Pflügers Arch (1993) 424:478–487

Pflügers Archiv
European Journal of Physiology

Force-velocity relation and isomyosins in soleus muscles from two strains of mice (C57 and NMRI)

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Received September 14, 1992/Received after revision March 1, 1993/Accepted March 30, 1993

Abstract. We compared soleus muscles from two strains of mice, NMRI and C57. Soleus muscles from NMRI mice produced slower twitches and lower maximum tetanic force (F_o) but higher maximum tetanic stress (S_o), (owing to their smaller weight). Their Hill's velocity constant (b) was lower, but their force constant (a/S_o), their maximum velocity of unloaded shortening (V_u) and their maximal mechanical power (P_{max}) were similar. All soleus muscles contained two isomyosins (SM_2 and IM) and the two myosin heavy chains (MHC1 and MHC2A) corresponding to type I fibres and type IIA fibres; however, soleus muscles from NMRI strain had higher proportions of isomyosin SM_2 and of myosin heavy chain 2A. Regression equations were computed between the mechanical variables and the myosin heavy chain content. Using a simple hypothesis, the results were used to estimate the mechanical properties of type I and type IIA fibres. We conclude that type IIA fibres from soleus muscle are mechanically more similar to slow-twitch type I fibres than to fast-twitch type II fibres. The results also suggest a hypothesis to account for the diversity of isomyosins, by a matching diversity of mechanical properties based on a separate physiological control of the three factors that control P_{max}.

Key words: Mechanical power – Force-velocity relations – Isomyosins – Mouse strains NMRI C57 – Muscle fibre types – Myosin heavy chains

Introduction

When stained by the histochemical adenosine triphosphatase (ATPase) method [6], soleus muscle of mouse, a slow-twitch striated muscle, is found to contain two types of muscle fibres: type I and type IIA [2, 18, 25]. When analysed by polyacrylamide gel electrophoresis (PAGE), it is found to contain two isoforms of myosin, slow-twitch isomyosin 2 (SM_2) and intermediate isomyosin (IM). Isoform SM_2 is composed of myosin light chains 1s and 2s ($LC1_s$, $LC2_s$) and myosin heavy chains type 1, MHC1 (identical to β-cardiac MHC), whereas isoform IM is composed of a mixture of myosin light chains 1s, 1f, 2s and 2f ($LC1_s$, $LC1_f$, $LC2_s$, $LC2_f$) and myosin heavy chain type 2A, MHC2A ([14, 17, 23], review in [26]). Muscle fibres contain the myosin isoform corresponding to the histochemical fibre type [36], and, therefore, the proportion of isomyosins SM_2 and IM is equivalent to that of type I and type IIA fibres, at least to the extent that hybrid fibres are in a minority [4, 25, 36].

We have observed that soleus muscles from NMRI mice have a higher proportion of slow-twitch isomyosin SM_2, and have thus, presumably, a higher proportion of type I fibres, whereas the opposite was true in the soleus muscles from C57 mice. We found, as expected, that the twitch is faster in soleus muscles from C57, since type IIA fibres are fast-twitch fibres [18]. But we found only a small, although significant, difference in the force-velocity relation between the soleus muscles from the two strains, and little difference in their maximum mechanical power (P_{max}). This finding was unexpected, since type IIA fibres from rat fast-twitch muscle are mechanically more similar to high-power type IIB fibres than to low-power type I fibres from rat slow-twitch soleus muscle [3]. It indicates that type IIA fibres from slow-twitch soleus muscle are mechanically different from type IIA fibres from fast-twitch muscle. Preliminary results have been published elsewhere [20].

Materials and methods

Muscle preparation. Experiments were performed on whole soleus muscles isolated from NMRI white mice (n = 46 a local outbred strain) and C57/BL10SnSc black mice (n = 11 an inbred strain, obtained from the animal house of Charing-Cross and Westminster University, London, UK). Due to a difference in growth rate, mice of strains NMRI and C57 cannot be matched simultaneously for body weight (W_b) and for age; we chose to match them for body

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weight (Table 1), on the basis of the report of Brooks and Faulkner [7], who observed no differences in force-velocity relation between young (2–3 months) and adult (9–10 months) C57 mice.

The animal was killed by exposure to ether and the soleus muscle was dissected free. The distal tendon was tied to a fixed frame and the muscle belly was inserted between two parallel rows of five platinum electrodes spaced 2 mm apart, supported by the fixed frame. Finally the proximal tendon was tied to a glass rod (250 mg in weight) and the whole assembly was placed in a thermostat bath containing a buffered physiological salt solution (mM: NaCl, 118; $NaHCO_3$, 25; glucose, 5; KCl, 5; $CaCl_2$, 2.5; $MgSO_4$, 1; KH_2PO_4, 1; pH 7.4, gassed with 95% O_2 and 5% CO_2; temperature, 20 ± 0.5°C). The glass rod was attached to the force transducer of an electromagnetic puller described previously [21]. Force and length signals, sampled at a rate of 1 kHz and digitized (A/D conversion card RTI-815), were displayed on an AT IBM computer and stored for further analyses.

Mechanical experiments. After a recovery period of 15 min, the muscle was slightly stretched at rest until a small resting force developed of approximately 3–5 mN: this length was defined as the optimal length (L_o). A series of ten twitches was performed at 1/s using capacitor discharges of alternating polarity to avoid electrolysis (time constant: 5 ms; output impedance: 100 Ω; 1/s). The last twitch was recorded for further analysis. The muscle was tetanized isometrically for 1.2 s at 100Hz, shortened for 1.2 mm and tetanized again after an interval of 3 min, giving the tetanic force at longer and shorter lengths. Under the conditions used, the force at the shorter length was up to 20% less than the force at the longer length. Whenever this difference was larger, the length of the muscle was readjusted and the protocol was started over. This adjustment was critical: when stretched too far, the muscle produced so high a resting tension that it perturbed the measurements of the force-velocity relation, by boosting the phase of exponential decrease of the shortening (see below). On the other hand, if the initial muscle length was too short, the difference in isometric force developed at longer and shorter lengths was such that it depressed considerably the force maintained during the plateau phase of the shortening (see below).

After these initial adjustments, the soleus muscle was tetanized isometrically for 0.6 s, until the force remained approximately constant, and then it was released at a slow constant velocity (V) from L_o to (L_o–1.2 mm). During the release the force fell towards a slanting plateau (Fig. 1A). The initial curvature and the slope of the plateau increased with the velocity of the release. The initial curvature is probably due to the contribution of some elastic elements in series with the sarcomeres. In order to eliminate it, we tried to discharge the series elastic elements by shortening the muscle at very high velocity (300 mm/s) for a short extent (25–100 μm), a method proposed by Cecchi et al. [11]. This reduced the initial curvature, but did not suppress the slanting plateau. The slanting plateau depends on the fact that the isometric force developed by the muscle at L_o is higher than the force it could produce at (L_o–1.2 mm). The experimental protocol was repeated 18–22 times at different velocities arranged in a quasi-mirror order. Fatigue was minimized by allowing the muscle to rest for 5 min between two consecutive tetani: the ratio of maximum isometric force (F_o) between the last and the first tetanus of the series remained over 95%. The maximum velocity of shortening under zero load (V_o) was then determined by the slack test [2, 13].

After the mechanical experiment, the muscle was blotted and weighed (W_m). It was placed on a plastic sheet and stretched to L_o; under the binocular microscope, the lengths of 5–10 superficial fibres were measured and averaged, giving the fibre length, L_f. The ratio "muscle fibre length, L_f"/"muscle length, L_o" was similar in both strains and found to be 0.68, in agreement with previous reports [7, 19]. The muscle was rapidly frozen in liquid nitrogen and stored at −80°C until used for chemical analyses.

Analyses of myosin heavy chains and isomyosins. The frozen muscle was pulverized and extracted on ice for 20 min in 100 μl of a Guba-Straub solution (mM: NaCl, 300; NaH_2PO_4, 100; Na_2HPO_4, 50; $MgCl_2$, 1; $Na_4P_2O_7$, 10 and 0.1% of NaN_3, 0.1% of 2-mercaptoethanol; pH: 6.5), and centrifuged (10 min; 20 000 rpm 50 000 g; 4°C). One volume of glycerol was added to the supernatant. The extracts were analysed in duplicate and the results were averaged.

Myosin heavy chains were separated by SDS-PAGE [12], including 33% weight/volume glycerol both in separating (6% of polyacrylamide) and stacking gels (3% of polyacrylamide) [5, 9]. Two zones were present in all the samples. The faster moving zone was identified as myosin heavy chain of slow-twitch muscle, MHC1, because: (1) it co-migrates with the single zone observed in guinea-pig soleus, a pure slow-twitch muscle and, (2) it is labelled by a monoclonal antibody anti-slow F36 2C9 [24], using an immunoblot procedure. The slower moving zone was identified as MHC2A based on the following: (1) the zone reacts with monoclonal antibody F113 15F4 that in rat labels type IIA fibres, present in rat soleus, and a subset of type IIB fibres, IIB2, absent in rat soleus [24], (2) its electrophoretic mobility corresponds to that of the slower moving zone identified as MHC2A [12], HCIIa [42] or MHC2S [10, 32]. A third zone was present in one-third of the samples, with an intermediate electrophoretic mobility; we believe it to be MHC2B, because its mobility is equal to that of the MHC2B zone of the extensor digitorum longus (EDL), a fast-twitch muscle rich in type IIB fibres and by analogy with the earlier reports [12, 42]. However, the possibility exists that this chain is neonatal (see HCneo in Fig. 6 in [26]).

Isomyosins were separated by PAGE in non-denaturing conditions (PPi-PAGE, [1, 22]). Since the absolute electrophoretic mobilities varied noticeably, not only in consecutive runs, but even in samples run simultaneously, we measured the electrophoretic mobilities (R_f) relative to that of an internal standard: taenia coli myosin in the case of isomyosins and β-galactosidase in the case of myosin heavy chains. R_f measured by this method were reproducible and had a low coefficient of variance (about 1%): the R_f values were helpful to identify the bands [23]. Densitometry of the peaks is described in detail elsewhere [22].

Extraction of the mechanical parameters. F_o (measured at L_o) was used to calculate the maximum isometric stress ($S_o = F_o \times L_o / W_m$). The force F maintained during a release was likewise expressed in units of stress: $S = F \times L_o / W_m$.

The force-velocity relation was obtained as follows. As the force decay was nearly linear with time during most of the release (Fig. 1A), it was possible to extrapolate the force back to the time at which the movement began, the isometric force also being measured at that time [21]. This was done by extrapolation of a regression line calculated by the computer. The stress, S, thus computed was expressed in units of S_o measured just prior to the release. The data pairs (S/S_o, V) were fitted to Hill's hyperbolic equation:

$$S/S_o = (b - a/S_o \times V)/(b + V) \quad (1)$$

yielding values for b, the velocity constant in mm/s per mm of muscle fibre length (L_f/s), and a/S_o, the force constant, dimensionless. In this equation, V is the velocity of shortening, also measured in mm/s per mm of muscle fibre length, L_f/s.

Maximum velocity of contraction (V_{max}) was computed as b (velocity constant) divided by a/S_o (force constant).

Normalized maximum power (M^2) is the maximum power computed when force and velocity are normalized respectively to S_o and V_{max}. M^2 is a function of the curvature G [43]:

$$M^2 = [(1 + G)^{1/2} - 1]/G \quad (2)$$

$$G = S_o/a = V_{max}/b \quad (3)$$

P_{max}, the maximum mechanical power, is then [43]:

$$P_{max} = S_o \times V_{max} \times M^2 \quad (4)$$

Given the units chosen for the variables, P_{max} is expressed in units of μW/mm³ muscle.

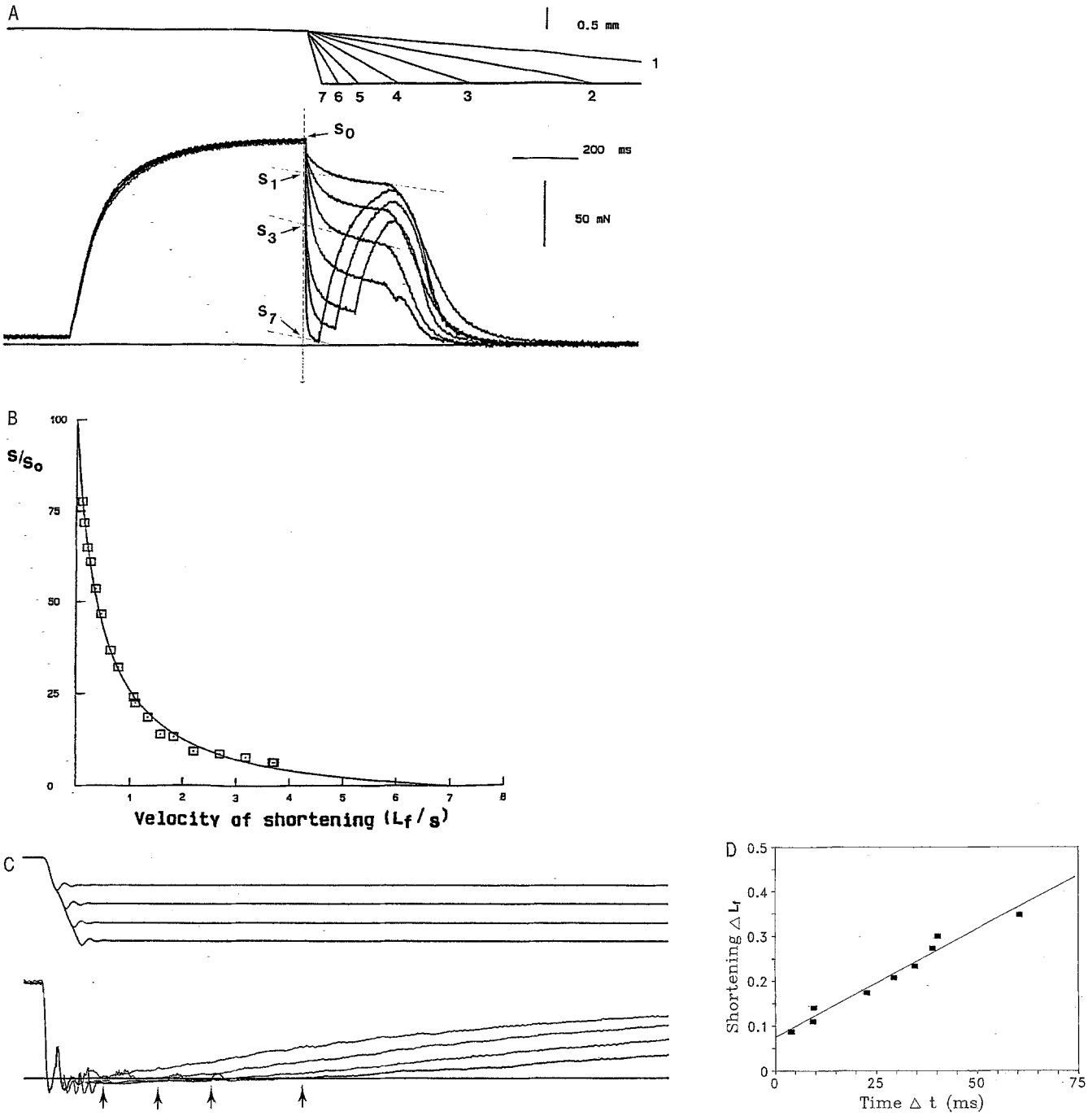

Fig. 1A–D. Soleus muscle of a normal mouse (strain C57). **A** Seven superimposed isometric tetani with isovelocity releases. *Upper panel* shows muscle length; the releases are numbered in order of ascending velocities. *Lower panel* shows muscle force. The seven tetani are nearly identical in their initial isometric portion. Data obtained from the right soleus muscle of a male mouse aged 115 days; W_b, 27 g; W_m, 13.2 mg; L_f, 6.2 mm; initial resting force: 6.4 mN; maximum F_o of the first tetanus: 197 mN; maximum F_o of the last tetanus (18th): 197 mN; velocities of the 18 releases ranged between 0.11 and 3.7 L_f, but 11 of them are not shown in order not to overcrowd the graph. The curves were analysed on line by computer during the experiment as follows. The *thin lines* illustrate the extrapolation procedure used to evaluate the force. First a *vertical line* is drawn at the time of release. Then *lines* are drawn through the linear section of the curves during the releases; the intersection between such *line* and the *vertical line* defines the force used to compute the corresponding stress (S_1, S_3 or S_7). The same procedure was used for all the other tetani with releases. The set of 18 couples of data (stress velocity) is plotted in Fig. 1B. In this way, the data for the force-velocity relation are estimated at a constant time of contraction and a constant muscle length. **B** Force-velocity relationship of the experiment shown in **A**. The data points are shown within a *square*. The curve is a Hill hyperbola [Eq. (1)] computed by a non-linear least squares procedure. The parameters of the curve are: $a/S_o = 0.06 \pm 0.01$; $b = 0.43 \pm 0.01$ L_f/s; $V_{max} = 7.0$ L_f/s; residual variance (SSH): 34. **C** Slack test. Four of a total of nine releases (70 mm/s) to final length (L_0) were applied during the plateau of an isometric tetanic contraction. Step sizes were 1.98, 1.54, 1.10, 0.66 mm. The duration of unloaded shortening associated with each release (t_L) was measured from records as the interval between the onset of the release and the intercept between a linear extrapolation of the rising force and the base line. Same muscle as **A**, **B**. **D** Determination of V_u. The unloaded shortening time intervals Δt increases linearly with release amplitudes ΔL_f, in percentage of L_f. The V_u value was calculated as the inverse of the *slope* of the resulting *line*

Statistical analyses. The constants b and a/S_o were evaluated using a non-linear least squares regression program based on the Gauss-Newton method [44] and rewritten in Truebasic. The "goodness" of fit can be estimated by three statistical parameters which are given by the mathematical analysis of the data obtained from each muscle: the estimates of a/S_o and of b produced by the analysis are in fact "means" of a population of estimates that are compatible with the data: the distribution of this population is described by "standard errors of means", SEa and SEb, respectively for a/S_o and b. The third statistical parameter is the residual variance (SSH) which is the portion of the data variance not accounted for by the fitted hyperbola. Table 1 contains the means and standard errors of the three statistical variables.

Our method of analysis constrains the force/velocity curve to pass through $S/S_o = 1.00$ for $V = 0$, an assumption questioned by some authors (review in [43]). We have not attempted to drop this assumption because it would require to fit three variables (a/S_o, b and S_o) to the force-velocity data, and the computation algorithm then becomes liable to large errors due to the relatively small number of data points.

Means (with their standard errors), t-tests, correlations and linear regressions were computed with the SPSS statistical package. All computations were performed on an IBM AT computer.

Results

The force-velocity relation

Figure 1A shows a typical experiment in which a mouse soleus muscle is tetanized and released at various controlled velocities. Figure 1B shows the data points for the force-velocity relation of the same muscle. The curve drawn through the data is a Hill's hyperbola [(Eq. (1), see Methods)] whose parameters a/S_o and b are fitted to the data by a non-linear least squares procedure. Figure 1C, D illustrates the measurement of V_u using the slack test [13].

Identical experiments were performed on soleus muscles of NMRI white mice ($n = 46$) and C57 black mice ($n = 11$) and the results are reported in Table 1. The W_b values of the two mouse strains are similar, but soleus muscles from NMRI mice are smaller, and therefore develop less F_o. However, their S_o value is larger, indicating that in NMRI soleus muscle the lower muscle weight is more than offset by a higher production of isometric force per unit area.

The velocity constant b depends on the strain of mice: it is larger in C57 than in NMRI by about 25% ($P < 0.01$). The force constant a/S_o shows no dependence on the strain. As the normalized maximal power M^2 is a function of $G = S_o/a$ [Eqs. (2, 3)], it is also similar in C57 and NMRI mice. The P_{max} values [Eq. (4)], as well as the V_u values are the same in NMRI and C57 mice.

The statistical parameters, residual variance (SSH), standard errors (SEb and SEa) of the individual estimates of b and a/S_o are not influenced by the strain. This indicates that on average the experimental data points are equally well described by the Hill's hyperbolic curve for both strains. It should be noticed that SEb and SEa are of the same order of magnitude than the standard errors of these parameters: this means that most of the variance of b and a/S_o arises from the uncertainty of the fitting of the hyperbolic curve to the data and not from biological variations.

Table 1. Force-velocity relations of the soleus muscle of the mouse

Parameter	Strain				
	NMRI $n = 46$			C57 $n = 11$	
W_b (g)	32.5 ± 0.8			35.7 ± 3.0	
W_m (mg)	7.8 ± 0.3	***		12.5 ± 0.5	
L_f (mm)	7.3 ± 0.1			7.2 ± 0.2	
F_o (mN)	166 ± 7	***		200 ± 14	
S_o (mN/mm²)	158 ± 6	**		115 ± 8	
b (L_f/s)	0.47 ± 0.01	**		0.60 ± 0.04	
a/S_o	0.12 ± 0.01			0.10 ± 0.02	
M^2	0.062 ± 0.001			0.057 ± 0.004	
P_{max} (μW/mm³)	37 ± 2			40 ± 6	
V_u (L_f/s)	6.3 ± 0.2			6.1 ± 0.5	
SEb	0.030 ± 0.002			0.037 ± 0.005	
SEa	0.029 ± 0.002			0.029 ± 0.005	
SSH	98 ± 11			212 ± 102	

Means ± SEM. W_b, Body weight; W_m, muscle weight, L_f, muscle fibre length; F_o, maximum isometric tetanic force; S_o, maximum isometric tetanic stress; b, velocity constant of the Hill equation; a/S_o, force constant of the Hill equation; M^2, normalized maximum power [Eq. (2)]; P_{max}, maximum mechanical power per unit volume [Eq. (4)]; V_u, maximum velocity of shortening under zero load (slack test); SEb, SEa, standard errors of b and a/S_o in individual experiments; SSH, residual variance (see text). Probability of a significant difference between two means: ** $P < 0.01$; *** $P < 0.001$

Table 2. Isometric twitch of the soleus muscle of the mouse

Parameter	Strain				
	NMRI $n = 44$			C57 $n = 11$	
S_{ot} (mN/mm²)	22 ± 1	***		14 ± 1	
S_{ot}/S_o	0.14 ± 0.08			0.12 ± 0.1	
t_p (ms)	67 ± 3	***		50 ± 3	
t_d (ms)	99 ± 12[a]	*		70 ± 6	

Means ± SEM. S_{ot}, Maximum stress of the isometric twitch; S_{ot}/S_o, twitch-to-tetanus ratio; t_p, twitch time-to-peak; t_d, twitch time-to-half-relaxation. Probability of a significant difference between two means: * $P < 0.5$; *** $P < 0.001$. [a] Mean based on 14 experiments

The isometric twitch

The properties of the isometric twitch are reported in Table 2. In comparison with the soleus muscle of the black mouse C57, the soleus muscle of the white mouse NMRI is both stronger (higher peak tension) and slower [higher time-to-peak (t_p) and half-relaxation (t_d) time]. However, the twitch-to-tetanus ratio is the same in both mouse strains.

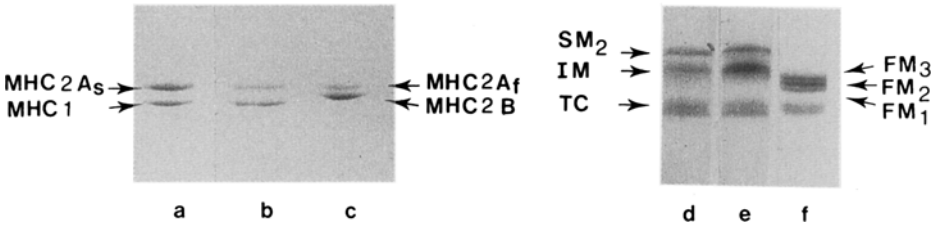

Fig. 2. The *left panel* shows myosin heavy chains of mouse soleus separated by glycerol-SDS-PAGE. NMRI soleus (*a*); C57 soleus (*b*); NMRI extensor digitorum longus (*c*). $MH2A_s$, Myosin heavy chain 2A of slow-twitch soleus muscle; $MHC2A_f$, myosin heavy chain of fast-twitch extensor digitorum longus muscle; MHC2B, myosin heavy chain 2B. The *right panel* shows isomyosins of mouse soleus separated by PPi-PAGE. NMRI soleus (*d*); C57 soleus (*e*); NMRI extensor digitorum longus (*f*). The lowest zone TC is a marker (isomyosin of a smooth muscle, guinea-pig taenia coli) used as an internal standard to measure the electrophoretic mobility of the isomyosins. FM_1, FM_2, FM_3, Fast-twitch isomyosin 1, 2 and 3

Table 3. Myosin heavy chains and isomyosins of the soleus muscle of the mouse

Parameter	Strain		
	NMRI $n = 46$		C57 $n = 11$
MHC1	64.5 ± 1.3	***	36.4 ± 2.7
MHC2A	33.2 ± 1.3	***	60.3 ± 2.3
MHC2B	2.2 ± 0.6		3.4 ± 1.6
SM_2	65.9 ± 1.8	***	34.7 ± 4.6
IM	34.1 ± 1.8	***	65.3 ± 4.6

Means ± SEM in percentage of total myosin heavy chain or total isomyosin. MHC1, Myosin heavy chain 1; MHC2A, myosin heavy chain 2A; MHC2B, myosin heavy chain 2B; SM_2, slow-twitch isomyosin 2; IM, intermediate isomyosin. Probability of a significant difference between two means: *** $P < 0.001$

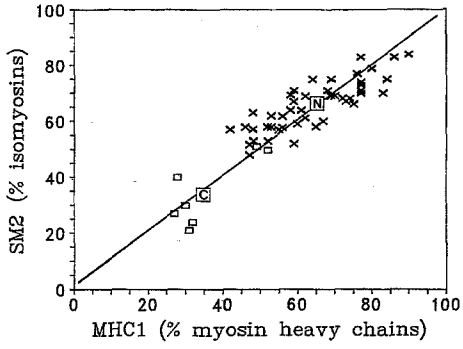

Fig. 3. Relations between SM_2 and MHC1. NMRI soleus muscles are shown by *crosses* and C57 soleus muscles are shown by *empty squares*. The means of each strain is indicated by the *boxed letter*, *N* for strain NMRI, *C* for strain C57. The *line* is the identity line of slope 1, expected if SM_2 contains MHC1

Myosin heavy chains and isomyosins

Two zones, MHC1 and $MHC2A_s$, were observed in 36 samples (Fig. 2, panels a, b); the remaining 21 muscles (not shown) contained a third zone, MHC2B, of a relatively low density: only in 7 cases was its proportion higher than 5% of the total myosin heavy chains content.

The soleus muscles were also analysed for their undissociated (i. e. native) isomyosins contents (Fig. 2, panels d, e). All samples displayed two zones identified as SM_2 and isomyosin IM based on their electrophoretic mobilities [$R_f = 100$ for SM_2 and 113 for IM (see also [2, 23])]. Fast-twitch isomyosins FM_1, FM_2, FM_3, or slow-twitch isomyosin SM_1 were not observed.

In comparison with the soleus muscles of black mice C57, the soleus muscles of white mice NMRI have larger proportions of MHC1 and isomyosin SM_2 (Table 3). The proportion of MHC1 is equal to that of SM_2: this is true for both strains (Table 3, Fig. 3). The same can be said for MHC2A and IM. These observations confirm our earlier results that isomyosin SM_2 only contains MHC1 whilst isomyosin IM only contains MHC2A [23].

Correlations between myosin heavy chains and mechanical parameters

Since soleus muscles of mice contain two forms of myosins in variable proportions, it is sufficient to study the correlation between one of these forms and mechanical variables. Some soleus muscles contain a third component, MHC2B, but we shall neglect this in a first approximation, since this fast myosin heavy chain is a minor component and it is absent from most soleus muscles. We decided to use the myosin heavy chain content as a parameter rather than the native isomyosin content as the former can be measured slightly more accurately (Table 3). More explicitly, we focused on MHC1.

Correlations with force-velocity curves

We first examined whether b depends on the fraction of MHC1. To this end the regression equation between MHC1 and b was computed for all data (NMRI + C57):

$$b = 0.68\ (\pm\ 0.05) - 0.0032\ (\pm\ 0.0008) \times [\text{MHC1}]$$
$$r = -0.478,\ n = 57,\ P < 0.001 \qquad (5)$$

This relation is presented in Fig. 4A with the mean values indicated for both NMRI and C57. The regression equations computed for each strain separately do not differ significantly.

a/S_o and M^2 are not correlated with MHC1 neither within each strain nor when the two strains are pooled (Fig. 4B). Figure 4B shows the experimental data, the means for the two strains and the general mean for a/S_o (thick line).

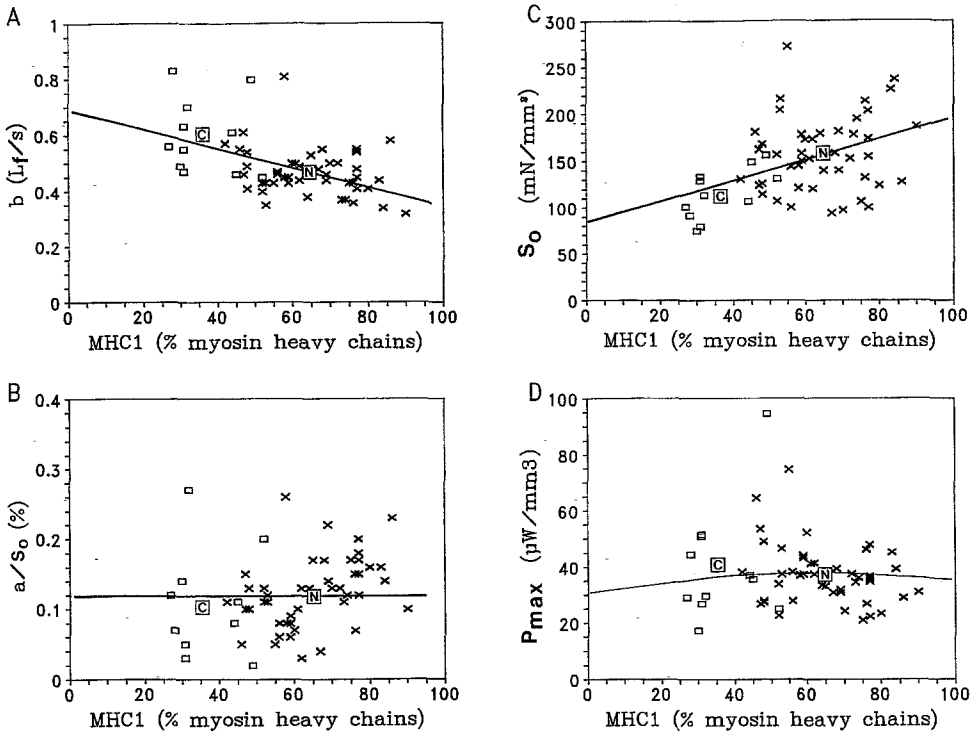

Fig. 4A–D. Relations between the fraction of MHC1 and the velocity constant b (**A**), the force constant a/S_o (**B**), the maximum isometric stress S_o (**C**) and the maximum mechanical power P_{max} (**D**). Soleus muscles from strain NMRI are shown by *crosses*; soleus muscles of strain C57 are shown by *empty squares*. The mean for each strain is indicated by the *boxed letter*, *N* for strain NMRI, *C* for strain C57. The *thick lines* are regression lines. The *thin* curve on Fig. 4D represents Eq. (7)

The regression equation for S_o is:

$$S_o = 87\ (\pm\ 19) + 1.07\ (\pm\ 0.31) \times [\text{MHC1}]$$
$$r = 0.421,\ n = 57,\ P < 0.01 \qquad (6)$$

S_o is thus moderately correlated with the fraction of MHC1. Figure 4C shows its regression line and the means for the two strains.

P_{max} is the product of three factors [Eq. (4)] which are linear functions of the MHC1 content; therefore, P_{max} itself is a function of MHC1. Substitution of Eqs. (5) and (6) into Eq. (4) with $M^2 = 0.056$ yields:

$$P_{max} = 30 + 0.23 \times [\text{MHC1}] - 0.0017 \times [\text{MHC1}]^2 \qquad (7)$$

This relation is shown in Fig. 4D by the thin line: the parabolic curve accounts satisfactorily for the data points and the means for both strains. Note that P_{max} is maximum (37.8 µW/mm³) at [MHC1] = 65.5: these values are remarkably close to the mean obtained empirically for soleus muscle of NMRI strain (37 and 65, respectively). We have examined whether muscles containing MHC2B can be more powerful on average and the answer appears to be that they are not: the correlation coefficient between P_{max} and MHC2B content is very low ($r = 0.092$, $n = 57$); their mean P_{max} is close to the general mean and, moreover, none of them have P_{max} values exceeding 50 µW/mm³. Thus if, as it is likely, muscle fibres containing fast-twitch MHC2B are more powerful than the other slower muscle fibres, their number is too small to bring a measurable contribution to the force/velocity relation measured on the whole muscle.

V_u is not correlated with MHC1 content ($r = -0.064$, $n = 57$).

Correlations with twitch parameters

Three parameters of the isometric twitch are correlated with the MHC1 content. The corresponding regression equations are for the maximum isometric twitch stress (S_{ot} in mN/mm²):

$$S_{ot} = 12\ (\pm\ 4) + 0.13\ (\pm\ 0.06) \times [\text{MHC1}]$$
$$r = 0.410,\ n = 32,\ P < 0.05 \qquad (8)$$

for the time-to-peak (in ms):

$$t_p = 36\ (\pm\ 9) + 0.48\ (\pm\ 0.14) \times [\text{MHC1}]$$
$$r = 0.436,\ n = 32,\ P < 0.05 \qquad (9)$$

and for the time of half-relaxation (in ms):

$$t_d = 15\ (\pm\ 19) + 1.4\ (\pm\ 0.4) \times [\text{MHC1}]$$
$$r = 0.626,\ n = 32,\ P < 0.01 \qquad (10)$$

The twitch-to-tetanus isometric ratio S_o/S_{ot} is an exception: it shows no correlation with the MHC1 content ($r = 0.140$).

Correlations with age and W_b

MHC1 and SM_2 are negatively correlated with age ($r = -0.644$, -0.761, respectively, $P < 0.001$). The fraction of MHC1 of C57 soleus corrected for age 80 days (the mean age of NMRI mice) is equal to 44 ± 4. Thus the decrease of MHC1 with age accounts for 29% [(44 − 36.4)/(64.5 − 36.4)] of the difference in MHC1 observed between strains, leaving 71% for the effect of the difference in strain itself. Among the mechanical variables, only two are influenced by age: S_o ($r = -0.394$, $P < 0.05$) and b ($r = 0.450$, $P < 0.01$). W_b in-

creases with age ($r = 0.524$, $P < 0.01$) and has no influence on the mechanical parameters of the tetanus ($r < 0.3$ in all cases), in spite of the fact that heavier mice have lesser proportions of MHC1 or SM_2 ($r = -0.453$ and -0.505, respectively, $P < 0.01$). Heavier mice have shorter twitches but of similar strength: the correlation coefficient between W_b and twitch t_p and t_d are significant ($r = 0.50$ in both cases, $P < 0.01$), but the correlation coefficient with S_{ot} is not significant ($r = -0.313$, $n = 28$).

Discussion

Diversity of soleus muscles of mouse

Soleus muscles from two strains of mice, the white mice NMRI and the black mice C57 display large difference between them. Even though the mice were matched for W_b, soleus muscles from NMRI mice were smaller and developed less F_o. However the force per unit area of NMRI mice was higher. NMRI mice contracted more slowly: not only their twitch was longer and slower but they also shortened more slowly during a tetanus with release, resulting in a lower b value. In the circumstances, it is remarkable to note that the P_{max} was similar for both strains.

It is important here to recall the difference between the M^2 and P_{max}. M^2 is a function of the force constant a/S_o [Eqs. (2, 3)]. Since both mouse strains have the same force constant, they both have normalized force-velocity curves of the same shape. The force-velocity relation per unit muscle volume is obtained by scaling M^2, using S_o and V_{max} as scaling factors [Eq. (4)]. The scaling factors are different for the two strains of mice, NMRI mice being stronger (higher S_o) and slower (lower b and thus lower V_{max}), but they are balanced in such way that P_{max} is the same for both strains. This is possible because the higher S_o is counteracted by a lower V_{max} (a lower b), M^2 remaining constant. One may wonder which physiological advantage is gained by changing the factors composing P_{max} without modifying V_{max} itself. The answer is that changing the factors composing P_{max} changes the velocity of shortening at which P_{max} is produced, the optimal velocity of shortening V_{opt}. We computed V_{opt} for both strains: it is lower (0.9 L_f/s) for the NMRI mice than for the C57 mice (1.4 L_f/s). It is tempting to conclude that the difference in V_{opt} at constant P_{max} is a meaningful physiological adaptation when we consider that NMRI mice are less active and move more slowly than C57 ones. This suggests that at least two of the three factors of Eq. (4) can be independently controlled to adjust P_{max} and V_{opt} to the physiological needs. If this inference is true, it implies that the muscle cell is able to control not only its velocity constant (or P_{max}), but also its maximum stress S_o. A possible mechanism, explained and discussed below, is by controlling the proportions of two isomyosins of different V_{max} and S_o.

The fractions of MHC1 and MHC2A are reversed in the two mouse strains (Table 3) and, by inference, the fractions or type I and type IIA fibres are also reversed. There are however large variations in the fractions of the isomyosins: for instance, the fraction of MHC1 varies widely between the lowest value of 26% and the highest value of 90%. As expected, we observed parallel variations of several mechanical variables so that we could compute the linear regressions between MHC1 fraction and each of the mechanical variables. The coefficients of correlation of b and S_o with the fraction of MHC1 are low. The variation in MHC1 accounts for only 25% (r^2) of the variations observed in the mechanical parameters. The remaining variance is mostly explained by the large sampling errors of the mechanical parameters during the curve fitting procedure (compare SEa and SEb to the SEM of b and a/S_o) and, to a lesser extent, to errors in estimating the myosin heavy chains fraction (see Fig. 3). In addition, some of the unexplained variance must also arise from biological factors (additional protein isoforms of myosin light chains or heavy chains; phosphorylation of contractile proteins; differential effect of fatigue, variation in pennation angle of muscle fibres, etc.). In spite of this high statistical noise, the probability is very strong ($P < 0.001$) that the fraction of myosin heavy chains influences the mechanical parameters.

Mechanical properties of isomyosins in mouse soleus

The regression parameters can be used as a tool to estimate the mechanical properties of type I or type IIA fibres. The deduction is as follows. If a soleus muscle is homogeneous in type I fibres, the fraction of MHC1 is 100%; the value of a mechanical variable for type I fibres is estimated by solving the regression equation of that variable for [MHC1] = 100. If a soleus muscle is homogeneous in type IIA fibres, then the fraction of type I fibres is 0%, and the value of a mechanical variable for type IIA fibres is obtained by solving the corresponding regression equation for [MHC1] = 0. This procedure implies the assumption that the muscle fibres are parallel in the soleus muscle, and therefore simply add their forces when they are released at a controlled velocity. Table 4 reports the results of these computations, together with some of the biochemical properties of the isomyosins obtained from the literature.

Values reported in Table 4 for type I fibres can be taken as typical for a slow-twitch fibre, given the conditions under which the experiments were performed (isolated muscle at 20°C in a Krebs solution). They are to be compared with the values obtained for type IIA fibres.

The twitch t_p and t_d values are much shorter in type IIA fibres, compared to type I fibres. Thus type I fibres are "slow-twitch" fibres, and type IIA fibres are "fast-twitch" fibres. This result is of course expected from current knowledge. It also confirms earlier direct measurements by Lewis et al. [18] who stimulated motor units via the ventral roots and reported that type IIA motor units of mouse soleus have twitch t_p values twice as short as type I motor units, but equal to that of type II motor units of mouse EDL, a "fast-twitch" muscle. On the other hand, the twitch isometric force (or stress)

Table 4. Isomyosins and mechanical properties of type I and type IIA fibres of mouse soleus muscle

	Fibres		
	Data source	Type I	Type IIA
Isomyosins:			
Isomyosin	a	SM_2	IM
Myosin heavy chains	a	MHC1	MHC2A
	b		MHC2s
Myosin light chains	a	$LC1_s$ $LC2_s$	$LC1_s$ $LC1_f$ $LC2_s$ $LC2_f$
Mechanical properties:			
S_o (mN/mm^2)	Eq. (6)	194	87
b (L_f/s)	Eq. (5)	0.36	0.68
a/S_o	c	0.12	0.12
M^2	Eq. (2)	0.056	0.056
P_{max} (μW/mm^3)	Eqs. (4, 7)	36	30
V_{opt} (L_f/s)	d	0.9	1.4
V_{max} (L_f/s)	e	3.0	5.7
S_{ot} (mN/mm^2)	Eq. (8)	25	12
S_{ot}/S_o	f	0.14	0.14
t_p (ms)	Eq. 9	84	36
t_d (ms)	Eq. 10	155	15

$LC1_s$, $LC2_s$, $LC1_f$, $LC2_f$, Myosin light chains 1 and 2 of slow and fast type; V_{opt}, velocity of shortening at P_{max}; V_{max}, maximum velocity of shortening; all other variables as given in legends to Tables 1–3. Sources: a, this work, [23] see also review in [26]; b, [32]; c, mean of the values reported in Table 1; d, computed from the derivative of Eq. 1; e, computed from the equation $V_{max} = b/(a/S_o)$, using values of Table 4; f, mean of the values reported in Table 2. Equations 2–10 refer to the equations reported in the text and used to compute the values of Table 4, using [MHC1] = 100 for type I fibre and [MHC1] = 0 for type IIA_s fibre

is low, both in type I and in type IIA fibres: its proportion to the isometric tetanic value, the "isometric twitch-to-tetanus ratio", is typical for a "slow-twitch muscle" [7]. Here is a first indication that type IIA fibres from soleus muscles are not exactly like type IIA fibres from fast-twitch muscles.

During a fused tetanus, type IIA fibres from soleus muscles shorten at a V_{max} approximately twice that of type I fibres. Many authors have reported that V_{max} (equal to b divided by a/S_o) or V_u (measured by the slack test method) is 2–6 times larger in type II fibres than in type I fibres [2, 3, 14, 16, 28–31, 34, 35, 39, 40]. In whole muscle, V_u should equal the V_{max} of the fastest fibres [2]. By this argument, V_u of soleus muscles from both NMRI and C57 mice should be independent of the fraction of the myosin heavy chains and be equal to V_{max} of type IIA fibres, since in both cases the fastest fibres are type IIA fibres, a prediction supported by our results (compare Tables 1 and 4).

The other mechanical properties of type IIA fibres shown in Table 4 make them rather similar to slow-twitch type I fibres and quite different from fast-twitch type II fibres. The values of a/S_o, M^2 and P_{max} are identical in type I and type IIA fibres, being 2–5 times lower than the values of type II fibres from typical fast-twitch muscles (on whole muscles: [8, 27]; on single fibres: [3]). However, the velocity V_{opt} at which P_{max} is produced is 50% higher in type IIA compared to type I fibres and their b value is twice as high, but these differences are still less than the 4–6 times differences reported between slow and fast muscles by many authors (review in [43]). Finally, S_o is lower by half in type IIA fibres. On the other hand, Bottinelli et al. [3] found that type IIA fibres from fast rat muscle are mechanically little different from type IIB fibres, but quite different from type I fibres: their observations taken together with ours suggest that type IIA fibres from slow-twitch soleus muscle are mechanically different from type IIA fibres from fast-twitch muscle. To summarize our findings, type IIA fibres from soleus muscle resemble type I fibres, but for four major differences: their twitch is fast-like, their S_o value is lower, their b value is higher and, what we believe is the most significant physiological difference, they shorten at a higher V_{opt} to produce P_{max}. We conclude that type IIA fibres from soleus muscle are mechanically more similar to slow-twitch type I fibres than to fast-twitch type II fibres.

Type IIA fibres from soleus muscle are also different from fast-twitch type IIA fibres by their myosin isoforms. Subjected to gel electrophoresis in non-denaturing conditions, myosin from fast muscle like EDL muscle or tibialis anterior (TA) muscle separates into three bands, the three FM isoforms (FM_3, FM_2, FM_1), according to the myosin light chains present, combined in the myosin molecule, $LC1_f$, a mixture $LC1_f$ and $LC3_f$ or $LC3_f$, whereas myosin from soleus type IIA fibres gives a single band IM (review in [26]). Each of the FM bands contains myosin fast light chains, whereas isomyosin IM is composed of a mixture of fast and slow light chains [14, 17, 23]. MHC2A from slow-twitch muscles differs from that isolated from fast-twitch muscles by its peptide map [17, 32]. There are thus biochemical and mechanical evidences to believe that the fibres identified as type IIA by the histochemical ATPase reaction are not identical in slow-twitch and fast-twitch muscles.

There remains the question whether type IIA fibres of soleus muscle correspond to the recently discovered fibre types, IIX or IID (review in [26]). MHC2d migrates slightly faster than MHC2a (see Fig. 6 in [26]), and the monoclonal antibody used by us to identify MHC2A in mouse soleus cross reacts with sub-type IIB2 fibres [24], which might be identical to IID/IIX fibres. Therefore the sub-unit which we identified as MHC2A could be in fact MHC2d or MHC2x. However, we believe that this is not the case for the following reasons. First, holomyosin from IIA fibres of soleus muscle migrates as a single zone IM when electrophoresed in non-denaturing conditions (this work) whilst isomyosin from IID fibres migrates as a fast triplet myosin [41]. Second, rat soleus has no IID fibres [39], except if chronically stimulated [33]. We could not find a report stating clearly that mouse soleus also lacks IID/IIX fibres. However Gorza [15] states that in mouse muscle IIX fibres stain like IIB fibres when treated by the method of Brooke and Kaiser [6]; since mouse muscle has no type IIB fibres, we may deduce that mouse soleus has no type IIX fibres. Third, the force-velocity relation of IIX fibres is similar to that of IIB fibres and quite different from

that of type I fibres [3], whilst we show in this paper that IIA fibres from soleus muscle display a force-velocity relation similar to that of type I fibres. These arguments strongly suggest that type IIA fibres from soleus muscle are not identical to type IIX/IID fibres. Since they are also biochemically and mechanically different from IIA fibres located in fast muscle (see above), there is a distinct possibility that they contain a special form of myosin.

Physiological meaning of the variety of myosin isoforms

Animals use their muscle within a wide range of loads and velocity of shortening. It is a basic principle of engineering that the transfer of power is optimal when force and velocity of the engine are matched to load and velocity of the object to be moved. This principle would require that animals recruit in their muscles the motor units that can precisely move the load at the velocity optimal to produce peak power at the velocity of shortening required by circumstances. It is not known whether mammals actually use their muscle fibres in this way. In fishes, it has been shown that the slow fibres shorten during slow locomotion at a velocity that gives peak mechanical power and efficiency, and the fast fibres shorten at their V_{opt} when powering maximal movement [33]. In this work, we have found that soleus muscles of white NMRI mice produce the same peak power as the soleus muscles of black C57 mice, but the V_{opt} values are different. White mice produce peak power at a markedly lower velocity of shortening. They are also much less active in their cages. We surmise that the difference in V_{opt} values between the two strains is an adaptation of the muscles to the different behaviour of the mice of the two strains. Our work presents a relatively simple example of how the fractions of two types of muscle fibres can be changed to adapt peak power and V_{opt} to the physiological requirements.

More generally, it is seen that a large number of combinations of optimal velocities and forces could be necessary in order to adapt the muscle to the numerous physiological requirements. We propose that each mechanical combination can be produced by a specific myosin isoform, in which S_o, V_{max} and M^2 could be independently determined. This hypothesis can easily account for a large number of isomyosins, since if only three levels are designed for each of the three parameters, it predicts the existence of $3^3 = 27$ isomyosins, producing a mechanical diversity that could distribute the fibres in a mechanical continuum matched to the biochemical continuum of the distribution of myosin isoforms so well described by Staron and Pette [37, 38] and reviewed in Pette and Staron [26].

Acknowledgement. This work has been supported by grants n° 1.5.234.90F from the "Fonds National de la Recherche Scientifique" (Belgium), n° 3.4516.89 from the "Fonds de la Recherche Scientifique Médicale" and n° 7.12676 and n° 6.12849 from the "Fonds de développement de la Recherche Scientifique" of the "Université Catholique de Louvain". We thank Dr J. J. Léger (Montpellier) for the gift of the monoclonal antibodies used in this work to identify the myosin heavy chains and Drs P. Baatsen and P. Moens for reading and criticizing the manuscript.

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