Power output of skinned skeletal muscle fibres from the cheetah (Acinonyx jubatus)

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SUMMARY

Muscle samples were taken from the gluteus, semitendinosus and longissimus muscles of a captive cheetah immediately after euthanasia. Fibres were "skinned" to remove all membranes leaving the contractile filament array intact and functional. Segments of skinned fibres from these cheetah muscles and from rabbit psoas muscle were activated at 20°C by a temperature jump protocol. Step and ramp length changes were imposed after active stress had developed. The stiffness of the non-contractile ends of the fibres (series elastic component) was measured at two different stress values in each fibre; stiffness was strongly dependent on stress. Using these stiffness values, the speed of shortening of the contractile component was evaluated, and hence the power it was producing. Fibres were analysed for myosin heavy chain content using gel electrophoresis, and identified as either slow (Type I) or fast (Type II). The power output of cheetah Type II fibre segments was 92.5 ± 4.3 W kg⁻¹ (mean ± s.e., 14 fibres) during shortening at relative stress 0.15 (=stress during shortening/isometric stress). For rabbit psoas fibre segments (presumably Type IIX) the corresponding value was significantly higher (P<0.001), 119.7 ± 6.2 W kg⁻¹ (mean ± s.e., 7 fibres). These values are our best estimates of the maximum power output under the conditions used here. Thus the contractile filament power from cheetah was less than that of rabbit when maximally activated at 20°C, and does not account for the superior locomotor performance of the cheetah.
INTRODUCTION
The cheetah is the fastest running animal with a top speed of 29 m s\(^{-1}\) (Sharp, 1997), considerably faster than racehorses (Spence et al., 2012) and racing greyhounds (Usherwood and Wilson, 2005), which can only achieve about 20 m s\(^{-1}\). Cheetah hunting also involves extremely rapid acceleration and the power required for this acceleration has been measured in wild cheetahs (Wilson et al., In Review). Averaged across three gait cycles the power can be as high as 90 W kg\(^{-1}\) of body mass. About half of the cheetah's body mass is muscle, so this is equivalent to 180 W kg\(^{-1}\) of muscle. This is one of the highest recorded values for a terrestrial mammal and suggests that cheetah muscle may have unusually powerful characteristics that contribute to its high running speed and acceleration. It has already been reported (Hyatt et al., 2010; Goto et al., in press) that the muscles of the cheetah contain a very high proportion of the fastest and most powerful mammalian muscle fibre types (Type IIB and IIx).

We had the opportunity to obtain post-mortem cheetah muscle fibres from a captive animal and have measured their power output while shortening under different loads. The aim was to investigate whether the maximum power was large compared with that of muscle from a animal that is less specialized for running speed; for this comparison experiments were done on fast fibres from rabbit. Skinned muscle fibres were used for two reasons: their power output could be measured after the unavoidable delay between collecting cheetah samples and testing them, and skinned fibres allow comparison of power output of the contractile filaments themselves.

Cheetah and rabbit fibres contracted under identical conditions of activation, temperature, movement pattern. To activate the fibres we used the temperature-jump method (Bershitsky and Tsaturyan, 1989, which reliably produces repeated contractions at temperatures closer to \textit{in vivo} temperature than those conventionally used with Ca\(^{2+}\) activation of skinned fibres. We found that under the conditions used here, notably 20\(^{\circ}\)C and constant velocity shortening, the cheetah fast fibres did not produce higher power than rabbit fast fibres. The results suggest that either the superior locomotor performance of cheetah is due to factors other than the contractile filament function itself, or that if there is a difference in contractile filament power output \textit{in vivo}, the difference does not exist under the conditions used here. More \textit{in vivo}-like conditions that could be explored including, higher temperature, submaximal activation, and/or a more life-like pattern of movement.
METHODS

Muscle fibre preparation and storage

A captive cheetah (male, age: 6.5 years, weight: 41 kg), with probable renal failure, was euthanized at the Royal Veterinary College with an overdose of barbiturates. It was a mature, but not aged animal. Bundles of skeletal muscle fibres (10 mm in diameter, 40 – 50 mm long) were dissected from gluteus medius, semitendinosus and longissimus muscles. Functionally, gluteus medius is a hip extensor, semitendinosus is a hip extensor and knee flexor (Hudson et al., 2012) and longissimus extends the spine. Fibres were also obtained from the psoas muscles of two female New Zealand White rabbits as previously described (He et al., 1999).

The cheetah became available unexpectedly, so experiments were done on skinned muscle fibres because they can be stored for later study. Intact mammalian fibres must be used within hours after removal from the animal, which was not possible on this occasion. Fibre bundles were permeabilised at 4°C in a ‘skinning solution’ (Table 1) containing increasing concentrations of glycerol, as described previously (Thirlwell et al., 1994; He et al., 1997; He et al., 1998). The permeabilised fibre bundles were stored at -20°C in skinning solution made up in 50% glycerol and were used within 8 weeks. Single muscle fibre segments were dissected away from the fibre bundle in a relaxing solution (Table 1) containing 0.5% Triton X100 at 7°C on a thermostated stage of a dissecting microscope. Aluminium foil ‘T-clips’ (Photofabrication Ltd, St Neots) were placed on the ends of fibre segment (length between 0.8 and 1.5 mm). The clips were attached with shellac to stainless steel wire hooks, one connected to a force transducer (AE801; HJK Sensoren + Systeme, Friedberg) and the other to a purpose-built motor as described by Bershitsky et al. (1996). The fibre segment was suspended in 30 µlitre of relaxing solution in the quartz trough of an aluminium temperature-controlled stage.

Fibre dimensions and units

While in relaxing solution, the sarcomere length (SL) was set to 2.4 µm using laser diffraction; the filament lengths in mammalian muscle are such that this SL is optimal for force development. The segment length at SL of 2.4 µm was our reference length (L0). Fibre segment length, width and depth were measured using a Zeiss (Jena, Germany) ACM microscope with 40x water-immersion objective. Cross-sectional area (CSA) was calculated
from the measured width and depth assuming an elliptical cross-section; no correction was
made for possible swelling as a result of skinning.

The values of $L_0$ and CSA were used to take account of variations in the size of the fibre
segments. Density was assumed to be 1 kg dm$^{-3}$ when converting from muscle fibre volume
to mass. Force is reported either as stress ($= \text{force}/\text{CSA}$, units kPa) or in some contexts as
relative stress (stress during shortening/isometric stress produced before shortening in the
same contraction). Velocity of shortening is reported in relative units: change in
length/$L_0$ per s. Stiffness ($=\text{stress change}/\text{relative length change}$) is reported in units MPa.

Power is reported in units of W/kg muscle. Relative power values were multiplied by
isometric stress (kPa) to give power in units of W kg$^{-1}$, assuming 1 dm$^{-3}$ corresponds to 1 kg.

$$\text{Power} = \frac{F_s}{F_i} \times \frac{\Delta L}{L_0} \times \frac{1}{\Delta t} \times F_i$$

Units for power $= \frac{kPa}{kPa} \times \frac{m}{m} \times \frac{1}{s} \times kPa = \frac{W}{kg}$

Fibre activation

Fibre segments were activated by the temperature-jump (T-jump) method (Bershitsky and
Tsaturyan, 1989). The principle of the method is that at low temperature, the active fibre
segment produces little force because few cross-bridges are attached and many of these
produce low force. At the higher temperature, the active fibre segment gives more force both
because more cross-bridges are attached and because more of them produce a high force.
The T-jump apparatus, based on the design by Linari et al. (1998), consisted of two
temperature-controlled stages that were moved horizontally by a stepper motor. The low T
was $\sim 2^\circ$C (range 1.6 to 2.1°C) and the high T was 20°C. Each stage carried a quartz platform
on which were placed two separate drops (30 µlitre) of solution. The stages were moved so
that the fibre segment was immersed successively in the following solutions: (1) pre-
activating at low T for 2 minutes, (2) activating at low T, (3) activating at high T, and finally
(4) relaxing at high T. See Figure 1A.

LabView software was used to control the stage position and fibre segment length and
recorded the digitised output signals from the force transducer and motor at 1 or 10 kHz.

Protocol for measuring power output by the contractile component (CC)

The fibre segments were activated by increasing $[\text{Ca}^{2+}]$ at low T; temperature was then
rapidly increased (T-jump) and when isometric stress had been developed the fibre length
was decreased in a “step & ramp” pattern (Figure 1B). Each fibre segment performed a
series of contractions with different ramp velocities and different step sizes.
Our aim is to measure the power produced by the CC, which is the product of stress and the velocity of CC shortening. In the next section we will describe how we analysed the records to obtain the CC power, which involves separating the total shortening of the fibre segment into the part due to the CC and the part due to non-contractile component(s) using the methods previously described by Sonnenblick, 1964; Curtin et al., 1998; Mellors and Barclay, 2001. The stiffness of the non-contractile components will be evaluated as part of the analysis; Figure 1B and Figure 2 show respectively an overview and an example record. It should be noted that this stiffness is, to a large extent that of the damaged ends of the skinned fibre segment (Figure 1C); in contrast \textit{in vivo} and in isolated, intact muscle fibres the non-contractile stiffness is dominated by the tendons. The mechanical properties of these damaged ends were found to be different than those of the tendons of muscle \textit{in vivo} and of isolated, intact muscle fibres. In particular, the stiffness of the skinned fibre SEC (damaged ends) varied with stress, whereas the stiffness of tendon is independent of stress except at very low stress.

In the following description SEC (series elastic component(s)) refers to the non-contractile parts of the skinned fibre segment. The principles underlying separation of SEC and CC shortening are (1) the stress in SEC and CC is the same and equal to the observed stress, in other words they are mechanically in series, (2) the total length change is the sum of the SEC length change and the CC length change, and (3) the length of the SEC depends solely on stress (and not on velocity of movement). Thus the method assumes that the SEC can change its length much more quickly than the CC. For this reason the SEC stiffness can be measured when there is a sudden alteration in length change. The dependence of SEC stiffness on stress could be evaluated using the two parts of each record where there as an abrupt change, that is, at the step and at the end of the ramp. Two values of the SEC stiffness were measured in each record (Figure 2A&B), and the corresponding relationship between stiffness and stress is shown in Figure 2C. From this relationship the SEC stiffness at any stress (and thus at any time during the record) can be read off the graph. At each time point during the ramp shortening, the rate of SEC shortening was \((dF/dt) / S_F\), where \(dF/dt\) is the rate of change of stress and \(S_F\) is the stiffness at stress = \(F\). The time-course of SEC length change is found by integration and is shown in Figure 2D. The shortening of the CC is found by subtracting the SEC shortening from the total shortening. Power produced by the CC was evaluated as the product of relative force (or stress) and the rate of change of the CC.
Successful mechanical experiments were done on 18 cheetah and 7 rabbit fibre segments; of
the cheetah fibres, 15 were typed as slow or fast on the basis of gel electrophoresis as
described below.

**Curve fitting to evaluate peak power**

Peak power was evaluated by considering two functions fitted to the observed values of
power vs relative stress. (1) Regression lines for the relation between power and relative
stress (Fs/Fi) were calculated by a robust method (MatLab "robustfit") that minimises the
influence of outlying points as described by Holland and Welsch, (1977). The regression
gave values for the mean power across the range of stress values investigated and also gave
an intercept on the relative stress axis (F*/Fi). (2) The observed power was also compared to
the following function, describing the relation between stress during shortening and power
(W kg\(^{-1}\)), which is based on Hill's hyperbolic force-velocity relationship as modified by

\[
\text{Power} = Fs \times V_{\text{max}} \times \left(1 - \frac{Fs/Fi}{F*/Fi}\right) / \left(1 + G \times \frac{Fs/Fi}{F*/Fi}\right) \quad \text{Eqn (1)}
\]

where Fs is the stress during shortening (kPa), Fi is the isometric stress (kPa) and F*/Fi is the
intercept of the regression analysis described above, Vmax is the maximum shortening
velocity (dL/Lo per s), and G is a constant. This function, assuming differing values of
Vmax and G, neither of which could be determined in the experiments reported here, was
compared with the experimental results.

**Fibre typing**

Fibre segments 1-3 mm in length from cheetah muscles were dissolved in 20 µlitre of sample
buffer (Laemmli, 1970) and heated at 100°C for 3 minutes. 4 µlitre samples were loaded into
0.75 mm thick gels in a Hoefer ‘mighty small’ apparatus. Two different stacking-separating
gel combinations were used, a 4.5% stacking gel with a 7.5% separating gel containing 30%
glycerol (Picquet and Falempin, 2003) and a 4% stacking gel with a 9% separating gel both
containing 30% glycerol (Blough et al., 1996). The gels were run using the method of Picquet
and Falempin (2003) with minor modifications. Over a 12 hour period, a constant voltage of
100 V was set until the bromophenyl blue marker had migrated significantly (~2 cm) into the
separating gel, at which point the voltage was increased to 180 V for the remainder of the
run. Gels were subsequently silver stained (Morrissey, 1981) to reveal the myosin heavy
chain bands. A sample of gluteus muscle, which contains both slow-twitch and fast-twitch
fibres, was treated as the fibre segments and used as a standard. Two clearly separated bands
were seen in this standard, which we assume to be from the fast and slow fibres. We did not
see any convincing subdivisions of the fast band and thus are not able to identify the two
types of fast fibres which these muscles of the cheetah are known to contain (Goto et al., in
press).

RESULTS

Of the 15 cheetah fibre segments analysed by gel electrophoresis, 4 were identified as slow-
twitch and 11 as fast. As will be described below, the power output from the fibre segments
identified as slow-twitch on the gels was considerably less than that of those identified as
fast. The 3 segments for which there is no gel data were assumed to be fast because their
mechanical performance matched that of the fibre segments identified as fast.

Analysis of all the individual mechanical records, like the example shown in Figures 1B and
2, indicated that SEC stiffness, which is probably due to the non-contractile material at the
ends of the fibres segments, was proportional to stress during a contraction. This rather
unexpected variation of stiffness with stress was not unique to this example record. The same
dependence was seen when results from different contractions and from different fibre
segments were pooled (Figure 3).

As described in the methods we used the stiffness of the SEC (non-contractile parts of the
skinned fibre segment) to evaluate the velocity of shortening of the contractile component
(CC). The relationship between stress and the velocity of CC shortening is shown for cheetah
fast and slow fibre segments and for rabbit fibre segments in Figure 4. As expected the slow-
twitch cheetah fibre velocities (Figure 4C) are well below those for the fast fibres (Figure
4A). For both cheetah and rabbit the relative stress-velocity results for the fast fibres fall
along the expected hyperbolic relationship. Cheetah fibres from different anatomic sites
(shown by different colours in the figure) behaved similarly. It is clear that the range of
velocities we used was not sufficient to cover the entire stress-velocity curve and we are
unable to evaluate the maximum velocity of shortening (Vmax). For an explanation of the
lines in Figure 4, see Discussion.
The relationship between power output and relative stress as measured in individual records is shown in Figure 5. Linear regression analysis was done in each case using a robust method (see Methods) which reduces the influence of outlying points. The residuals about the fitted lines are also shown for each case and their distributions indicate that these regression lines are a reasonable summary of the results. The 95% population confidence intervals derived from the regression analysis are also shown. Equation 1, which is based on the usual hyperbolic stress vs velocity relationship, did not fit the data better than the linear regression line, as judged either by the correlation coefficient or by the distribution of the residuals (not shown). In the Discussion we will return to the problem of estimating the peak power from the results shown in Figure 5.

The isometric stress values and power at relative stress of 0.15 from the regression analysis for the different fibre types are summarised in Table 2. Within the range of relative stresses that were tested the power decreases as relative stress increases. The slopes of the regressions lines for the fast fibres from cheetah and rabbit are not significantly different, whereas the intercepts are. Thus the line for the cheetah fast fibres lies below that for the rabbit results. The power for the slow cheetah fibres is only about 20% of that of fast fibres at all relative stress values.

**DISCUSSION**

*Estimate of peak power output for skinned fibres*

We were unable to obtain satisfactory measurements of power when the relative stress was below 0.1. Power is zero when stress is zero and, as stress increases, power rises to a peak and then falls at higher stresses. The linear regression (Figure 5) thus cannot give a complete description of power output. The regression analysis gives values for the maximum observed power within the range of stresses that we tested (Table 2). These estimates of the maximum power may be less than, but cannot be greater than, the true peak power. Equation 1, which is based on a hyperbolic force vs velocity relationship, is useful here because it provides a function that passes through the origin and has a defined peak power. This equation was used to explore the range of power curves that lie within the confidence intervals of the linear regression analysis and are thus compatible with our observations.
To assess how much our estimated maximum power falls short of the true peak power, we have considered for each of the muscle types, cheetah fast and slow and rabbit fast, a range of power vs stress curves that are compatible with the observations. As can be seen from equation 1, power is determined by the values of constants: Vmax, G and F*. The value of F* was taken to be equal to the intercept of the regression line on an x-axis (see Figure 5). The range of values of G and Vmax that give curves that fit within the confidence intervals of the regression was identified, subject to the constraint that Vmax was less than 70 Lo/s for fast fibres and 20 Lo/s for the slow fibres. The peak powers calculated from equation 1 using these G and Vmax values fall within the small cyan area shown in each panel of Figure 6. The maximum power from the regression line (red point in Figure 6) is close to this area, showing that our best estimates of peak power are unlikely to be much less than the actual peak power. The broken blue line for each muscle type is an example of the complete power vs stress curve for the lowest Vmax value that is compatible with the data. We show in Figure 4 the stress vs velocity curves calculated using the lowest Vmax and its corresponding G value tabulated in the legend.

Comparison of rabbit and cheetah muscle.

The results reported here do not show that cheetah muscle is more powerful than rabbit, as would reasonably be expected from their locomotor performance. In fact our best estimate of cheetah muscle power is significantly less than that of rabbit muscle (P<0.001, Table 2). We will discuss three factors (fibre types, temperature, and skinned-vs-intact fibres) that affect power and are likely to mean that in vivo power is higher than that observed here. These factors have not been fully explored for muscle from cheetah and rabbit; if they were, it might emerge that cheetah muscle is more powerful than rabbit muscle under conditions close to those in vivo. Alternatively, it could be that the muscles' capacity to produce power is very similar in these two species, and that the cheetahs' superior locomotor performance depends on other aspects of the animals' design.

Fibre types

The rabbit fibres we used were most likely the fast IIX (=IID) type which makes up the majority of the psoas muscle (Hamalainen and Pette, 1993;Ducomps et al., 2004). The cheetah muscles we used contain both IIA and IIX fibres (Goto et al., in press). We were unable to distinguish between these two fast fibre types by gel electrophoresis; the group of fibres used was probably a mixture of both types. Thus the power output of cheetah IIX
fibres is likely to be greater than the value we report which is probably from a mixed group of IIA and IIX fibres. We speculate that the power output of cheetah IIX fibres is greater than that of rabbit fibres of the same type. Confirmation of this point will need immuno-staining techniques to identify the fibre types, combined with measurements of their power output.

**Temperature**

Most previously published measurements of the power output of skinned mammalian muscle fibres have been made at temperatures below 20°C. A summary of these values, and the values reported here, is shown in Figure 7A. Power output is known to be very temperature sensitive (Ranatunga, 1984, 1998, 2010), so it is not surprising that most of the earlier measurements are well below the value of about 100 W kg⁻¹ at 20°C which we have found for both the rabbit and cheetah fibres (red square and red cross). The only published value for skinned fibres at temperature higher than 20°C is for rat fibres at 30°C, which produced power of 166 W kg⁻¹ (Knuth et al., 2006).

For intact rat and mouse muscle fibres there are published values of power output covering a wide temperature range (filled triangles, Figure 7B). It can be seen that the temperature dependence of power is steeper at temperatures below 20°C than at higher temperatures. This can be illustrated by drawing two straight lines through the rat data points; for the lower temperature range the slope is 5 (i.e. corresponds to a Q₁₀ = 5) and for higher temperatures the slope is 2.25. To make a comparison of our skinned fibre results with other published values, we have assumed that these Q₁₀ values also apply to the skinned fibres. The solid line in Figure 7A has the same slopes as in B, but has been shifted vertically to pass through our measured power values for rabbit and cheetah. The line passes close to the values at 12°C previously reported for rabbit and for large felids. However, a substantial number of the previously reported power values lie below this line, including all the values reported for human muscle, and in particular the one value for human muscle at 20°C. This difference suggests that cheetah and rabbit muscles are inherently more powerful than human muscle, in agreement with the conclusion of Kohn and Noakes (2013), and have a higher glycolytic capacity (Williams et al., 1997).

**Comparison of skinned and intact fibres**

It has previously been reported that the power output of skinned muscle fibres is less than that of intact muscle fibres of the same species at the same temperature. For example, Curtin and Woledge (1988) found that intact fibres gave a maximum power output 65% greater than
that of skinned fibres reported by Bone et al. (1986); both studies used white muscle fibres of the dogfish at 12°C. A similar comparison of skinned and intact fibres has been made for frog. Ferenczi et al. (1984) reported experiments on skinned frog fibres showing that the curvature of the force vs velocity relationship was greater than literature values for intact muscle fibres also from frog (Edman et al., 1976; Edman and Hwang, 1977; Cecchi et al., 1978). A more curved force vs velocity relationship would mean a lower maximum power output if F* (intercept on the force axis) and Vmax (the maximum velocity of shortening) remained unchanged. Ferenczi et al. (1984) report that neither the isometric force nor the Vmax values were significantly different between their skinned fibre and these intact fibre studies. Maximum power was, therefore, less in the skinned than in the intact fibres. We calculate from the parameters of the force vs velocity relationships given in these publications that the intact fibres produced on average 45% more power than the skinned fibres used by Ferenczi et al. (1984).

A similar difference can be seen in Figure 7B where the measured power outputs for intact rat fibres (filled down triangles) is greater than that of skinned rat fibres (open triangles): by 76 % at 15°C and by 31 % at 30°C. Would the same difference be found for the muscle from the species investigated here? As discussed above we do not have enough observations at low force to establish the full force vs velocity range for the cheetah and rabbit skinned fibres. However, we did find the G values which gave peak powers compatible with our observations (Table 2). The G values are high, between 24 and 42, indicating that force vs velocity relationships are very curved. In contrast, the corresponding values for intact fibres from rat and mouse are for fast fibres less than 4 and for slow fibres less than 7.5 (Ranatunga, 1982, 1984 and Barclay 1996). Thus, it seems likely that the force vs velocity relation for intact cheetah fibres would be less curved and the power output greater than for skinned fibres as has been found for frog, dogfish and rat muscle. Experiments with intact fibres from cheetah and rabbit are required to test this conjecture and make a more complete comparison of these species.

Power output of cheetah muscles in vivo.
We report our best estimate of the average maximum power output by skinned cheetah muscles fibres of the fast types is 92.5 W kg⁻¹. How much power might be produced in vivo? At the in vivo temperature of 38°C power would be increased to 400 W kg⁻¹, using a Q₁₀ for power output of 2.25 as illustrated in Figure 7. It is likely that intact fibres would produce about 50 % more power than the skinned fibres used here: ~600 W kg⁻¹. This is an estimate.
of the power output of a mixed population of the two fast fibres types. The power output from the IIX fibre type would be higher, possibly approaching 1 kW kg\(^{-1}\). Such large power outputs have been previously reported for two small land vertebrates: 0.95 kW kg\(^{-1}\) for lizard muscle at 39°C (Curtin et al., 2005) and 1.15 kW kg\(^{-1}\) from quail muscle \textit{in vivo} (~40°C) (Askew et al., 2001). The similarity in the power output of the fibres from animals of such different sizes (40 kg cheetah, 5 g lizard, and 40 g quail) agrees with the summary reported by Askew et al (2001) for a flying animals ranging in size from bee to turkey based on \textit{in vivo} measurements. In contrast, Seow & Ford, (1991) using skinned muscle fibres at low temperature (~5°C) found a strong negative correlation of power output with body mass across a range of mammalian species. Clearly temperature and the type of preparation both have a big influence on the relationship between muscle power and body mass.

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**Figure Legends**

Fig. 1. A. Diagram showing contraction protocol. Top panel shows the imposed length changes; middle panel shows record of stress; bottom panel shows the solutions (\(p = \) pre-activating solution, \(a = \) activating solution, \(r = \) relaxing solution) and temperature. The arrow indicated the time of the length step and ramp shortening. The spikes labelled c indicate artefacts that occurred during solution change.

B. Expanded section of the stress and length records giving an over-view of the measurements used to get \(S_1\), the SEC stiffness during the step = \(\Delta F/\Delta L\), and \(S_2\), the SEC stiffness at the end of the ramp, \(\Delta (dF/dt) / \Delta (dL/dt)\). Stress changes are shown in more detail in Figure 2.

C. Diagram of a segment of a skinned fibre. Contractile part in red, non-contractile ends gray.

Fig. 2. A. Expanded section of the stress around the step. Vertical broken line marks the time of the step. \(Fi = \) isometric stress before step, \(Fs = \) stress value at end of step found by extrapolation. \(\Delta F = Fi – Fs\).
B. Expanded section of the stress around the end of the ramp. Vertical broken line marks the end of the ramp. \((dF/dt)_a\) is the rate of stress change at the end of the ramp, and \((dF/dt)_b\) is the rate of stress change after the end of the ramp. \(\Delta(dF/dt) = \text{abs}[(dF/dt)_a - (dF/dt)_b]\).

C. The relationship between stress and SEC stiffness measured at step (S1) and at the end of ramp shortening (S2) in record shown in A and B, respectively. S1 is plotted against the average of Fi and Fs. The gradient of the broken line, 0.0648 MPa/kPa, describes the variation in SEC stiffness with stress.

D. The time-course of total length change of the fibre segment (black) and also the change in length of the sec (green) and of the cc (red) calculated using the stress and the SEC stiffness as described in the text.

Fig. 3. The relationship between the SEC stiffness and stress during step shortening (closed symbols) and at the end of ramp shortening (open symbols). A. Pairs of SEC stiffness values from 70 contractions of cheetah fibre segments measured by the method described in the text. Results for 4 fibre segments from gluteus, black; 4 from semitendinosus, pink; 6 from longissimus, blue. Between 1 and 8 contractions per fibre. Different symbols indicate results for different fibre segments. Regression line: \(y = 0.0502 x + 0.1156, r^2 = 0.8329\). B. Pairs of SEC stiffness values from 41 contractions of 7 fibres segments from psoas muscle of rabbit. Between 1 and 12 contractions per fibre segment. Regression line: \(y = 0.0431 x - 0.0852, r^2 = 0.6528\).

Fig. 4. Velocity-relative stress relationships. Relative stress is the average produced during the first part of the ramp shortening, Fs, expressed relative to the isometric stress just before shortening, Fi. A. Cheetah fast fibres. Gluteus, black; semitendinosus, pink; longissimus, blue. Fs from first 33% of shortening. B. Rabbit fast fibres from psoas. Fs from first 50% of shortening. C. Cheetah slow-twitch fibres. Gluteus, black; semitendinosus, pink; longissimus, blue. Fs from first 50% of shortening. Different symbols indicate results for different fibres.

Lines are hyperbolic Hill relationships: \(\text{Vel} = V_{\text{max}} \times \frac{\frac{F_s}{F_i}}{1 + \frac{F_s}{F_i}}\)

With constants shown below fitted as described in the text.

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<thead>
<tr>
<th>(F_s, \text{Relative Stress})</th>
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</table>

Figure 5. Power–relative stress relationships. Power = relative stress $\times$ velocity $\times$ isometric stress for each record. Units W/kg = (kPa/kPa) $\times$ (m/m) $\times$ (1/s) $\times$ kg; assuming 1 dm$^3$ = 1 kg. The relative stress and velocity values are shown in legend of Figure 4. A. Cheetah fast fibres. B. Rabbit fibres. C. Cheetah slow-twitch fibres. Symbols as in Figure 4. Solid line is the regression line and broken lines are the 95% confidence intervals (see text and Table 2). The residuals are shown in the lower set of graphs.

Figure 6. Power–relative stress relationships. The solid black lines are the linear regression lines and gray areas show the 95% confidence intervals; see Figure 5. The red filled circles on the regression lines show the power at relative stress 0.15. The broken blue lines show power calculated from equation 1, which is based on the hyperbolic Hill relationship, using the constants shown in Figure 4. The cyan area encloses the peak power values calculated using the velocity given by the hyperbolic Hill relationship (equation 1) subject to the following constraints: $V_{\text{max}} < 70 L_0/s$ for fast fibres and <20 $L_0/s$ for the slow fibres, and power within the 95% confidence intervals of the regression line.

Fig. 7. Relationship between power output (log scale) of muscle from different mammalian species and temperature. A. Skinned fibre results. Red square = cheetah (this report); red cross = rabbit (this report); white up triangle = mouse (Seow and Ford, 1991); white down triangle = rat (Bottinelli et al., 1991; Seow and Ford, 1991; Knuth et al., 2006); cyan circle = rabbit (Seow and Ford, 1991; He et al., 1999; Sun et al., 2001); green circle = goat (Hanes et al., 2008); yellow circle = sheep (Seow and Ford, 1991); orange diamond = lion and caracal (Kohn and Noakes, 2013); pink circle = horse (Rome et al., 1990); grey circle = cow (Seow and Ford, 1991); blue circle = human (Bottinelli et al., 1996; Widrick et al., 1996; He et al., 2000; Gilliver et al., 2009; Claflin et al., 2011; Kohn and Noakes, 2013). Broken lines join values from the same study. Solid lines, which are drawn through the cheetah and rabbit results reported here, are calculated for $Q_{10} = 5.0$ between 0 and 20$^\circ$C, and $Q_{10} = 2.25$ at $T>20^\circ$C. B. Intact fibres: black up triangle = mouse (Barclay et al., 2010); black down
triangle = rat (Ranatunga, 1998). Skinned fibres: white down triangle = rat (Knuth et al., 2006). Solid lines summarize the relationship for rat intact fibres; slopes as in A.

REFERENCES


Figure 1

A

Stress (kPa)
-100
0
100
200
dL/Lo
-0.1
0.0

D(h/dt)
D(F/fig)
D(L)
D(F)

B

Time from step (s)
0.000 0.025 0.050
Stress (kPa)
0
100
200
300

C

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Figure 2

A

Stress (kPa)

Time from step (s)

0.00 0.01 0.02

B

Stress (kPa)

(dF/dt)\textsubscript{a}

(dF/dt)\textsubscript{b}

Time from step (s)

0.02 0.03 0.04

C

Stiffness (MPa)

Stress (kPa)

0 50 100 150 200

S1

S2

D

dL/Lo

0.00 0.01 0.02

total

sec

cc

-0.10 -0.05 0.00
Figure 3

**A**

SEC stiffness (MPa) vs Stress (kPa)

**B**

SEC stiffness (MPa) vs Stress (kPa)
Figure 4

A

B

C

Relative Stress

Relative Stress

Relative Stress

Velocity (dL/Lo per s)

Velocity (dL/Lo per s)

Velocity (dL/Lo per s)
Figure 5

A: Correlation between Relative Stress and Power (W/kg).
B: Another correlation showing a different trend.
C: A third correlation highlighting residual analysis.

The graphs illustrate the relationship between Relative Stress and Power, with a focus on residual analysis to assess the model fit.
Figure 6

A  B  C

Relative Stress

Relative Stress

Relative Stress

Power (W/kg)

0.00 0.25 0.50 0.75

0.00 0.25 0.50 0.75

0.00 0.25 0.50 0.75

0 50 100 150

0 50 100

0 10 20 30

0.00 0.25 0.50 0.75

0.00 0.25 0.50 0.75

0.00 0.25 0.50 0.75
Figure 7

A

B

Power (W kg$^{-1}$)

Temperature (°C)

Power (W kg$^{-1}$)

Temperature (°C)
Table 1. Constituents of the solutions.

<table>
<thead>
<tr>
<th>Component</th>
<th>Skinning solution*</th>
<th>Relaxing solution</th>
<th>Pre-activating solution</th>
<th>Activating solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>TES</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Imidazole</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Magnesium acetate</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>0</td>
<td>7.8</td>
<td>6.8</td>
<td>6.5</td>
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<tr>
<td>Potassium propionate</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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<td>ATP</td>
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<td>5.7</td>
<td>5.7</td>
<td>5.7</td>
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<tr>
<td>EGTA</td>
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<td>25</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>Calcium-EGTA</td>
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<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>HDTA</td>
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<td>0</td>
<td>24.9</td>
<td>0</td>
</tr>
<tr>
<td>Creatine phosphate</td>
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<td>21.2</td>
<td>21.2</td>
<td>21.2</td>
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<tr>
<td>Glutathione</td>
<td>0</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>PMSF</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Leupeptin</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Trypsin inhibitor</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

All concentrations in units mmol litre$^{-1}$, except Leupeptin and Trypsin inhibitor which are in mg litre$^{-1}$.

*Skinning solutions were made up with 12.5, 25, and 50% glycerol for the permeabilization process; permeabilized fibres were stored in 50% glycerol skinning solution. Solution pH was 7.1 at 20°C. $^1$TES = N[Tris(hydroxy-methyl)methyl]-2-aminoethanesulfonic acid, $^2$ATP = Adenosine 5’ Triphosphate disodium salt hydrate, $^3$EGTA = ethylene glycol-bis(2-aminoethylether)-N,N,N’,N’-tetraacetic acid, $^4$[Ca$^{2+}$] = 32 µmol litre$^{-1}$, $^5$HDTA = 1,6 Diaminohexane- N,N’,N’-tetraacetic acid, $^6$PMSF = Phenylmethanesulphonyl fluoride.
Table 2A. Mechanical properties of skinned fibres from cheetah and rabbit.

<table>
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<th></th>
<th>mean</th>
<th>s.e.m.</th>
<th>N</th>
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</thead>
<tbody>
<tr>
<td>Isometric stress (kPa)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheetah (Type I)</td>
<td>132.4</td>
<td>32.7</td>
<td>3</td>
</tr>
<tr>
<td>Cheetah (Type II)</td>
<td>195.0</td>
<td>12.2</td>
<td>14</td>
</tr>
<tr>
<td>Rabbit (Type IIX)</td>
<td>194.5</td>
<td>14.9</td>
<td>7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>mean</th>
<th>s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum power (W kg⁻¹)</td>
<td></td>
<td></td>
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<tr>
<td>Cheetah (Type I)</td>
<td>19.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Cheetah (Type II)</td>
<td>92.5</td>
<td>4.3</td>
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<tr>
<td>Rabbit (Type IIX)</td>
<td>119.7</td>
<td>6.2</td>
</tr>
</tbody>
</table>

Table 2B. Linear regression of power (W kg⁻¹) on relative stress for skinned fibres from cheetah and rabbit. Power = (slope × relative stress) + intercept.

<table>
<thead>
<tr>
<th></th>
<th>slope</th>
<th>s.e.</th>
<th>intercept</th>
<th>s.e.</th>
<th>r²</th>
<th>n</th>
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<tr>
<td>Linear regression</td>
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<tr>
<td>Cheetah (Type I)</td>
<td>-30.9</td>
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<td>24.3</td>
<td>2.9</td>
<td>0.384</td>
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<tr>
<td>Cheetah (Type II)</td>
<td>-139.7</td>
<td>16.5</td>
<td>113.5</td>
<td>6.4</td>
<td>0.728</td>
<td>66</td>
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<tr>
<td>Rabbit (Type IIX)</td>
<td>-147.7</td>
<td>19.2</td>
<td>141.9</td>
<td>8.7</td>
<td>0.775</td>
<td>41</td>
</tr>
</tbody>
</table>

Table 2C. Hill equation parameters for skinned fibres from cheetah and rabbit. See Discussion.

<table>
<thead>
<tr>
<th></th>
<th>F*</th>
<th>G</th>
<th>Vmax (dL/Lo s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheetah (Type I)</td>
<td>0.787</td>
<td>31.3</td>
<td>9.86</td>
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<tr>
<td>Cheetah (Type II)</td>
<td>0.813</td>
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<tr>
<td>Rabbit (Type IIX)</td>
<td>0.961</td>
<td>42.4</td>
<td>39.4</td>
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</table>

** Maximum power Rabbit > Cheetah, P < 0.001.