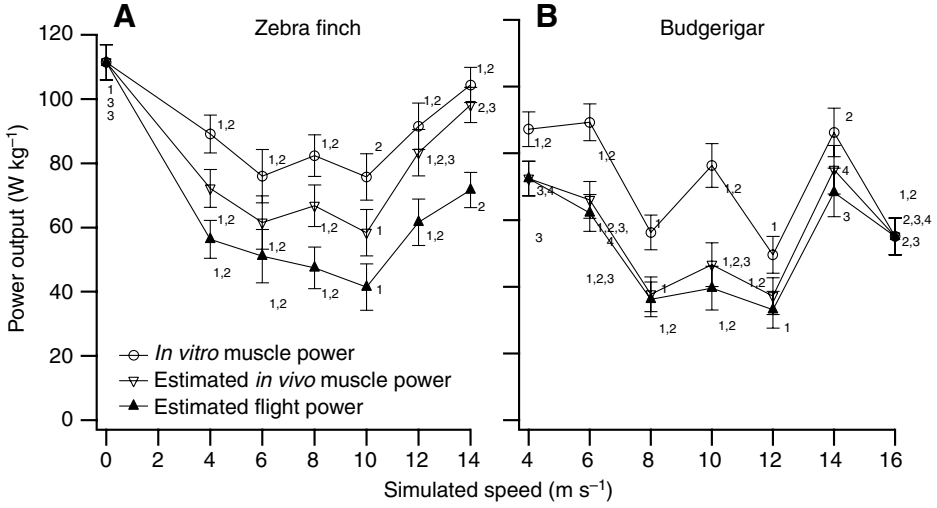

Fig. 4. Relationship between mechanical power output of (A) zebra finch and (B) budgerigar pectoralis muscle fascicles and simulated flight speed. Open circles show the power output of supramaximally stimulated muscle measured *in vitro*. Open triangles show these values corrected to account for *in vivo* changes in EMG intensity with flight speed. Closed triangles show the values from supramaximally stimulated muscle corrected to account for changes in EMG intensity and the relative duration of non-flapping flight with flight speed. For each species and within each set of conditions (*in vitro*, estimated *in vivo* and flight power), mean values designated by the same number were not significantly different (Scheffé P>0.05). Values are means ± s.e.m. (budgerigars: N=11, 9, 9, 6, 9, 5, 7 for speeds 4, 6, 8, 10, 12, 14, 16 m s^{-1}, respectively; zebra finches: N=7, 6, 3, 5, 4, 4, 7 for speeds 0, 4, 6, 8, 10, 12, 14 m s^{-1}, respectively).

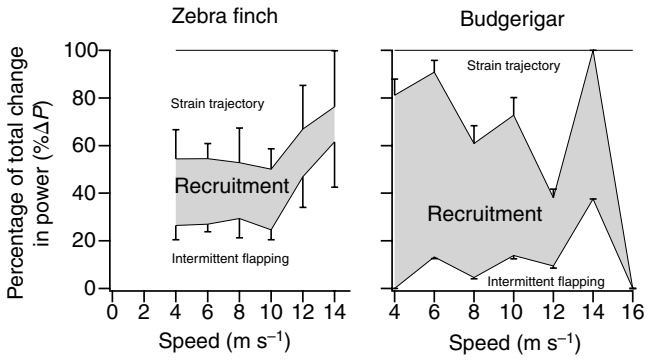

Fig. 5. Relative changes in pectoralis power output (%ΔP) due to changes in muscle strain trajectory, recruitment intensity and intermittent flapping duration in (A) zebra finches and (B) budgerigars with flight speed. Values are means ± s.e.m. (zebra finches: N=6, 3, 5, 4, 4, 7 for speeds 4, 6, 8, 10, 12, 14 m s^{-1}, respectively; budgerigars: N=11, 9, 9, 6, 9, 5, 7 for speeds 4, 6, 8, 10, 12, 14, 16 m s^{-1}, respectively).

131–200 kN m^{-2} in quail pectoralis muscle (Johnston, 1985; Askew and Marsh, 2001)]. However, the isometric stress is not very relevant to the cyclical contractions that are performed *in vivo*, in which the muscle needs to be activated and deactivated during approximately the time available for shortening. Mean stress difference (Askew and Marsh, 2002b; Casey and Ellington, 1989) is a more appropriate measurement of stress under *in vivo* conditions that incorporates length–force, force–velocity and the time course of muscle activation and deactivation.

There are few physiological data on muscles that operate at *in vivo* cycle frequencies comparable to budgerigar and zebra finch pectoralis muscle. In order to assess the performance and viability of our muscle preparations, we have used scaling relationships for a number of physiological measurements taken from other power generating muscles. Mean stress difference ($\overline{\sigma}$) is a measure of muscle performance that directly relates to locomotion (Fig. 3). In a wide range of aerobic muscles during maximal performance *in vivo* or under *in vitro* conditions optimised to maximise power output, $\overline{\sigma}$ is dependent on cycle shortening duration (Askew and Ellerby, 2007). During maximal *in vivo* power output in budgerigar and zebra finch muscle preparations, $\overline{\sigma}$ falls within the 95% confidence limits of this relationship (Askew and Ellerby, 2007). The work generated by budgerigar and zebra finch muscle also falls within the 95% confidence limits for the relationship between work and shortening duration (Fig. 6). The similarity between the data we have collected here and the scaling relationship for two physiological measurements derived from previous studies, gives us confidence in the viability of our muscle preparations.

The force–velocity curves for the pectoralis muscle are flatter than those of many other locomotor muscles. The power ratio for amphibian and mammalian hindlimb muscles is 0.08–0.12 (Askew and Marsh, 1997; Marsh, 1999) compared with 0.22 in budgerigar, 0.17 in zebra finch (Table 1) and 0.17 in blue-breasted quail (G.N.A. and R. L. Marsh, unpublished data) pectoralis muscles. Tree frog external oblique muscles also have a low degree of curvature of their force–velocity relationships, indicated by the high power ratio [0.15–0.16 (Girgenrath and Marsh, 1999)]. Flattening of the force–velocity curve increases the maximum instantaneous power output and the optimal relative shortening velocity at which it is attained (Askew and Marsh, 2002a) and may be a feature of power generating muscles that operate at high cycle frequencies.

Maximum *in vivo* power output was approximately 14% and 20% of the maximum isotonic power output in budgerigar and zebra finch pectoralis muscles, respectively. This compares with 24% and 39% in the external oblique muscles from *Hyla chrysoscelis* and *H. versicolor*, respectively (Girgenrath and Marsh, 1999), and 21% in the scallop adductor muscle (Olson and Marsh, 1993; Marsh and Olson, 1994), under simulated *in vivo* conditions. During sawtooth cycles (saw50% and saw75%) optimised to maximise net power output, mouse hindlimb muscles generated 38–60% of the maximum isotonic power (Askew and Marsh, 1997). It seems likely that while the potential exists to generate a higher fraction of the maximum isotonic power output there must be a trade-off in terms of muscle fatigue. The need to sustain muscle performance over a large number of cycles (e.g. wing strokes or vocalisations) may limit the extent to which this occurs *in vivo*.

The relative shortening velocity during flight ranged from 0.23–0.28 V_{max} in budgerigars and 0.27–0.37 V_{max} in zebra finches [calculated from shortening velocities given in the

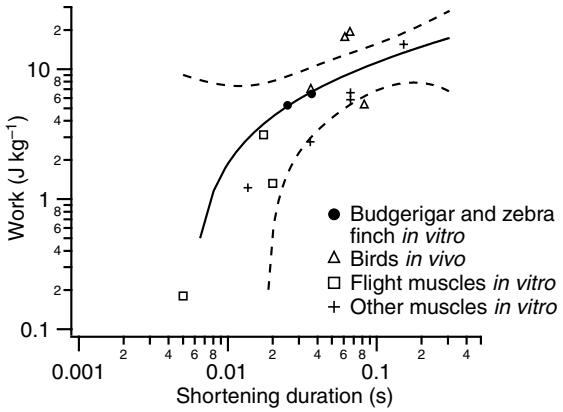

Fig. 6. The effects of cycle shortening duration on work output for a range of aerobic power generating muscles. Data are from zebra finch *Taeniopygia guttata* pectoralis muscle (present study) and budgerigar *Melopsittacus undulatus* pectoralis muscle (present study); 'birds *in vivo*' are European starling *Sturnus vulgaris* pectoralis muscle (Biewener et al., 1992), mallard *Anas platyrhynchos* pectoralis muscle (Williamson et al., 2001), cockatiel *Nymphicus hollandicus* pectoralis muscle (Hedrick et al., 2003) and pigeon *Columba livia* pectoralis muscle (Biewener et al., 1998); 'flight muscles *in vitro*' are tettigonid (*Neoconocephalus triops*) wing muscles (Josephson, 1985b) and hawkmoth dorsoventral muscle (Stevenson and Josephson, 1990); 'other muscles *in vitro*' are *Hyla versicolor* and *H. chrysoscelis* external oblique muscles (Girgenrath and Marsh, 1999), mouse (*Mus*) and rat (*Rattus*) diaphragm muscle (Altringham and Young, 1991), mouse soleus muscle (Askew and Marsh, 1997) and rat soleus muscle (Swoap et al., 1997). The solid line indicates scaling relationships for data excluding the data from the present study, and broken lines the upper and lower 95% confidence limits.

accompanying paper (Ellerby and Askew, 2007)]. The optimal shortening velocity during isotonic contractions was $0.38V_{max}$ in budgerigar and $0.46V_{max}$ in zebra finch muscle. In quail pectoralis muscle (Askew and Marsh, 2001), peak isotonic power also occurs at higher shortening velocities than during *in vivo* contractions. It has been shown that the optimal relative shortening velocity varies depending upon the strain trajectory that the muscle is subjected to (Askew and Marsh, 1998). This previous study showed that the optimal relative shortening velocity was lower than that at which power was maximal during isotonic shortening contractions during asymmetrical strain trajectories in which the proportion of the time spent shortening was greater than that spent lengthening. The reduction in optimal V/V_{max} is due to the reduction in force that occurs as a result of operating at lengths below the plateau off the length–force relationship (Askew and Marsh, 1998).

Partitioning metabolic costs during flight

The metabolic energy required by the flight muscles to generate power has previously been estimated by subtracting basal metabolism, multiplied by a postural cost factor, from total energy expenditure (Tucker, 1973; Rayner, 1999). The mechanical power requirements of flight have previously been calculated from this estimate of flight metabolic cost using an estimate of the efficiency of the flight muscles in converting metabolic to mechanical power (Pennycuick, 1989; Ward et al., 2001). The correct level for the costs not associated with power production by the pectoralis when making this type of calculation is not known. Our direct measurements of muscle mechanical power, combined with physiological estimates of muscle efficiency, allow the non-power costs to be estimated.

Comparisons of the additional energy costs incurred in tilted wind tunnels with the additional power requirements of a vertical velocity component yield efficiency estimates of 20–30% (Tucker, 1972). However, during such experiments gait changes, and the concomitant changes in muscle recruitment that occur, mean that such data are difficult to relate to the efficiency of power production at the muscle level. No data are available for bird muscle, but mammalian muscle *in vitro* efficiencies in converting metabolic into mechanical power range from 10 to 19% (Smith et al., 2005). It is reasonable to assume that avian muscle efficiencies are similar. Measurements of total metabolic flight costs during wind tunnel flight are available for budgerigars (Tucker, 1973; Bundle et al., 2007). At a flight speed of 10 m s^{-1} the total measured metabolic power input ranges from 125 W kg^{-1} body mass (Tucker, 1973) to 184 W kg^{-1} body mass (Bundle et al., 2007). These represent increases in metabolism above rest [resting metabolic rate, 18.4 W kg^{-1} (Tucker, 1968)] of 107 and 165 W kg^{-1}, respectively. At this speed the power output of the pectoralis muscles was approximately 6 W kg^{-1} of body mass for a relative pectoral muscle mass of 15% of total body mass. This would require between 32 and 60 W kg^{-1} metabolic power, based on efficiencies of 19 and 10%, respectively. This suggests that the cost of mechanical power production by the pectoralis muscles constitutes between 19 and 56% of the total increase in metabolic cost above rest at this flight speed. In flying birds a substantial mass of muscle is associated with controlling the shape and orientation of lifting surfaces and in the case of the supracoracoideus muscles, doing work to elevate the wing and lengthen the pectoralis (Dial, 1992; Gatesy and Dial, 1993). These muscles account for approximately one-third of the total flight muscle mass (K. M. C. Tjørve and G.N.A., unpublished data). Increased energy expenditure above rest associated with increased cardiac outputs and ventilation rates during flight is also expected. A relatively high cost associated with functions other than power production by the pectoralis is therefore unsurprising.

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