Shortening Velocity and Power Output of Skinned Muscle Fibers from Mammals Having a 25,000-fold Range of Body Mass

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ABSTRACT The shortening velocities of single, skinned, fast and slow skeletal muscle fibers were measured at 5–6°C in five animal species having a 25,000-fold range of body size (mouse, rat, rabbit, sheep, and cow). While fiber diameter and isometric force showed no dependence on animal body size, maximum shortening velocity in both fast and slow fibers and maximum power output in fast fibers were found to vary with the $-1/8$ power of body size. Maximum power output in slow fibers showed a slightly greater ($-1/5$ power) dependence on body size. The isometric force produced by the fibers was correlated ($r = 0.74$) inversely with fiber diameter. For all sizes of animal the average maximum velocity was 1.7 times faster in fast fibers than in slow fibers. The large difference in mechanical properties found between fibers from large and small animals suggests that properties of the contractile proteins vary in a systematic manner with the body size. These size-dependent changes can be used to study the correlations of structure and function of these proteins. Experimental results also suggest that the different metabolic rates observed in different sizes of animals could be accounted for, at least in part, by the difference in the properties of the contractile proteins.

INTRODUCTION

It has been known since the time of Galileo that small animals have higher power/weight ratios than large animals (for discussion, see McMahon, 1984). Several observations of animal ability suggested that the muscle power/weight ratios vary in the same proportion as the body length/weight ratios (Hill, 1950; Heglund et al., 1974; McMahon, 1977) and that both ratios vary with (body weight)$^{-0.25}$ (for a review see Ford, 1984). The present study was undertaken for two reasons. The first was to determine the extent to which the size dependence of the shortening velocity and power output of the whole animals can be correlated with the same properties of the myofibrils. The second reason was to define the mechanical properties of the

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contractile proteins that have different shortening abilities. Other laboratories are working to produce mutations of muscle proteins that will alter the contractile properties of muscle (Peckham et al., 1989; White and Sparrow, 1989). By comparing the structural and functional properties of the normal and mutant muscle, they hope to gain further insight into the mechanism of force generation by actomyosin. The animal size dependence of the power/weight ratio suggests that natural selection may have already provided mutations of actomyosin with very specific alterations of function. A possible advantage of studying these naturally occurring variations is that the power/weight ratios vary smoothly over a wide range of animal size, suggesting that natural selection might have provided relatively fine gradations of shortening velocity and power output among animals of different sizes. Accordingly, we examined the maximum shortening velocity and maximum power output of skinned fibers from a group of animals spaced about a decade apart in weight, and having a 2.5×10^4-fold size range.

It is well known that skeletal muscle contains at least two isoforms of myosin (Close, 1972) and that the intrinsic shortening speed of the muscle varies with the relative proportion of these isoforms. In the present experiments the possibility of varying proportions of isoforms had to be considered as a cause of the variations in the velocity of muscle taken from different animals. Ideally, the individual fiber used in mechanical experiments would have been assayed for isoform composition and the velocities rated accordingly. Unfortunately, this was technically impossible for us. The assays available to us require much larger tissue samples than are present in the single fiber. Since the isoform composition may vary among fibers in the same muscle, assays of muscle bundles would not accurately reflect the composition of the individual fibers. For this reason we chose instead to make a functional distinction. If fibers from muscle that contains either predominantly fast or predominantly slow isoforms retain the same speed relative to each other across species, it would seem unlikely that this difference in speeds among species is due to changes in the relative proportions of the isoforms. Accordingly, we compared fibers taken from fast (usually extensor digitorum longus [EDL]) and slow (usually soleus [SOL]) muscles in all animals.

METHODS

Preparation

Animals. The animals used in the experiments (mice, rats, rabbits, sheep, and cows) were at a stage of puberty or early adulthood. Mice and rats were killed by decapitation. Rabbits were first anesthetized with intramuscular injections of 50 mg ketamine and 20 mg xylazine, followed by an intracardiac injection of saturated KCl solution to stop the heart. Muscle tissues from sheep and cows were obtained from local abattoirs immediately after the animals were slaughtered. Table I lists some general characteristics of the animals.

Dissection of muscle bundles. Strips of fast and slow muscles (~10–20 mm long and 1 mm in diameter) were removed from the hindlimbs of the animals, tied to glass rods, and kept overnight at 0°C in skinning solution (see Solutions). In small laboratory animals the fast EDL

and the slow SOL muscles were easily identified, but in larger animals the identification of specific muscles was less certain, as described below.

Tissue storage. After the overnight skinning at 0°C in skinning solution, the tissue was soaked for 2 h in a solution containing 25% (by volume) of glycerol and 75% of relaxing solution (see Solutions) at 0°C. The tissue was transferred to the storage solution containing equal portions of glycerol and relaxing solution. The pH of the storage solution was set to 6.5 to provide a pH of 7.0–7.5 at the storage temperature of −20°C. The precise pH of the solution below the normal freezing temperature was uncertain.

Dissection of single fibers. Every few days a muscle strip was briefly taken out of the storage freezer to have a small bundle of fibers dissected free. The bundle was then separated into single fibers (~2.5 mm long) in a dissecting tray containing glycerol and relaxing solution under a binocular microscope. The dissecting tray was maintained near 0°C by circulating coolant. Aluminum foil clips (Ford et al., 1977) were attached to the fiber segment at the ends, leaving an average fiber length of ~1.5 mm between the clips. Some clipped fibers were used immediately, while others were stored at −20°C in the glycerol relaxing solution for several days.

Fiber mounting. The fiber was held horizontally in a covered trough into which precooled solution could be injected (Chiu et al., 1985). Both ends of the clipped fiber were connected to

TABLE I

Animal Strain and Age

Species	Strain	Age	Weight
		mo	*kg*
Mouse	CBA/J	1.5–2	0.02
Rat	Sprague-Dawley	3	0.2
Rabbit	New Zealand White	2.5	2
Sheep	Unknown	8–9	55
Cow 1	Holstein	4	160
Cow 2	Angus-Herford	18	500

Ages and weights are approximate.

the wire hooks extending from the force-transducer and servo motor (see Apparatus). The trough was kept at 5–6°C by circulating coolant during the experiment. Solution change in the trough was accomplished by injecting solution into one end of the covered trough and removing solution from the other end by suction.

Apparatus

Except for the sarcomere length sensor, the apparatus has been described previously in detail (Ford et al., 1977; Chiu et al., 1982, 1985).

Sarcomere length sensor. Changes in sarcomere length were monitored in ~50% of the fibers used in the experiments. Sarcomere length change was measured by tracking the position of the first order diffraction pattern of a laser beam passed through the fiber. The incident angle of the laser beam was set to one-half the expected diffracted angle to maximize the Bragg angle effect (Goldman and Simmons, 1984). A series of cylindrical lenses were used between the laser source and the fiber to draw the beam along the fiber axis and narrow it onto the fiber diameter. A single cylindrical lens above the fiber narrowed the beam onto the

photosensor (LSC/5D; United Detector Technology, Hawthorne, CA). This system assured that all of the sarcomeres were included within the laser beam and that much of the beam was narrowed onto the fiber diameter. A resistor shaping network was used to convert the position of the first order beam to a linear function of sarcomere length over the region of 2.27–2.5 μm (Goldman and Simmons, 1984). The system was calibrated using the 10th and 11th orders of an eyepiece reticle having 40 divisions/mm. Because the sensitivity of the sensor varies inversely with the background light scattered from the zero order beam, which was not well controlled, the calibration was not exact with the fiber in place. The system was, therefore, used to measure only the relative changes in sarcomere length and not the absolute changes.

Data recording. Analog signals from the force-transducer, servo motor, and sarcomere length sensing device were converted to digital signals with the use of a Labmaster interface board (Tekmar Co., Cincinnati, OH) in an IBM PC computer. Experiment timing and control were provided by the SALT software package (Fenster and Ford, 1985; Wirth and Ford, 1986).

Solutions

All solutions except storage solution were adjusted to pH 7.0 at 25°C.

Skinning solution contained 150 mM K-propionate, 1 mM $MgCl_2$, 5 mM Na_2ATP, 5 mM K_2EGTA, 0.1 mM PMSF, and 10 mM imidazole.

Relaxing solution for storage had the same composition as the skinning solution except that PMSF was not added and it contained 6 mM instead of 1 mM of $MgCl_2$ and 5 mM NaAzide.

Solutions for experiment all contained 90 mM K-propionate, 10 mM imidazole, 6 mM $MgCl_2$, 5 mM Na_2ATP, and 20 mM creatine phosphate (PCr). In addition, there was 5 mM K_2EGTA in "relaxing" solution, 0.1 mM K_2EGTA in "rinse" solution, and 5 mM EGTA plus 4.95 mM $CaCl_2$ in "activating" solution. Dextran T-70 was added in a concentration of 76 g/liter to compress the skinned fibers to their physiological diameter (Godt and Maughan, 1977). The appropriate amount of dextran added to the solution was suggested by our recent study (Ford et al., 1990) showing that velocity achieves a plateau at concentrations between 56 and 105 g/liter. Dextran was used because it does not contain an acid impurity, as does polyvinylpyrrilidone (Godt and Maughan, 1977). Also, the T-70 size dextran is sufficiently large that it does not appear to penetrate the filament lattice (Godt and Maughan, 1977), and it is much less viscous than larger size dextran.

Experimental Protocols

A fiber was mounted horizontally in the trough in relaxing solution and the sarcomere spacing was adjusted so that it would be 2.4–2.5 μm when the fiber was activated. Activation was achieved by injecting precooled activating solution into the trough. To avoid dispersion of sarcomere pattern, the fiber was subjected to repetitive ramp shortening and rapid restretch every 2.5 s (Brenner, 1983; Sweeney et al., 1987).

Isotonic steps. The method for obtaining force–velocity data is illustrated in Fig. 1. Isotonic steps were imposed on the activated fiber in groups of nine. The first five steps proceeded from the lowest to the highest load, and the last four proceeded back to the lowest load. For fast fibers the time interval between steps was 2.5 s, long enough for the tension to recover to a plateau. For the cow slow fibers, the time interval was 5 s. The duration of the isotonic steps was 150 ms for all fibers except the slow fibers from cows, for which a duration of 300 ms was used. This longer period was used because the fibers displayed very slow velocity transients. Usually four sets of nine isotonic steps were conducted to collect data for a single force–velocity curve, but the number of sets varied from two to six. The fibers were relaxed and reactivated between two sets of nine steps.

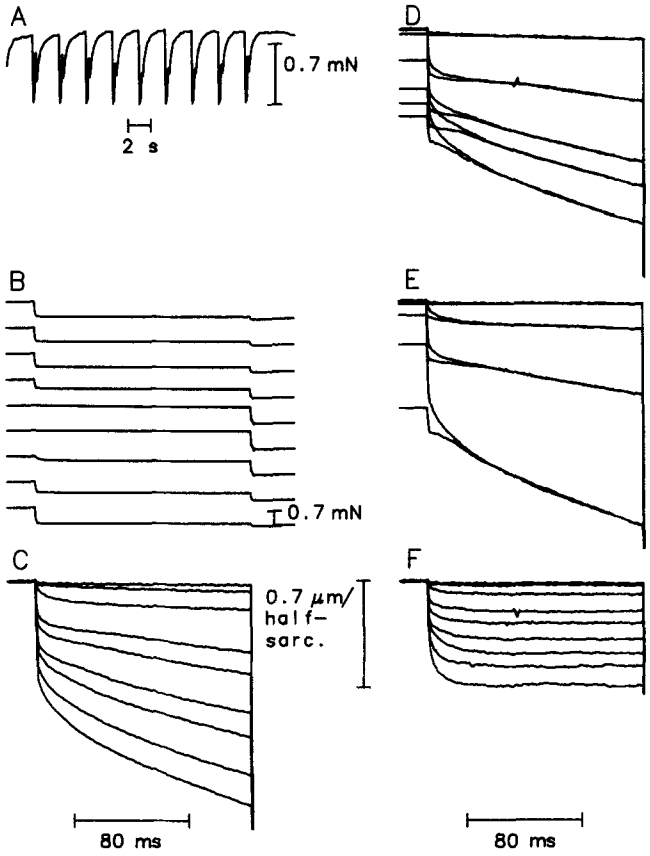

FIGURE 1. Physiological parameters recorded during a set of nine consecutive isotonic steps. (*A*) Force record on the time scale indicated to show isometric force recovery after each step. The fiber was maximally activated throughout. Recording began immediately after a ramp release and quick restretch. (*B*) Force recorded on a fast time scale during the isotonic steps. The first 20 ms of the record indicates the isometric force before the isotonic steps. The isotonic period lasted 150 ms and was followed by 30 ms of recording after the fiber was made slack by a quick length release of 0.3 μm per half-sarcomere length. The zero force baseline was recorded during the last 20 ms of this slack period. The time sequence in which the steps were applied is shown in order from top to bottom in these force traces. (*C*) Fiber length during the isotonic steps. Steps to make muscle go slack are off scale. (*D* and *E*) Sarcomere length records superimposed on the overall muscle length records for the first five (*D*) and the last four (*E*) isotonic steps. The fiber length traces are the same as in *C*. The sarcomere length traces are scaled to match the fiber length traces during the steady state of shortening. (*F*) Difference between fiber length and sarcomere length. Temperature 5–6°C. Exp. G2589.012.

Data Analysis

All the force–velocity data for a single fiber were fitted to the Hill (1938) hyperbolic equation using a nonlinear least-squares (Newton-Raphson) technique to determine the maximum velocity and relative maximum power (maximum power divided by the extrapolated isometric

force). Isometric force was determined as the force immediately before the step. In fitting the force–velocity data to the Hill equation, the data points where isotonic force fell below 2.5% of the isometric level were excluded because there was a great deal of scatter in data obtained at these low loads.

For statistical purposes the values from each fiber were treated as a single observation. These single observations were then averaged to obtain a mean value for each animal, which in turn was used as a single observation in assessing the size dependence of each parameter. This averaging was done because there was substantial variation in the numbers of fibers studied in each animal. If the values from each fiber were used individually, undue weight would have been given to a few fiber types (e.g., sheep fast muscle).

Significance of the slopes of the linear regression lines was tested at the 95% confidence level by the t test. In situations where multiple means were compared, Duncan's multiple range test was used.

Use of sheep fibers as a standard. A total of 181 skinned single fibers from five animal species were used in this study. After preliminary studies it took about two months to collect the final data presented here. To monitor the constancy of the experimental conditions during that period, sheep fast fibers were used as a "standard." We chose the sheep fiber because it seemed to be more robust than the others. Data from sheep fast fibers were collected throughout the experimental period on six separate dates. The force–velocity parameters showed no significant difference (Duncan's multiple range test) among the six groups of data. This procedure helped us to identify a several-day period of cooling malfunction when both temperature and shortening velocity varied widely. Data collected during that period were discarded.

Notes on Dissection

The EDL and SOL muscles in small animals (mouse, rat, and rabbit) were easily identified on the basis of their function and anatomy. In addition, the EDL was much paler than the SOL. Identifying the same muscles in sheep and cow was more difficult in the slaughterhouses. When the animals became available, their hooves had been removed and the carcasses were hung by the Achilles' tendons. Extensive dissections were not possible. Although all the muscles of the larger animals were redder than those of the smaller animals, the fast muscle was recognizably paler than the slow muscle. In general, we attempted to take samples from the EDL and SOL muscles of the animals, but in the case of the one large cow that we were able to dissect the presumed EDL was slower and redder than the presumed SOL muscle. For this reason we refer to the muscles as "fast" and "slow" rather than EDL and SOL muscles.

We encountered in great deal of difficulty in obtaining specimens of cow muscle because the local abattoirs are large assembly line factories. The factory foremen would not let us interrupt their operations long enough for an adequate dissection. Only once were we successful in gaining access to a large cow. From this animal we obtained several samples of what we believed were EDL and SOL muscles, but as explained above, the muscles were reversed in their expected speeds and appearances. We later also obtained samples from smaller "veal." These smaller animals were nearly sexually mature and very near their adult weight, although they were about one-third the size of the larger cows. We were also told that they had been raised in pens and we found that their muscle fibers were of recognizably smaller diameter than those of the large cow, suggesting that the smaller animal might have had less well-developed muscles, either because of less exercise or because they were less mature.

Because both the small cow and the young mice yielded small fibers, we developed the suspicion that fiber diameter might be correlated with the animals' age or activity. To examine this hypothesis, for the purpose of control experiments on diffusion, we dissected muscles from two 2-yr-old male mice that had been used as studs in a breeding colony. The diameter of the fast fibers from these animals were slightly but not significantly smaller (mean = 50.2 μm ±9.0

SD, $n = 24$) than the fast fibers from the younger mice used for the main part of the study (mean = 53.2 μm ±9.9 SD, $n = 11$), suggesting that the small fiber diameter, at least in mice, was not correlated with immaturity or inactivity.

We dissected samples from four separate bundles of SOL muscle from sheep. All four of these were redder than the bundles taken from EDL, but only one consistently yielded fibers that were substantially slower than those taken from the EDL. All of the data presented for slow sheep fibers here were taken from that one bundle.

RESULTS

Velocity Transients

Velocity transients were observed in many of the length records and were most prominent at intermediate loads, as described previously (Ford et al., 1990). At lighter loads the transients may be obscured by recoil of a damped series elastic element in the fiber preparation. To determine the effect of these transients on velocity measurements, we made simultaneous records of sarcomere shortening and overall muscle shortening in about half the fibers used in the experiments. Velocity transients were most prominent in the sarcomere length records, especially at low loads (Fig. 1).

Damped Series Elastic Recoil

In Fig. 1, *D* and *E*, the sarcomere length traces have been scaled so that they match the fiber length traces at 50 and 142 ms of shortening. During this period of steady isotonic shortening the traces match very closely. Mismatch of the two traces occurs mainly within the first 30 ms of shortening. This early disparity is better illustrated in Fig. 1 *F*, where the difference between the two records is plotted. As shown, the difference declines to zero at a progressively declining rate over the first 30 ms of shortening. This pattern can be explained by damped recoil of a series elastic element in the preparation, possibly at the two ends where the fiber was crushed in the clips. As shown in Fig. 1, *D* and *E*, the transients can be quite prominent at low loads, even though they are not obvious in the fiber length record.

Force–Velocity Measurements

The velocity transient consisted of a period of rapid shortening during and immediately after the step (phases 1 and 2), and a period of very slow shortening (phase 3) followed by a quasi-steady state (phase 4) (see Huxley and Simmons, 1973, for a description of these phases). Shortening was not constant during phase 4, but declined progressively (Brenner, 1986). To make comparable measurements in different fibers and different loads, we made force–velocity measurements early in phase 4 under all conditions.

The velocity measurements were made on the fiber length records because they were less noisy and more accurate than the sarcomere length records. The latter records were used to assess the validity of this practice. The durations of the velocity transients were different both for different fibers and for different isotonic loads. This load and species dependence is illustrated in Fig. 2, which shows that the transients lasted longer with heavier loads, and that the faster fibers had briefer transients. To

accommodate these differences and accurately measure the velocity in different fibers shortening under different loads, the time of the velocity measurement was individualized for each condition. The time of measurement for each fiber type and load range is shown in Table II. For simplicity, the isotonic forces were divided into four separate ranges for each type of fiber and the time of measurement was determined by the load range for each fiber type. The object of this procedure was to make the force–velocity measurements early in phase 4 of the transients. The durations of the intervals over which the velocities were measured in the four load ranges were also different. They were 18, 20, 20, and 66 ms, respectively, for force ranges 1–4. These time intervals were centered around the time points given in Table II. The purpose

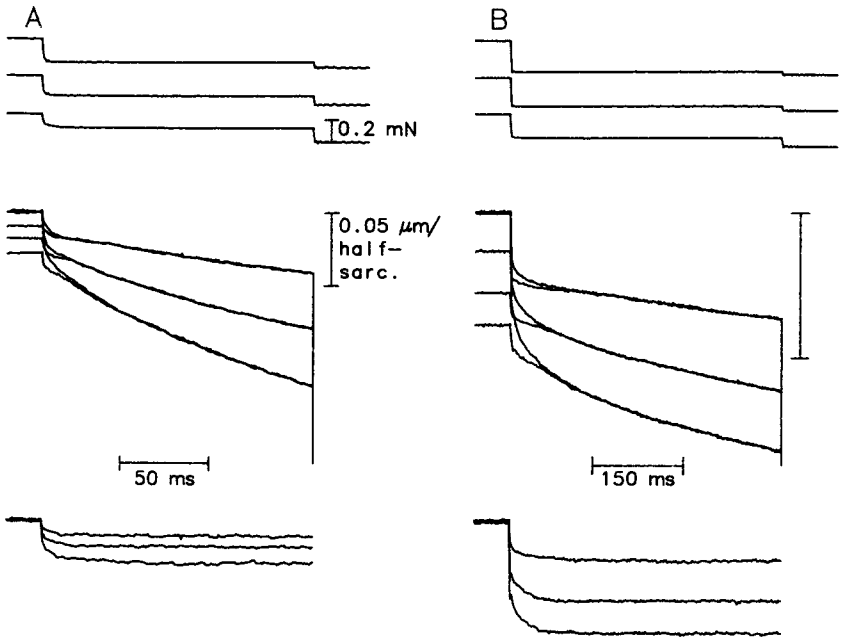

FIGURE 2. Superimposed fiber length traces and sarcomere length traces for rat fast fibers (*A*) and cow (cow 2) slow fibers (*B*). The velocity transients are briefer with light loads and with fast fibers.

of lengthening the interval was to reduce noise in the low velocity measurements made at higher loads. Shorter intervals were used for traces obtained at low loads because the traces were more curved.

An observation made after examining the experimental records was that the damped series elastic recoil for all types of fibers lasted ~30 ms. This implies that accurate measurement of the shortening velocity cannot be made before 30 ms after the onset of the isotonic step. For fibers with a very high speed of shortening, such as rat EDL, the velocity transient was finished in <30 ms (Fig. 2 *A*). Therefore, measurements taken after 30 ms would give velocities later in phase 4 and underes-

TABLE II

Times for Measuring Force–Velocity Data

		Force range 1	Time	Force range 2	Time	Force range 3	Time	Force range 4	Time
		%Po	*ms*	*%Po*	*ms*	*%Po*	*ms*	*%Po*	*ms*
Mouse	Fast	2.5–40	20	40–60	30	60–70	70	70–100	133
	Slow	2.5–30	35	30–40	50	40–50	80	50–100	133
Rat	Fast	2.5–35	30	35–50	45	50–60	80	60–100	133
	Slow	2.5–25	40	25–35	60	35–45	80	45–100	133
Rabbit	Fast	2.5–40	35	40–50	45	50–60	60	60–100	133
	Slow	2.5–20	50	20–30	70	30–40	100	40–100	133
Sheep	Fast	2.5–20	45	20–30	55	30–40	80	40–100	133
	Slow	2.5–10	45	10–20	55	20–30	80	30–100	133
Cow 1	Fast	2.5–25	40	25–35	60	35–45	80	45–100	133
	Slow	2.5–10	45	10–25	60	25–35	150	35–100	266
Cow 2	Fast	2.5–30	40	30–40	70	40–50	85	50–100	133
	Slow	2.5–10	55	10–15	65	15–20	50	20–100	266

The time values given represent midpoints of the time intervals. Duration of the measurement intervals were 18, 20, 20, and 66 ms, respectively, in ranges 1–4.

timate the initial steady-state velocity. If the measurements were taken earlier, apparent velocity would be increased by the damped series elastic recoil. With the faster fibers velocity was measured slightly before 30 ms, when the two opposing effects on velocity would approximately cancel each other. For most fibers the velocity measurements were taken much later than 30 ms and were not likely to be influenced by the damped recoil of the series elastic element.

An example of a force–velocity curve is shown in Fig. 3. The data points were collected from a single sheep fast fiber during five sets of nine isotonic steps, including the set shown in Fig. 1. The two values obtained from the plotted curves were the maximum velocity, determined by extrapolating the force–velocity curve to

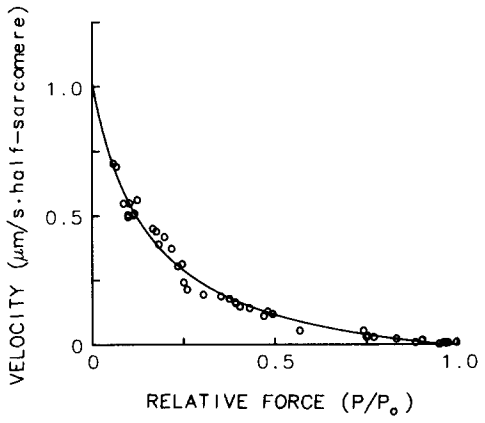

FIGURE 3. Typical force–velocity curve from a single sheep fast fiber. Data taken from five groups of nine isotonic steps, including the set shown in Fig. 1.

zero load, and relative maximum power obtained by interpolation among the force–velocity points. This latter value is a good measure of the shortening velocity at intermediate loads (Podolin and Ford, 1986). Maximum velocity, maximum power, and relative maximum power all showed significant dependence on body size (Figs. 4 and 5). The slope of the linear regressions fitted through the mean logarithmic value of each parameter vs. the logarithmic values of body size gives an estimate of this

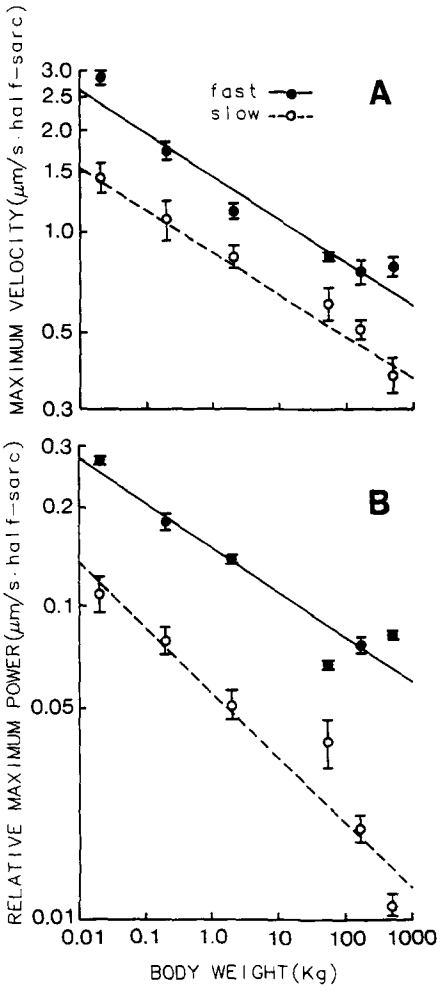

FIGURE 4. (*A* and *B*) Maximum shortening velocity and relative maximum power vs. body size on logarithmic scales. Filled and open circles represent the mean values for fast and slow fibers, respectively. Error bars represent SEM. Equations for the linear regression lines and the correlation coefficients (r) are listed in Table III.

dependence in terms of the power of body size. The slopes of the lines relating maximum velocity for both fast and slow fibers and maximum power (both absolute and relative) for fast fibers differed by $< 5\%$. These parameters varied almost exactly with the $-1/8$ power of body size. There was a slightly steeper dependence of absolute and relative maximum power in slow fibers, varying approximately with the $-1/5$ power of body size. However, this slope was not significantly different from $-1/8$. All

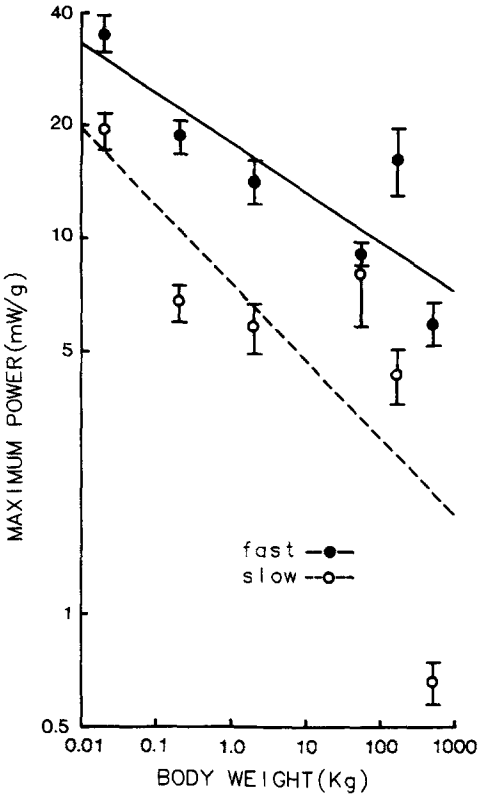

FIGURE 5. Maximum power output vs. body size on logarithmic scales. Filled and open circles represent the mean values for fast and slow fibers, respectively. Error bars represent SEM.

TABLE III

Summary of Linear Regression on Experimental Data

Plot	Equation	Type	a	b	Coefficient (r)	Significant difference of b from 0	Significant difference of b from $-1/4$
Maximum velocity (V_{max}) vs. body weight (W)	$V_{max} = aW^b$	Fast	1.49	−0.126	0.97	$P < 0.05$	$P < 0.05$
		Slow	0.94	−0.130	0.98	$P < 0.05$	$P < 0.05$
Maximum power (PV_{max}) vs. body weight (W)	$PV_{max} = aW^b$	Fast	17.8	−0.129	0.85	$P < 0.05$	$P < 0.05$
		Slow	7.6	−0.205	0.74	$P < 0.05$	NS
Maximum relative power (PV'_{max}) vs. body weight (W)	$PV'_{max} = aW^b$	Fast	0.15	−0.132	0.96	$P < 0.05$	$P < 0.05$
		Slow	0.06	−0.187	0.96	$P < 0.05$	NS
Isometric stress (σ) vs. body weight (W)	$\sigma = aW^b$	Fast	141.1	0.020	0.02	NS	—
		Slow	151.6	−0.03	0.25	NS	—
Fiber diameter (D) vs. body weight (W)	$D = aW^b$	Fast	60.8	0.007	0.20	NS	—
		Slow	54.3	0.036	0.375	NS	—
Isometric stress (σ) vs. fiber diameter (D)	$\sigma = a + b \cdot D$	Fast + Slow	320.2	−2.70	0.74	$P < 0.05$	—

a and b are constants obtained from curve fitting.

the slopes are significantly different from zero ($P < 0.05$). A summary of the slopes, correlation coefficients, and P values for the linear regressions are presented in Table III. The equations used in the curve fitting and the test for significance against zero and $-1/4$ slopes are also given in the same table.

Isometric Force

The fiber diameter and stress (force normalized to cross-sectional area) showed no dependence on body size (Fig. 6 and Tables III and IV). The linear regression lines

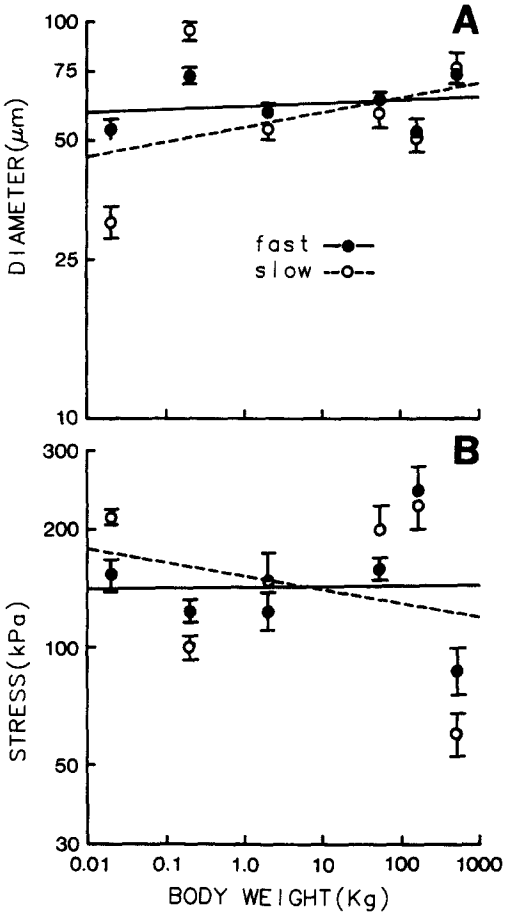

FIGURE 6. (*A* and *B*) Fiber diameter and isometric stress (isometric force normalized to the fiber cross-sectional area) vs. body size on logarithmic scale. Filled and open circles represent fast and slow fibers, respectively. Error bars represent SEM.

fitted through the mean values had slopes that were not significantly different from zero and their values were very low. There was, however, a large scatter of mean diameters and isometric forces from different animals. The source of this scatter may have arisen from inaccuracies in measurement of fiber diameter. Diameter was measured only to the nearest 5 μm, ~10% of the average diameter, causing ~20%

uncertainty in estimates of cross-sectional area. A much greater source of variation in stress was found when the influence of fiber diameter on stress was examined, as described below.

Absolute Power

Absolute maximum power in milliwatts per gram was obtained by multiplying the relative maximum power (Fig. 4 *B* converted to dimensions of centimeters per centimeter per second) by the fiber stress (Fig. 6 *B* in dimensions of newtons per square centimeter). Since fiber stress did not show any dependence on body size, the absolute maximum power varied in the same manner as the relative maximum power. This similarity is shown in Fig. 5 and Table III, which also shows that there is somewhat more scatter in the data due to the greater scatter in the isometric stress.

TABLE IV

Summary of Means and Standard Deviations of Parameters Studied

Species	Fiber type	Fiber diameter	Isometric stress	Maximum velocity	Maximum power	Maximum relative power	Number of fibers
		μm	*kPa*	*μm/half-sarc. per s*	*mW/g*	*μm/half-sarc. per s*	
Mouse	Fast	53.2 ± 9.9	153.3 ± 52.4	2.85 ± 0.46	34.9 ± 13.6	0.27 ± 0.033	11
	Slow	31.1 ± 6.4	212.9 ± 20.8	1.61 ± 0.47	19.4 ± 5.6	0.11 ± 0.024	6
Rat	Fast	72.7 ± 14.4	123.2 ± 41.2	1.73 ± 0.48	18.6 ± 9.1	0.18 ± 0.048	23
	Slow	95.1 ± 13.9	99.9 ± 25.0	1.09 ± 0.47	6.67 ± 2.59	0.079 ± 0.019	10
Rabbit	Fast	58.9 ± 12.2	123.1 ± 61.5	1.15 ± 0.22	14.3 ± 7.8	0.14 ± 0.017	19
	Slow	53.4 ± 12.6	146.8 ± 54.1	0.84 ± 0.22	5.8 ± 3.2	0.051 ± 0.018	13
Sheep	Fast	62.4 ± 16.1	159.4 ± 72.1	0.86 ± 0.14	9.1 ± 4.9	0.067 ± 0.007	49
	Slow	59.3 ± 18.4	198.0 ± 105.6	0.62 ± 0.22	8.1 ± 8.3	0.04 ± 0.025	13
Cow 1	Fast	52.2 ± 11.0	248.0 ± 112.3	0.77 ± 0.20	16.3 ± 9.3	0.08 ± 0.011	8
	Slow	51.6 ± 14.1	232.7 ± 86.1	0.52 ± 0.12	4.4 ± 2.1	0.022 ± 0.003	9
Cow 2	Fast	73.6 ± 15.3	87.8 ± 39.8	0.79 ± 0.17	6.0 ± 2.7	0.082 ± 0.007	11
	Slow	77.1 ± 40.8	60.1 ± 62.4	0.38 ± 0.39	0.66 ± 0.72	0.013 ± 0.006	9

Control Observations on Diffusion

The recent reports by Elzinga and colleagues (1989*a*, *b*) that fiber stress is inversely dependent on fiber diameter raised the question of whether the large variation in isometric force seen here could be correlated with diameter. A plot of stress vs. diameter (Fig. 7) shows a significant correlation ($r = 0.74$), suggesting that developed force was strongly influenced by delayed diffusion of some substance. This suggestion raised further questions of which substance was responsible and whether the delayed diffusion of that substance was also affecting velocity. The diffusible substances that are known to participate in the contractile reaction scheme are the substrate, MgATP, and the reaction products, ADP, inorganic phosphate, and protons. Separate control experiments to investigate the influence of buffers on all but inorganic phosphate

were undertaken. The experiments were done on fast fibers from two species, mouse and rabbit. Mouse fibers were used because they had the highest shortening velocity. We also tried to obtain larger fibers by taking muscles from older and more active male animals, but these fibers were still among the smallest studied (mean diameter = 50.2 μm ±9.0 SD, n = 24). To obtain larger fast fibers for these control experiments we also studied rabbit psoas fibers (mean diameter = 62.8 μm ±12.5 SD, n = 18).

Creatine kinase. The solution used in the main set of experiments included creatine phosphate but no creatine phosphokinase. We relied instead on the

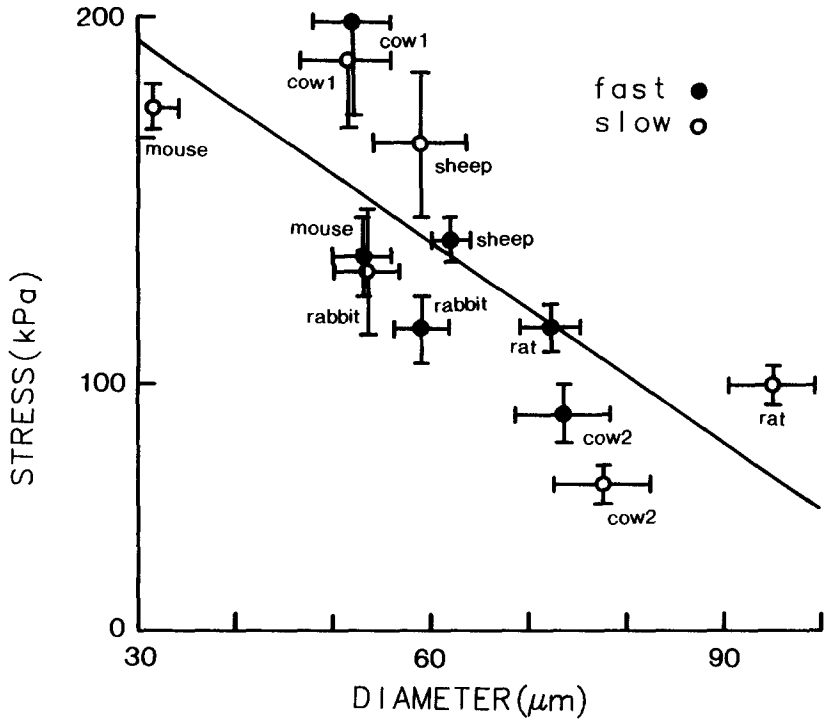

FIGURE 7. Isometric stress vs. fiber diameter on linear scales. Filled and open circles represent fast and slow fibers, respectively. The linear regression line fits through all 12 mean values, irrespective of the fiber type. See Table III for the fitting equation and the correlation coefficient. Error bars represent SEM.

presence of endogenous creatine kinase associated with the M-line and on the low temperature that reduced ATP hydrolysis rate. To test the validity of this practice, and to determine either the accumulation of ADP or the depletion of ATP were affecting contractile kinetics, we assessed the effect of added creatine kinase on isometric force and the relative force–velocity curves. These control experiments were done using a bracketing protocol; the force–velocity data in the presence of the exogenous creatine kinase were compared with similar data obtained both before and after the addition of creatine kinase. In each fiber two sets of nine isotonic releases

were made in the absence of the exogenous creatine kinase. The fibers were then immersed in the low EGTA rinse solution containing creatine phosphokinase (60 U/ml, plus 0.1% bovine albumin to avoid inactivation of the enzyme due to dilution) for at least 3 min to allow the enzyme to diffuse into the fiber. After this equilibration period, two sets of nine isotonic steps were made in the presence of the creatine phosphokinase. The relaxed fibers were then immersed in relaxing solution without

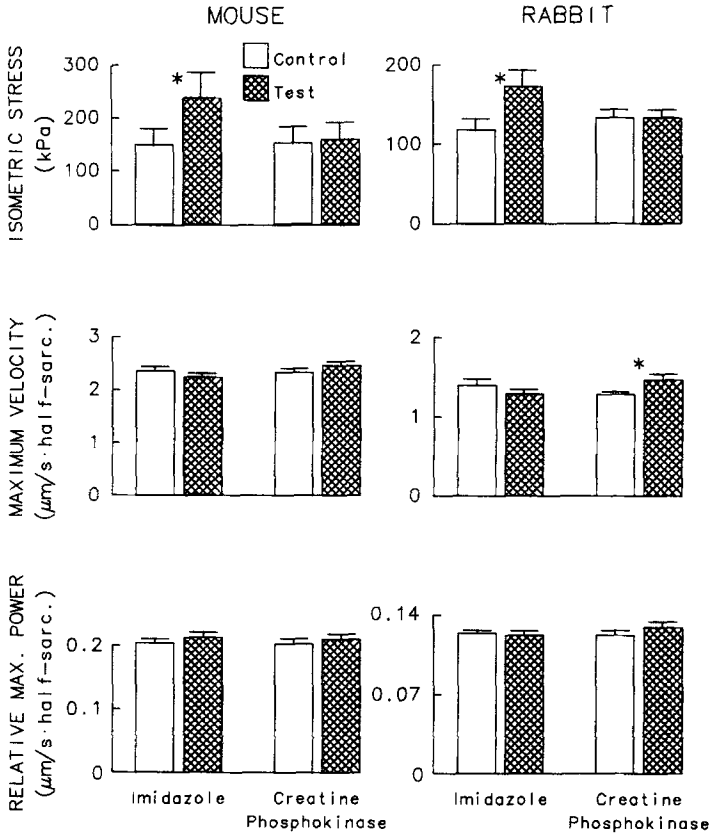

FIGURE 8. Comparison of isometric stresses, maximum velocities, and maximum power in control and test experiments. For the controls, the concentration of imidazole was 10 mM, and no creatine phosphokinase was added. For the imidazole test the concentration of imidazole was increased to 40 mM. For the creatine phosphokinase test, 60 U/ml (Sigma Chemical Co., St. Louis, MO) of the kinase was added to the solution. *Significantly different from control. Error bars represent SEM.

the kinase for 3 min before another two sets of nine isotonic steps were made. The procedure was repeated once more so that in the final analysis the control force–velocity curve contained data with six sets of nine steps and the test (creatine phosphokinase) contained four sets. The parameters of the fitted curves, maximum velocity, and maximum power output, together with the average isometric force measured before the isotonic steps, are compared in Fig. 8. As shown, there is no

significant difference in all paired parameters of the fitted curves from mouse and rabbit fast fibers except a small increase ($P < 0.05$) in shortening velocity in the presence of exogenous creatine kinase in rabbit fibers. The results suggest that neither the limitation of the substrate ATP nor accumulation of ADP would have substantially altered the measured values of maximum velocity or maximum power.

Increased pH buffering. The protons liberated during contraction should bind immediately to any pH buffer present and diffuse away bound to the buffer. The extent of the pH change that occurs during contraction will therefore depend upon the amount, molecular size, and pKa of the buffers present. Thus, raising the concentration of the relatively low molecular weight imidazole molecule should reduce the pH change during contraction and hasten the diffusion of protons from the fiber. The effect of increasing imidazole concentration four times, from 10 to 40 mM (with K-propionate concentration reduced from 90 to 75 mM to maintain the same ionic strength in the solution) was assessed in mouse and rabbit fast fibers (Fig. 8). The same type of bracketing conditions described for the creatine kinase were used here, except that the equilibration period for diffusion of imidazole into the fiber was reduced to a few seconds. As shown, there was a 59 and 46% ($P < 0.05$) increase in isometric force for mouse and rabbit fast fibers, respectively. The maximum velocity and maximum relative power were, however, not changed. This finding strongly indicates that a pH decline within the fiber during contraction had a large influence on the developed force with no detectable effect on velocity.

DISCUSSION

The main conclusion of this study is that shortening velocity and power output of skinned muscle fibers are inversely related to animal body size. This size dependence is probably a result of the presence of different forms of contractile proteins in different animal species. Because animals are known to have an inverse relation between body size and muscle power/weight ratio, it seemed likely that the power output of their contractile proteins would show a similar relationship. One of the major reasons for undertaking this study was to define this relationship so that further correlations between protein structure and shortening velocity could be made in the future. Other laboratories are presently engaged in the development of mutant muscles of *Drosophila* (Peckham et al., 1989; White and Sparrow, 1989). The promise of these attempts is that they will provide mutants in which protein structure and function can be correlated to learn more about contractile mechanisms. The observation that animals have size-dependent variations in their power/weight ratios suggests that natural selection may have provided mutations that are very specific in their effects on contractile processes. The present experiments confirm that such specific mutations have occurred.

Different Myosins vs. Different Ratios of Isoforms

It is well known that skeletal muscle myosin from a single animal contains several isoforms having different ATPase rates (Close, 1972). These observations raise the question of whether the differences in shortening speeds and power outputs described here are due to the differences in the myosins among the different species

or simply to differences in the ratios of fast and slow isoforms. The difference in speeds over the whole range of animal size is so large as to make it seem unlikely that all of it could be accounted for by different mixtures of the same two isoforms. Such an explanation would require the fast isoform to be ~10-fold faster than the slow isoform.

A related but more difficult question is whether the smaller differences among the more similarly sized animals are due to variations in the ratios of isoforms. The observation that fast and slow fibers from each species differed in speed by the same relative amount suggests that each muscle type contained predominantly one myosin isoform. The apparently continuous variation in shortening speeds and power outputs with animal size thus suggests a mechanism that allows closely spaced differences in shortening speeds. Unambiguous proof of such a mechanism will require measurements of myosin isoform composition in the same fibers used to make the mechanical measurements.

Relations to Early Experiments

The −0.126 to −0.130 power relationship between muscle shortening velocity and body size is very similar to the −0.13 to −0.17 power relationship found by Close (1972) in intact fibers at 35°C over a much smaller range of animal size (mouse, rat, and cat). This size range was less because the longer muscles from much larger animals would require very different techniques for study than the small muscles that he studied. A major advantage of studying skinned fibers is that identical techniques can be used for all animals. Another advantage of this preparation is that it gives more direct evidence about the contractile proteins.

Relations to Studies in Intact Animals

It is well known that smaller animals have higher power/weight ratios than large animals. Both the basal metabolic rate and the muscle power output during running vary with the ¾ power of body weight (Kleiber, 1932; Heglund et al., 1974; McMahon, 1977). These findings can be explained by the elastic scaling theory of McMahon (1973, 1975). According to this theory, both basal metabolic rate and muscle power output are determined by the intrinsic muscle power, which should vary with the −¼ power of body weight (for review, see Ford, 1984). In a variation of this theory, McMahon (1984) has proposed that muscle speed should vary with the −⅛ power of body weight and that the increased power output during running in smaller animals is due to a greater angular excursion of the limbs to make total power output proportional to the −¼ power of body weight. This variation of the theory is in almost exact agreement with the present results. The size independence of fiber stress is in agreement with Biewener's (1989) measurements in running animals.

Influence of Diffusion Limitation

The observation that there was a diameter dependence of developed stress in the absence of a body size dependence was surprising. The diameter dependence suggests that force generation was being limited by diffusion of some substance, while

the absence of a body size dependence suggests the absence of a dependence on ATP hydrolysis rate. For example, the small, fast fibers from mice produced three times more stress than the large, fast fibers from the large cow, even though its velocities were three times greater. The three times higher velocity suggests that the ATP hydrolysis rate was three times greater (Barany, 1967). If the fibers were the same in diameter, the concentration of reaction products at the center of the fiber would be three times greater. The 38% greater diameter of the cow fast fibers (Table IV) would not be expected to increase the concentration of reaction products at the center of the fiber to the same extent. In the steady state, the concentration of products at the center of the fiber should increase with the square of the fiber diameter (Cooke and Pate, 1985). A 38% increase in diameter would increase the concentration at the center by only 90%, insufficient even to match the influence of the higher hydrolysis rate in mouse. These disparate findings could be reconciled, however, if the slower fibers from larger animals were more sensitive to the accumulation of reaction products.

The absence of a large effect of creatine kinase on the contractile behavior of the fibers suggests that neither ATP depletion nor ADP accumulation was responsible for the diameter dependence of developed stress. Such an effect seems unlikely because both lowering ATP (Ferenczi et al., 1984) and raising ADP (Cooke and Pate, 1985) concentrations increase isometric force. It is more likely that accumulation of either or both of the other reaction products, proton and phosphate, was responsible. The increase of force at the higher pH buffer concentration argues in favor of proton accumulation as the responsible agent. The additional finding that the maximum velocity was not altered by the higher buffer concentration further suggests that this was not the only mechanism. It has been shown previously that lowering pH decreases maximum velocity (Metzger and Moss, 1987; Cooke et al., 1988). A possible explanation of the large effect of pH buffer on force with no effect on velocity is that both proton and phosphate accumulation contribute to the decreased force. An increased phosphate concentration has been shown to decrease force without altering velocity (Cooke and Pate, 1985), while only the monobasic form of phosphate has been shown to alter force (Nosek et al., 1987). The present results would be explained if the protons released in the absence of a high imidazole concentration bound to the accumulated phosphate, shifting more of it to the monobasic form, without lowering pH enough to alter velocity. Whatever the mechanism, the finding that velocity was not altered by the increased buffer or the presence of exogenous creatine kinase suggests that the experimental conditions used here did not artifactually decrease the apparent body size dependence of velocity.

Conclusion

The body size dependence of shortening velocity and muscle power described here for skinned fibers will account for about half the body size dependence in the intact animal. The higher myosin ATPase activity observed in small animals may, at least partly, account for the higher metabolic rate associated with small animals. The much weaker dependence of shortening velocity on body size (compared with that of metabolic rate on body size), however, suggests that the difference in metabolic rates

observed in animals with different body size cannot be entirely due to the difference in the intrinsic properties of the muscle fibers.

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