

Constraints on muscular performance: trade-offs between power output and fatigue resistance

Robbie S. Wilson^{1*} and Rob S. James²

¹Department of Zoology and Entomology, The University of Queensland, St Lucia, QLD 4072, Australia

²School of Science and the Environment, Coventry University, James Starley Building, Priory Street, Coventry CV1 5FB, UK

*Author for correspondence (rvilson@zen.uq.edu.au).

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An important functional and evolutionary constraint on the physical performance of vertebrates is believed to be the trade-off between speed and endurance capacity. However, despite the pervasiveness of physiological arguments, most studies have found no evidence of the trade-off when tested at the whole-animal level. We investigated the existence of this trade-off at the whole-muscle level, the presumed site of this physiological conflict, by examining inter-individual variation in both maximum power output and fatigue resistance for mouse extensor digitorum longus (EDL) muscle using the work-loop technique. We found negative correlations between several measures of *in vitro* maximum power output and force production with fatigue resistance for individual mouse EDL muscles, indicating functional trade-offs between these performance parameters. We suggest that this trade-off detected at the whole-muscle level has imposed an important constraint on the evolution of vertebrate physical performance.

Keywords: trade-off; constraint; performance; muscle; power output; fatigue resistance

1. INTRODUCTION

Identification of the physiological mechanisms underlying constraints on the evolution of vertebrate physical performance represents an important research initiative for contemporary evolutionary-focused physiologists (Garland & Carter 1994; Feder *et al.* 2000). The vertebrate locomotor system is ideal for studying the physiological mechanisms underlying performance trade-offs because the same functional network must often perform disparate tasks (e.g. sprinting, climbing, endurance, high mobility, etc.). An important functional and evolutionary constraint on the vertebrate locomotor system is believed to be the trade-off between speed and endurance capacity (Bennett *et al.* 1984; Garland 1988; Vanhooydonck *et al.* 2001; Van Damme *et al.* 2002). The physiological basis of this trade-off is considered to emanate from individual differences in the ratio of fast, powerful muscle fibres to the slow more fatigue-resistant fibres (Bennett *et al.* 1984; Garland 1988; Bonine *et al.* 2001). However, despite the pervasiveness of physiological arguments when tested at the level of the

whole animal, most intraspecific studies have found no evidence of the trade-off between speed and endurance (e.g. Ford & Shuttlesworth 1986; Garland & Else 1987; Garland 1988; Huey *et al.* 1990; Jayne & Bennett 1990; Sorci *et al.* 1995; Dohm *et al.* 1996; Wilson *et al.* 2002; but see Van Damme *et al.* 2002).

Lack of evidence supporting a speed/endurance trade-off at the whole-animal level may indicate that the presumed physiological conflict at the muscular level may not be as ubiquitous as previously suggested. A detailed test of the validity of the speed/endurance trade-off at the muscular level should involve analyses of inter-individual variation in whole-muscle performance, especially using realistic muscle-length-change cycles. However, few studies have either investigated *in vitro* muscular fatigue during realistic length-change cycles (Stevens & Syme 1993; Askew *et al.* 1997; Wilson *et al.* 2002) or individual variation in the contractile dynamics of whole heterogeneous-fibred muscles (Ashley-Ross & Barker 2002). In the only study to examine the trade-off between fatigue and power of whole-muscle performance using the work-loop technique, Wilson *et al.* (2002) found only mixed support for the trade-off. Further investigations of inter-individual variation in whole-muscle performance will indicate whether this functional trade-off is as ubiquitous at the whole-muscle level as previously suggested.

Mouse extensor digitorum longus (EDL) has often been used as a representative vertebrate fast-twitch muscle and represents an ideal candidate for an inter-individual analysis of the physiological mechanisms underlying muscular-level trade-offs. In this study, we investigated inter-individual variation in the maximum power output and fatigue resistance of mouse (*Mus musculus*) EDL muscle using the work-loop technique. Fatigue resistance was tested by observing changes in the power output of an individual muscle during a continuous set of work-loop cycles that simulated extended locomotor activity. We predicted that relatively high levels of power output for an individual EDL muscle would be associated with poor fatigue resistance.

2. MATERIAL AND METHODS

Ten-week-old female white mice were euthanased by cervical dislocation. Muscles were dissected out ($n = 17$, one muscle per mouse) at room temperature (18–23 °C) in constantly aerated (95% O₂ : 5% CO₂) Krebs–Henseleit solution with the following composition (in mM): NaCl, 118; KCl, 4.75; MgSO₄, 1.18; NaHCO₃, 24.8; glucose, 10.0; KH₂PO₄, 1.18; CaCl₂ 2.54; pH 7.6 at 20 °C. Aluminium foil clips were wrapped around the tendons and mounted on stainless steel hooks, with one end attached to a force transducer (Dynamometer UF1, Pioden Controls Ltd; 39.1 mN V⁻¹) and the other to a servomotor (V201, Ling Dynamic Systems; 1.35 mm V⁻¹). The muscle preparation was mounted inside a Perspex bath with circulating Krebs–Henseleit solution saturated with 95% O₂ and 5% CO₂ at a constant 35 ± 0.5 °C.

A series of twitches delivered via two parallel platinum wire electrodes was used to determine the stimulation amplitude and muscle length (L_0) that generated the greatest isometric twitch force (stimuli of 1.5–1.8 ms). Frequency of stimulation was optimized during tetanic contractions (typically 200–210 Hz). Each muscle was then subjected to sinusoidal length-change cycles (a total strain of 0.10 was used, which is the strain eliciting maximum power output; James *et al.* (1995)) using the work-loop technique (Josephson 1985). Both maximum power output and fatigue resistance were assessed at the cycle frequency of 5 Hz, representing a stride frequency used during sustainable activities (James *et al.* 1995). Maximum power output was also assessed at 10 Hz, corresponding to the cycle frequency yielding maximum power output for mouse EDL muscle and an ecologically relevant shortening velocity. The number of stimuli and timing of stimulation were optimized (stimulation phase usually –5 ms; burst duration usually 100 ms) and a 10 min recovery period was allowed between each tetanic contraction and work-loop run.

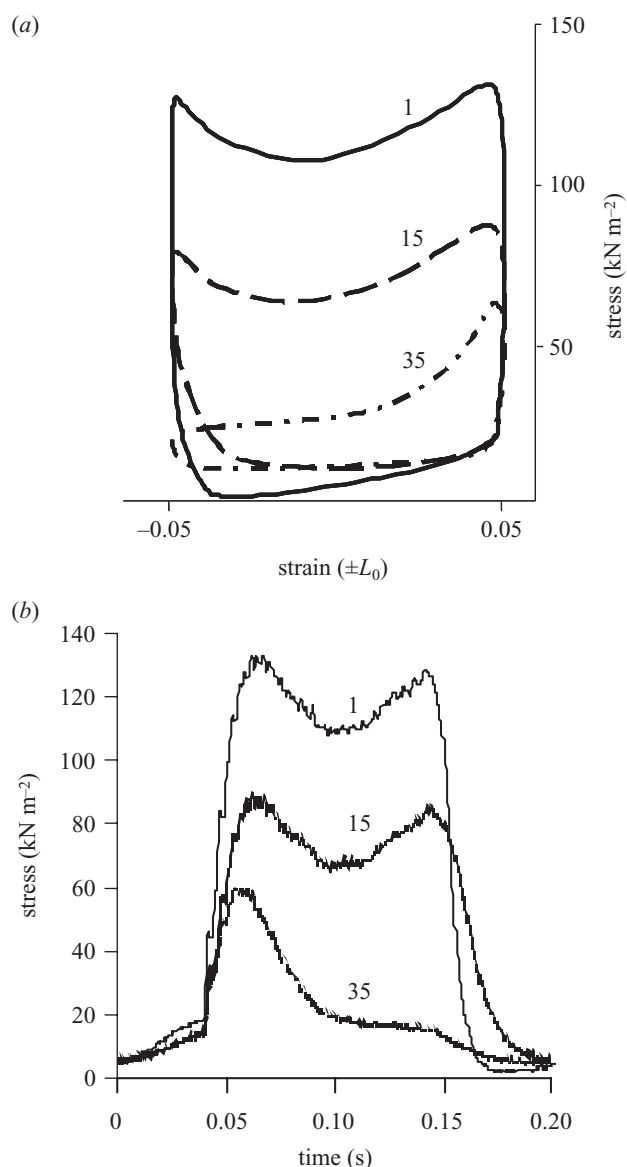


Figure 1. (a) Typical changes in work-loop shape, and (b) force-time plots, of mouse (*Mus musculus*) EDL muscle during a fatigue run at a cycle frequency of 5 Hz. L_0 , muscle length yielding the greatest twitch force. Numerals represent work-loop numbers.

Muscle stimulation and length changes were controlled via a D/A board and a customized program using TESTPOINT software (CEC, MA, USA). Force and length data were recorded at 2000 Hz and used to calculate the net work of the muscle (Josephson 1985). For active work-loop cycles, the work done (negative work) on the muscle during lengthening from L_0 to peak length (W_{NEG2}), the total work done by the muscle during shortening (W_{POS}), the work done on the muscle during lengthening from minimum length to L_0 (W_{NEG1}) and the peak force produced during the work-loop (F_{max}) were all calculated. Net work output was calculated as total work output - total work input. Power outputs and inputs were then calculated as work multiplied by the frequency of length-change cycles.

Assessment of the fatigue resistance was determined by recording the changes in power production and force during 50 cycles of work at 5 Hz using the optimized stimulation parameters, with only one run per muscle. Fatigue resistance of power production (FR_{POW}) and force (FR_{FOR}) for each muscle was estimated by recording the power/force of the muscle at cycle 25 as a proportion of the original value at cycle 1.

At the end of the fatigue runs, tendons were removed from the muscle and excess Krebs solution was removed by blotting the muscles. Muscle wet mass was determined with an electronic balance (A&D Instruments, Oxford, UK; ± 0.01 mg) and used to calculate

isometric stress and normalized muscle power output. Average muscle cross-sectional area was calculated by dividing muscle mass by fibre length, with muscle density assumed to be 1060 kg m^{-3} (Méndez & Keys 1960).

Correlations between measures of whole-muscle performance were performed on mass residuals using the Pearson's product-moment correlation. Muscle mass residuals were used to account for the potentially confounding effects of muscle mass. Significance values were adjusted for multiple correlative tests using the Bonferroni method.

3. RESULTS

We found that the maximal isometric stress of EDL was $225.0 \pm 9.7 \text{ kN m}^{-2}$ ($n = 17$), a value similar to those previously reported for EDL muscle (James *et al.* 1995). Maximum power output of EDL was 51.9 ± 3.7 and $71.6 \pm 3.7 \text{ W kg}^{-1}$ at the cycle frequencies of 5 and 10 Hz ($n = 17$), respectively. During a typical optimized work-loop for EDL at 5 Hz, force began to rise near the end of muscle lengthening and reached a peak just after reaching maximum muscle length, i.e. during shortening (figure 1). Muscle force decreased only slightly throughout muscle shortening and then increased again near its shortest length, after which the muscle rapidly relaxed before returning to L_0 (figure 1).

Changes in work-loop shape of EDL during a fatigue run reflected both the rapid decrease in maximum force production and the inability of the muscle to maintain force during shortening (figure 1). During a fatigue run, the F_{max} of EDL decreased rapidly during the first 25 cycles, reaching a plateau that was less than 50% of the original force production at cycle 1 (figure 2). Net power output (P_{NET}) also decreased rapidly during the first 20–25 cycles, but reached a plateau that was *ca.* 10% of initial net power production (figure 2). We found that P_{NET} for EDL had returned to greater than 85% of pre-fatigue values following 30 min of rest, indicating that changes in muscle power output during fatigue runs were the result of muscle fatigue rather than damage (Stevens & Syme 1993; Askew *et al.* 1997).

We found several significant correlations between measures of whole-muscle performance for the EDL muscles taken from different individual mice. Several significant positive correlations were detected among measures of maximum power output and force production (table 1). In addition, several negative correlations were detected between measures of both maximum power output and force production with fatigue resistance, indicating functional trade-offs between these pairs of performance traits. For example, fatigue resistance of maximum work-loop force was negatively correlated with both maximum power output at 5 Hz ($n = 17$, $r_p = -0.78$; $p < 0.0002$) and net power output of the muscle at the beginning of the fatigue run ($n = 17$, $r_p = -0.793$; $p < 0.001$).

4. DISCUSSION

The biophysical and biochemical properties of vertebrate skeletal muscle differ between fast and slow fibre types (for reviews see Goldspink 1996; Bottinelli & Reggiani 2000). Because most vertebrate muscles are composed of a mixture of fibre types, a functional trade-off is expected between power output and fatigue resistance in vertebrate whole-muscle performance. We tested this prediction by examining inter-individual variation in

Table 1. Pearson product-moment correlations between measures of isolated whole-muscle performance for mouse (*Mus musculus*) EDL^a.

(For each correlation between performance parameters, a correlation (above) and probability (below) value are given. Statistically significant correlations are given in bold text. The Bonferroni-adjusted significance level was taken as $p < 0.002$. Significant negative correlations indicate a trade-off between measures of muscle performance.)

	P_{\max} (5 Hz)	P_{\max} (10 Hz)	P_{NET} (cycle 2)	FR_{POW}	F_{\max} (cycle 2)	FR_{FOR}
F_{TET}	0.610 0.00925	0.516 0.0339	0.367 0.147	0.178 0.493	0.348 0.171	-0.219 0.398
P_{\max} (5 Hz)		0.778 0.000238	0.864 0.00000763	-0.163 0.532	0.771 0.000291	-0.784 0.000193
P_{\max} (10 Hz)			0.824 0.0000468	-0.174 0.505	0.818 0.0000604	-0.545 0.0237
P_{NET} (cycle 2)				-0.457 0.0649	0.933 0.00000005	-0.793 0.000147
FR_{POW}					-0.450 0.0697	0.476 0.0534
F_{\max} (cycle 2)						-0.626 0.00721

^a Maximum tetanic force (F_{TET}); maximum power output at the cycle frequency of 5 Hz (P_{\max} (5 Hz)); maximum power output at the cycle frequency of 10 Hz (P_{\max} (10 Hz)); maximum power output at the second cycle of a fatigue run (P_{NET} (cycle 2)); fatigue resistance of net power output (FR_{POW}); maximum force produced during the second work-loop cycle of a fatigue run (F_{\max} (cycle 2)); fatigue resistance of maximum work-loop force (FR_{FOR}).

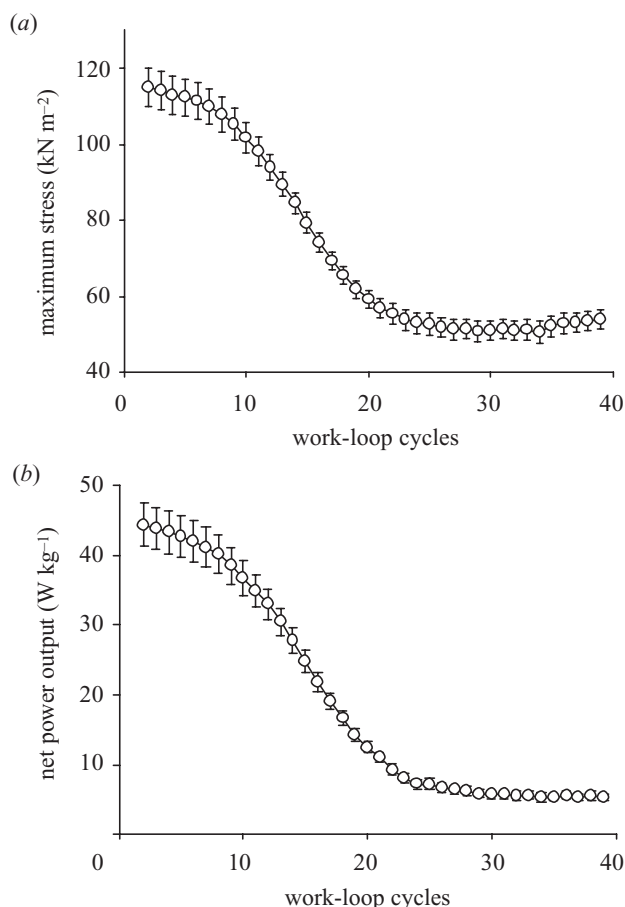


Figure 2. Changes in (a) maximum work-loop stress, and (b) total net work, for mouse (*Mus musculus*) EDL muscle during a fatigue run at a frequency of 5 Hz.

maximum power output and fatigue resistance of mouse EDL using the work-loop technique. We found significant negative correlations between several measures of

maximum power output and force generation with fatigue resistance, representing some of the most convincing support to date for the existence of a functional trade-off in vertebrate whole-muscle performance.

Interpreting the *in vivo* significance of studies of the contractile properties of vertebrate muscle using the work-loop technique can be difficult (for a review see Marsh 1999). During *in vivo* function, the activation patterns imposed on the EDL may alter as the muscle fatigues, whereas for *in vitro* studies it is not possible simultaneously to optimize all stimulation conditions during a continuous set of work-loop cycles. The shape of the strain waveform during *in vitro* studies can also have marked effects on the net power output and optimal cycle frequency (Askew & Marsh 1998). When the strain waveform used by mouse EDL *in vivo* is simplified to a sawtooth waveform, with ca. 25% of the cycle used for shortening (James *et al.* 1995), both net power output and the optimal cycle frequency for power production decrease relative to a sine waveform (Askew & Marsh 1998). However, despite these limitations, it is likely that the present study provides a reasonable indication of the effects of fatigue on mammalian muscle performance during dynamic muscle actions. It also avoids the problems of overestimation of power output encountered by earlier studies of muscle fatigue that used less realistic strain waveforms (Josephson 1993; James *et al.* 1995; Askew & Marsh 1998).

Despite detecting a clear trade-off between power output and fatigue resistance at the whole-muscle level in mice, few intraspecific studies implicitly support the existence of the speed/endurance trade-off at the whole-animal level (e.g. Ford & Shuttlesworth 1986; Garland & Else 1987; Garland 1988; Huey *et al.* 1990; Jayne & Bennett 1990; Sorci *et al.* 1995; Dohm *et al.* 1996). Even an extensive quantitative genetic analysis of sprinting and endurance capacity in the house mouse (*Mus domesticus*) found no significant phenotypic correlation between these traits (Dohm *et al.* 1996), although a negative genetic

correlation was detected. By contrast, interspecific analyses of speed and endurance capacity appear to support the existence of this trade-off (Huey *et al.* 1984; Garland *et al.* 1988; Vanhooydonck *et al.* 2001). Using an interspecific phylogenetic comparison of lizard locomotion, Vanhooydonck *et al.* (2001) reported a trade-off between speed and endurance capacity among several species of lacertid lizards.

Lack of support for the speed/endurance trade-off from studies at the whole-animal level when it occurs at the whole-muscular level is intriguing. One interesting possibility is that during locomotion an individual animal could partially compensate for the functional constraints imposed upon each muscle by cycling the activation of several agonistic muscles (with differing fibre populations). Other possible factors masking a trade-off at the whole-animal level may include the influence of motivation on measures of locomotor performance (Wilson *et al.* 2002), individual variation in the size and quality of the muscles or even an individual's overall general quality (Van Damme *et al.* 2002). In an analysis of decathlete performance, Van Damme *et al.* (2002) found significant negative correlations between sprinting and endurance events only when an athlete's general quality was factored into the analysis. Despite the limited evidence from whole-animal studies, we suggest that the functional trade-off between maximum power output and fatigue resistance at the muscular level has imposed an important constraint on the evolution of vertebrate physical performance.

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