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CONTRACTILE PROPERTIES OF SKELETAL MUSCLES FROM YOUNG, ADULT AND AGED MICE

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SUMMARY

1. Comparisons were made *in vitro* at 25 °C among soleus and extensor digitorum longus (EDL) muscles from young (2–3 months), adult (9–10 months), and aged (26–27 months) male mice. We tested the hypotheses that, compared with soleus and EDL muscles of young and adult mice, those from aged mice develop decreased maximum tetanic force (P_0, mN) and specific P_0 (N/cm^2), and that no significant differences occur for contraction time, half-relaxation time, or force–velocity relationship.

2. For the aged mice, the P_0 of the soleus muscles and EDL muscles were 78 and 73% respectively of the values for adult mice. The specific P_0 of EDL muscles of aged mice was 78% of the value of 23 N/cm^2 obtained for young and adult mice. For soleus muscles, the specific P_0 of 21 N/cm^2 did not change with age.

3. Compared to values for young and adult mice, the contraction and half-relaxation times of soleus muscles from aged mice were increased, but the overall force–velocity relationships of soleus and EDL muscles did not change. The pooled values for the maximum velocity of unloaded shortening extrapolated from the force–velocity relationship of soleus and EDL muscles were 4·6 and 10·1 fibre lengths/s, respectively.

4. The decrease in the specific P_0 of the EDL muscle with ageing must result from either a decrease in the number of cross-bridges in the driving stroke or a decrease in the force developed by each cross-bridge.

INTRODUCTION

During the past 150 years, investigators have studied many different muscles, but the basic observation of Quetelet (1835) remains that between 30 and 80 years of age muscle strength decreases 30–40%, with men showing a greater loss than women (Larsson, Grimby & Karlsson, 1979; Grimby & Saltin, 1983; Young, Stokes & Crowe, 1984, 1985). The limited observations on the maximum isometric tetanic force (P_0, mN) developed by small whole muscles of rodents studied *in vitro* (Gutmann & Carlson, 1976; Fitts, Troup, Witzmann & Holloszy 1984; Larsson & Edström, 1986)

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support the premise that a decrease in P_0 with age is not unique to humans. In a 1983 review, Grimby & Saltin concluded that the decrease in muscle strength with age correlated highly with the decrease in muscle mass and there was no need to propose changes in the composition of skeletal muscle with ageing. More recent estimates of maximum specific strength suggest a decrease of maximum specific strength with age for men (Young *et al.* 1985) but no change for women (Young *et al.* 1984). For muscles from rats, Fitts *et al.* (1984) and McCarter & McGee (1987) reported no significant differences in the specific P_0 (N/cm^2) with age. Consequently, the issue of a decrease in the specific P_0 of muscle in aged compared to young animals remains controversial.

The effect of age on the velocity of shortening of human muscles has not been studied extensively. Larsson *et al.* (1979) initially reported that the maximum knee extension velocity of 65 year old men was 66% that of young men, but Grimby & Saltin (1983) reassessed the data of this and other studies and concluded that 'the steepness of the force–velocity curve did not differ markedly' between young and old subjects. In a study of soleus and EDL muscles from 9- and 28-month-old rats, Fitts *et al.* (1984) found no change with age for the maximum velocity of unloaded shortening (V_{max}) extrapolated from the force–velocity relationship. For small bundles of fibres and single skinned fibre segments obtained from 9-month- and 29- to 30-month-old male rats, Eddinger, Cassens & Moss (1986) concluded that soleus muscles became 'faster and stronger'. No significant difference was observed with ageing for the P_0 or V_{max} of bundles from EDL muscles, but the velocities at intermediate after-loads were lower.

The absence of adequate data on the contractile properties of whole skeletal muscles from young, adult and aged rodents measured *in vitro* prevents an unequivical statement as to the deficit for P_0 and specific P_0 and a resolution of the conflicting data on V_{max} and the force–velocity relationship of slow and fast muscles. To resolve these issues, data were collected on the soleus and EDL muscles from 3–4 month (young), 9–10 month (adult), and 26–27 month (aged) male mice. We tested the hypotheses that: (1) muscles from aged mice develop a lower P_0 and specific P_0 than muscles from young or adult mice; and (2) with ageing, no significant differences occur in contraction or relaxation times or force–velocity relationships. Our results support both hypotheses, with the exceptions that for soleus muscles of aged mice, compared to those from young or adult mice, the specific P_0 did not change, the contraction and relaxation times were prolonged and the frequency–force relationship was shifted upwards and to the left. A brief report of these results was presented to the American Biophysical Society (Brooks & Faulkner, 1987).

METHODS

Experiments were performed on fifty-five male mice of the C57BL/6 strain. All experiments were conducted in accordance with the policy statement outlining the care and use of animals published by the American Physiological Society. Mice were anaesthetized with an initial intraperitoneal injection of sodium pentobarbitone (40 mg/kg). Supplemental injections were given as needed. Contractile properties were measured *in vitro* and in most cases on two soleus and two EDL muscles from each young, adult or aged mouse. The small 8–11 mg muscles of mice met all of the criteria chosen previously for a viable and stable muscle preparation (Segal & Faulkner, 1985). No muscle showed a decrease in P_0 greater than 15%.

Following the removal of the muscles, the anaesthetized mice were killed by an overdose of sodium pentobarbitone. The muscles were isolated and 5-0 silk suture was tied securely to the proximal and distal tendons. Each muscle was then removed and immersed in a horizontal bath containing buffered physiological salt solution (composition in mM: NaCl, 137; $NaHCO_3$, 24; glucose, 11; KCl, 5; $CaCl_2$, 2; $MgSO_4$, 1; NaH_2PO_4, 1; and tubocurarine chloride, 0·025). The solution was maintained at 25 °C, and the pH was maintained at approximately 7·4 by buffering with a gas mixture of 95% O_2 and 5% CO_2. The distal tendon of the muscle was attached to an inflexible post and the proximal end was tied directly to the lever arm of a position feed-back servomotor (Cambridge Technology Inc., Model 300H). The motor and associated electronics provided position and force information as well as variable electromagnetic after-loads.

Muscles were stimulated directly by an electrical field generated between two platinum electrodes. All muscle fibres in a muscle were activated simultaneously by stimulation with 200 μs pulses of supramaximal intensity. Muscle length was adjusted until a single stimulus pulse elicited maximum force during an isometric twitch. The twitch force (P_t) and contraction and half-relaxation times were measured during each isometric twitch for each muscle. Although for individual soleus and EDL muscles of mice the optimal length for P_t and P_0 might vary by a maximum of $\pm$2% of muscle length, the mean optimal lengths for P_t and P_0 of each of these muscles were not significantly different. Furthermore, a change of this magnitude for muscle length had no significant effect on mean P_0 of either muscle for any of the three age groups. Other investigators (Larsson & Edström, 1986) have reported no significant differences in optimal length for P_t and P_0 for slow and fast muscles of adult and old rats. The determination of optimal length by twitches is energetically less demanding than determination by tetanic contractions, consequently, the optimal muscle length (L_0) for the development of twitch force was determined and then held constant for all subsequent isometric measurements.

A frequency–force curve was constructed for each muscle from records of the force exerted during periods of stimulation at increasing frequencies. The frequency–force curves for soleus and EDL muscles from young, adult and aged mice are shown in Fig. 1. P_0 was defined as the maximum isometric tetanic force on the frequency–force curve. The twitch/tetanus ratio was calculated (P_t/P_0). The stimulus frequency that produced the maximum rate of force development (dP/dt_0) was determined by increasing the stimulus frequency from that necessary to elicit P_0 until a plateau was observed in the frequency–dP/dt relationship (Buller & Lewis, 1965). A stimulus frequency of 350 Hz was usually required to elicit a maximum dP/dt_0.

Shortening velocities were measured during twelve different after-loaded isotonic contractions (P/P_0 = 0·05–0·50). The same relative after-loads were used for each muscle tested. Shortening velocities were measured in order of increasing after-loads and were determined from the straight, maximum velocity segment of the displacement signals (Fig. 2). The stimulation frequency that resulted in the maximum dP/dt_0 was used for measuring velocities and was held constant during the experiment. As reported by others (Jewell & Wilkie, 1958; Lannergren, 1978; Ranatunga, 1982) the velocity observed with an after-loaded isotonic contraction was not significantly different from the velocity after a quick release from P_0 to a given after-load (D. R. Claflin & J. A. Faulkner, unpublished observation). Consequently, the conclusion of Jewell & Wilkie (1958) that 'this presumably indicates that sufficient time had elapsed before the load was lifted for the muscle to become fully active' is valid. The P_0 was monitored throughout the procedure, and after-loads were calculated based on the most recently measured P_0. After determination of shortening velocities, a final measurement of P_0 was made. The P_0 measured after the determination of the force–velocity relationship averaged 102% of pre-test value for the soleus muscle and 87% for the EDL muscle.

Following the measurements of force and velocity, the tendons were removed from the muscle, the muscle was blotted, and immediately weighed. The muscle was pinned at L_0 and viewed under a dissection microscope. With microdissection, five to ten small bundles of fibres were isolated from their origin on the proximal fascial sheath to their insertion on the distal fascial sheath. The lengths of the bundles of fibres were measured and a mean length for the bundles of fibres was obtained for each muscle.

The fibre lengths of soleus and EDL muscles from young, adult and aged mice were determined using the nitric acid digestion technique (Close, 1964; Maxwell, Faulkner & Hyatt, 1974). For these experiments, the muscle was removed and isometric contractile properties were measured as described previously. The muscle was pinned at L_0 and the lengths of five to ten fibres were

measured under a dissecting microscope. Still pinned at L_o, the muscle was fixed overnight in 10% formaldehyde solution in 0·9% saline. After fixation, the muscle was immersed in 20% HNO_3 for 8–12 h to dissolve the connective tissue. The acid was replaced by 50% glycerine solution. For each digested muscle, the resting length was measured and the muscle was separated into several bundles. Bundles were selected from the proximal, distal and middle portions of muscles. Single fibres were teased from the bundles, and the lengths of approximately five fibres from each bundle were measured at 100× magnification. A total of eighteen to twenty fibres from each muscle were measured, and the mean fibre length was determined. The fibre length/muscle length ratio was determined by dividing the mean fibre length by the resting length of the digested muscle.

The velocities of shortening were converted from mm/s to fibre lengths/s. For each muscle, the data on force (P/P_0) and velocity (L_f/s) were fitted by a rectangular hyperbola of the form $(V+b)(P/P_0 \pm a/P_0) = b(1+a/P_0)$ where V is the velocity of shortening (Hill, 1938). The constants a/P_0 and b were determined from an algorithm which minimized the sum of the squares of the differences between the resultant hyperbola and the measured velocities (Claflin & Faulkner, 1985). V_{max} was calculated from $b/(a/P_0)$. For each of the two muscles and each of the three age groups, the mean ± 1 S.E.M. was calculated for the velocities at each of the twelve after-loads. The force–velocity relationship was then plotted for soleus and EDL muscles of young, adult and aged mice (Fig. 2).

The dry masses of four to six soleus and EDL muscles for each age group were determined. The muscles were dehydrated at 80 °C for approximately 30 min. After 30 min, the muscles were weighed at 5 min intervals until the masses stabilized. Dry mass/wet mass ratios were calculated. The mean cross-sectional area of each muscle was estimated by dividing muscle wet mass by the product of fibre length and 1·06 g/cm^3, the density of mammalian skeletal muscle (Méndez & Keys, 1960).

For each variable, significant differences among groups were determined by a univariate one-way analysis of variance. If the F statistic of the analysis of variance was significant, differences between groups were assessed by a Scheffe comparison. A paired t test was used to determine the difference between the two techniques for measuring fibre lengths. The accepted level of significance for all statistical tests was set at $P \leqslant 0{\cdot}05$. All data are reported as a mean ± 1 S.E.M.

RESULTS

The body mass of the mice increased 25% from 3 to 10 months of age and decreased by 13% from 10 to 27 months of age (Table 1). Compared to soleus and EDL muscles from adult mice, significant decreases of 20 and 13% respectively were observed for the muscle wet masses of soleus and EDL muscles from aged mice (Table 1). The dry mass/wet mass ratio did not differ among the three age groups for either muscle nor was the ratio different between the muscles. The pooled dry mass/wet mass ratio was 0·25 ± 0·005.

As reported previously by Segal, White & Faulkner (1986), no significant difference was observed between the fibre lengths measured on bundles of fibres *in situ* and on single fibres after nitric acid digestion. Furthermore, the fibre lengths from soleus and EDL muscles did not change significantly with age (Table 2). The fibre length/muscle length ratio determined on intact soleus muscles was not different among the three age groups and the pooled mean equalled 0·69 ± 0·006. With the nitric acid digestion technique, in spite of no differences in the mean fibre length with age, the value of 0·68 for the fibre length/muscle length ratio of the soleus muscles of adult mice was significantly smaller than the values of 0·71 and 0·70 for young and aged mice, respectively. This difference of 3–4% is not likely to be of physiological significance. For EDL muscles, the fibre length/muscle length ratio did not change with age and the pooled ratio was 0·45 ± 0·004. Although muscle wet mass decreased with age, small differences in individual fibre lengths and muscle wet

TABLE 1. Data are given (mean ± 1 S.E.M.) for body mass, muscle wet mass, dry mass/wet mass ratio, fibre length and total cross-sectional area of muscle fibres, as estimated by dividing muscle mass by the product of fibre length and 1·06 g/cm³, the density of mammalian skeletal muscle, of soleus and extensor digitorum longus (EDL) muscles of young (2–3 months), adult (9–10 months), and aged (26–27 months) mice

	Soleus muscle			EDL muscle		
	Young ($n = 11$)	Adult ($n = 14$)	Aged ($n = 14$)	Young ($n = 15$)	Adult ($n = 18$)	Aged $n = 18$)
Body mass (g)	27·0 ± 1·04*	35·4 ± 1·44	29·7 ± 1·09*	27·5 ± 0·75*	33·3 ± 1·02	30·0 ± 0·59*
Muscle wet mass (mg)	8·75 ± 0·61	10·3 ± 0·48	8·27 ± 0·50*	10·74 ± 0·22	10·66 ± 0·40	9·28 ± 0·37*†
Dry mass/ wet mass	0·24 ± 0·003	0·25 ± 0·02	0·24 ± 0·01	0·26 ± 0·01	0·24 ± 0·01	0·25 ± 0·02
Fibre length (mm)	7·84 ± 0·22	8·15 ± 0·22	7·45 ± 0·17	5·44 ± 0·12	5·76 ± 0·15	5·37 ± 0·08
Fibre area (mm^2)	1·05 ± 0·06	1·17 ± 0·03	1·03 ± 0·06	1·82 ± 0·06	1·74 ± 0·04	1·63 ± 0·08

* Value for muscle of young or aged mice significantly different from value for adult mice ($P \leqslant 0·05$).

† Value for muscle of aged mice significantly different from young mice ($P \leqslant 0·05$).

TABLE 2. Fibre lengths and fibre length/muscle length ratios (L_f/L_o) as measured from intact muscles (intact) and following nitric acid digestion (digested), are given for soleus and EDL muscles from young (2–3 months), adult (9–10 months) and aged (26–27 months) mice

	Soleus muscle			EDL muscle		
	Young ($n = 3$)	Adult ($n = 6$)	Aged ($n = 5$)	Young ($n = 3$)	Adult ($n = 5$)	Aged ($n = 5$)
L_f intact (mm)	8·00 ± 0·15	7·37 ± 0·20	7·11 ± 0·21	5·46 ± 0·07	5·67 ± 0·07	5·70 ± 0·14
L_f/L_o intact	0·71 ± 0·02	0·69 ± 0·01	0·69 ± 0·01	0·45 ± 0·003	0·44 ± 0·004	0·45 ± 0·01
L_f digested (mm)	8·08 ± 0·27	7·28 ± 0·20	7·21 ± 0·21	5·52 ± 0·19	6·03 ± 0·21	5·61 ± 0·17
L_f/L_o digested	0·71 ± 0·01*	0·68 ± 0·002	0·70 ± 0·003*	0·45 ± 0·01	0·47 ± 0·02	0·44 ± 0·004

* Value for muscle of young or aged mice significantly different from value for adult mice ($P \leqslant 0·05$).

masses resulted in no significant differences in the cross-sectional area of either muscle with ageing (Table 1).

Between 3 and 10 months of age, the soleus showed an increase in P_0, whereas the EDL muscle showed no change in P_0 (Table 3). The P_0 of both muscles decreased significantly with ageing, the soleus muscles to 78% of the adult value and that of the EDL muscles to 73%. The specific P_0 of 21 N/cm² for soleus muscles did not differ among the three age groups. For EDL muscles, the specific P_0 of 23 N/cm² did not change between 3 and 10 months of age, but that of the aged mice decreased significantly to 78% of the value for adult mice (Table 3).

For the soleus muscles, the contraction and half-relaxation times were prolonged significantly in adult compared to young and aged compared to young mice. The

TABLE 3. *In vitro* contractile properties at 25 °C are presented for young (2–3 months), adult (9–10 months) and aged (26–27 months) mice. Data (mean ± 1 S.E.M.) include: time to peak twitch tension (TPT), half-relaxation time ($RT_{1/2}$), maximum isometric tetanic force (P_0), maximum specific tetanic force (specific P_0), and twitch tetanus ratio (P_t/P_0), extrapolated maximum velocity of unloaded shortening (V_{max}), and the parameter a/P_0 that describes the force–velocity relationship

	Soleus muscle			EDL muscle		
	Young ($n = 11$)	Adult ($n = 14$)	Aged ($n = 14$)	Young ($n = 15$)	Adult ($n = 18$)	Aged ($n = 18$)
TPT(ms)	28 ± 1*	34 ± 1	39 ± 2†	16 ± 1	15 ± 1	16 ± 1
$RT_{1/2}$ (ms)	35 ± 2*	45 ± 2	50 ± 2†	18 ± 1	17 ± 1	19 ± 1
P_0(mN)	213 ± 6*	259 ± 11	202 ± 9*	413 ± 11	411 ± 13	299 ± 14*†
Specific P_0(N/cm²)	20·6 ± 0·67	22·1 ± 0·65	18·7 ± 1·05	23 ± 0·80	23·8 ± 0·67	18·6 ± 0·86*†
P/P_0	0·14 ± 0·01	0·15 ± 0·01	0·19 ± 0·02	0·23 ± 0·01	0·26 ± 0·01	0·28 ± 0·01†
$V_{max}(L_t/s)$	4·8 ± 0·19	4·2 ± 0·26	4·6 ± 0·18	10·4 ± 0·25	9·8 ± 0·2	10·0 ± 0·39
a/P_0	0·22 ± 0·02	0·20 ± 0·01	0·19 ± 0·02	0·34 ± 0·02	0·38 ± 0·01	0·34 ± 0·02

* Value for muscle of young or aged mice significantly different from value for adult mice ($P \leqslant 0·05$).

† Value for muscle of aged mice significantly different from young mice ($P \leqslant 0·05$).

significant prolongations of the contraction and half-relaxation times of the soleus muscle with ageing were supported by significant increases in the tetanic force developed at submaximum frequencies of stimulation by soleus muscles of aged mice compared to the forces developed by soleus muscles of young and adult mice (Fig. 1). In contrast, the EDL muscles showed no significant differences in any of these variables with ageing (Table 3), except for one significant Scheffe comparison between young and aged mice in the frequency–force relationship at 50 Hz.

Neither the force–velocity relationships (Fig. 2) nor the V_{max} (Table 3) of soleus or EDL muscles showed any significant change with age. The lack of a significant change in the force–velocity relationships of soleus and EDL muscles with ageing was supported by no evidence of significant differences in the values for a/P_0 (Table 3).

DISCUSSION

The conclusion that human beings lose a significant amount of muscle mass with ageing is based primarily on cross-sectional studies and indirect estimates of muscle mass, but the magnitude of the estimated loss, greater than one-third of the muscle mass, attests to the validity of the observation (Grimby & Saltin, 1983). For rats, decreases in mass of 13–26% have been reported for soleus muscles and 15–22% for EDL muscles (Gutmann & Carlson, 1976; Fitts *et al.* 1984; Larsson & Edström, 1986). The aged mice in our study had a decrease of 20% in the mass of the soleus muscle and of 13% in the mass of the EDL muscle. These decreases in muscle mass with ageing are similar to the values of 10 and 18% for soleus and EDL muscles, respectively, reported by White, Clarke & Kandarian (1986) for mice of the same strain. The lack of any change in the dry mass/wet mass ratio with ageing is consistent with a previous report on soleus and omohyoid muscles of rats (McCarter & McGee, 1987). The stable value of approximately 0·25 in both muscles at all ages

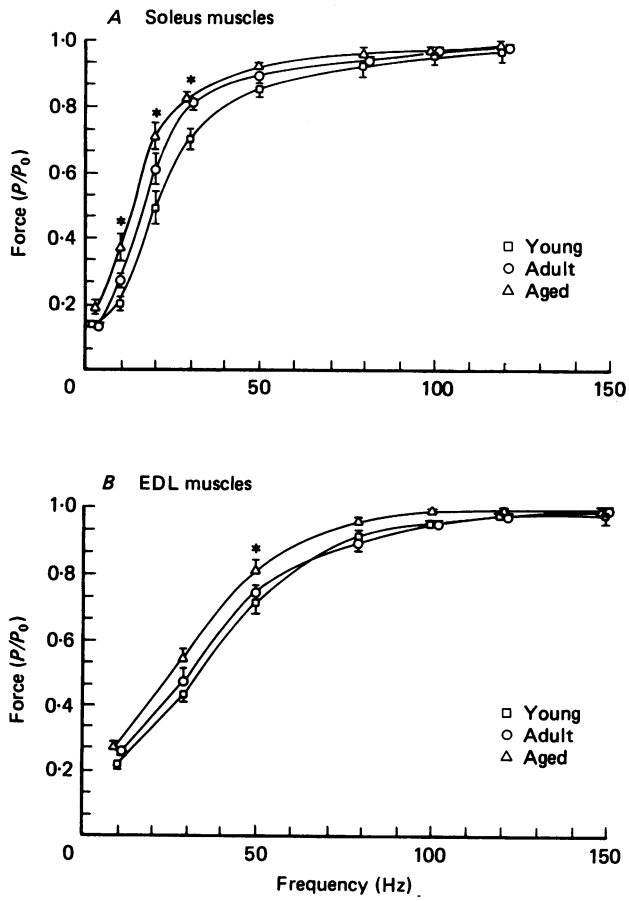

Fig. 1. Frequency–force relationships for soleus muscles (*A*) and extensor digitorum longus (EDL) muscles (*B*) from young, adult and aged mice. The values on the ordinate are normalized as the force at each frequency (*P*) divided by the maximum isometric tetanic force (P_0). Plots are for means ± 1 S.E.M. Error bars are omitted when they fall within the symbol. The asterisks indicate significant differences in the *F* ratio of the univariate one-way analysis of variance among the three groups and a significant Scheffe comparison between the muscles from the young and aged mice ($P \leqslant 0{\cdot}05$).

in spite of significant decreases in mass with age indicates that losses in protein and water were proportional.

For male Wistar rats 26 months of age, the EDL muscle developed a P_0 24% less than that of EDL muscles from 5-month-old rats (Gutmann & Carlson, 1976). In a similar comparison of soleus muscles in 6-month- and 20- to 24-month-old rats, the deficit in P_0 was 19% (Larsson & Edström, 1986). Fitts and his associates (1984) did not present data on the P_0 of muscles in their study of ageing of Long–Evans rats, so values for P_0 were calculated from their data on specific P_0 using predetermined fibre length/muscle length ratios of 0·60 for soleus and 0·40 for EDL muscles (Segal & Faulkner, 1985). Compared to data for 9-month-old rats, the deficit in P_0 for 28-month-old rats was 14% for soleus muscles and 21% for EDL muscles. In the

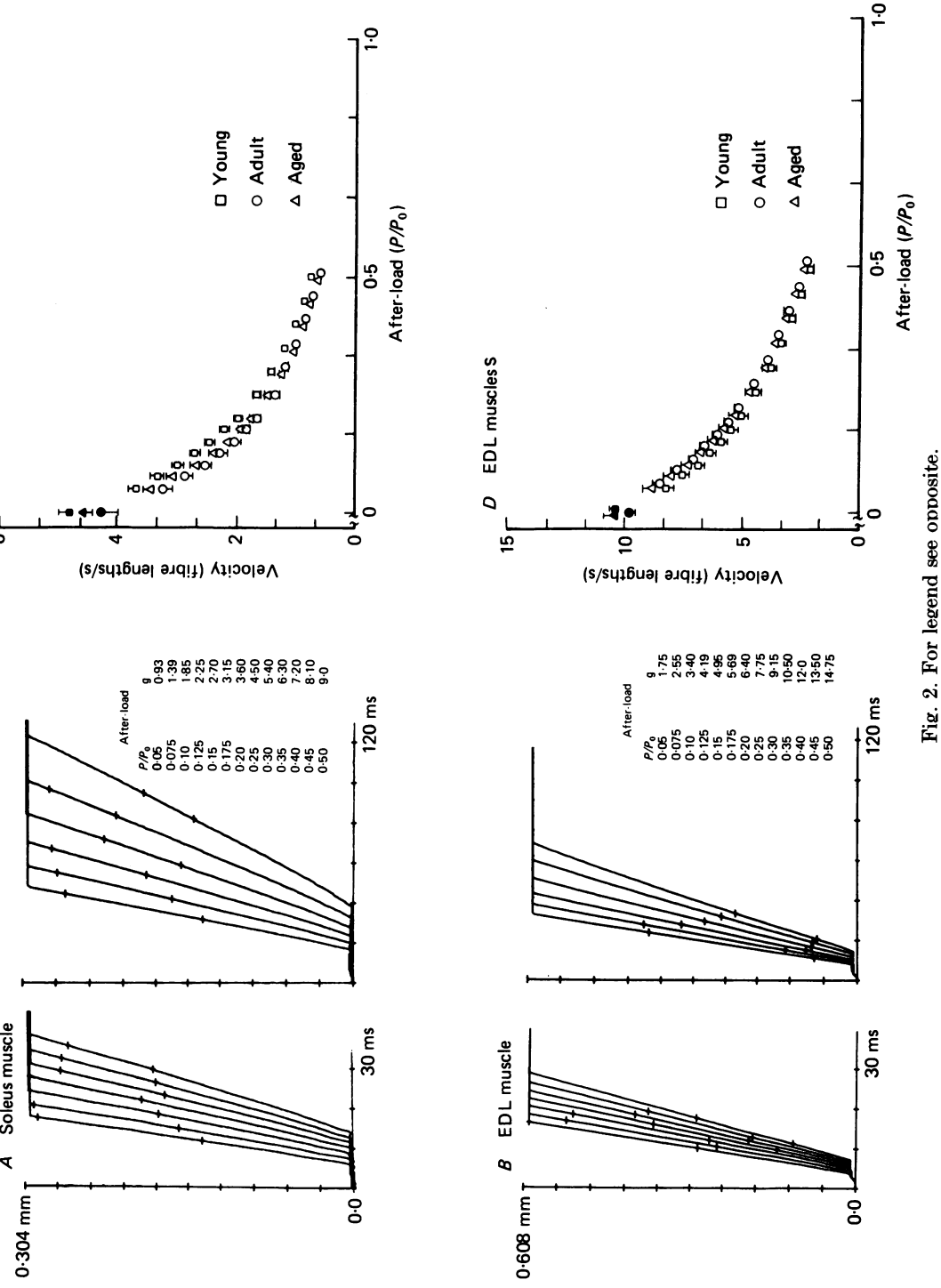

Fig. 2. For legend see opposite.

present study, soleus and EDL muscles from aged male mice showed a deficit in P_0 of 22 and 27% respectively. Although the deficits for the P_0 of soleus and EDL muscles of aged compared to young rodents are lower than the deficits of 30–40% reported for the maximum strength developed by aged compared to young men and women (Grimby & Saltin, 1983), these data support the hypothesis of a significant deficit in the development of maximum force by whole skeletal muscles of aged animals.

The omohyoideus of the rat, a parallel-fibred muscle with the fibres parallel to the long axis of the muscle (McCarter, Radicke & Yu, 1977), has a specific P_0 of approximately 28 N/cm^2 (McCarter & McGee, 1987). In contrast, in the present study of mice the soleus muscle had a specific P_0 of 22 N/cm^2 and the EDL muscle of 24 N/cm^2 with similar values reported previously for rats (Close, 1972). The hierarchy in the values for specific P_0 from soleus to EDL to omohyoideus is explained in part by the angle of pennation of 5 deg for the soleus, 3·5 deg for the EDL muscles (Close, 1964), and 0 deg for the omohyoideus muscle (McCarter *et al.* 1977). Although Close (1964) calculated that the effect of the angles of pennation on the force developed by the soleus and EDL muscles was less than 0·1%, our data indicate the effect is between 15 and 20%. Since the fibre length/muscle length ratio does not change with ageing for either the soleus or the EDL muscles, the pennation remains constant and therefore does not contribute to the decrease in the development of force that occurs with ageing.

Ageing does not appear to influence the specific P_0 of the anterior tibial, soleus, or omohyoideus muscles of rats (Fitts *et al.* 1984; Larsson & Edström, 1986; McCarter & McGee, 1987). In the present study, the specific P_0 for soleus muscles of all ages and EDL muscles of young and adult mice were within the normal range for the specific P_0 of young animals (Close, 1972). In contrast, the specific P_0 of EDL muscles from aged mice was significantly lower than the values for young and adult mice. Fitts *et al.* (1984) reported no difference in the specific P_0 of EDL muscles of rats, but an adequate description of the method for normalization of the P_0 was not presented. The deficit in the specific P_0 of 22% for the EDL muscles from aged male mice is in reasonable agreement with the 20% deficit observed in the maximum voluntary contraction/cross-sectional area of aged men (Young *et al.* 1985), although the mechanisms responsible are potentially quite different.

Fig. 2. A sample of the displacement records at different after-loads for one soleus muscle (*A*) and one extensor digitorium longus (EDL) muscle (*B*) from an aged mouse, and the mean force–velocity relationship for soleus (n = 11, 14, 14) (*C*) and EDL (n = 15, 18, 18) (*D*) muscles from young, adult and aged C57BL/6 male mice, respectively. On the abscissa of the displacement curves (panels *A* and *B*), two different time scales are used to accommodate the variations in velocity at different after-loads. The velocity for each after-load was measured between the plus signs on each displacement curve based on a computer program that selected the section of the displacement record with the highest velocity. Open symbols (panels *C* and *D*) are velocities measured at each of the twelve after-loads, and the maximum velocity of unloaded shortening (V_{max}: closed symbols) is extrapolated from the hyperbola which best fits the data. No significant differences were observed for the overall relationship, for V_{max}, or for a/P_0 (see Table 3 for V_{max} and a/P_0). Plots are for mean ± 1 S.E.M. Error bars are omitted when they fall within a symbol.

Single skinned fibres from soleus and EDL skeletal muscles of aged rats do not show significant deficits in specific P_0 (Eddinger *et al.* 1986). During measurements of fibre length after nitric acid digestion, most of the fibres in muscles of aged mice appeared to be of normal size, but a significant population of small, atrophic fibres was observed in each soleus and EDL muscle. White *et al.* (1986) have noted such a population of fibres in their frequency histogram for fibre cross-sectional areas of soleus and EDL muscles of mice. These observations suggest that the deficit of 22% observed for the specific P_0 of EDL muscles of aged mice results from impairments in the force development of select fibres, but the possibility of differences between species and observed differences between soleus and EDL muscles raise issues that preclude a clear resolution of the problem.

Regardless of whether some or all of the fibres are involved, the significant decrease in the force-generating capacity per unit cross-sectional area of whole EDL muscles from aged male mice must result from a decrease in the number of cross-bridges per unit area, fewer cross-bridges in the driving stroke at any one time, or a decrease in the force developed by a cross-bridge during the driving stroke. Fragments of the S_1 and S_2 portions of the myosin molecule have been observed in rested muscles of young chickens (Ball, Krus & Alizadeh, 1987). An increased susceptibility to the disruption of cross-bridges in muscles of aged animals would reduce the total number of cross-bridges. A decrease in the number of cross-bridges in the driving stroke occurs in the presence of high concentrations of inorganic phosphate (Hibberd, Dantzig, Trentham & Goldman, 1985) or hydrogen ions (Metzger & Moss, 1987). Consequently, an increased concentration of the end-products of metabolism could reduce the force per cm^2 developed by skeletal muscle fibres. A possible mechanism unsupported by evidence is a decrease in the force developed by a cross-bridge during the driving stroke. Conformational changes in proteins with ageing could impair movement of the cross-bridge during the driving stroke.

Our observation of no significant difference with age for the V_{max} of either soleus or EDL muscles of male mice is consistent with previous data for whole muscles of rats (Fitts *et al.* 1984). The lack of any significant change in the whole force–velocity relationship of either soleus or EDL muscles, supports the contention of Grimby & Saltin (1983) that the force–velocity relationship of humans was not influenced by age. In a muscle composed of heterogeneous fibre types, the force–velocity relationship at low velocities is a function of all of the fibres, but at high velocities of shortening, velocities exceed the V_{max} of the slower fibres and only the faster fibres contribute to the relationship (Claflin & Faulkner, 1985). The stability of the total force–velocity curve suggests that no significant changes occur in the distribution of the fibre types in either soleus or EDL muscles.

In the present study, the prolongation of the time characteristics of isometric twitches of soleus muscles appeared to be of physiological significance. Fitts *et al.* (1984) and Larsson & Edström (1986) also report significant increases in the contraction and relaxation times of soleus muscles of aged compared to adult rats. The changes observed with ageing in the soleus muscle are consistent with a decrease in the rate of uptake of calcium by the sarcoplasmic reticulum, whereas the lack of change with age for the overall force–velocity relationship and V_{max}, indicate no

significant modifications in myosin ATPase activity (Barany, 1967), or in the light or heavy chains of myosin (Hoh, McGrath & White, 1976; Reiser, Moss, Giulian & Greaser, 1985; Sweeney, Kushmerick, Mabuchi, Gergely & Stréter, 1986). Close (1965, 1972) considered the product of V_{max} and contraction time to be a constant for slow and fast muscles from a wide variety of species and the correlation coefficient for the means of these two variables for eight mammalian species (Close, 1965) was 0·99. For soleus muscles from young and adult mice, the correlation coefficient between V_{max} and 1/contraction time is 0·76. The significant change in the contraction time of the soleus muscle without a change in the force–velocity relationship results in a decrease in the correlation to 0·17 which is not significantly different from zero. Apparently, with ageing the biochemical events underlying V_{max} and contraction time vary independently of one another.

Eddinger and his associates (1986) conclude that the decrease in strength observed in old age does not reside in changes intrinsic to skeletal muscles. Similarly, McCarter & McGee (1987) report that their major finding was the 'age-related decline in motor performance is most likely unrelated to deficits in the functional properties of fibers'. In contrast, for whole skeletal muscles from mice studied *in vitro*, we found significant decreases with ageing in skeletal muscle mass, P_0, and for the EDL muscle in the specific P_0. A species difference is not a likely explanation of the differences since muscles of rats and mice show the same magnitude of deficit in mass and P_0. The studies of bundles of fibres (Eddinger *et al.* 1986; McCarter & McGee, 1987) and single skinned fibres (Eddinger *et al.* 1986) show considerable variability in the values for specific P_0 within and between groups which precludes the recognition of any age-associated change. Under these circumstances, comparisons between values for specific P_0 of whole muscles with those of bundles and single fibres are difficult. The measurements of time characteristics and force–velocity relationships of bundles and single skinned fibres show little change with age and our observations are consistent with these data.

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