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Regional variation in the mechanical properties and fibre type composition of the rat extensor digitorum longus muscle

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New Findings:

What is the central question of this study?

Mammalian muscle is typically heterogeneous in fibre type distribution, with distinct regional variation in composition: the effects this may have on mechanical performance are largely unknown.

What is the main finding and its importance?

Contractile properties vary regionally within a heterogeneous muscle. The mixed extensor digitorum longus (EDL) muscle has phenotypically distinct compartments that differ in their isometric twitch kinetics, the optimum cycle frequency for maximum power generation and fatigue resistance. The mechanisms underpinning the decline in performance during fatigue differ between compartments. Regional variation in mechanical performance suggests that regions of the EDL may be differentially recruited during locomotion, depending upon functional demand.

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Abstract

Fibre type composition is heterogeneous and distribution varies spatially in many muscles, indicating there may be regional variation in recruitment and mechanical output. The rat extensor digitorum longus muscle is composed of predominantly fast-twitch fibres, and exhibits a gradient in phenotype, resulting in oxidative medial (areal composition 24.3% Type I/IIa) and glycolytic lateral (92.4% Type IIx/IIb) compartments. Here, we investigated the variation in mechanical performance between the medial and lateral compartments during isometric, isotonic and cyclical contractions. Isometric tetanic stress and force-velocity relationships were similar in both compartments, but isometric twitch kinetics were slower in the medial compared to the lateral compartment. The medial compartment also had a lower optimum cycle frequency for maximum net power generation (11Hz *vs*. 15Hz; $P < 0.05$) due to slower isometric kinetics, resulting in a lower level of activation and reduced net work generation at higher cycle frequencies, compared to the lateral compartment. The more oxidative, medial compartment had higher fatigue resistance, maintaining net power 26% longer than the lateral compartment. The predominant mechanisms underpinning the decrease in net power varied between the compartments, resulting from an increase in the work to extend the muscle and from a reduction in work during shortening in the medial and lateral compartments, respectively. Regional variation in mechanical performance and resistance to fatigue within a mixed muscle suggests a differential recruitment pattern is likely during locomotion, with the medial compartment being utilised during slow speed locomotion and the lateral compartment during burst activities.

Introduction

The force developed by a muscle and the duration for which it can be sustained depends on the number of motor units recruited and the type(s) of muscle fibre they contain. Muscle fibre contractile properties vary in their rates of force development and maximal shortening velocity, as well as on the metabolic pathways (oxidative or glycolytic) that supply high-energy phosphates. Motor unit recruitment often follows a pattern that depends on motorneuron excitability (Henneman size principle), but it may also track mechanical demands (Wakeling *et al.*, 2006; Hodson-Tole & Wakeling, 2007). Many muscles are composed of a mixture of fibre types in proportions that relate to activity of the muscle during locomotion. However, the distribution of these fibre types often varies regionally, indicating that there may be variation in recruitment and mechanical output within a single muscle (De Ruiter *et al.*, 1996). Much of our knowledge about contractile properties has been obtained using supramaximally activated whole muscle preparations. Measurements of this type yield information about the average mechanical performance of a muscle, but potentially obscure regional variations in mechanical output.

The extensor digitorum longus (EDL) muscle has been widely used as an example of a 'fast' muscle in many contractile studies (Close, 1964; Luff, 1981; James *et al.*, 1995; Askew & Marsh, 1997). The rat EDL contains approximately 5% Type I, 53% Type IIa and 42% Type IIb (Schiaffino *et al.*, 1970; Pullen, 1977; Windisch *et al.*, 1998) while in mice the proportions are 46% Type IIa and 54% Type IIb (Askew & Marsh, 1997). In both of these species, the fibre type is predominantly fast, with a similar number of both oxidative and glycolytic phenotypes. Fibre type distribution within the EDL is non-uniform with a decrease in the proportion of fatigue resistant fibre phenotype across the medial-lateral axis (Pullen, 1977; Egginton, 1990). In additional to regional variation in fibre type, an additional indicator that there may be regional variation in function arises from the fact that the

peroneal nerve diverges into two extramuscular (K and F) branches, which innervate the medial and lateral compartments of the muscle, respectively (Balice-Gordon & Thompson, 1988). A differential activation pattern of muscle compartments has been confirmed by glycogen depletion histology (Balice-Gordon & Thompson, 1988; Deveci *et al.*, 2001).

Regional differences in fibre type composition are expected to result in differences in contractile characteristics and susceptibility to fatigue within the same muscle. Previous work has demonstrated regional variation in muscle mechanical properties using isometric and isovelocity contractions (De Ruiter *et al.*, 1995). However, relating such differences to functional differences is challenging given the substantially different way in which muscles operate during locomotion (submaximally activated cycles of work during locomotion *vs.* supramaximally activated constant length/velocity contractions *in vitro*). The work loop technique was developed to simulate more closely the way in which muscles operate, by subjecting muscles to cyclical length changes with phasic stimulation (Josephson, 1985; James *et al.*, 1995; Askew & Ellerby, 2007) allowing regional contractile differences to be investigated in a functionally relevant way. The importance of such an approach can be highlighted by considering, for example, muscle fatigue. The mechanical consequences of fatigue are a reduction in force, a slowing of relaxation and a reduction changes in the force-velocity relationship (Askew *et al.*, 1997; Tallis *et al.*, 2014), all of which impact on a muscle's power generating capacity. The consequences of these combined effects on muscle performance can be quantified using the work loop technique but not using isometric/isovelocity protocols.

The aim of this study was to characterise the morphometric and mechanical properties of skeletal muscle compartments in the rat EDL. Mechanical properties were measured under isometric, isotonic and work loop conditions. We hypothesised that, compared to the lateral compartment, the medial compartment would have slower isometric twitch kinetics but that the force-velocity properties would not differ between the muscle compartments (Josephson & Edman, 1988), reflecting the predominance of 'fast' fibre types in both compartments. However, given the gradient in fatigue resistant fibre types across the muscle, we reasoned that fatigue resistance would be higher in the medial compartment compared with the lateral compartment.

Material and methods

Ethical approval

This research was approved by the University of Leeds Animal Welfare and Ethics Committee, and was conducted in accordance with UK Animals (Scientific Procedures) Act 1986 (ASPA). The investigators understand the ethical principles under which this journal operates and confirm that our work complies with their animal ethics checklist.

Animals

Twenty in-house bred male Wistar rats, aged 6-8 weeks and weighing 249 ± 2g were used in this study. Animals were reared under conditions specified in the UK's Animal Welfare Act 2006 and The Welfare of Farm Animals (England) Regulations 2007. Animals were housed under a 12:12 hour light:dark cycle at 21°C and had *ad libitum* access to food and water.

Muscle preparation

Animals were anaesthetised with Isoflurane: induced with 5% IsoFlo® in O_2 (0.4 L min kg^{-1}), and maintained at 0.28 L min kg^{-1} during the dissection of the muscle. The fusiform EDL comprises a single proximal tendon originating on the lateral epicondyle of the distal femur; the distal portion subdivides into four tendons that insert onto the distal phalanx of digits 2-5. The hind limb extensor muscle tendons were exposed, released by transecting the retinaculum ligament in the ankle, allowing the tibialis anterior muscle to be reflected, exposing the EDL. A ligature (4-0 Mersilk, Ethicon) was attached to the proximal tendon of the EDL to enable the muscle to be manoeuvred, allowing it to be freed from connective fascia. Following its removal, the EDL was immediately placed in chilled (4°C), oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit solution [117 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 24.8 $NaHCO_3$, 1.2 KH_2PO_4 and 11.1 glucose; concentrations in mmol L^{-1} (Burton, 1975)] and the animal was humanely killed by cervical dislocation in accordance with ASPA Schedule 1 requirements. The whole EDL was pinned out in a Petri dish, and the medial and lateral compartments separated from the intermediate compartments, and pinned out at approximately their resting length in the animal until required for contractile measurements.

One of the muscle compartments (randomised order of lateral or medial) was transferred to a Perspex flow-through muscle chamber, through which oxygenated Krebs-Henseleit solution at 37°C was circulated. The distal tendon of the muscle was secured to the base of the chamber using stainless steel clips, and the proximal tendon was attached to an ergometer (series 300B-LR, Aurora Scientific Inc., Ontario, Canada) that controlled muscle strain trajectory *via* a light-weight (100 mg) stainless steel rod. Muscle resting length was controlled using a moveable stage on which the ergometer was mounted. Following mounting, the muscle compartment was allowed 30 minutes recovery in fresh, oxygenated Krebs-Henseleit solution before measurements were taken.

Mechanical properties of EDL

Isometric and cyclical contractions were controlled using custom written software (CEC Tespoint version 7, Norton, MA, USA) *via* a D/A board (DAS1801AO, Keithley Instruments, Theale, UK), and the computer generated wave was converted into an analogue signal by a 16-bit A/D converter that was used to control the ergometer (series 300B-LR, Aurora Scientific Inc., Ontario, Canada). Data was sampled at 10 kHz during isometric twitch kinetics, and at 1000 × cycle frequency during the work loop experiments.

Isometric contractile properties

Optimal muscle length (L_0, defined as the muscle length that generated maximal isometric twitch force) was determined using a series of isometric twitches performed at a range of muscle lengths varied incrementally by 0.5 mm. The muscle was activated using a supramaximal stimulus with a 0.2 ms pulse width (Askew & Marsh, 2001) delivered using parallel, platinum electrodes running the length of the muscle. L_0 was used as the mean length in all subsequent experiments. An isometric tetanus was performed using a train of stimuli delivered at 200 Hz for 200 ms; the maximum isometric tetanic force was defined as P_0.

Force-velocity characteristics

The force-velocity relationship was determined using a series of afterloaded isotonic contractions (*ca.* 5-80 % P_0). Force and velocity were plotted and a hyperbolic-linear function was fitted to the data (Marsh & Bennett, 1986). Maximum shortening velocity (V_{max}) was determined from the force-velocity relationship by extrapolating to zero force, and expressed relative to muscle fibre length ($L_{0\text{-}F}$; see below for methodological details). Peak instantaneous isotonic power (W_{max}) and the curvature of the force-velocity relationship (defined as a power ratio calculated as the ratio of W_{max} to the product of V_{max} and P_0) were also determined. The condition of the muscle preparation was monitored by performing control isometric tetanic contractions every fourth isotonic contraction, to allow correction for any decline in force generating capacity (assuming a linear decline in P_0 between control contractions). Data were excluded if the predicted P_0 fell below 70 % of the initial P_0.

Muscle mechanical power output during cyclical contractions

The work loop technique (Josephson, 1985) was used to quantify the net power output of each muscle compartment over a range of cycle frequencies (3-25 Hz) using a sinusoidal length trajectory and a strain amplitude of ± 5 % of fibre length ($L_{0\text{-}F}$). At each cycle frequency the timing and duration of stimulation were optimised to yield maximum net power output. Five cycles were performed at each cycle frequency and the average of the two highest net power outputs was calculated. Control work loop cycles were performed at 7 Hz every fourth run, to assess the condition of the preparation and to allow for correction of any decline in net power (assuming a linear decline in net power between control contractions). Data were excluded once predicted control net power had declined below 70 % of the initial net power. A fatigue run of 30 cycles at 7 Hz was performed to analyse the rate of decline in net power. Net power was normalised to the net power output of the first cycle, and an index of fatigue was determined as time taken for net power to drop below 50 % of initial net power (T_{W50}). The mechanisms involved in net power decline were subsequently derived from the fatigue test by calculating: (1) the decline in maximum force production (presented as time taken for peak cycle force to drop below 70 % of initial peak force, T_{P70}); (2) changes in the force-velocity relationship, indicated by the change in the ratio of peak cycle force to force at mid-shortening (P_{L0}/P_{max}); and (3) changes in the relaxation kinetics, estimated from the work done on the muscle during lengthening.

Muscle fibre type composition and capillarity

Mid-portions of each muscle segment were mounted on cork discs and snap frozen in isopentane cooled in liquid nitrogen, and stored at -80 °C. Serial cryostat sections (10 µm) were cut at -20 °C and attached to polysine coated slides (VWR International), and stored at -20 °C until staining. Muscle fibre types were distinguished using two monoclonal anti-MHC antibodies (Developmental Studies Hybridoma Bank, University of Iowa): BA-D5 for Type I fibres (slow MHC) and SC-71 for Type IIa (fast oxidative, glycolytic). The remaining unstained fibres are considered to be Type IIx/IIb (fast glycolytic) (Elliott *et al.*, 2016). Muscle fibre boundaries were labelled with an anti-laminin antibody (Sigma, L9393), a glycoprotein integrated in the basement membrane. Finally, capillaries were labelled with a carbohydrate-binding protein (lectin) specific to rodent endothelial cells, *Griffonia simplicifolia* lectin I (GSL I, Vector Labs, FL-1101). Numerical capillary to fibre ratio (C:F), capillary density (mm^{-2}), capillary domain area (the region of tissue supplied by an individual capillary based

on tessellating Voronoi Polygons; μm^2), and heterogeneity of capillary distribution described by LogSD of domain area were calculated (Al-Shammari *et al.*, 2014).

Relative muscle fibre length

Muscle fibre length was determined following fixation in 10% neutrally buffered formalin for 24 hours at L_0. Once fixed, individual muscle fibres were freed from any connective tissue following a 48 hour digestion in 30 % nitric acid, and separated in a 50% glycerol solution; 10-20 fibres were measured from five medial and lateral compartment, and mean muscle fibre length ($L_{0\text{-}F}$) determined relative to L_0.

Statistical analysis

All data are expressed as mean ± SD (n). Differences between the medial and lateral muscle compartments were analysed using a Student's t-test, with statistical significance defined as $P < 0.05$.

Results

Muscle morphology

The morphological characteristics of the medial and lateral compartment of the rat EDL are presented in Table 1. The fibre length relative to muscle compartment length was higher in the medial compartment compared to that of the lateral compartment, 0.66 ± 0.07 and 0.60 ± 0.02 (t_8 = -2.016, P = 0.079) of L_0, respectively. Relative to the length of the whole muscle, the fibre lengths were short in the medial compartment and located towards the proximal end of the EDL, whereas in the lateral compartment the fibres run almost the entire length of the muscle. The relative mass of the lateral compartment was 2.84× that of the medial compartment ($P < 0.05$). The medial compartment had a significantly higher numerical and areal composition of oxidative fibres compared to the lateral compartment, comprising both Type I and Type IIa fibres, while the lateral oxidative fraction comprised only Type IIa fibres (Figure 1). Mean Type IIx/IIb fibre area was significantly lower within the medial compartment (t_8 = 4.487, P = 0.002), with Type IIa fibres tending towards a smaller fibre area within the lateral compartment.

The medial compartment had a significantly higher capillary density to that of the lateral (1000.62 ± 51.89 *vs.* 762.18 ± 85.67 mm^{-2}; $t_{6.587}$ = 6.587, P = 0.001) and significantly smaller capillary domain area (Figure 1). The distribution of the capillary domain areas differed in the two compartments, with the medial compartment having a lower median domain area compared to the lateral (796.18 μm^2 *vs.* 1139.69 μm^2) with a shift in distribution to comparably smaller capillary domain areas (Figure 1E, F) while also having a more homogeneous distribution of those capillaries (LogSD = 0.18 ± 0.02 *vs.* 0.20 ± 0.05; $t_{5.642}$ = -0.579, *P = 0.585*, Figure 1G).

Table 1. Morphometric characteristics of medial and lateral compartment of rat EDL

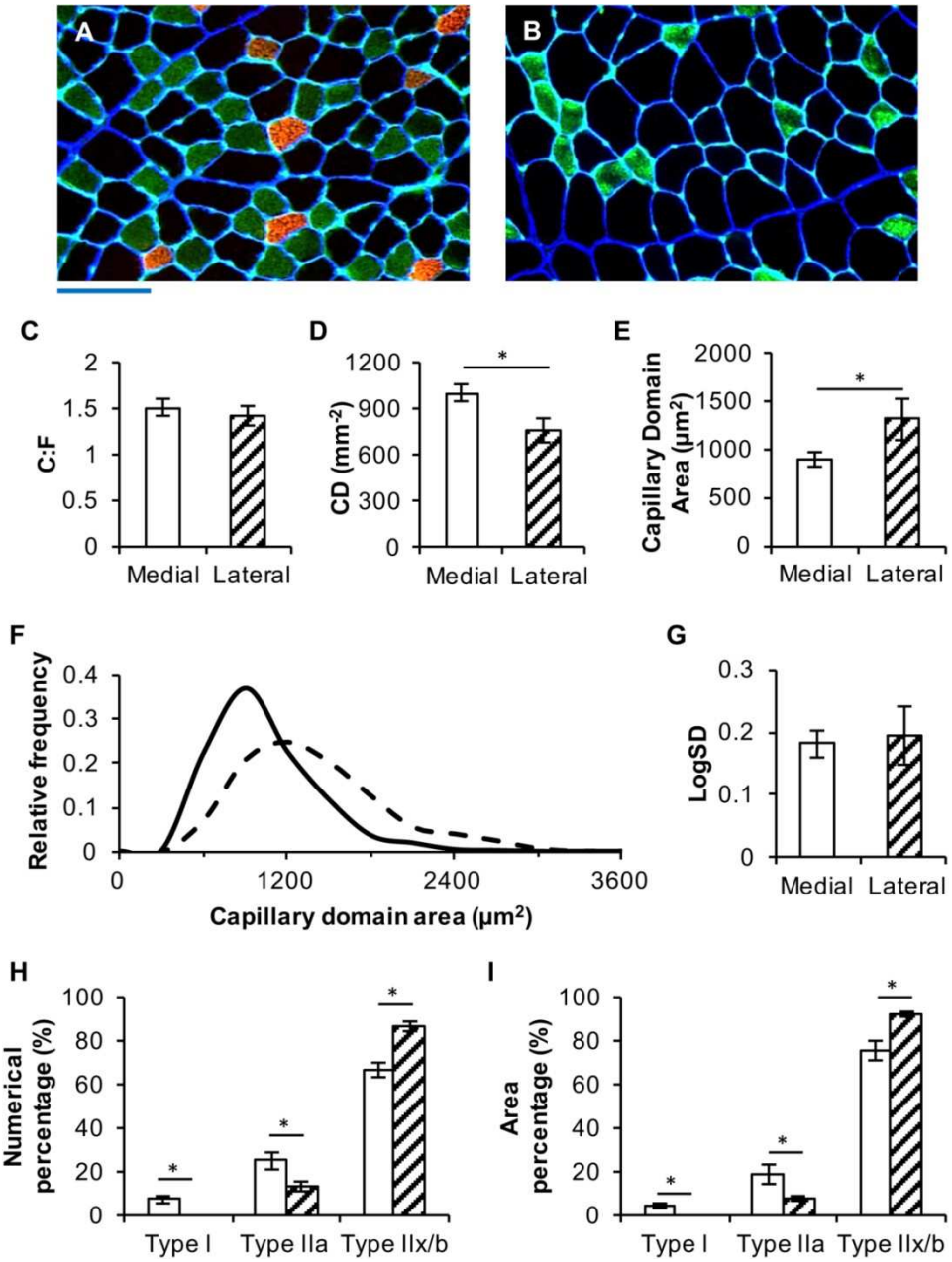
	Medial	Lateral	t	*P*

Mass (mg)	22.23 ± 4.25 *(12)*	63.12 ± 6.88 *(13)*	18.027	<0.001
Optimal muscle length, L_0 (mm)	19.35 ± 0.77 *(12)*	25.05 ± 1.31 *(13)*	13.117	<0.001
Relative fibre length ($L_{0\text{-}F}$ /L_0)	0.66 ± 0.07 *(5)*	0.60 ± 0.02 *(5)*	-2.016	=0.079
Mean Type I fibre area (μm^2)	922 ± 85 *(5)*	n/d (*5*)	-24.246	<0.001
Mean Type IIa fibre area (μm^2)	1049 ± 128*(5)*	1213 ± 114 *(5)*	2.135	=0.065
Mean Type IIx/IIb fibre area (μm^2)	1572 ± 54 *(5)*	2218 ± 318 *(5)*	4.487	=0.002
Numerical composition	7.48 ± 2.07 *(5)*	n/d *(5)*	-8.073	<0.001
Type I fibre area (%)				
Numerical composition	25.54 ± 3.96 *(5)*	13.07 ± 2.34 *(5)*	-6.061	<0.001
Type IIa fibre area (%)				
Numerical composition	66.98 ± 3.44 *(5)*	86.93 ± 2.34 *(5)*	-6.061	<0.001
Type IIx/IIb fibre area (%)				
Area composition	4.90 ± 1.26 *(5)*	n/d *(5)*	10.719	<0.001
Type I fibre area (%)				
Area composition	19.38 ± 4.47 *(5)*	7.56 ± 1.19 *(5)*	10.719	<0.003
Type IIa fibre area (%)				
Area composition	75.72 ± 4.34 *(5)*	92.44 ± 1.19 *(5)*	-8.676	<0.001
Type IIx/IIb fibre area (%)				

Values are means ± SD (n); n/d = not detected

Figure 1. Heterogeneity of fibre type and capillary supply within the EDL. Type I fibres – Red, Type IIa fibres – Green, Type IIx/IIb fibres – Unstained. Transverse cross section for the (A) medial and (B) lateral compartments. Capillary content and distribution described by the capillary to (C) fibre ratio, (D) capillary density, (E) capillary domain area, relative frequency of (F) capillary domain area and (G) the heterogeneity of the capillary distribution. Fibre type composition are presented as (H) numerical fibre type

composition, and (I) areal fibre type composition. The data is presented for the medial (open bars) and lateral compartment (hatched bars). Data presented as means ± SD *(n=5)*. * $P < 0.05$.



Isometric properties

The medial and lateral compartments both generated comparable maximal isometric tetanic stresses, 280.08 ± 49.55 kN m^{-2} and 300.19 ± 54.23 kN m^{-2}, respectively (t_{23}, $P = 0.345$), with similar twitch:tetanus ratio (0.24 ± 0.03 *vs.* 0.24 ± 0.04, respectively; $t_{15} = -0.519$, P = 0.611), but had distinct isometric twitch kinetics. The medial compartment had both a slower twitch-rise time (10.21 ± 0.91 ms *vs.* 9.14 ± 0.0.62 ms; $t_{15} = -2.865$, $P = 0.012$), and slower rate of relaxation when compared

to that of the lateral compartment, with half-relaxation times of 11.93 ± 1.62 ms *vs.* 8.55 ± 0.88 ms, respectively (t_{15} = -5.421, P < 0.001).

Force-velocity relationship

W_{max} was not significantly different between the medial and lateral compartments, generating 364.29 ± 57.49 W kg^{-1} and 347.32 ± 46.35 W kg^{-1}, respectively (t_6 = -0.459, P = 0.662). The force-velocity relationships for both muscle compartments studied were similar, with no significant differences in V_{max} or curvature, as characterised by the power ratio (Table 2; Figure 2). Consequently, both the medial and lateral compartments have equivalent V/V_{max} and P/P_0 values at which maximum isotonic power was generated (Table 2).

Figure 2. Force-velocity relationship for the medial (circles, solid line) and lateral (triangle, dashed line) muscle compartments. All data points are displayed, with the force-velocity curve plotted using the coefficients of a hyperbola-linear equation generated and averaged across the four animals (Marsh & Bennett, 1986).

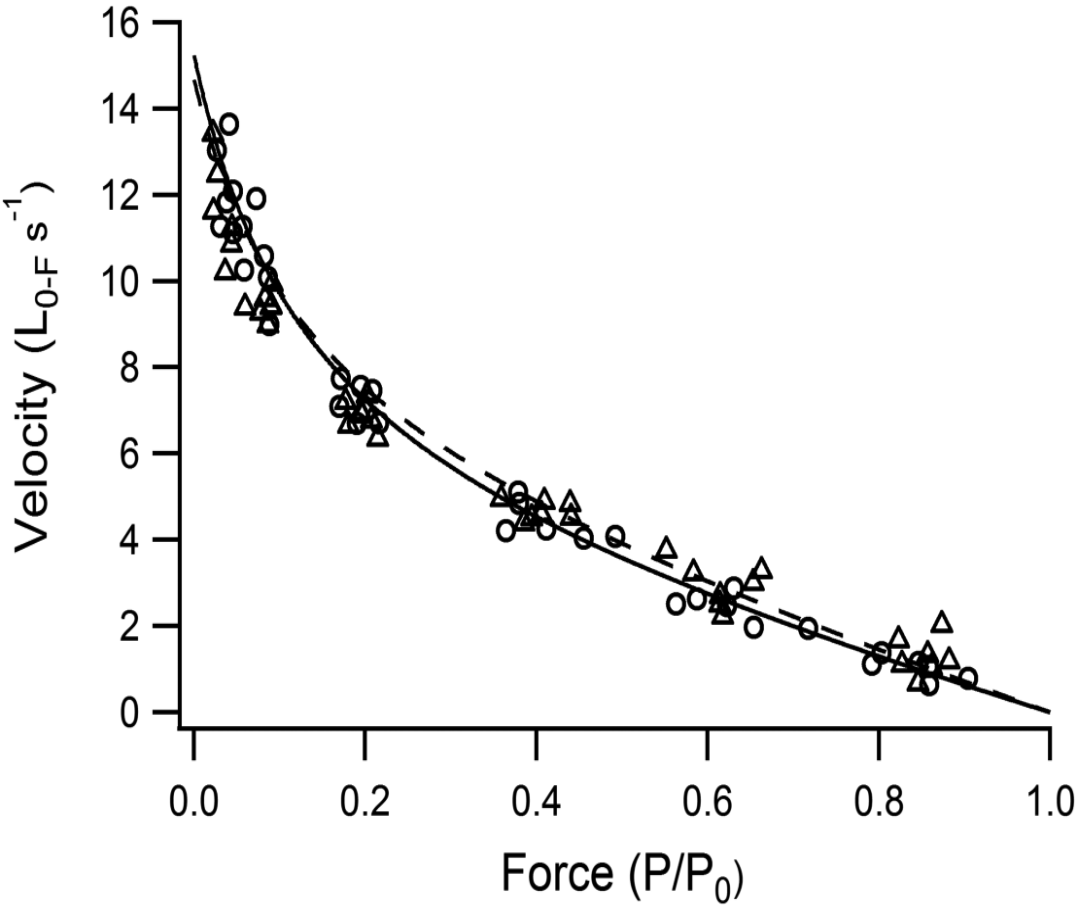


Table 2. Isotonic properties of the medial and lateral muscle compartments of rat EDL

	Medial	Lateral	t	P
W_{max} (W kg^{-1})	364.3 ± 57.49 *(4)*	347.3 ± 46.35 *(4)*	-0.459	= 0.662
V_{max} ($L_{0\text{-}F}$ s^{-1})	15.3 ± 1.5 *(4)*	14.8 ± 2.5 *(4)*	-0.353	= 0.736
V/V_{max} at max power	0.28 ± 0.02*(4)*	0.29 ± 0.05 *(4)*	0.259	= 0.805
P/P_0 at max power	0.43 ± 0.02 (*4*)	0.46 ± 0.04 (*4*)	1.536	= 0.176
Power ratio	0.12 ± 0.02 *(4)*	0.13 ± 0.0.02 *(4)*	0.999	= 0.356

Values are means ± SD (*n*).

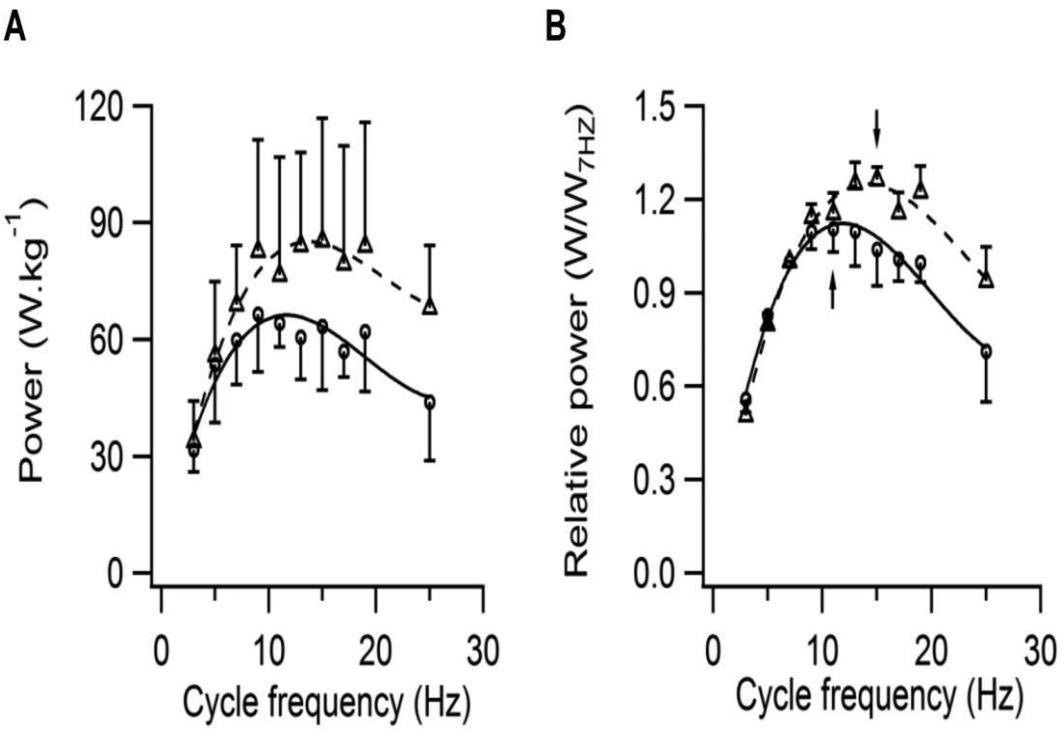
V/V_{max}, ratio defining the velocity that generates maximum isotonic power compared to V_{max}; P/P_0, ratio defining the force that generated maximum isotonic power divided by maximum isometric force; Power ratio, [$W_{max}/(P_0V_{max})$].

Cyclical muscle contractions

The power-frequency relationship is presented for both absolute net power (Figure 3A) and as relative power (Figure 3B; where net power, W, was normalised to net power at a cycle frequency of 7 Hz, W_{7Hz}). The frequency yielding maximum relative power differed between the two compartments, with maximum relative power generated at 11 Hz for the medial compartment, and 15 Hz for the lateral compartment (1.11 W/W_{7Hz} ± 0.08 *vs. 1.26* W/W_{7Hz} ± *0.04,* $P < 0.05$) (Figure 3). The maximum absolute net powers of the medial and lateral compartments were similar, 64.19 W kg^{-1} ± 6.18 and 85.29 W kg^{-1} ± 31.46, respectively ($t_5 = -1.342$, $P = 0.237$). The maximum net power for the medial compartment was not significantly different across 7-19 Hz, while they were not significantly different across 9-19 Hz in the lateral compartment.

Figure 3. Power-frequency relationship for the medial and lateral compartments of the EDL. (A) The absolute power-frequency relationship is presented and (B) the relative power-frequency relationship normalised to 7 Hz. Medial (circles, solid line) and lateral (triangles, dashed line) data

are presented as mean ± SD with a forth-order polynomial fitted. Arrows depict optimum frequency for the medial compartment (11 Hz) and the lateral compartment, (15 Hz).

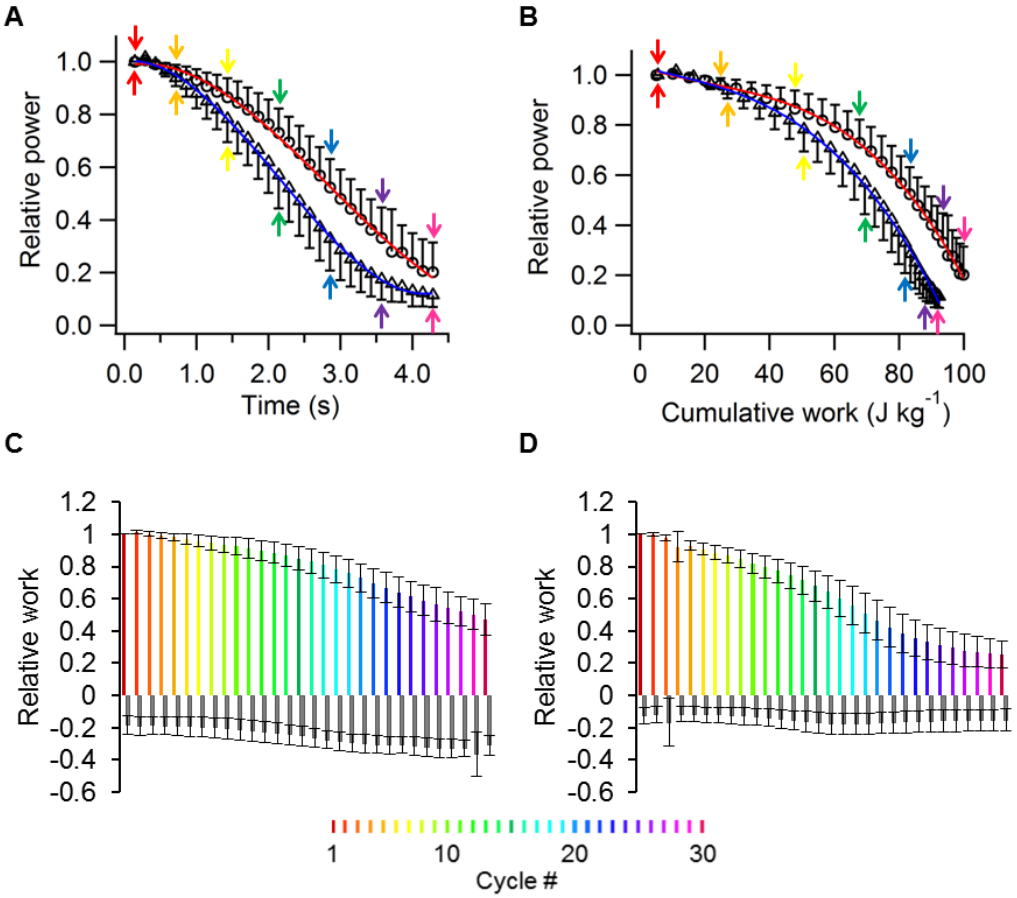


Muscle fatigability

T_{W50} for the medial compartment was 2.95 ± 0.37 s, maintaining power significantly longer than the lateral compartment, where T_{W50} = 2.34 ± 0.37 s (t_{15} =-3.440, P = 0.004, Figure 4A). Over the 30 cycle fatigue run, the medial compartment produced more cumulative work 99.79 ± 35.85 J kg^{-1} than the lateral compartment 93.69 ± 35.65 J kg^{-1}, though the difference was not significant (t_{15} = -0.49, P = 0.631, Figure 4B). The cycle-by-cycle changes in positive and negative work differed between the two compartments. As the muscle fatigued, the decline in positive work was lower in the medial compartment (better maintained) compared to the lateral compartment, however, more work was required to re-lengthening the muscle (i.e. greater increase in negative work; Figure 4C, D).

Figure 4. Relative power output during a 7 Hz fatigue run and changes in positive and negative net work. (A) Relative power output against time and (B) cumulative work for the medial (circles, red line) (*n*=8) and lateral (triangles, blue line) (*n*=9) compartments. Net work broken down into positive (calculated during shortening, coloured fill) and negative (calculated during lengthening, grey fill)

work during the fatigue work loop cycles for the (C) medial and (D) lateral compartment. Data are presented as mean ± SD with a fourth-order polynomial fit.



The work loop shapes for the medial and lateral compartments changed differently over the 30 cycles (Figure 5A, C). The medial compartment had a greater capacity to maintain force during shortening as the muscle fatigued (Figure 5B, D). Conversely, the medial compartment showed an impaired capacity to relax during shortening, resulting in force generation continuing into lengthening, thus requiring an increased amount of work to re-lengthen the muscle (i.e. negative work): the increase in force during lengthening was less apparent in the lateral compartment. The relative mid-cycle force (P_{L0}/P_{max}) decreased as the muscle fatigued in both compartments, however the decline was greater in the lateral compartment compared to the medial compartment (Figure 5E-H, 6B). The peak relative force decreased in both compartments over the course of the fatigue test with a greater decline in the lateral compartment (Figure 6A), and is indicated by the significantly longer T_{P70} for the medial compartment (3.43 ± 0.59 s) compared to the lateral compartment (T_{P70} = 2.38 ± 0.44 s; t_{15} = 4.197, P = 0.001).

Figure 5. Effect of fatigue on work loop shape and force-time characteristics for the medial and lateral compartment. Work loop shape and force-time characteristics displayed for (A, B) medial and (C, D) lateral compartments, respectively. Overlaid on the relative force trace is the sinusoidal strain the muscle is subjected to (black line). Individual cycles normalised to cycle specific peak force,

allowing the visualisation of changes in force-velocity characteristics, evident by decreasing force at mid-length through shortening, for the (E, F) medial and (G, H) lateral compartments.

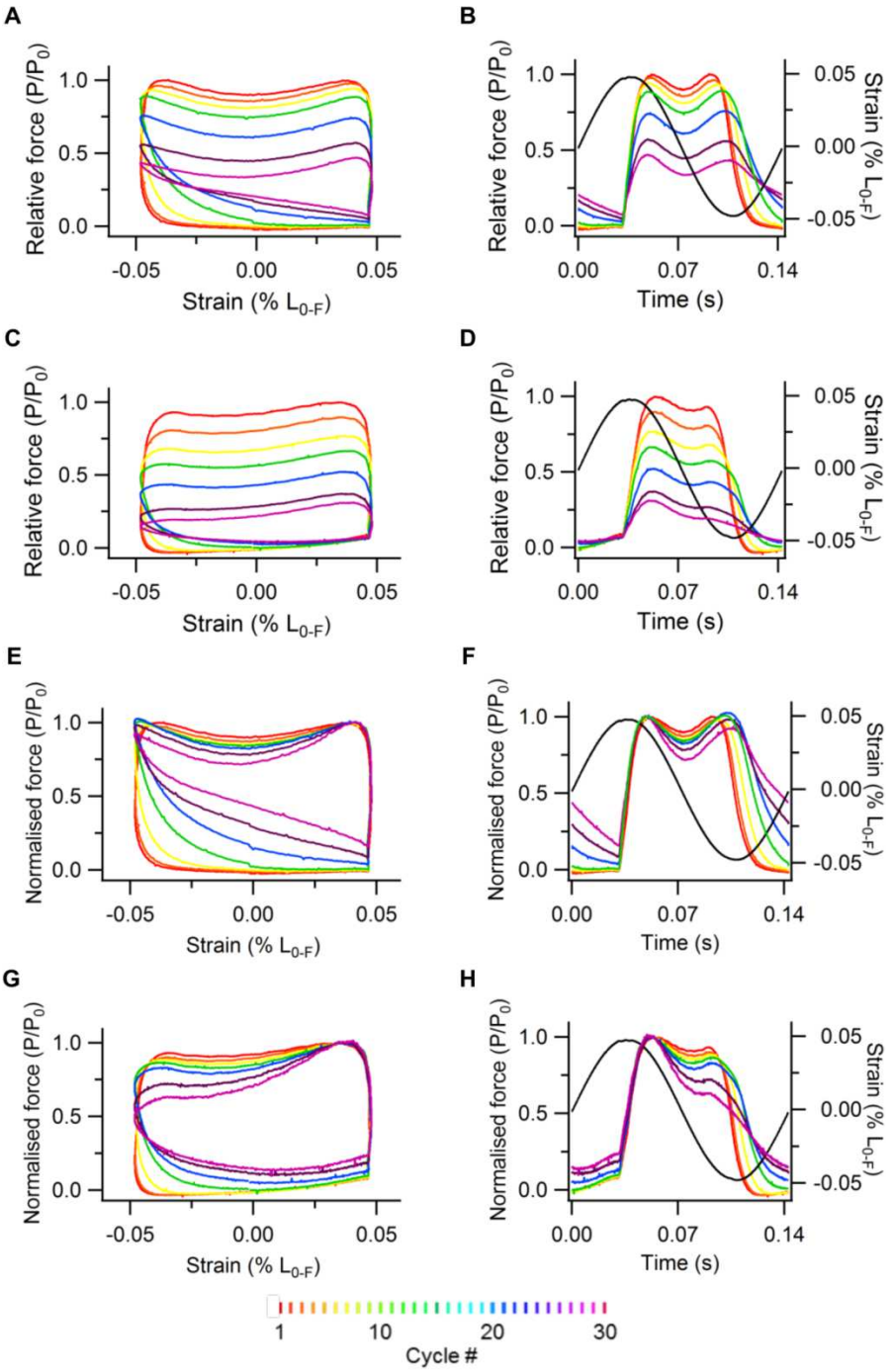
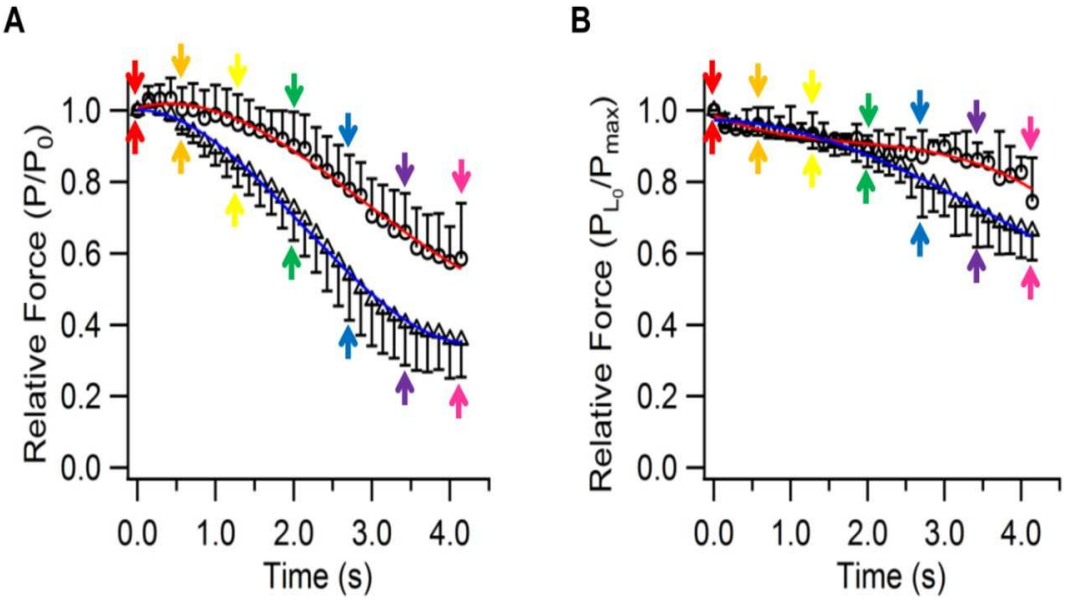


Figure 6. Mechanisms involved in the reduced power generating capacity of the medial and lateral compartment. (A) Peak force generated per cycle, relative to the force generated in cycle one, plotted against time. (B) Individual cycles normalised to cycle specific peak force, presented as a ratio to force at mid length. Data are presented

for the medial (circles, red line) (n=8) and lateral (triangles, blue line) (n=9) compartments as mean ± SD with a fourth-order polynomial fit.



Discussion

The main aim of this study was to quantify the effect of spatial fibre type heterogeneity on mechanical properties of a skeletal muscle, utilising the compartmental differences in innervation of the EDL. It was hypothesised that the predominance of fast-twitch fibres in both the lateral and medial EDL compartments would result in minimal differences in contractile characteristics under isometric and dynamic conditions, but that the presence of Type I fibres in the medial compartment would result in a higher resistance to fatigue. Regional variation in fibre type had no bearing on the force-velocity properties between the medial and lateral compartments of the EDL. However, the greater composition of Type I and IIa fibres within the medial compartment resulted in slower isometric twitch kinetics and a significantly higher fatigue resistance compared to that of the lateral compartment. Finally, the identification of differential fatigue responses among distinct muscle compartments has never before been reported.

Regional variation in fibre type distribution and capillarity

In this study, as in previous studies (Pullen, 1977; Deveci *et al.*, 2001), it was found that the medial compartment of the EDL contains all major muscle fibre types, whereas the lateral compartment only contains the fast phenotypes (Type IIa and IIx/IIb). In addition, there was a higher proportion of oxidative fibre types (Type I and IIa) in the medial compartment compared to the lateral compartment. The heterogeneity in fibre type composition across the EDL was matched by a heterogeneous distribution in capillarity. The medial compartment contained more capillaries per fibre area that were more homogeneously distributed, when compared to the lateral compartment. These structural data suggest a gradient in oxidative capacity, where the demand for oxygen (i.e. oxidative fibre phenotype) is matched with supply of oxygen (i.e. higher capillary density and homogeneity of supply).

Unlike previous studies that have reported muscle composition by fibre number, we additionally determined regional variation in relative area of fibre types as this provides a better index of muscle functional capacity since force generation is proportional to myofibrillar cross-sectional area, which in turn varies between different fibre types. The importance of reporting areal composition is emphasised by the observation that size of Type IIx/IIb fibres varied regionally, with those in the medial compartment being 29.2 % smaller than those within the lateral compartment. The proportion of oxidative fibres (Type I and IIa) based on fibre number is 13.07 % in the lateral compartment compared with 33.02 % in the medial compartment whereas the comparable proportions by fibre area are 7.56 % and 24.28 %, respectively. The spatial heterogeneity of fibre type composition within the EDL enabled potential correlation with functional capacity to be assessed.

Regional variation in isometric and isotonic contractile properties in EDL

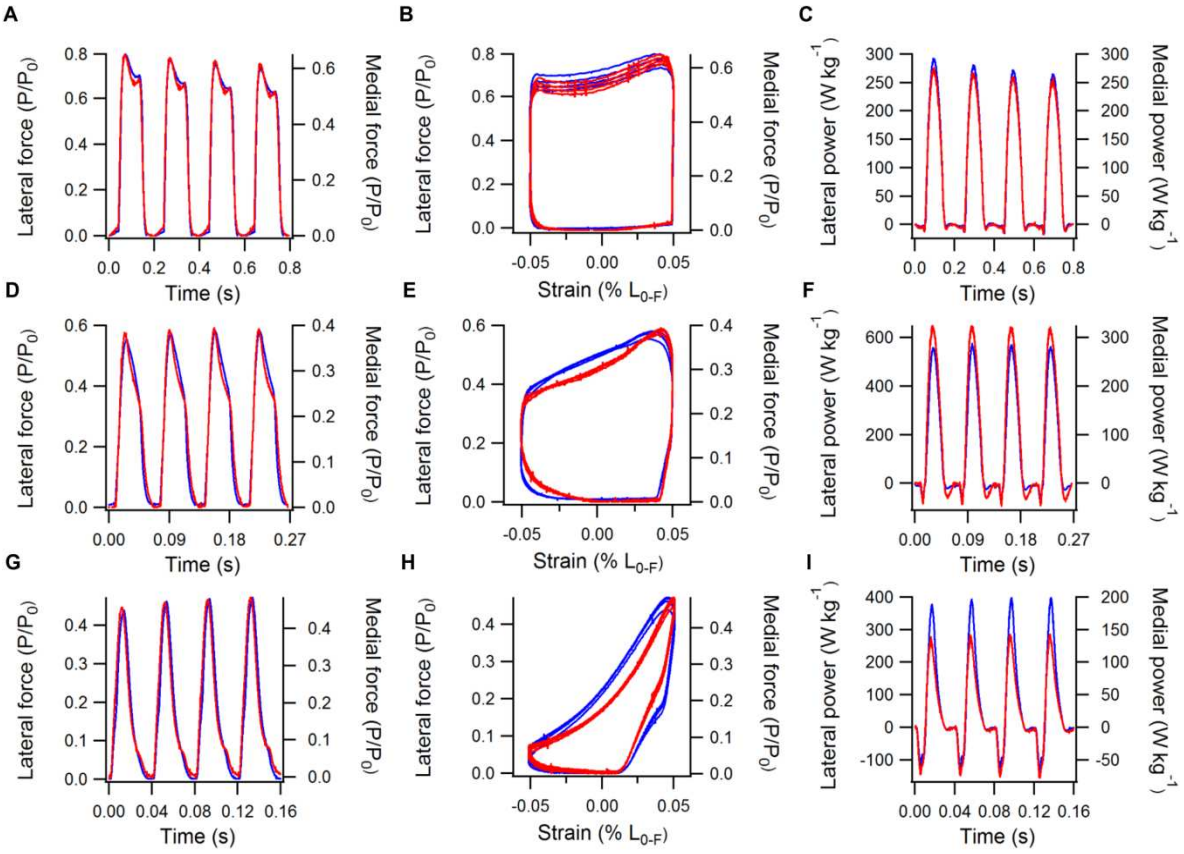
The medial and lateral compartments both generated comparable isometric stresses, with similar values to those previously reported [e.g. isometric tetanic stress: 250-300 kN m^{-2} (Van Der Meulen *et al.*, 1997; Plant *et al.*, 2001) and twitch:tetanus ratio ranging from 0.19 to 0.26, in whole rat EDL (Close, 1964, 1967; Frischknecht & Vrbová, 1991; Buffelli *et al.*, 1997)]. The major differences in isometric twitch contractile characteristics between the two compartments were in twitch-rise and twitch half-relaxation times. Isometric twitch rise time for the medial compartment was 11.7 % longer than that of the lateral, with a 39.5 % longer twitch half-relaxation time. These slower twitch kinetics are likely the result of differences in myosin heavy chain isoform composition, which determine cross bridge cycling rates together with differences in the magnitude and time-course of the myoplasmic [Ca^{2+}] (Bottinelli *et al.*, 1996; Baylor & Hollingworth, 2012). There were no regional differences in isotonic force-velocity properties detected between the two compartments of the EDL (as assessed by V_{max}, W_{max}, power ratio, optimal P/P_0 and optimal V/V_{max}), and maximum shortening velocities of the lateral and medial compartments were comparable to values reported for whole EDL preparations (Ranatunga, 1984). There is a dependence of force-velocity characteristics on myosin heavy-chain isoform (Ranatunga, 1984; Bottinelli *et al.*, 1991), although within each muscle fibre type there is a broad distribution in the maximum shortening velocity that may overlap between different fibre types (Bottinelli *et al.*, 1991). Clearly, V_{max} of the maximally activated muscle compartments is largely dictated by the fastest fibre type (Josephson & Edman, 1988). While the curvature of the force-velocity relationship has been shown to vary in relation to fibre type, differences are largely observed between fast and slow fibre types rather than between Type IIa and IIx/IIb (Bottinelli *et al.*, 1991). Given the predominance of Type II fibres in both compartments, the absence of any significant differences in the force-velocity characteristics between the two compartments is therefore not surprising. *In vivo*, motor units can be differentially recruited and it is therefore possible to recruit just slow motor units (with a slower V_{max}) as well as faster motor units (generating a higher V_{max}); under such circumstances there could be regional differences in mechanical performance of the EDL.

Regional variation in net power output during cyclical contractions

The absence of a difference in maximum net power output between lateral and medial EDL compartments is consistent with their similar force-velocity characteristics. However, there was a shift in the optimum cycle frequency for maximum net power to a lower frequency in the medial

compartment, compared to the lateral compartment. This is likely to result from the slower activation and deactivation kinetics of the medial compartment, and therefore slower rate of force development and relaxation, as indicated by the slower twitch rise and half-relaxation times. For a given cycle frequency, slower contractile kinetics result in a muscle spending a greater proportion of the cycle submaximally activated than one with faster twitch kinetics. In addition, slower contractile kinetics mean that the medial compartment had to be stimulated with an earlier phase and reduced duration during shortening compared to that of the lateral compartment to ensure sufficient relaxation occurs. At all cycle frequencies, stimulating the medial compartment at an earlier phase avoided increasing negative work on the muscle. However, at higher cycle frequencies the slower rates of force development and relaxation meant that the muscle was less completely activated during shortening, reducing the positive work generated (see Figure 7C, F, I) in comparison to the lateral compartment. It is likely that a lower level of activation in the medial compartment and a concomitant reduction in positive work generation is the primary reason for the lower mass-specific net power at high cycle frequencies, and a shift in optimal frequency for maximum net power output to lower cycle frequencies compared to the lateral compartment (Figure 3A). Despite the shift in optimal cycle frequency, both compartments generated in excess of 90 % of the maximum power across a broad range of cycle frequencies.

Figure 7. Representative (B, E, H) work loop traces with (A, D, G) force- and (C, F, I) power-time plots for at (A, B, C) 5 Hz, (D, E, F) 15 Hz and (G, H, I) 25 Hz for both the medial (red line) and the lateral compartment (blue line).



Fatigability of intramuscular compartments

Muscle fatigue results in a reduced capacity to generate force and power during sustained activity, underpinned by changes in mechanical components such as a decline in force generating capacity, changes in the force-velocity properties and a slowing of relaxation (Edman & Mattiazzi, 1981; Curtin & Edman, 1994; Barclay, 1996). Many studies have quantified fatigue using repeated isometric tetanic contractions (Cady *et al.*, 1989; Lännergren & Westerblad, 1991). This approach allows any reduction in force generating capacity to be quantified but this is only one of a number of factors that lead to a reduction in power, and therefore gives incomplete insight into the decline in muscle performance during fatigue. Some studies have assessed changes in power following fatigue by measuring changes in the force-velocity relationship (Jones, 2010), but this protocol is also far removed from the normal operating conditions of many muscles during locomotion, where repeated cycles of work are performed and during which, changes in the ability to generate force, the force-velocity relationship and relaxation characteristics simultaneously impact on performance. The use of repeated cycles of work to induce and quantify fatigue overcomes the limitations of using isometric and isotonic/isovelocity protocols to assess fatigue (Askew *et al.*, 1997; Tallis *et al.*, 2014). The rate of decline in net power output was significantly lower in the medial compartment, as assessed by T_{W50}, which resulted in a larger cumulative amount of net work done during the 30 cycles of work, compared to the lateral compartment. A novel finding of this study is that the relative importance of the physiological changes that reduce net power varied between the two muscle compartments, indicated by changes in work loop shape. A number of physiological changes may lead to a reduction in net power with the onset of fatigue. First, a reduction in force generated during shortening, likely through impaired cross-bridge cycling resulting from interactions of free phosphate (generated through hydrolysis of ATP and PCr) and hydrogen ions (resulting from lactic acid accumulation), which impairs calcium binding with troponin and lowers ATPase activity (Cady *et al.*, 1989; Westerblad *et al.*, 1998). The reduction in ability to generate force was greater in the lateral compartment compared to the medical compartment. Second, an increase in the force produced during lengthening, particularly at the onset of lengthening, which reflects a slowing of relaxation (Stevens & Syme, 1993; Askew *et al.*, 1997) and an increase in the work required to extend the muscle. The slowing of relaxation during fatigue, thought to be related to slowing of Ca^{2+} uptake into the sarcoplasmic reticulum (Holloway *et al.*, 2006; Allen *et al.*, 2008; Jones, 2010), occurred to a greater extent in the medial compartment compared to the lateral compartment. Third, a change in the force-velocity relationship (slowing of V_{max} and increased curvature of the force-velocity relationship), evident from the change in P_{L0}/P_{max}. Mid-shortening, when the muscle is at L_0, the shortening velocity is constant (both strain and cycle frequency are constant), therefore any change in P_{L0}/P_{max} (which accounts for the reduction in force-generating capacity) indicates an additional depression in force that results from a slowing of V_{max} and increased curvature of the force-velocity relationship, with the latter effect likely to play a more significant role in the decrease in power (Jones, 2010). The reduction in force due to changes in the force-velocity relationship was greater in the lateral compartment compared to the medial compartment. The overall effects of fatigue are more pronounced in the lateral compartment of the EDL, due to the glycolytic fibre type composition, whereas the more oxidative, medial compartment is more resistant to fatigue. Differential fatigue resistance among compartments within the same muscle undergoing the same fatigue protocol has not been reported previously.

Fibre type heterogeneity and EDL function

Characterising the regional variation in the contractile properties can help interpret task-dependent regional variation in muscle recruitment (De Ruiter *et al.*, 1995). Currently there are limited data available on *in vivo* activity patterns in rat EDL in relation to limb kinematics, and none in relation to specific regions of the muscle. The EDL in rats is active throughout the swing phase of the hindlimb during walking (Nicolopoulos-Stournaras & Iles, 1984). This activity coincides with a decrease in joint angle of the ankle (Gruner *et al.*, 1980; Wilson *et al.*, 1997; Thota *et al.*, 2005), suggesting that the EDL could play a role in ankle dorsiflexion during walking. This has been shown in cats (Goslow *et al.*, 1973; Goslow *et al.*, 1977; Perret & Cabelguen, 1980; Abraham & Loeb, 1985), and dogs (Tokuriki, 1973a) where it has also been shown that the EDL plays a similar role in swing at higher speeds of locomotion (Tokuriki, 1973b; Abraham & Loeb, 1985). It is currently unknown how regional variation in recruitment of the EDL varies with speed during locomotion.

The fatigue resistance of the medial compartment of EDL indicates that it may have a role during sustained, sub-maximal activation, e.g. during low speed locomotion, whereas the glycolytic compartment of the EDL is more likely to be recruited during burst activities, such as high-speed locomotion. Goslow *et al*. (1973) identified a bimodal fatigue response in the cat EDL, and suggested that this was an adaptive mechanism due to differences in duty cycle between walking and high-speed galloping. Therefore, the fatigue resistant medial compartment of the EDL is likely to be active during the slower duty cycle (walking) and the more fatigable lateral compartment would be used during pursuit of flight activities (together with the medial compartment if the size principle is followed). The utilisation of predominantly Type I and IIa fibres during walking has been shown in cats and rats (Armstrong *et al.*, 1977; Smith *et al.*, 1977; Sullivan & Armstrong, 1978) with an increasing prevalence of Type IIx/IIb recruitment as the speed of locomotion increases (De Ruiter *et al.*, 1996). Further data on muscle length change and recruitment are required to test these hypotheses.

In conclusion, we have demonstrated that within a single muscle, phenotypically distinct compartments exist. These intramuscular compartments exhibit similar maximal shortening velocities and generate comparable maximum isometric stresses. However, under dynamic conditions they differ in the optimum cyclical frequencies for maximum net power generation and in their response to fatigue during repeated cycles of work. This suggests that these compartments are likely to be differentially recruited and to have different mechanical roles during locomotion, in particular during burst and sustained behaviours.

Competing interest

The authors confirm there are no conflicts of interest.

Author contributions

RWPK participated in the design of the work, acquisition, analysis and interpretation of the data, and

drafting and revising the manuscript. SE participated in the design of the work, interpretation of the data and revision of the manuscript. GNA participated in the design of the work, acquisition, analysis and interpretation of the data, and revision of the manuscript. All authors approved the final version of the manuscript, agree to be accountable for all aspects of the work and all persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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