

RESEARCH ARTICLE

Skinned fibres produce the same power and force as intact fibre bundles from muscle of wild rabbits

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ABSTRACT

Skinned fibres have advantages for comparing the muscle properties of different animal species because they can be prepared from a needle biopsy taken under field conditions. However, it is not clear how well the contractile properties of skinned fibres reflect the properties of the muscle fibres *in vivo*. Here, we compare the mechanical performance of intact fibre bundles and skinned fibres from muscle of the same animals. This is the first such direct comparison. Maximum power and isometric force were measured at 25°C using peroneus longus (PL) and extensor digiti-V (ED-V) muscles from wild rabbits (*Oryctolagus cuniculus*). More than 90% of the fibres in these muscles are fast-twitch, type 2 fibres. Maximum power was measured in force-clamp experiments. We show that maximum power per volume was the same in intact ($121.3 \pm 16.1 \text{ W l}^{-1}$, mean \pm s.e.m.; $N=16$) and skinned ($122.6 \pm 4.6 \text{ W l}^{-1}$; $N=141$) fibres. Maximum relative power (power/ $F_{\text{IM}} L_0$, where F_{IM} is maximum isometric force and L_0 is standard fibre length) was also similar in intact (0.645 ± 0.037 ; $N=16$) and skinned (0.589 ± 0.019 ; $N=141$) fibres. Relative power is independent of volume and thus not subject to errors in measurement of volume. Finally, maximum isometric force per cross-sectional area was also found to be the same for intact and skinned fibres ($181.9 \text{ kPa} \pm 19.1$; $N=16$; $207.8 \text{ kPa} \pm 4.8$; $N=141$, respectively). These results contrast with previous measurements of performance at lower temperatures where skinned fibres produce much less power than intact fibres from both mammals and non-mammalian species.

KEY WORDS: Muscle, Power, Force, Skinned fibre, Rabbit

INTRODUCTION

Skinned fibres are commonly used for comparing the muscle properties of different animal species. They are an attractive choice because they can be prepared from a needle biopsy – a minimally invasive procedure possible under field conditions – and skinned fibres can be stored for many months, allowing time for return to the laboratory and for testing. However, it is not clear how well the contractile properties of skinned fibres reflect the properties of the muscle fibres *in vivo*. At present, the best information about this question comes from comparing published data for skinned fibres with data for intact fibres from the same species of animal but from experiments performed in different laboratories.

Much of the published data, which we summarise in the Discussion, suggest that skinned fibres produce less power and

force than intact fibres. However, these comparisons are not completely satisfactory for at least two reasons: (1) most experiments on skinned mammalian fibres have been done at 15°C or less, and it is unclear how relevant comparisons at these low temperatures are to *in vivo* function; (2) comparison of the data from studies made in different laboratories may not be reliable, since values measured under ostensibly similar experimental conditions vary widely. For example, the maximum specific isometric force reported for human Type 2A fibres at 12°C ranges from 22 to 250 kPa (Harridge et al., 1996; Larsson et al., 1997; see also Kalakoutis et al., 2014). Similarly, for this fibre type and temperature, the maximum power output ranges from 1.6 to 7.3 W l^{-1} (Bottinelli et al., 1996; He et al., 2000). Thus, for both force and power, the indications are that the details of the methods used in a particular laboratory have a strong influence on the quantitative results.

We report here the experiments designed to deal with, as far as possible, these two problems and thus give a more robust comparison of skinned and intact fibres. (1) The experiments were done at 25°C, a temperature closer to that *in vivo* than is often used. (2) We used skinned and intact fibres from the same two muscles, peroneus longus (PL) muscle and extensor digiti-V (ED-V). All fibres were from the same population of wild European rabbits (*Oryctolagus cuniculus* Linnaeus 1758) and in many cases, skinned and intact fibres from the same individual were used.

RESULTS

Intact fibre bundles

An example of a force-clamped contraction from a bundle of intact fibres from an ED-V muscle is shown in Fig. 1. The active force and stimuli are shown in Fig. 1A. Once force reached the chosen level, the force-clamp was started and the controller regulated motor velocity to maintain constant force. The record of shortening is shown in Fig. 1B. Power (Fig. 1C) was calculated as the product of active force and velocity. This protocol was repeated for several different levels of force-clamp and the results are summarised in Fig. 2A,B. The line in Fig. 2A was fitted through the power versus force data points using Hill's relationship (as described in the Materials and methods) and the force versus velocity curve in Fig. 2B was calculated with the parameters from the fit. This particular fibre bundle has a rather straight force versus velocity curve and a relatively large value of F_0^* , the fitted intercept on the force axis.

Such experiments were done with fibre bundles from ED-V and PL muscles. Useable results were obtained from 16 fibre bundles (9 ED-V and 7 PL). In 5 cases, we used bundles from both of these muscles from the same rabbit. The mean values of isometric force and the parameters of the fitted curves were not significantly different ($P>0.125$) for the two muscles, so all the results were combined. Table 1 summarises the mean values for the 16 fibre bundles of isometric force, maximum power, force and velocity at which maximum power was produced, V_{max} and F_0^* ; all of these values, except isometric force, are from the fitted curves.

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List of symbols and abbreviations

a	$-1 \times$ asymptote of force of the force versus velocity relationship
ATP	adenosine 5' triphosphate disodium salt hydrate
BDM	2,3-butanedione monoxime
CSA	cross-sectional area
EDL	extensor digitorum longus
ED-V	extensor digiti-V
EGTA	ethylene glycol-bis(2-aminoethylether)- <i>N,N,N',N'</i> -tetraacetic acid
F	force during shortening
F_{IM}	isometric force
F_o^*	fitted force intercept of the power versus force relationship
F_{Qmax}	force at maximum power
FHB	flexor hallucis brevis
HDTA	1,6-diaminohexane- <i>N,N,N',N'</i> -tetraacetic acid
L_o	standard fibre length
PL	peroneus longus muscle
PMSF	phenylmethanesulphonyl fluoride
Q	normalised power
Q_{max}	normalised maximum power
SL	sarcomere length
T	temperature
TES	<i>N</i> [tris(hydroxy-methyl)methyl]-2-aminoethanesulfonic acid
V	velocity during shortening
V_{max}	velocity intercept of the force versus velocity relationship

Since there is evidence that force per cross-sectional area (CSA) is greater for small than large fibres in human skinned fibres (Gilliver et al., 2009) and because we are aiming to compare the performance of skinned and intact fibres, we examined the dependence of force on CSA in both intact fibre bundles and skinned fibres (see below). Fig. 3A summarises the relation between isometric force and CSA of bundles of intact fibres from ED-V and PL. We fitted lines through the values for each of these muscles assuming proportionality, that is, with the intercept=0, and found

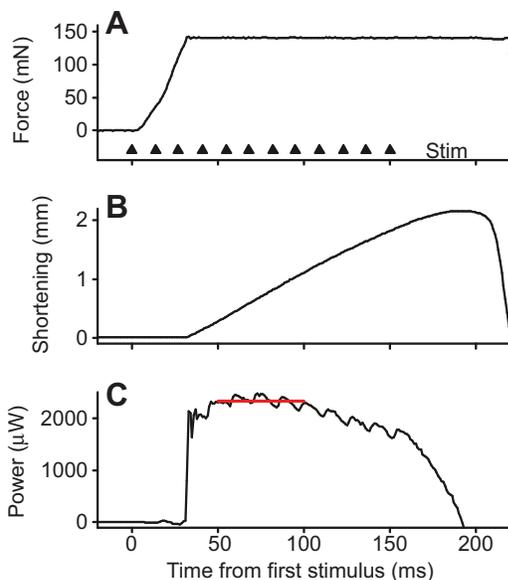


Fig. 1. An example recording from a bundle of intact fibres from rabbit ED-V muscle. (A) Active force during a force-clamp contraction. Stimulus times are marked. (B) Length change. (C) Power calculated from active force and velocity obtained by differentiating the record shown in B. The red line shows the period selected for measuring the power output.

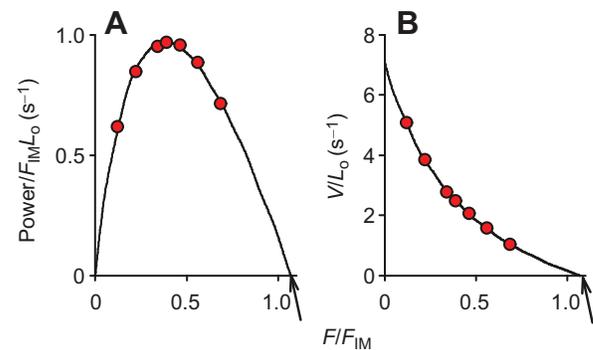


Fig. 2. Force, velocity and power data from an intact fibre bundle from rabbit ED-V muscle. (A) Power normalised by F_{IM} , isometric force, and L_o , the fibre length giving maximum isometric active force, as a function of force during shortening normalised by F_{IM} . The line is fitted to the data points (see Materials and methods). (B) Velocity of shortening normalised by L_o , as a function of force during shortening normalised by F_{IM} . The line is drawn with the parameters fitted to the curve in A. The arrows indicate the intercept (F_o^*) of the lines on the abscissa.

that the slopes were not significantly different for the two muscles ($P=0.87$). Therefore the line fitted through all the data points is shown in the graph. Fig. 3B shows the analogous graph of maximum power versus volume of the bundles of intact fibres. Again the slopes were not different for fibres from ED-V and PL ($P=0.78$) and therefore a single line is shown.

Skinned fibre results

An example record from a single skinned fibre from an ED-V muscle is shown in Fig. 4. The fibre dimensions were measured and zero-force baseline set in relaxing solution at 25°C. From there, the fibre was transferred through the sequence: pre-activating solution, activating solution at low temperature (~1°C), activating solution at 25°C. The fibre was then relaxed at 25°C. Note that the free Ca^{2+} concentration in the activating solution is sufficient to produce maximum force (Millar and Homsher, 1990). In this high-temperature solution, force develops to a plateau in about 1 s. At this point, the force-clamp was started and the controller adjusted motor velocity to hold force constant during shortening for about 20 ms. The lower part of the figure shows, on an expanded time scale, the force, length change, velocity and power during the period from just before to shortly after the force-clamp. During the first 5 ms of the force-clamp, the force is falling rapidly and the velocity and power are very high. During this time, the elasticity in series with the fibre is shortening with falling force and the series elasticity is delivering power. Once the force has become constant, the shortening and power are delivered overwhelmingly by the contractile component, i.e. by filament sliding driven by crossbridge cycles. This is the power we measured by averaging the recorded values over a period of about 10 ms (between the blue vertical lines). We also measured the isometric force just before shortening starts (between the red vertical lines).

In this experiment, the protocol was repeated for 7 different force-clamp levels. Fig. 5 shows how power and velocity varied with force. The curve was fitted through the power versus force points (see Materials and methods). The maximum power and the force and velocity at which maximum power is exerted were identified on the fitted curve. We calculated the velocity versus force curve from the fitted parameters.

Such experiments were done on skinned fibres from ED-V and PL muscles. In total, 141 fibres from 16 rabbits gave useable

Table 1. Mechanical properties of rabbit muscle intact fibre bundles and skinned fibres

	Skinned fibres ^a			Pooled skinned fibres ^b (N=141)	Intact fibre bundles ^c (N=16)	<i>P</i> ^d
	ED-V (N=88)	PL (N=53)	<i>P</i>			
F_{IM} (kPa)	221.5±4.8	190.1±4.9	<0.001	207.8±4.8	181.9±19.1	NS
Max power/ $F_{IM}L_o$ (s ⁻¹)	0.599±0.014	0.577±0.024	NS	0.589±0.019	0.645±0.037	NS
F/F_{IM} at max power	0.300±0.004	0.285±0.003	0.006	0.294±0.004	0.373±0.005	<0.001
V/L_o at max power (s ⁻¹)	2.014±0.048	2.010±0.081	NS	2.012±0.065	1.61±0.088	<0.001
F_o/F_{IM}	1.00±0.01	0.97±0.02	NS	0.99±0.01	1.07±0.03	0.013
Max power (W l ⁻¹)	133.6±4.5	108.3±4.7	<0.001	122.6±4.6	121.3±16.1	NS
V_{max}/L_o (s ⁻¹)	6.85±0.21	6.94±0.36	NS	6.89±0.28	4.99±0.32	<0.001

^aValues are means±s.e.m.

^bThe skinned fibre results for ED-V and PL combined, with the ED-V results weighted at 9 and the PL results at 7, corresponding to the *N* values for intact fibre bundles shown. These values are therefore weighted means±s.e.m.

^cMean values±s.e.m. from 16 fibre bundles, 9 from ED-V and 7 from PL.

^dComparison of pooled skinned fibre data with intact fibre bundle data.

NS, not significant. In calculating *P* values, the degrees of freedom for the skinned fibres were reduced to allow for the fact that the number of rabbits was smaller than the number of fibres (Welch, 1947). F_{IM} , ratio of maximum isometric force/cross-sectional area of the bundle or fibre; L_o , standard fibre length (see text); F , force during shortening; F_o and V_{max} , intercepts on the force and velocity axes of the fitted curve (see Fig. 2B and Fig. 5B).

results. Table 1 shows the isometric force and the parameters of the power versus force fit for fibres from each of these muscles. The isometric force (F_{IM} ; kPa) and maximum power (W l⁻¹) are significantly different between the two muscles, with ED-V fibres producing more force per CSA and more power per volume than those from PL.

We examined the dependence of force on CSA in our skinned fibres to see whether force per CSA is greater for small than large fibres, as has been reported for skinned fibres from humans (Gilliver et al., 2009). Fig. 6A summarises the relation for ED-V and PL, where lines were fitted through the values for the fibres from each of these muscles assuming proportionality, that is, with the intercept=0. The slopes were significantly different for the two muscles. Fig. 6B shows the residual errors, which do not indicate any deviation from proportionality, in other words force per CSA did not vary with CSA. Fig. 6C shows the analogous graph of maximum power versus volume of the skinned fibres. Again the slopes were different and the residual plot (Fig. 6D) supports proportionality.

None of the force versus velocity properties, normalised as shown in Table 1, was correlated with rabbit mass. However, the fibres from male rabbits produced 12% more isometric force per CSA than fibres from female rabbits [$P=0.027$, two-way ANOVA with anatomical muscle (ED-V, PL) and rabbit sex as the factors].

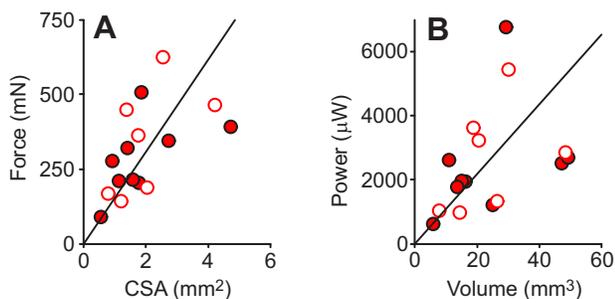


Fig. 3. Dependence of force and power on size of intact fibre bundles. (A) Maximum isometric force for PL (open symbols) and ED-V (filled symbols) as a function of bundle cross-sectional area (CSA). The line is: force (mN)=CSA (mm²)×154 (kPa). (B) Maximum power for PL (open symbols) and ED-V (filled symbols) as a function of bundle volume. The line is: power (µW)=volume (mm³)×109 (W l⁻¹).

Comparison of bundles of intact fibres with skinned fibres

The comparison between intact and skinned force and power data is shown in Fig. 7A,B. The skinned fibres and intact fibre bundles are very different in size, so the values are plotted on a log–log scale to ensure that the individual points are visible. In each figure, the blue line was fitted through the results for skinned fibres from both ED-V and PL muscles and can be compared with the red line for the intact fibre bundles from ED-V and PL. In both graphs, the two lines are almost continuous with each other. The constants of proportionality for the skinned and for intact fibres (given in the figure legend) are not significantly different. Note that on this log–log plot the assumption of proportionality results in all the lines being at 45 deg and the constant of proportionality sets the vertical position of each line.

The results for skinned fibres from ED-V and PL are combined in Table 1 (weighted as described in the footnote) and Fig. 7, where they are compared with the intact fibre bundle results. The isometric force and maximum power are not significantly different between skinned and intact fibre preparations. However, the force at which maximum power is produced is significantly greater in intact fibre bundles than in skinned fibres. Also the force intercept of the power versus force curve is significantly greater for intact fibre bundles than for the skinned fibres.

DISCUSSION

The main aim of this study was to compare the mechanical performance of intact fibres and skinned fibres under as similar conditions as possible. Maximum power output and isometric force were chosen because they are aspects of mechanical performance particularly relevant to muscle function *in vivo* and whole animal performance. Both of the muscles we used, PL and ED-V, are composed largely of fast-twitch, type 2 fibres with less than 10% slow twitch, type 1 fibres (Curtin and Woledge, 2014). Thus, the results can be taken as mainly representative of the population of fast-twitch fibres.

We found that maximum isometric force produced by intact and skinned fibres is the same within the limits of experimental errors. This result is based on comparison of the combined results from ED-V and PL using the mean values of the simple ratio of force/CSA for each preparation (Table 1). It is worth noting here that the value of force/CSA for a group of preparations can be evaluated in other ways. Here, we report one such other method: the slope of the

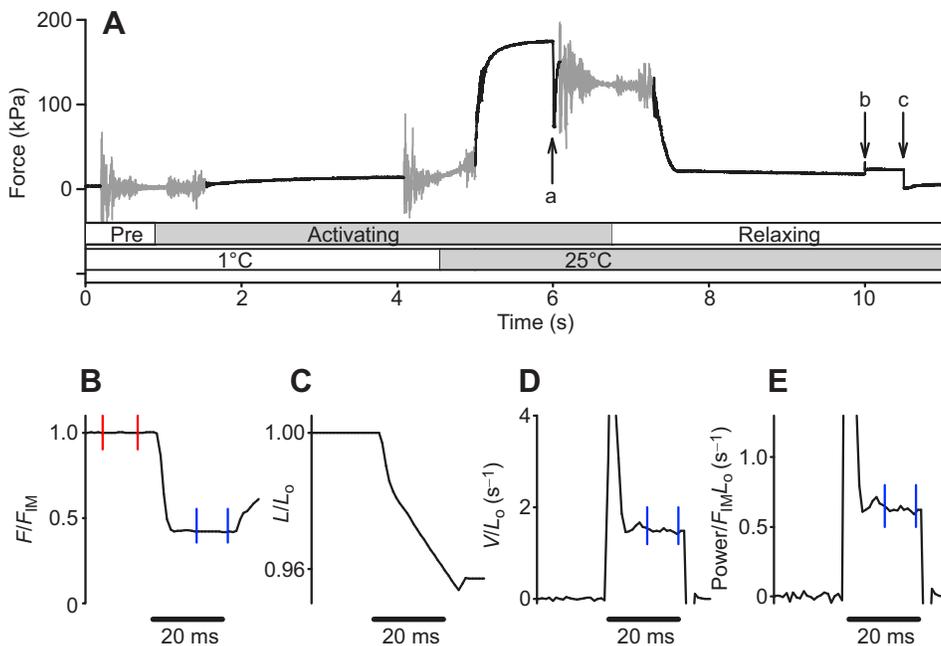


Fig. 4. An example recording from a skinned fibre experiment. (A) Complete force record on a compressed time scale. The shaded bars indicate the sequence of solutions (Pre, pre-activating; activating and relaxing) and temperatures. The noisy parts of force record during movement between solutions are shown in grey. At (a), the force-clamp occurred. At (b), the fibre is restored to L_0 , the fibre segment length at sarcomere length 2.5–2.6 μm . At (c), the fibre is released to slack length at which force=0. The lower panels show, on an expanded time scale, (B) force, (C) length, (D) velocity and (E) power during the period from just before to shortly after the force-clamp. Mean values between the vertical lines are reported. F_{IM} is the isometric force.

force versus CSA relationship (Fig. 3A, Fig. 6A and Fig. 7A) when we were testing whether smaller preparations have more force/CSA than larger ones. This method gives a value for the group of force and CSA values that is different from the mean of the ratios for the individual preparations. The difference arises because when the slope of force versus CSA is calculated, preparations with large CSA and/or large force have greater influence than smaller preparations. Whereas the mean of the simple force/CSA values is influenced equally by all preparations, we prefer this method as a summary of our findings and the basis for comparing skinned fibres with intact fibre bundles.

We found that the maximum power (W l^{-1}) produced by intact and skinned fibres from wild rabbit muscle is the same within the limits of our errors. However, it is worth noting that the accuracy of such comparisons of power between intact and skinned fibres is limited by a number of factors. First, the methods used to estimate the amount of contractile material is different for intact and skinned preparations. For intact fibre bundles, volumes were obtained from

the masses of the blotted muscle and the density of muscle (Mendez and Keys, 1960). Whereas, for skinned fibre segments the volumes were obtained from measurements under the microscope of width, depth and length. Second, each of these methods is an imperfect estimate of the amount of myofibrillar material. The volume of an intact fibre bundle includes extracellular space. Also damaged and non-excitabile muscle fibres in the bundle add volume but do not produce power. Fibres swell during skinning and so have a volume greater after skinning than before. The amount of swelling is highly variable (Godt and Maughan, 1977). A better comparison between intact and skinned preparations might be achieved based on the mass of the contractile proteins. A possible method is to measure dry mass of the fibres or fibre segment after fixation in alcohol and washout of salts and use this as an estimate of mass of contractile proteins. We measured dry mass in this way for the intact fibre bundles in this study and found that the coefficient of variation ($\text{CV}=\text{s.d./mean}$) of the power output is reduced by 25% if the results are expressed as W mg^{-1} dry mass rather than based on W mm^{-3} volume. We have used this method successfully on large single skinned fibres from dogfish (West et al., 2005). However, the CSA of rabbit fibres is much smaller and we only used a short segment of fibre. The dry masses of the smallest skinned fibre segments used in the experiments reported here were estimated to be less than 1 μg , which is beyond the accuracy of the methods we use. Another possible method is SDS-PAGE with stain-free technology, which may provide accurate quantification of fibre protein content and thus the size of skinned fibre segments.

For the purpose of comparing maximum power output of skinned and intact fibres, we have also used power expressed in relative units, which is less subject to the problem of measuring the amount of contracting myofibrillar material. Our definition of relative power is the ratio, maximum power/maximum isometric force \times standard fibre length ($\text{max power}/F_{IM}L_0$; units: $\text{W/N m} = \text{s}^{-1}$). The relevant point here is that relative power does not require measurement of fibre CSA or bundle volume, but instead uses maximum isometric force as measure of the amount of contracting myofibrillar material per CSA. Thus, it is not surprising that the coefficient of variation of relative power is less

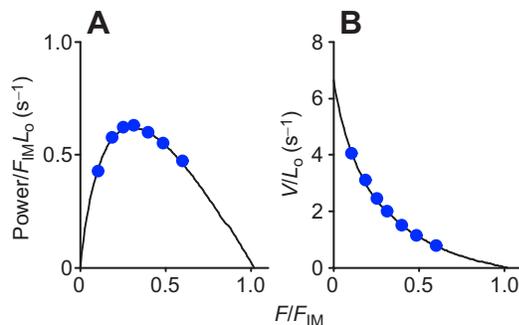


Fig. 5. Force, power and velocity data obtained from one rabbit ED-V fibre. (A) Power normalised by F_{IM} , isometric force, and L_0 , the fibre segment length at sarcomere length 2.5–2.6 μm , as a function of force during shortening normalised by F_{IM} . The line is fitted to the data points (see Materials and methods). (B) Velocity of shortening normalised by L_0 , as a function of force during shortening normalised by F_{IM} . The line is drawn with the parameters fitted to the curve in A.

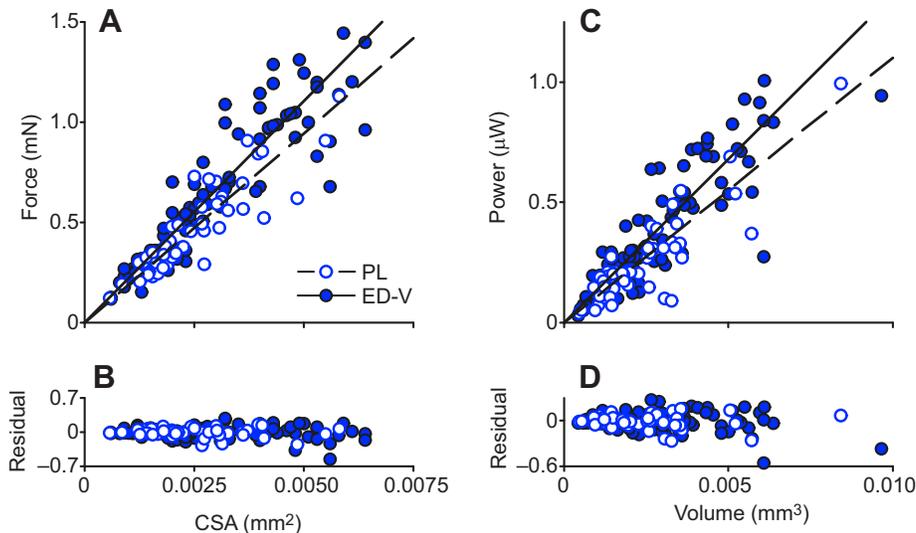


Fig. 6. Dependence of force and power on size of skinned fibres. (A) Maximum isometric force for ED-V (filled symbols, solid line) and for PL (open symbols, dashed line) as a function of fibre cross-sectional area (CSA). The full line is: force (mN)=CSA (mm²)×221 (kPa), and the dashed line is: force (mN)=CSA (mm²)×189 (kPa). (B) Residual scatter about the lines in A versus CSA. (C) Maximum power for ED-V and PL versus fibre volume (symbols and lines as in A). The solid line is: power (μW)=volume (mm³)×136 (W l⁻¹) and the dashed line is: power (μW)=volume (mm³)×110 (W l⁻¹). (D) Residual scatter about the lines in C versus fibre volume.

than that of power in W l⁻¹ for both skinned fibres and intact fibre bundles. CV for skinned fibres was 0.387 for power in relative units (s⁻¹) and 0.446 for power in W l⁻¹; for intact fibre bundles, the corresponding values were 0.229 and 0.530. Our conclusion that maximum power output by intact and skinned fibres from wild rabbit muscle is the same within the limits of the errors is supported by comparison of the maximum power expressed in relative units whether based on all results or on only those with isometric force above the rejection threshold of 75 kPa.

Other comparisons of intact and skinned fibre performance can be made on the basis of literature values. A maximum power of 166±8.9 W l⁻¹ (mean±s.e.m.) at 30°C has been reported for Type 2 skinned fibres from the rat (Knuth et al., 2006). This value can be compared with 177±22 W l⁻¹, measured with intact fibre bundles from rat flexor hallucis brevis (FHB) muscle, which is ~90% type 2 fibres (Roots and Ranatunga, 2008) and 174±12 W l⁻¹ calculated by Ranatunga (1998) from his earlier experiments (Ranatunga, 1982, 1984) using whole extensor digitorum longus (EDL) muscle and intact fibre bundles from EDL; all at 30°C. Our results also show that skinned and intact fibres produce the same power at around 25°C, and provide stronger evidence on this point because our intact and skinned fibres were from the same animals and the experiments were undertaken in parallel in the same laboratory.

This agreement contrasts with results obtained at lower temperatures, where skinned fibres maintain their performance well enough that reliable measurements of maximum power can be made. Comparisons of published data show that intact fibre preparations consistently produce more power than skinned fibres from the same species. For white muscle fibres of the dogfish at 12°C (its normal physiological temperature), Curtin and Woledge (1988) found that intact fibres produce a maximum power 65% greater than the value reported for skinned fibres by Bone et al. (1986). As reviewed by West et al. (2013), intact fibres from frog muscle at ~2°C produce about 45% more power than the skinned fibres investigated by Ferenczi et al. (1984). Similar large differences are seen also in mammalian muscle at low temperature. At 15°C, intact fibres from rat (Ranatunga, 1998; Roots and Ranatunga, 2008) produce 76% more power than rat skinned fibres (Knuth et al., 2006).

Thus, our results and the published data show that the large discrepancies between intact and skinned fibres that occur at low temperatures are not present in mammalian muscle at temperatures

of 25°C and 30°C. This prompts the question of how the powers compare at even higher temperatures. There are no data from which the comparison could be made for the highest muscle temperatures achieved during exercise; both human and horse muscle reach up to 39°C during intense exercise (Saltin and Hermansen, 1966; Hodgson et al., 1993). However, there is extensive evidence that intact mammalian fibres maintain their contractile performance *in vitro* at temperatures between 30 and 37°C (Barclay, 1994; Close, 1964, 1965; Close and Luff, 1974; Luff, 1981; McCrorey et al., 1966; Ranatunga, 1982) and lizard muscle performs well at 40°C (Curtin et al., 2005). In contrast, skinned fibre performance declines rapidly with repeated activation, which prevents reliable measurements of maximum power (Bottinelli et al., 1996; Stienen et al., 1996). We encountered the same problem in the current set of experiments with skinned rabbit fibres at 33°C. Taken together, these comparisons suggest that there is an unidentified factor(s) or mechanism(s) in the intact fibres that preserves their ability to produce force and power at low temperature and prevent deterioration at temperatures above 30°C. We speculate that the factor is lost, or the mechanism is inactivated in skinned fibres, thus compromising their ability to produce force and power at these higher temperatures.

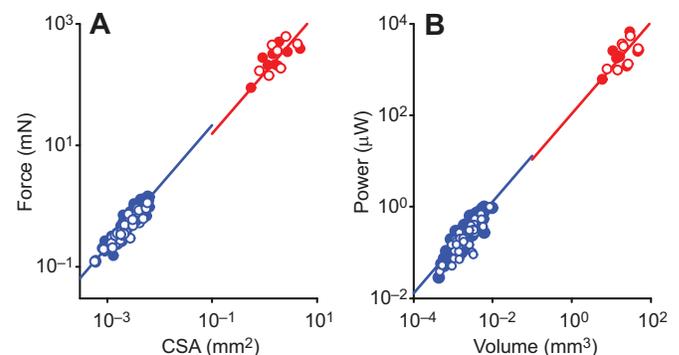


Fig. 7. Dependence of force and power on size of skinned fibres and intact fibre bundles. (A) Isometric force as a function of cross-sectional area (CSA) of skinned fibres (blue) and intact fibre bundles (red). Filled symbols are ED-V and open symbols are PL. The data are plotted on log scales. Blue line: force (mN)=CSA (mm²)×212 kPa. Red line: force (mN)=CSA (mm²)×154 kPa. (B) Power versus volume. Colours and symbols as in A. Blue line: power (μW)=volume (mm³)×129 kPa. Red line: power (μW)=volume (mm³)×109 kPa.

The match between the performance of intact and skinned fibres that we find at 25°C strengthens confidence in results of skinned fibres in situations where, for example, only needle-biopsy material can be obtained as is the case for some wild and/or rare animals. Skinned fibres have some advantages over intact fibres; for example, effects of metabolite levels and Ca²⁺ sensitivity can be measured. However, it is important to recognise that the *in vivo* behaviour of muscle cannot be duplicated in skinned fibre experiments, at least with current methods. In particular, it is not possible to rapidly turn off contraction of skinned fibres. Intact fibres have a definite advantage in this context because at the end of electrical stimulation action potentials cease and contraction relaxes, as *in vivo*. Consequently, the normal cycles of length change with intermittent contraction that occur during most locomotion can be duplicated with intact, but not with skinned fibres.

MATERIALS AND METHODS

Rabbits

Wild rabbits were live-trapped on the Hawkshead Campus of the Royal Veterinary College with ethical approval from the Royal Veterinary College Ethics Committee. They were killed according to supplementary material Appendix 1 of Schedule 1 (revised 1997) of the Animals (Scientific Procedures) Act 1986. Eighteen rabbits were used (9 male and 9 female). Their mass ranged from 0.4 to 2.0 kg (mean 1.1 kg). Successful experiments were done on bundles of intact fibre from 11 rabbits. Skinned fibres were prepared from 16 rabbits. The ED-V and PL muscles were usually dissected from both legs and then a part of each muscle was prepared for experiments.

Intact fibre bundles

Fibre bundles [average length 12.3±0.5 mm (mean±s.e.m.) and average CSA 1.92±0.29 mm²] were dissected and kept in physiological saline (mmol l⁻¹): NaCl 135, KCl 4.0, CaCl₂ 2.35, MgCl₂ 0.85, NaH₂PO₄ 1, NaHCO₃ 20 and glucose 5.5 and equilibrated with 95% O₂, 5% CO₂. Experiments were done at 25°C. A fragment of tendon at each end of the fibre bundle was held in an aluminium foil clip. At one end, the clip was attached to a fixed hook and the other to the motor/force transducer (Series 300B Lever System, Cambridge Technology, Inc. Watertown, MA, USA). A MultiStim System D330, with modules D334, D332, D333 and D342 (Digitimer Ltd, Welwyn Garden City, UK) provided electrical stimulus pulses. Supra-maximal stimulus strength (*I*, 1.0 ms pulse⁻¹) and *L*₀, the standard fibre length, were found for each fibre bundle at the start of the experiment. *L*₀ is the fibre length at which maximum isometric active force is produced. Brief tetanic contractions (stimulus frequency 100 Hz) were used, either isometric or force-clamped to a predetermined level. Passive force (i.e. without stimulation) was recorded during movements matching those in each force-clamped contraction, and was subtracted from that recorded with stimulation to give the active force. The experiments were controlled by a custom-written LabView program which sent trigger pulses to the stimulator, provided appropriate force-control and length-control signals to the motor and acquired the data at 1 kHz.

At the end of the experiment, the fibre bundle was fixed in alcohol while pinned at *L*₀ so that fibre length could be measured. Tendons were removed and the preparation was then blotted and weighed. Preparation volume was calculated from the blotted mass assuming a density of 1.064 (value for 25°C from Mendez and Keys, 1960).

Preparation of skinned fibres

Thin fibre bundles (4–5 mm in diameter) were permeabilised on ice in solution A (Table 2) containing 2% Triton X-100. For fibres from 10 of the 16 rabbits, this solution also contained 20 mmol l⁻¹ BDM (2,3-butanedione monoxime). BDM is used to prevent contraction which can occur during skinning. We found part way through this set of experiments that such contractions do not occur in the absence of BDM under the conditions we used and therefore we discontinued using BDM. Exposure to BDM did not affect mechanical properties: maximum values of isometric force, power and shortening velocity were not significantly different between the groups (96

Table 2. Constituents of the solution for preparing skinned fibres

Component	Concentration
Imidazole	6
Magnesium acetate	8
Potassium propionate	70
ATP	7
EGTA	5
PMSF (μmol l ⁻¹)	10
Leupeptin (mg l ⁻¹)	4
Trypsin inhibitor (mg l ⁻¹)	50

All concentrations in mmol l⁻¹, except as indicated. Solution pH 7.1 at 20°C. ATP, adenosine 5' triphosphate disodium salt hydrate; EGTA, ethylene glycol-bis(2-aminoethylether)-*N,N,N',N'*-tetraacetic acid; PMSF, phenylmethanesulphonyl fluoride.

fibres with BDM, 45 fibres no BDM). After 30 min, the fibre bundles were transferred to solution A for a further 30 min to remove the Triton X-100. Fibre bundles that were to be used within 4 weeks were stored at -20°C in a 50% glycerol solution containing the components at the concentrations shown in Table 2. Fibre bundles to be stored for longer than 4 weeks were stored at -80°C in 0.5% glycerol solution containing the cryoprotectant trehalose (500 mmol l⁻¹). In this case, a gradual transition from solution A was achieved by holding the skinned fibre bundles on ice for 30 min in the following solutions: (1) 50:50 mixture of solution A and 500 mmol l⁻¹ trehalose in 0.5% glycerol; (2) 500 mmol l⁻¹ trehalose in 0.5% glycerol. The bundles were then frozen in liquid nitrogen and stored in cryo-tubes at -80°C for up to 20 months. When thawed, they were thawed in solution 2 above, then transferred to solution 1 and finally to a 50% glycerol solution containing the components at concentrations shown in Table 2. These thawed bundles were then stored at -20°C in this solution, and used for experiments within 4 weeks. Fibres used within 4 weeks of skinning (*N*=21) and those used between 4 weeks and 20 months of skinning (*N*=120) did not have different mechanical properties; maximum values of isometric force, power and shortening velocity were not significantly different between these groups.

Small segments of the skinned fibre bundles (2–3 mm long) were dissected and used to prepare several single fibres in solution A at 7–10°C on the thermostat stage of a dissecting microscope. Aluminium foil 'T-clips' (Photofabrication Ltd, St Neots, UK) were placed on the ends of fibre segments (length between 0.6 and 1.5 mm). Clips were attached to several single fibres at a time, and some were stored again at -20°C in the glycerol-based solution A (described above) for use within 3 days. Individual fibres were transferred to solution A, containing 0.5% Triton-X 100, on the Aurora 600A permeabilised fibre apparatus, where they were 'set-up' for activation. The 600A apparatus was positioned on the *x,y* moving stage of an inverted microscope.

Fibre dimensions

While in relaxing solution at 25°C, the sarcomere length (SL) was set to 2.5–2.6 μm using either laser diffraction or by visualising the striation pattern (under 40× objectives) and using the Aurora 900B FFT imaging protocol. *L*₀, the standard length, is the fibre segment length at which SL is 2.5–2.6 μm. *L*₀ was measured using the eye-piece graticule under low magnification (10×). Fibre depth and width were measured using the 900B camera software. The fibre depth was visualised by using a prism built in the bath of the 600A apparatus. Each value of depth and width was recorded as the mean of three separate measurements along the fibre length. CSA was calculated from the fibre width and depth assuming an elliptical cross-section. Skinned fibre CSA was correlated with the mass of the rabbits.

We did not make a correction to the CSA for swelling that may have occurred as a result of skinning for the following reasons. (1) We did not measure the extent of swelling. (2) Literature values for the extent of swelling are highly variable. The published values range from 20% (Moss, 1979) to 52% (Godt and Maughan, 1977) increase in diameter. (3) The published values are for frog fibres at low temperature (5°C) and may not be relevant to mammalian fibres at high temperature. The variability is likely to be dependent on factors such as sarcomere length (Matsubara and Elliott, 1972) and solution ionic strength (West et al., 2005).

Table 3. Constituents of pre-activating, activating and relaxing solutions for skinned fibre experiments

Component	Low- <i>T</i> solutions		High- <i>T</i> solutions	
	Pre-activating solution	Activating solution	Relaxing solution	Activating solution
TES	60	60	60	60
EGTA	0	0.01	25	0.15
Calcium-EGTA	0	24.99	0	24.85
HDTA	25	0	0	0
Magnesium chloride	7.56	7.56	8.31	7.97
Potassium propionate	16.88	18.24	18.08	16.16
Glutathione	10	10	10	10
ATP	4.88	4.94	4.47	4.50
Creatine phosphate	20	20	20	20
Creatine phosphokinase (U ml ⁻¹)	300–400	300–400	300–400	300–400

All concentrations, except creatine phosphokinase, are in mmol l⁻¹. Solution pH 7.1 and ionic strength 200 mmol l⁻¹. TES, *N*[Tris(hydroxy-methyl)methyl]-2-aminoethanesulfonic acid; ATP, Adenosine 5' Triphosphate disodium salt hydrate; EGTA, ethylene glycol-bis(2-aminoethylether)-*N,N,N',N'*-tetraacetic acid; HDTA, 1,6-diaminohexane-*N,N,N',N'*-tetraacetic acid. Free Ca²⁺ in activation solutions, 32 μmol l⁻¹; free Mg²⁺, 2 mmol l⁻¹; free ATP, 4 mmol l⁻¹.

Fibre activation and power measurement

The Aurora 600A apparatus was modified for 'temperature-jump' (T-jump) activation of fibre segments (Goldman et al., 1987; Bershtitsky and Tsaturyan, 1989) using the solutions shown in Table 3. The low temperature (*T*) was 0–1°C and the high *T* was 25°C. For this protocol the fibre segment was immersed successively in: (1) pre-activating solution at low *T* for 45 s; (2) activating solution at low *T* for 3 s; (3) activating solution at high *T* for 1–2 s; and finally, (4) relaxing solution at high *T*; the baseline force at 0.8 *L*₀ was recorded before changing length back to the original *L*₀.

While in solution 3, force developed to a plateau at constant length (isometric force) and then was clamped for 20 ms to a predetermined level (see Fig. 4 for an example record). The shortening velocity during the force-clamp was measured and used to calculate power output. To accumulate a data series with a range of shortening velocities, the T-jump activation was repeated on each fibre between 5 and 8 times, each time with force clamped to a different level.

Rejection criteria

Some of the intact fibre bundles and some skinned fibres produced low isometric forces, probably because of damage during dissection and mounting. We rejected the data from preparations of either type that produced isometric force less than 75 kPa. Out of 26 intact fibre bundles, 10 were rejected and from 143 skinned fibres, 2 were rejected. In skinned fibre experiments the isometric force in successive activations decreased at an average rate of 2.1% per activation. After the isometric force fell below 80% of the maximum value for that fibre, further records were not used.

Data normalisation

Force values (in mN) were divided by the corresponding isometric force (*F*_{IM}). Power values were divided by the product (*F*_{IM} *L*₀); power normalised in this way has units of s⁻¹. Velocity data was divided by *L*₀ and also has units of s⁻¹.

Linear regression

For examining the dependence of isometric force on CSA and of power on volume, we used the Model 2 regression method (York et al., 2004) in which the value of the slope is found by minimizing the errors in both the dependent and independent variable.

Curve fitting for maximum power

The relation between normalised power (*Q*, s⁻¹) and normalised force (*F*, dimensionless) was fitted with the following version of Hill's equation:

$$Q = \frac{F \cdot V_{\max} \cdot (1 - (F/F_0^*))}{1 + (F/a)}, \quad (1)$$

Where *V*_{max} and *F*₀^{*} are, respectively, the velocity and force intercepts on a plot of normalised velocity against normalised force, and $-a$ is the asymptote of force on this plot. *F*₀^{*} is also the force intercept on the plot of

normalised power against normalised force. Note that *F*₀^{*} is not the same as the isometric force (Edman et al., 1976). The three parameters adjusted in Eqn 4 to obtain the best fit are the maximum power (*Q*_{max}), the force at maximum power (*F*_{Qmax}), and the force intercept on the power versus force curve (*F*₀^{*}). *V*_{max} and *a* as functions of these parameters are:

$$V_{\max} = \frac{Q_{\max} \cdot F_0^*}{F_{Q_{\max}}^2}, \quad (2)$$

$$a = \frac{F_{Q_{\max}}^2}{F_0^* - 2 \cdot F_{Q_{\max}}}. \quad (3)$$

Substituting (2) and (3) into (1) gives the relation between *Q* and *F* in terms of the three fitted parameters:

$$Q = \frac{((Q_{\max} \cdot F_0^*)/F_{Q_{\max}}^2) \cdot F \cdot (1 - (F/F_0^*))}{1 + F \cdot ((F_0^* - 2 \cdot F_{Q_{\max}})/F_{Q_{\max}}^2)}. \quad (4)$$

The relation between velocity, *V*, and force in terms of the same three parameters is:

$$V = \frac{((Q_{\max} \cdot F_0^*)/F_{Q_{\max}}^2) \cdot (1 - (F/F_0^*))}{1 + F \cdot ((F_0^* - 2 \cdot F_{Q_{\max}})/F_{Q_{\max}}^2)}. \quad (5)$$

The maximum power per unit volume in W l⁻¹ is

$$\frac{Q_{\max} \cdot F_{IM} \cdot L_0}{CSA \cdot L_0} = \frac{Q_{\max} \cdot F_{IM}}{CSA}, \quad (6)$$

where CSA is the cross sectional area of the preparation in mm².

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Competing interests

The authors declare no competing or financial interests.

Author contributions

A.M.W., R.C.W., N.A.C. and T.G.W. developed the concept. R.A.D., T.G.W., R.C.W. and N.A.C. performed experiments. R.C.W. and N.A.C. did data analysis, and wrote the manuscript with input from other authors.

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