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MAXIMUM VELOCITY OF SHORTENING OF THREE FIBRE TYPES FROM HORSE SOLEUS MUSCLE: IMPLICATIONS FOR SCALING WITH BODY SIZE

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SUMMARY

1. To explore how maximum velocity of shortening (V_{max}) of fibres varies within one muscle and how V_{max} varies with body size, we measured V_{max} of muscle fibres from soleus muscle of a large animal, the horse.

2. V_{max} was determined by the slack test on skinned single muscle fibres at 15 °C during maximal activation (pCa = 5·2). The fibre type was subsequently determined by a combination of single-cell histochemistry and gel electrophoresis of the myosin light chains.

3. V_{max} values for the type I, IIA and IIB muscle fibres were $0·33 \pm 0·04$ muscle lengths/s (ML/s) ($\pm$S.E.M., $n = 6$), $1·33 \pm 0·08$ ML/s ($n = 7$) and $3·20 \pm 0·26$ ML/s ($n = 6$), respectively. It is likely that the large range in V_{max} is due to differences observed in the myosin heavy chains and light chains associated with the three fibre types.

4. Comparison of V_{max} over a 1200-fold range (450 kg horse *vs.* 0·38 kg rat) of body mass (M_b) suggests that slow fibres scale more dramatically ($M_b^{-0·18}$) than do fast glycolytic fibres ($M_b^{-0·07}$). This difference may enable the slow fibres to work at high efficiencies in the large animal while the fast fibres can still generate a large mechanical power when necessary.

INTRODUCTION

It has been known for many years that animals have different muscle fibre types. Fibres differ in maximum velocity of shortening (V_{max}) and it is thought that recruiting fibre types with different V_{max}s enables animals to generate force and power efficiently over their full range of movements. Using fish as a model, Rome, Funke, Alexander, Lutz, Aldridge, Scott & Freadman (1988) demonstrated that both fast and slow fibres are necessary: the slow ones enable animals to locomote efficiently at slow swimming speeds, the fast ones at high speeds. This provided

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experimental evidence that V_{max} is an important design parameter of muscle. Rome and colleagues also showed that V/V_{max} (where V is the velocity of shortening of muscle fibres during locomotion) is an effective design constraint; that is, animals switch muscle fibre types so that the fibres powering a movement are shortening at the appropriate V/V_{max}. Thus, to generate a wide range of Vs, the animal must use fibres with a wide range of V_{max}s.

It is therefore of considerable interest to determine the range of V_{max} values that can be called upon within a given muscle and how this varies among animals. Two problems slowed progress in this regard in mammals. First, until recently there was no proven method of determining V_{max} of single fibres. A reliable method for measuring V_{max} in skinned fibres (the slack test) had to be developed (Edman, 1979) and verified (Julian, Rome, Stephenson & Striz, 1986*a*). Second, there existed no totally satisfactory method to determine the fibre type of a skinned fibre following mechanics measurements. The analysis of myosin light chains (MLC) and myosin heavy chains (MHC) by gel electrophoresis does not always differentiate between two subtypes of fast fibres. We have recently developed a technique of exposing serial sections of single skinned fibres to multiple histochemical reactions in combination with gel electrophoresis, which has allowed us to clearly distinguish the three major mammalian fibre types (Sosnicki, Lutz, Rome & Goble, 1989). Thus with these techniques, we have, for the first time, measured V_{max} of three fibre types within a single mammalian muscle.

We studied horse muscle in these experiments to explore implications of the scaling of V_{max} with body size. It was first predicted by Hill (1950) that (a) as body size increases, V of the muscles during locomotion decreases and (b) to keep the mechanical and energetic properties of muscle matched to function, V_{max} should decrease by the same proportion (i.e. so that V/V_{max} remains constant). Hill's model predicts, for instance, a 10-fold difference in V_{max} between rat and horse muscle.

Although V_{max} has been measured in small mammals (mice to cats; see Close, 1972), only recently has it been measured in a larger mammal, man (Fitts, Costill & Gardetto, 1989). To explore the theories of scaling, we made measurements of V_{max} on the largest animal on which it was feasible to work, the horse. Preliminary reports of this work have been published in abstract form (Rome, Sosnicki, Lutz & Goble, 1989*a*, *b*).

METHODS

Preparation

Experiments were conducted on soleus muscle taken from two clinically normal adult thoroughbred horses (average weight = 420 kg). The horse soleus, unlike the soleus of the cat, is heterogenous in fibre type. Analysis of seven fascicles (27–130 fibres/fascicle) from the region (middle) from which the biopsies were taken showed that the soleus muscle is composed of $22{\cdot}8 \pm 1{\cdot}7$% ($\pm$S.E.M. $n = 7$), $43{\cdot}2 \pm 1{\cdot}8$% ($n = 7$) and $34{\cdot}0 \pm 1{\cdot}9$% ($n = 7$) of type I, type IIA and type IIB fibres, respectively.

Muscle biopsies were taken by a veterinary surgeon. Ten minutes prior to anaesthetic induction, each horse was given Xylazine (tranquilizer/analgesic) through a pre-placed intravenous catheter. The hair was clipped from the intended surgical site and a non-sterile presurgical scrub was performed utilizing Povidone Iodine surgical soap. The oral cavity was flushed with tap water to remove any organic matter from previous ingestion of hay or grain.

The horses were placed in a padded anaesthetic induction room following onset of

tranquilization. Ketamine hydrochloride (1 mg/kg) was administered through the intravenous catheter as a bolus dose. Utilizing manual support, the horses were lowered to lateral recumbency as induction occurred. The horse was placed on 6 in thick high-density foam pads to protect and cushion them during the surgical procedure. An inflatable Silastic endotracheal tube was placed orally in the trachea. The endotracheal tube was then attached to a large-animal gas anaesthetic machine to maintain surgical anaesthesia. This was a semi-closed-circle system utilizing halothane with scavenging of excess gases outside of the room.

The intended surgical site was shaved and a sterile surgical field prepared using Povidone Iodine surgical scrub and a final spray of Povidone antiseptic solution. The surgical site was draped with sterile drapes. Fifteen minutes after a horse was anaesthetized, the muscle biopsy was obtained utilizing sterile surgical procedures. The incision was then closed utilizing absorbable polyglycolic sutures in the subcutaneous tissue and non-absorbable polypropylene to maintain skin apposition.

The muscle strip (biopsy; approximately 5 cm in length and 0·5 cm thick) was sutured to a wood applicator stick at its '*in situ*' length and then separated from the muscle. Small strips outside of the sutures were cut and frozen in isopentane cooled with liquid N_2 for subsequent histochemical analysis.

The biopsy was dissected and fibres skinned and stored as in Sosnicki *et al.* (1989) except that a different skinning solution was used (170 mM-K^+, 2·5 mM-Mg^{2+}-ATP, 5 mM-EGTA, 0·1 mM-PMSF (phenylmethylsulphonyl fluoride), 100 mM-imidazole and propionate as the primary anion, at a pH of 7·1; Goldman, Hibberd & Trentham, 1984).

The fibres were activated with the 'Ca^{2+}-jump' method using the same solutions as in Julian *et al.* (1986*a*, *b*). In some experiments, the fibres were osmotically shrunk by addition of 50 g/l Dextran T-500 to all of the solution (Maughan & Godt, 1981).

Experimental apparatus

A Cambridge Technology 300S servomotor was electronically linked to a Cambridge Technology Model 400 force transducer (natural frequency 2 kHz) permitting length steps in < 1 ms and clamping forces in about 7 ms (Rome & Sosnicki, 1990). Force and length signals were recorded on a Nicolet 4094 digital oscilloscope. The servosystem was controlled through an Analog Devices (RT-815) board installed in an IBM-XT computer.

The chamber had a movable spring-loaded central piece with narrow slots containing the three solutions. The fibre was transferred between the solutions as in Julian *et al.* (1986*a*, *b*). The temperature was maintai ed at 15 °C by a peltier device (Rome & Sosnicki, 1990). Sarcomere spacing and fibre diameter was measured by light microscopy as in Julian *et al.* (1986*a*, *b*)

Mechanics measurements

V_{max} was determined using the slack test (Edman, 1979; Julian *et al.* 1986*a*). Based on the technique of Brenner (1983) and Sweeney, Corteselli & Kushmerick (1987), we performed multiple shortening steps during a single activation. Figure 1 shows the length and force record from a typical slack test. The fibre was given a length step which caused the fibre to go slack and the isometric force (P_0) to fall to zero. The fibre then shortened at V_{max} until the slack had been removed and force started to redevelop. If the fibre were relengthened at this point, the stretching of the large number of attached cross-bridges would cause high forces and fibre damage. To decrease the number of attached cross-bridges, the fibre was given an additional length step (when the force had redeveloped to 0·20 P_0) and allowed to shorten at V_{max} for 2 ms. The fibre could then be relengthened to its original length without damage. It was subsequently held there for 2–3 s before being given the next shortening step. This procedure permitted relatively long activations without the deterioration associated with long *isometric* activations.

A series of five length steps (0·06, 0·08, 0·10, 0·12 and 0·14 of the muscle length, L_0) was used for a given slack test (the 0·06 L_0 step was approximately equal to the series elastic component and thus usually unreadable). For each step, the step length was plotted against the time necessary to take up the slack. The slope of the relationship is the V_{max} and the intercept is a measure of the series elastic component. Slack tests were repeated three times (i.e. fifteen to twenty length steps) during each activation. On several fibres, we also performed force clamps. Again the fibre underwent multiple shortenings during single activations. Force stabilized in about 7 ms following release and we read the velocity over the subsequent 10 ms when shortening at high velocities and 20 ms when shortening at lower velocities. For several reasons we found that this technique was not convenient

for determining the V_{max} of the fibres. First, loads often had to be repeated several times because the gain of the servosystem had to be adjusted to obtain good records. Second, in small diameter fibres (i.e. most type I and IIA fibres) we could not go to sufficiently low loads necessary for accurate determination of V_{max} (Julian *et al.* 1986*a*, *b*; note the force clamps shown for the type I fibre in Fig. 4 are from the largest diameter fibre of that type).

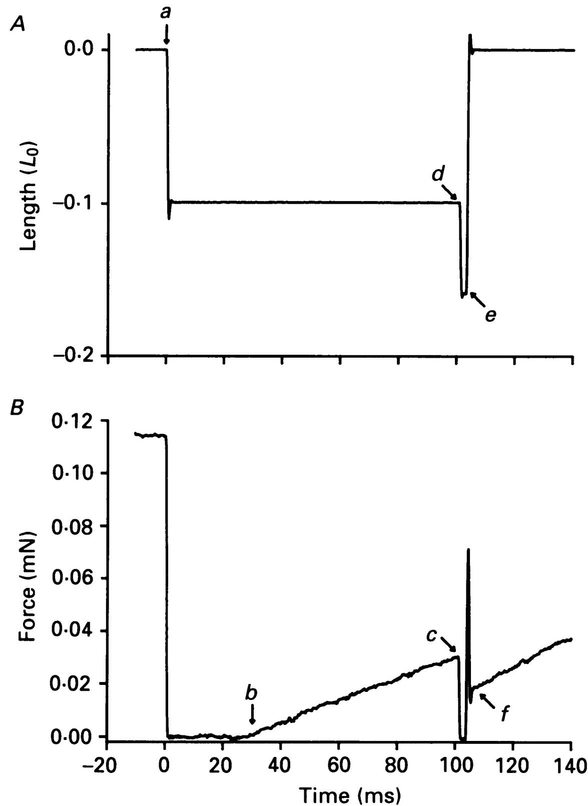

Fig. 1. The step and re-extension cycle during a slack test. The fibre is maximally activated and generating force isometrically prior to *a*. At *a*, the fibre receives a 10% length step which causes the fibre to go slack and force to drop to zero. The fibre shortens at V_{max} until *b* at which point the fibre has removed the slack and started redeveloping force. When the force is redeveloped to 20% of the initial force (*c*), the computer commands the servomotor to impose a second length step (*d*). The fibre is kept at the new length for 2 ms before being re-extended to the initial length (*e*). Over the next several seconds, force redevelops further (*f*) and returns to the initial value. Note that the force record is shifted slightly above the baseline so that it can be viewed more clearly. *A*, length *vs.* time; *B*, force *vs.* time.

Fibre type identification

Fibre type was distinguished by a combination of the histochemical and electrophoretic techniques (see Sosnicki *et al.* 1989). Briefly, single fibres were identified by comparing, on the same slide, the relative intensity in 6 μm sections of Ca^{2+}-ATPase following acid (pH = 4·35) and alkaline (pH = 10·35) pre-incubations and succinic dehydrogenase (SDH) reactions with that of a skinned bundle and a frozen muscle strip. Fibre types were identified as type I = SO (slow oxidative), type IIA = FOG (fast oxidative and glycolytic) and IIB = FG (fast glycolytic) based on the staining patterns.

Segments of the fibres were also analysed for myosin light chains (MLC) with (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) (Sosnicki *et al.* 1989).

Experimental protocol

Fibre length was adjusted to an initial sarcomere length of about 2·5 μm. At this length, resting tension was less than 0·01 P_0. We typically performed the slack test at two pCas: the first at a pCa of 5·75 or 5·5, the second at a pCa of 5·2. We used only V_{max} values determined at the maximally activating pCa of 5·2 for our analysis. In some fibres, we then made force clamp measurements or performed the slack test in Dextran solution. At the completion of the experiment we measured force production at several [Ca^{2+}] (including pCa = 4·4), showing that a pCa of 5·2 gave maximal activation in all fibre types. Following the mechanics experiments, the fibre was removed from the servomotor and its fibre type identified.

The force per cross-sectional area for each fibre was calculated from its width assuming the fibre was circular. Our procedure may have overestimated the cross-sectional area because the fibres were typically mounted so that a relatively large diameter would be measured. Analysis of the transverse sections of small frozen bundles of skinned fibres, however, shows that the fibres are quite circular. In the worst case (i.e. the *largest* diameter measured), this procedure would lead to a 20±0·43% (n = 17), 22±0·51% (n = 19) and 14±0·35% (n = 18) overestimation of the area in the type I, II A and II B fibres, respectively.

Unless otherwise stated, all values are given as means±S.E.M.

RESULTS

Fibre type identification

We were able to identify three distinct fibre types, type I, type II A and type II B, by staining intensities of Ca^{2+}-ATPase and SDH as in our previous study (Sosnicki *et al.* 1989; see this reference for table of staining intensities and plates). As in Sosnicki *et al.* (1989; see reference for gels), we found two myosin light chain patterns, one associated with slow and one associated with fast fibre types. In every case (n = 19), the histochemical identification of slow (type I) or fast (type II A or II B) fibres matched the electrophoretic identification. Because only a small difference in mobility between the type II B and II A myosin heavy chains was previously found (Sosnicki *et al.* 1989), SDS-PAGE of MHC was not performed in this study.

V_{max} *of different muscle fibre types*

The procedure of multiple length steps during a single activation resulted in reproducible slack tests. Slack tests for type I, II A and II B fibres are shown in Fig. 2. At maximal activation, slack tests were linear (average r^2 = 0·99) up to the largest length step used, 0·14 L_0 (in agreement with Metzger & Moss, 1988). V_{max} varied almost 20-fold among different fibres within the same muscle but there was no overlap in V_{max} values between fibre types (Fig. 3). The mean V_{max}s were 0·33±0·04 ML/s (n = 6, range = 0·21–0·48), 1·33±0·08 ML/s (n = 7, range = 1·16–1·72) and 3·20±0·26 ML/s (n = 6, range = 2–3·84) respectively for the type I, II A and II B fibres. The variation in V_{max} within each fibre type is typical of previous data (rat, Eddinger, Cassens & Moss, 1986; rabbit, Sweeney, Kushmerick, Mabuchi, Sreter & Gergely, 1988).

The mean intercepts of the slack tests were 0·056±0·008 L_0 (n = 6), 0·064±0·007 (n = 7) and 0·056±0·006 (n = 6), respectively for the type I, II A and II B fibres. These values are similar to the 0·058 value observed in the frog (Julian *et al.* 1986*a*).

The estimated force per cross-sectional area during maximal activation was

84 ± 10 kN/m^2 ($n = 6$), 97 ± 16 kN/m^2 ($n = 7$) and 120 ± 6 kN/m^2 ($n = 6$), respectively for the type I, type II A and type II B fibres. These values agree well with those of rat (Eddinger *et al.* 1986) but are somewhat smaller than those of rabbit (Sweeney *et al.* 1988) probably because our procedure overestimated cross-sectional area (see Methods).

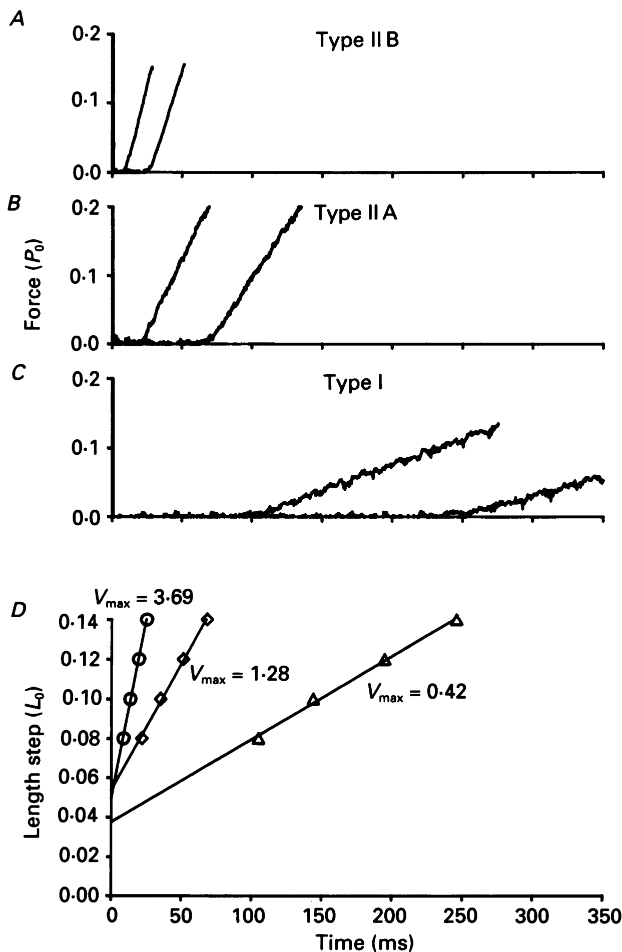

Fig. 2. Slack tests of type II B, II A and I fibres. Panels *A–C* show the force record of three fibres following length steps of 0·08 and 0·14 L_0 given at $t = 0$. Each force record has been normalized for the respective isometric force. Panel *D* shows the results of the slack tests for the same fibres. Length steps are plotted *versus* the time to take up slack. There is a linear relationship ($r^2 = 0·99$ for all three fibres), the slope of which is V_{max} (in ML/s). Note that the time scale in *A–D* is the same and thus the original records correspond to points on the graph. ○, type II B; ◇, type II A; △, type I.

V_{max} as a function of fibre diameter and isometric force

We analysed V_{max} as a function of fibre diameter and force generation to show that the large difference in V_{max} between slow and fast fibres is not a simple artifact of the fibre's relative ability to generate force (i.e. to overcome possible internal resistances

to shortening). Within a fibre type, diameter had no effect on V_{max}. Although on average the type I fibres had smaller diameters than the type II A and II B, there was considerable overlap. Thus a 50 μm diameter fibre could be type I, type II A or type II B and have a V_{max} which varied 10-fold. Figure 3 shows that there is also no

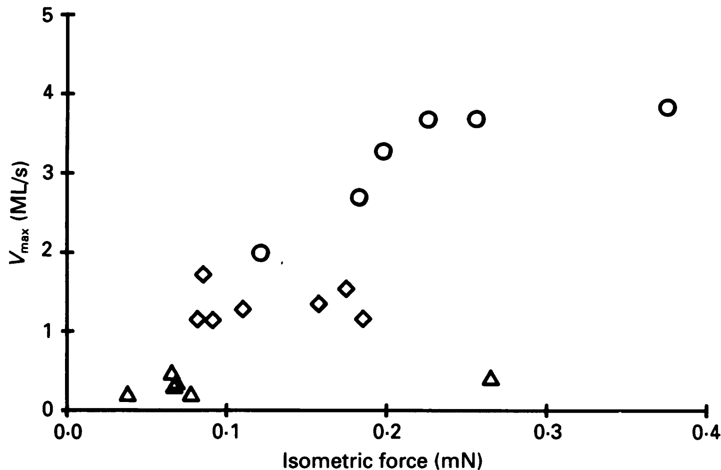

Fig. 3. V_{max} as a function of isometric force generation. For each fibre, V_{max} measured by the slack test is plotted as a function of isometric force generation. There was no correlation between force generation and V_{max} in type I and II A fibres. The correlation in type II B fibres was barely significant at the 0·05 level. Most importantly, a fibre generating a relatively large force (0·25 mN) can have a V_{max} varying 10-fold. ○, type II B (FG); ◇, type II A (FOG); △, type I (SO).

influence of force generation on V_{max} in the type I and II A fibres, and a barely significant influence on the type II B fibres. Most importantly, fibres which generate relatively large forces (0·25 mN) can have V_{max}s which vary over a 10-fold range. Thus, V_{max} is primarily associated with fibre type rather than diameter or force generation.

Influence of filament spacing on V_{max}

During skinning, fibres swell, resulting in increased filament spacing (Godt & Maughan, 1981). Goldman (1987) has suggested that this results in greater curvature in the force–velocity curve at low loads and higher measured V_{max} than occurs in intact fibres. To explore whether this process had a *larger* effect on the type II B than type I fibres, we have examined shortening velocity at low loads using force clamps and measured V_{max} after the filament lattice has been osmotically shrunk to the spacing found in intact fibres (Godt & Maughan, 1981).

Force clamps

Figure 4 shows original records for 0·07 and 0·18P_0 force clamps of a type I and a type II B fibre (same fibres as in Fig. 2). Figure 4*E* shows the plot of the force–velocity relationship as well as V_{max} determined by the slack test. The ratios of the velocities at a given load of the type II B fibre to the type I fibre are 8·78, 9·08 and 7·14 at 0, 0·07 and 0·18 P_0 respectively. The inset in Fig. 4*E* shows the

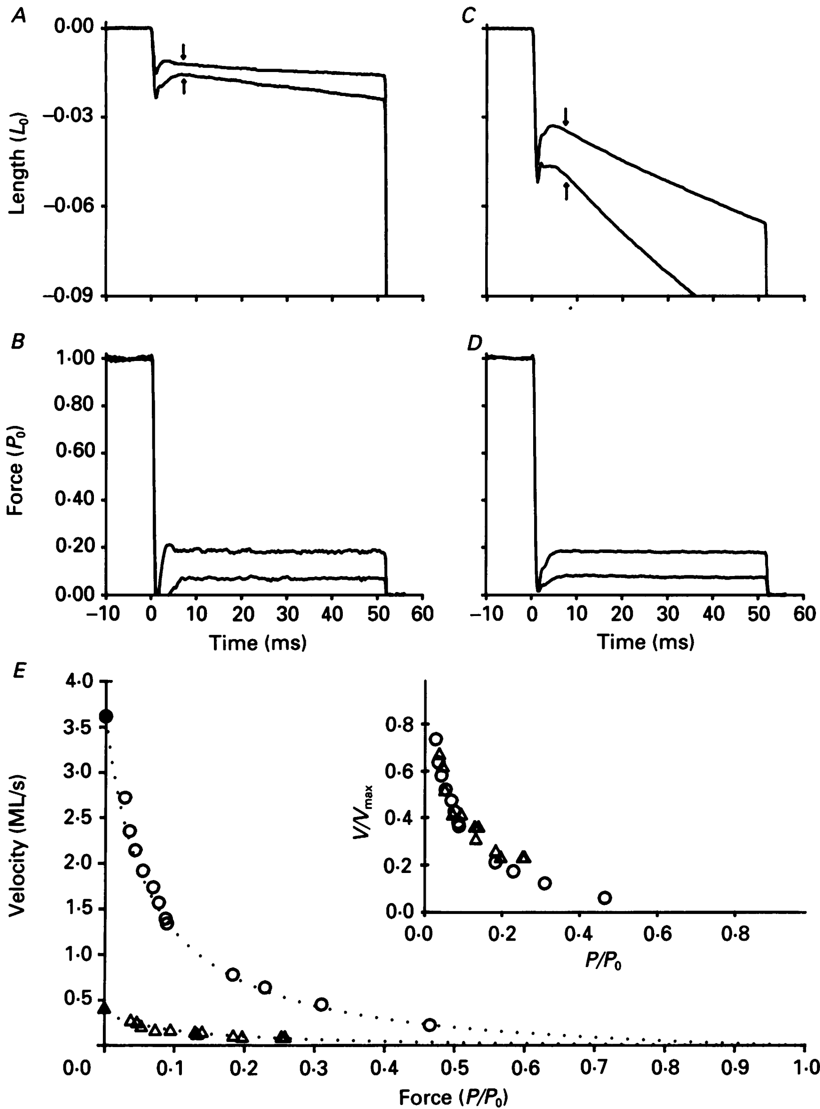

Fig. 4. Force clamps of type I and IIB fibres. Panels *A* and *B* represent the length and force records of force clamps at 0·18 and 0·07 P_0 of a type I fibre. Panels *C* and *D* show the same for a type IIB fibre. Force records have been normalized for the fibres' isometric tension. Likewise length records have been normalized for L_0. The arrows signify the beginning of the measurement period. The initial velocity (to the right of the arrows) was used in the force–velocity curves. Panel *E* shows the force–velocity relationship for these fibres. Note that these fibres are the same as those in Fig. 2. The dotted lines are the hyperbolic fits to the data calculated as in Rome & Sosnicki (1990) to pass through P_0. The V_{max}s determined by the slack tests are marked with the respective symbol, filled. The inset in *E* shows the force–velocity curve of the two fibre types after the velocity has been normalized for V_{max} determined by the slack test. Note the overlap of the points at low loads. ○, type IIB; △, type I.

force–velocity data after normalization for their respective values of V_{max} determined from the slack test. At low loads, the points from the type I and type IIB fibres become superimposed, suggesting that the large difference in V_{max} between slow and fast fibres is not an artifact of a higher curvature of the force–velocity curve in the type IIB fibres at near-zero loads.

V_{max} following osmotic shrinkage

Addition of 5% Dextran T-500 to solutions caused the fibres to shrink in diameter by $18{\cdot}2 \pm 1{\cdot}2$% ($n = 4$) and caused a $14 \pm 2{\cdot}8$% ($n = 4$) decrease in V_{max}. The change in V_{max} associated with osmotic shrinkage was similar in the type I and IIB fibres and thus the ratio of V_{max} of a type IIB to a type I fibre in normal solution (10·3) is hardly altered in Dextran solution (11·3). It appears from our small data set and a more extensive study in rats (Metzger & Moss, 1987*a*) that the large difference in V_{max} between type I and IIB fibres is not a result of a differential influence of filament swelling.

DISCUSSION

V_{max} of three fibre types in one muscle

There was a 10-fold difference in mean V_{max} between the three fibre types that make up the horse soleus muscle. Three lines of evidence suggest that this difference in V_{max} between the fibre types is not due to a mechanical artifact. First, V_{max} doesn't depend on the diameter of or the force generated by the fibre. Second, the shape of the force–velocity curve at low loads is similar among the different fibre types and thus the higher V_{max} of the type IIB fibres is not due to a far greater curvature at low loads. Finally, the difference in V_{max} between slow and fast fibres appears to be unaffected by the osmotic swelling associated with skinning; that is, the difference in V_{max} persists after the fibres are shrunk to diameters found in intact fibres. Excluding these mechanical artifacts, it is probable that the variation in V_{max} is caused by the differences in myosin heavy chains and light chains observed (Sosnicki *et al.* 1989) between different fibre types of the horse. A similar mechanism has previously been proposed in rabbit muscle (Greaser, Moss & Reiser, 1988; Sweeney *et al.* 1988.)

Functional role of different V_{max}s

The 10-fold difference in V_{max} between type I and type IIB fibres is more than the 3- to 5-fold difference in V_{max} between type I and type IIB fibres of small animals (Eddinger *et al.* 1986; Sweeney *et al.* 1988). This result poses interesting questions: what is the functional role of such a large difference and why is there a larger range in the horse than in smaller animals?

Using carp as a model, Rome and colleagues provided experimental evidence that V_{max} is an important design parameter and that V/V_{max} is an effective design constraint (Rome *et al.* 1988; Rome & Sosnicki, 1990). Thus, given the V at which the animal's muscle must shorten during locomotion, V_{max} is adjusted by recruitment so that the fibres are used over a narrow range of values of V/V_{max}. Although the 10-fold difference in V_{max} of horse fibres seems large, carp employ a 10-fold range in *effective*

V_{max} (2·6-fold change in V_{max} and 4-fold change in mechanical advantage) to power a 20-fold range of speed of movement while maintaining a V/V_{max} of 0·2–0·4. At present, the V/V_{max} of horse muscle fibres cannot be evaluated because the range of V is not known.

Implications for scaling

To understand why there may be a larger range in V_{max} in the horse than in small animals, it is useful to examine scaling of V_{max} with body size. Hill (1950) first hypothesized that the V (at the level of the sarcomere) at which the muscle shortens during locomotion should scale in proportion to limb length or to $M_b^{-0.33}$, where M_b is body mass. Because he believed that muscle should be used over the same range of V/V_{max}, Hill predicted that V_{max} should also scale with $M_b^{-0.33}$. Alternatively, McMahon (1975) suggested that V at a physiologically equivalent speed (trot–gallop transition) and V_{max} should both scale with $M_b^{-0.125}$. Based on a combination of anatomical and physiological measurements, Lindstedt, Hoppeler, Bard & Thronson (1985) calculated that V scales with $M_b^{-0.23}$, and similarly reasoned that V_{max} should scale with $M_b^{-0.23}$.

Before our study, the only experimental evidence for scaling of V_{max} had been based on data from whole EDL (extensor digitorum longus) and soleus muscles of small animals (mouse to cat). Based on Close's (1972) results, McMahon (1975) calculated that V_{max} of the EDL muscle (fast) scaled with $M_b^{-0.145}$ and V_{max} of the soleus muscle (slow) scaled with $M_b^{-0.194}$. The relatively small size range (100-fold) over which V_{max} was measured, and the problems associated with measuring V_{max} in a muscle containing more than one fibre type, make the validity of the scaling exponents questionable.

We can now obtain a 1200-fold size range by comparing V_{max} measured on the horse (full adult $M_b = 450$ kg) to that previously measured on the rat (full adult $M_b = 0.38$ kg; Eddinger *et al.* 1986) and on the rabbit (full adult $M_b = 5$ kg; Reiser, Moss, Giulian & Greaser, 1985; Moss, 1986) at the same temperature and using similar techniques. There is a relatively small effect of scaling on the type IIB fibres. The V_{max}s of type IIB fibres from rat, rabbit and horse are 5·38, 4·44 and 3·2 ML/s, respectively. Figure 5 shows that there is a linear relationship between log V_{max} and log M_b ($V_{max} = 5.01\, M_b^{-0.073}$). There appeared to be a larger scaling effect on the slow (type I) fibres. V_{max}s of type I fibres from rat, rabbit and horse are 1·16, 0·76 and 0·33 ML/s, respectively. Figure 5, again, shows a linear relationship but with a larger exponent ($V_{max} = 0.99\, M_b^{-0.179}$). The scaling of type IIA fibres is less certain because there are values for only two species: rat (5·27 ML/s; Metzger & Moss, 1987*b*) and horse (1·33 ML/s). These values suggest a similar scaling exponent (−0·194) to that of type I fibres. Recent V_{max} data from humans (4·85 and 0·86 ML/s for type IIB and type I, respectively; Fitts *et al.* 1989) fall above the scaling relationships in Fig. 5 (i.e. their V_{max} values are greater than those of rabbit). This discrepancy may represent a difference in scaling between bipedal and quadrupedal animals, or alternatively, differences in technique between laboratories (e.g. rat V_{max} data of Gardetto, Schluter & Fitts (1989) is 38–50% faster than those of Eddinger *et al.* 1986).

More definitive results will be obtained by analysing a greater number of species and by comparing V_{max} values measured in the same laboratory under *precisely* the

same conditions. None the less there appears to be a distinct difference between scaling of V_{max} of type IIB and I fibres and this difference is the reason for the larger range of V_{max} values found in large animals.

This difference in scaling exponent between type I and type IIB fibres may reflect the different biological constraints put on the use of the different fibre types. Slow

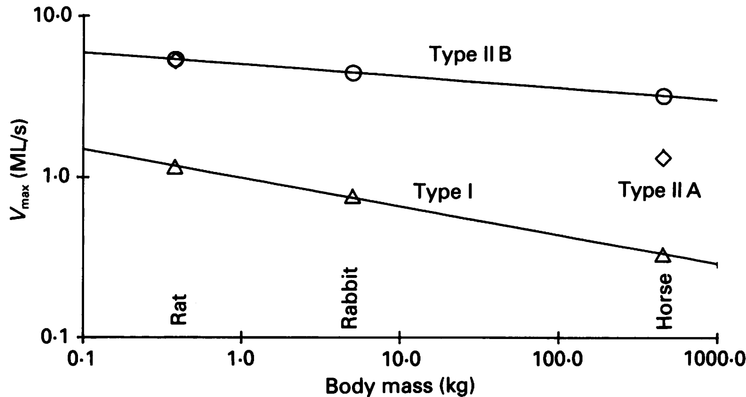

Fig. 5. Scaling of V_{max} with body size. V_{max} determined in skinned fibres at 15 °C using the slack test is plotted as a function of full adult body mass (S.E.M. for each point is smaller than the symbol). When plotted on log scales, there is a linear ($r^2 = 0.99$) relationship for both the type IIB and I fibres. The equations describing the relationship are $V_{max} = 5.01\, M_b^{-0.073}$ for the type IIB fibres and $V_{max} = 0.99\, M_b^{-0.179}$ for the type I fibres. The rat data is from Eddinger *et al.* (1986) and the rabbit data is from Reiser *et al.* (1985) and Moss (1986). The rat IIA data is from Metzger & Moss (1987*b*).

oxidative fibres are used continuously during locomotion so that their V_{max}s must be precisely matched to V to give maximal efficiency. Indeed, V_{max} has nearly the same scaling exponent as stride frequency at the trot–gallop transition ($M_b^{-0.15}$; Heglund & Taylor, 1988). Because sarcomere excursion appears to be nearly independent of body size (Lindstedt *et al.* 1985) this suggests that V scales in a similar manner to V_{max} thus keeping V/V_{max} constant. Fast glycolytic fibres, on the other hand, are probably used infrequently, only when the horse is jumping or running at maximum speed. In this case there is a sacrifice of efficiency for increased mechanical power. Because the mechanical power output is higher in fibres with a high V_{max} than with a low one, a smaller scaling factor for the type IIB fibres may be beneficial.

The scaling of V_{max} of type I fibres may also explain the increased energetic cost of locomotion per gram of tissue with decreasing body size ($M_b^{-0.30}$; Taylor, Heglund & Maloiy, 1982). Comparison of our results to those of smaller animals shows that force generation per cross-sectional area of a given fibre type is independent of body size. Thus the same volume of muscle must be recruited during locomotion in different sized animals (Biewener, 1989). It has been proposed by Taylor, Heglund, McMahon & Looney (1980) that the increased energetic cost of locomotion in small animals is due to the increased cost of generating force in their fibres. Our finding that small animals have a higher V_{max} combined with previous findings that force generation is more costly for fibres with high V_{max}s (Barany, 1968; Crow & Kushmerick, 1982, 1983), supports their hypothesis. Although the scaling exponent

for V_{max} is smaller than that for energetic cost of running, if the energetic cost of generating force increases more rapidly than V_{max} (Rome & Kushmerick, 1983), then scaling of V_{max} may provide a quantitative explanation of the increased locomotor costs of small animals. Direct measurements of the influence of scaling on the ATP usage during isometric tension generation must be performed to answer this fully.

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