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The Specific Force of Single Intact *Extensor Digitorum Longus* and *Soleus* Mouse Muscle Fibers Declines with Aging

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Abstract. In the present study we measured, for the first time, the isometric specific force (SF, force normalized to cross sectional area) generated by single intact fibers from fast- (*extensor digitorum longus*, EDL) and slow-twitch (*soleus*) muscles from young adult (2–6), middle-aged (12–14) and old (20–24 month-old) mice. SF has also been measured in single intact *flexor digitorum brevis* fibers from young mice. Muscle fibers have been classified into fast- or slow-twitch based on the contraction kinetics. Maximum SF recorded in EDL and *soleus* fibers from young and middle-aged mice did not differ significantly. A significant age-dependent decline in maximum SF was recorded in EDL and *soleus* fibers from young or middle-aged to old mice. The SF was 377 ± 18, 417 ± 20 and 279 ± 18 kPa for EDL fibers from young, middle-aged and old mice, respectively and 397 ± 17, 405 ± 24 and 320 ± 33 kPa for *soleus* fibers from age-matched mice, respectively. The frequency needed to elicit maximum force in EDL and *soleus* fibers from middle-aged to old mice did not differ significantly. In conclusion, the specific force developed by both fast and slow-twitch single intact muscle fibers declines with aging and more significantly in the former.

Key words: Aging — Skeletal muscle — Single fiber — *Soleus—Extensor digitorum longus* — *Flexor digitorum brevis* — Excitation-contraction coupling — Sarcopenia

Introduction

Age-related decreases in skeletal muscle mass, strength and composition, termed sarcopenia, contribute to physical disability and loss of independence in the elderly. Sarcopenia is associated with limitations in daily living activities, frequent falls, fractures related to increased susceptibility to higher impact, accelerated bone loss, reduced heat and cold tolerance and impaired glucose homeostasis (Dutta & Hadley, 1995; Dutta, 1997; Dutta, Hadley & Lexell, 1997). Despite the importance of muscle strength in preventing disability, the biological mechanisms responsible for these phenomena are only partially understood.

Decreases in muscle strength have been reported for different body segments in the elderly (Booth, Weeden & Tseng, 1994; Hurley, 1995; Schultz, 1995). Whether these age-dependent changes in physical performance result from alterations in muscle fibers, neuromuscular junction, peripheral nerve and/or central motor neurons is difficult to ascertain *in vivo. In vitro* contractility recorded in whole muscle, bundle of muscle fibers or skinned fibers in several mammalian species show discrepancies (Eddinger, Cassens & Moss, 1986; Edström & Larsson, 1987; Brooks & Faulkner, 1988; Holloszy, 1995). Therefore, fundamental questions to understanding muscle weakness associated with aging remain to be addressed. Do intact single skeletal muscle fibers devoid of neural influence develop less force at later ages? Does the age-related decline in muscle mechanical output result from alterations in single intact fast- and/or slow-twitch muscle fibers specific force? The answer to these questions demands the investigation of contractile properties of single intact fast- and slow-twitch muscle fibers from mammals at different ages.

The microscopic visualization of individual cells permits a more accurate calculation of fiber cross sectional area and consequently of specific force. Also, single fibers *in vitro* reproduce mechanical responses for many hours in contrast to whole muscles that deteriorate

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rapidly as a consequence of gradients in oxygen and pH between peripheral and core fibers. Multifiber preparations can reproduce mechanical responses only when the number of fibers is so small that all of them are exposed to the perfusing solution. Whole muscles contain a large number of muscle fibers whose characteristics and mechanical responses are not necessarily uniform and are composed of nerve fibers, connective tissue and blood vessels in addition to muscle fibers, complicating the analysis of functional tests (Sugi & Tsuchiya, 1998). In addition, skeletal muscle composition (relative proportion of muscle fibers, connective tissue and capillarity) changes with aging (Lexell, 1995; Davidson, 1999) making the analysis of whole muscle mechanical performance more difficult.

In the present study we have investigated isometric twitch and tetanic specific force (fiber force normalized to fiber cross sectional area) developed by single intact fast- and slow-twitch muscle fibers from young adult, middle-aged and old mice. We have selected *soleus* and *extensor digitorum longus* fibers for this work because they have been used for studies of excitation-sarcoplasmic reticulum calcium release coupling previously (Delbono & Meissner, 1996; Shirokova, Garcia & Rios, 1998; Wang, Messi & Delbono, 2000). Also, these mouse muscles exhibit a predominant fast- or slow-twitch fiber composition (Lännergren & Westerblad, 1991; Burkholder et al., 1994; Chleboun, Patel & Lieber, 1997). In addition, both EDL and *soleus* muscles have shown functional deficits with aging (Larsson & Edström, 1986; Brooks & Faulkner, 1988).

Maximum specific force has also been recorded in single intact fibers from *flexor digitorum brevis* muscle to compare the range of values measured in the present study with those reported in the literature (Lännergren & Westerblad, 1987; Westerblad & Allen, 1991).

Part of the results included in the present article has been presented at the Annual Meeting of the Biophysical Society (González & Delbono, 2000).

Materials and Methods

Fiber Dissection and Mounting

Single intact fibers from the *extensor digitorum longus* (EDL) and *soleus* muscles were dissected from 2–6 (young group; $n = 17$), 12–14 (middle-aged; $n = 7$) and 20–24-month-old (old; $n = 15$) DBA (Harlan-NIA colony) or FVB (our colony) mice. Single intact fibers from the *flexor digitorum longus* (FDB) from 2–6-month-old DBA or FVB mice were also dissected. DBA and FVB mice strain have been used previously as animal models of aging (Bakker et al., 1997; Renganathan, Messi & Delbono, 1998). Mice were housed in a pathogen-free area at Wake Forest University School of Medicine (WFUSM). Animal handling and procedures followed an approved protocol by the Animal Care and Use Committee of WFUSM. Mice sacrificed by cervical dislocation and hindlimb muscles were dissected. Single intact muscle fibers were manually dissected under stereoscopic observation. Debris from other fibers surrounded the intact contracting fiber. The solution for fiber dissection contained (mM): NaCl 145, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1, Hepes 10 and glucose 10 (pH adjusted to 7.4 with NaOH) (Delbono & Kotsias, 1987). The intact single fibers maintained the connection to the tendons allowing for a stable and safe mounting and recording for many hours (Lännergren & Westerblad, 1987). The tendons were gripped by microclips and transferred to the recording flow-through chamber (capacity 150 μl) where fibers were mounted horizontally on the stage of an inverted microscope. One clipped tendon was connected to a 403A force-transducer (Aurora Scientific, Ontario, Canada) (compliance: 19 μm/g, resonant frequency: 0.6 kHz) and the other was fastened to a fine micromanipulator allowing control on fiber position and length. The fiber was superfused permanently by means of a push-pull pump at a rate of ~12 ml/hr.

Contraction Recording and Analysis

Muscle fibers were stimulated directly by an electrical field generated between two parallel platinum electrodes connected to a stimulator. Fiber length was adjusted until a single stimulus pulse or a train of pulses elicited maximum force during a twitch or tetanus (Lo) under isometric conditions. Rectangular pulses (1.5× threshold and 0.5 msec duration) were applied to elicit twitch contractions. Trains of pulses of variable frequency, ranging from 5 to 300 Hz, were applied for 350 msec to eliciting unfused or fused contractions. Longer train duration was needed for *soleus* fibers to achieve maximum tetanic force (*see below*). All the experiments were performed at room temperature (20–21°C). The bath buffered physiological solution used for contraction recording contained (mM): NaCl 121, KCl 5, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.4, $NaHCO_3$ 24.0, glucose 5.5 and EDTA 0.1. This solution was continuously bubbled with a mixture of 5% CO_2 and 95% O_2 in a contiguous chamber to attain pH 7.4 in the recording chamber. The interval between trains of pulses was maintained constant (5 min). For data acquisition a personal computer, a D-A and A-D board, interface (Digidata 1200, Axon Instruments, Foster City, CA) and pCLAMP software (Axon) were used. Single pulses and different trains of pulses were acquired and digitized together with the contraction signal and stored for analysis offline. The diameter was measured in each fiber at Lo in the recording chamber at 200–400× magnifications by two investigators. Because single fiber diameter is not uniform, the largest and smallest diameter of each fiber was measured. These values did not change during the course of the experiments. The cross sectional area (CSA) was calculated as $\pi(d/2)^2$ where d is the mean diameter (Lännergren & Westerblad, 1987).

A typical experiment was developed as follows. A single intact fiber was mounted on the recording chamber and stretched until the optimal length (Lo) was attained with 0.5-msec supramaximal pulses. Lo was maintained throughout the experiment and confirmed later using train of pulses. After attaining Lo, fibers were allowed to rest (not pulsed) for 5–7-min and the force-frequency relationship was recorded using 350 msec trains of pulses of variable frequency for EDL fibers. Trains of pulses of 1–5 sec duration were applied systematically to *soleus* fibers to reach a steady-state or plateau force. When two consecutive trains of pulses elicited the same force, no further frequencies were applied, to reduce the duration of the experiment.

Myosin Heavy Chain (MHC) Isoforms Composition in EDL and *Soleus* Muscle Fibers

MHC composition of single fibers was determined by sodium dodecyl-polyacrylamide gel electrophoresis (SDS-PAGE), as described previously (Giulian, Moss & Greaser, 1983) with some modifications.

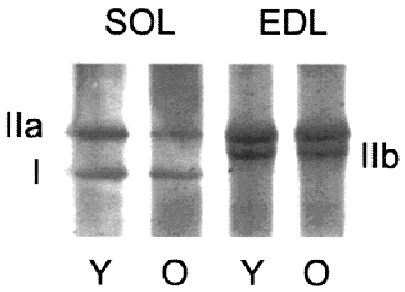

Fig. 1. Electrophoretic separation of myosin heavy chain (MHC) isoforms in *soleus* (SOL) and extensor digitorum longus (EDL) fibers. SDS-PAGE gel illustrating MHC soforms (IIa, IIb and I) composition of *soleus* and EDL fibers from young (Y) and old (O) mice.

MHC isoform composition might have contamination by cellular debris surrounding the contracting fiber. Briefly, single muscle fibers were dissolved in sample buffer in polypropylene centrifuge tubes (15 μl) after the contraction experiments. The sample tubes were incubated for 5 min at 95°C and were then ultrasonicated for 15 min. All muscle samples were stored at −80°C and were run on the electrophoresis system within 2 weeks. The acrylamide concentration was 4% (w/v) in the stacking gel and 6% in the running gel, and the gel matrix included 30% glycerol. For MHC detection in gels the Silver stain plus kit® and Silver Stain SDS-PAGE Standards, high range (Bio-Rad, Hercules, CA) were used. Figure 1 illustrates the MHC composition of *soleus* (SOL) and EDL fibers from young (Y) and old (O) mice.

Statistics

Values are given as mean ± SEM with the number of observation (n). Statistical analysis has been performed for multiple comparisons using analysis of variance (ANOVA) Bonferroni or DUNN test according to the distributions of the data ($P < 0.05$ was considered significant).

Results

Maximum Specific Force in Fast- and Slow-Twitch Muscle Fibers

Specific force for twitch and tetanic contractions was measured in FDB, EDL and *soleus* fibers. To this end, fiber diameter was measured at Lo at different times during the course of the experiment as explained before (*see* Materials and Methods). As this is the first study on the contraction properties of single intact EDL and *soleus* fibers, we determined the reproducibility of the maximum tension recordings at different times, during the course of the experiments, for the same fiber. The decline in maximum contraction tension was negligible over a period of 2–3 hr as demonstrated by the analysis of the ratio between tetanic force at the end over force at the beginning of the experiment for EDL and *soleus* fibers from young and old mice. These results were compared with measurements in FDB fibers from young mouse. The ratio for EDL fibers from young and old mice was 0.9 ± 0.2 and 0.99 ± 0.08, respectively ($P = 0.66$) and for *soleus* fibers 0.95 ± 0.09 and 0.91 ± 0.05, respectively ($P = 0.74$). The value for FDB fibers from young mice was 0.92 ± 0.08 ($P = 0.4$). The comparison of this ratio measured in FDB and EDL fibers from young mice did not show significant difference ($P = 0.94$). Similar results were obtained when FDB and *soleus* fibers ($P = 0.82$) or EDL and *soleus* fibers from young mice ($P = 0.86$) were compared. No significant differences were obtained when EDL and *soleus* ($P = 0.43$) fibers from old mice were compared. These results demonstrate that the tetanic force developed by single intact fibers from FDB, EDL and *soleus* muscles are stable over a period of 2–3 hr and the force decrement recorded during the course of the experiment was less than 10% regardless of the age group.

Because muscle fibers were dissected at different times after whole muscle dissection and the stability of the maximum contraction force in long intact single muscle fibers is not known, we measured the force generated by single EDL or *soleus* fibers from young, middle-aged or old mice at different times after whole muscle dissection. To this end, the maximum specific force — time after muscle dissection relationship was statistically analyzed for each muscle fiber type at different ages. The coefficient of determination (r^2) for EDL fibers from young, middle-aged and old mice was 0.002, 0.0002 and 0.01, respectively whereas for *soleus* fibers the value was 0.009, 0.17 and 0.06, respectively. For FDB the relation between both variables for fibers from young mice was 0.65. These results show that there is not a progressive decay in maximum force in EDL and *soleus* fibers with the time after whole muscle dissection. Therefore, based on this analysis, data recorded in different fiber types from mice of different ages were pooled and statistically compared as shown in Table 1.

The analysis of fiber diameter and cross sectional area demonstrated that fibers from *soleus* muscles from old mice were significantly reduced compared to fibers from young and middle-aged mice, whereas EDL fibers from old mice exhibited a smaller cross sectional area than fibers from middle-aged mice (Table 1).

Maximum specific force was measured in fibers from FDB, EDL and *soleus* from young mice. The values for FDB fibers from young mice were 418 ± 28 ($n = 8$). These values are not significantly different from those recorded in EDL and *soleus* fibers from age-matched mice (Table 1). Maximum specific force exhibited a pronounced decline in fibers from old compared to those from young and middle-aged mice. This decline in maximum tetanic force was associated with a significant decrease in twitch-specific force in EDL fibers from old compared with those from middle-aged mice. Twitch specific force recorded in *soleus* fibers from middle-aged

Table 1. Maximum specific force (SF) in intact single muscle fibers

	EDL			*Soleus*		
	Young	Middle age	Old	Young	Middle age	Old
Fiber diameter (μm)	38 ± 2.0 *n* = 12	45 ± 1.4 *n* = 8	40 ± 2.0 *n* = 25	41 ± 1.4 *n* = 16	41 ± 1.6 *n* = 11	35 ± 1.6*# *n* = 10
Cross sectional area (μm^2)	1187 ± 124 *n* = 12	1578 ± 97 *n* = 8	1217 ± 91# *n* = 25	1310 ± 100 *n* = 16	1362 ± 105 *n* = 11	958 ± 88*# *n* = 10
Twitch SF (kPa)	71 ± 13 *n* = 12	106 ± 11 *n* = 8	53 ± 4.6# *n* = 25	48 ± 8.7 *n* = 16	49 ± 7.2† *n* = 11	54 ± 11 *n* = 9
Maximum tetanic SF (kPa)	377 ± 18 *n* = 12	417 ± 20 *n* = 8	279 ± 18*# *n* = 25	397 ± 17 *n* = 16	405 ± 24 *n* = 11	320 ± 33*# *n* = 10
Frequency at maximum SF (Hz)	76 ± 6.8 *n* = 12	76 ± 19 *n* = 8	68 ± 4 *n* = 25	54 ± 4.7† *n* = 16	43 ± 2.7† *n* = 11	40 ± 4.5*† *n* = 10

Significant difference between fibers from middle age or old and young mice (*), old and middle age (#), EDL and *soleus* of the same age (†). Values are given in mean ± SEM.

mice was significantly reduced compared with that recorded in EDL fibers from age-matched mice, however no significant changes in *soleus* fibers twitch specific force was detected between middle-aged and old mice. No significant decrease in EDL or *soleus* maximum specific force was found when fibers from middle-aged and young mice were compared. Figure 2 shows representative records demonstrating that the maximum specific force of single intact EDL and *soleus* mouse muscle fibers declines with aging. Twitch and tetanic contractions recorded in EDL and *soleus* single intact muscle fibers have been plotted on the same force scale (expressed as specific force) to compare the magnitude of decline in fiber specific force across ages (*see also* Table 1). *Soleus* fiber contraction has been plotted in a slower time scale due to the more prolonged contractions than in EDL fibers. Twitches have been elicited by 0.5-msec supramaximal pulse whereas tetani have been evoked by 350-msec or 1 to 5-sec trains of pulses for FDB and EDL or *soleus* fibers, respectively. It is apparent that the specific force in EDL and *soleus* fibers is significantly lower in old than in young and middle-aged mice. Figure 3 shows the maximum specific force generated by EDL and *soleus* fibers from young, middle-aged and old mice. It is apparent that although some fibers from old mice developed similar tension to that recorded in young and middle-aged animals, a significant number of fibers developed less than 300 kPa in the older group.

The frequency–force curve was analyzed for each fiber. The sequence of frequencies tested was 5, 10, 20, 30, 40, 50, 75, 100, 150, 200 and 300 Hz. As it was explained above (*see* Materials and Methods), the sequence of fiber stimulation was interrupted when two sequential trains of pulses elicited similar force. This means that a fewer number of trains of pulses were applied to some fibers. Figure 4 shows the force-frequency curve for EDL and *soleus* fibers from young, middle-aged and old mice. The frequency needed to attain maximum force was lower in intact muscle fibers for the two muscles than that reported for whole muscle (*see below*). The force–frequency of stimulation relationship describes a single exponential function for the two muscle fiber types. *Soleus* fibers from young, middle-aged and old mice required lower frequencies to elicit maximum force than EDL fibers from animals of the same age. When we compared the frequency-force curve for a fiber type at different ages we found that EDL and *soleus* fibers from old required similar frequencies to elicit maximum force than fibers from young or middle-aged mice (Table 1; Fig. 4).

Myosin Heavy Chain (MHC) Isoforms Composition in EDL and *Soleus* Muscle Fibers from Young and Old Mice

Mouse EDL consists of 99% fast oxidative glycolytic and oxidative fibers and only 1% of slow oxidative fiber whereas mouse *soleus* muscle comprises 58% slow oxidative and 42% fast oxidative glycolytic fibers (Burkholder et al., 1994). These data indicate that there is a chance to dissect fast-twitch fibers from *soleus* and slow fibers from EDL muscle. Therefore, we measured MHC isoforms in a subset of intact muscle fibers used for functional recordings. In young mice, EDL fibers showed type II A and B or type II B MHC alone ($n = 5$) whereas *soleus* fibers showed type IIA and I or type I MHC alone ($n = 5$). We could not define the presence of type IIX MHC in fibers from old mice because this MHC isoform comigrates with the type IIA isoform in 6% SDS PAGE gels (Larsson et al., 1993). In old mice, EDL fibers showed type IIA and B or type IIB MHC alone ($n = 15$), whereas *soleus* exhibited only type IIA and I in all of the fibers studied ($n = 5$). In addition to MHC isoform determination, contraction kinetics were studied for each fiber (*see below*).

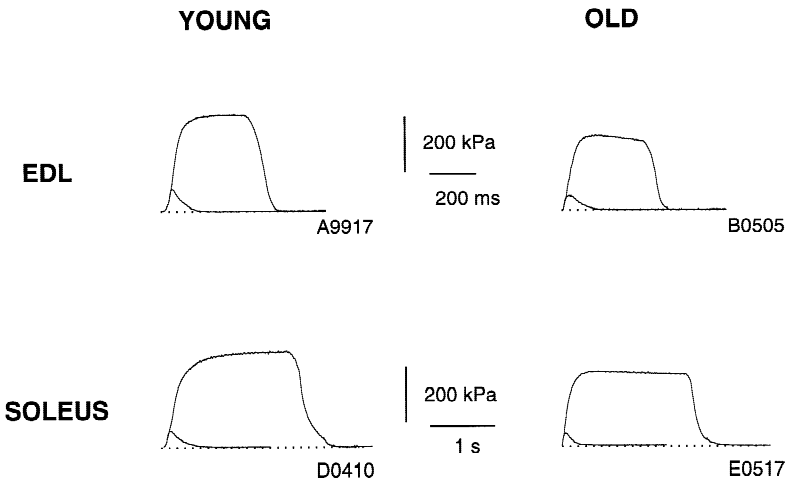

Fig. 2. The specific force of single intact *extensor digitorum longus* and *soleus* mouse muscle fibers declines with aging. Twitch and tetanic contractions recorded in EDL single intact muscle fibers have been plotted in the same force scale to compare the tetanus-twitch relationship and changes in fiber force across ages. *Soleus* fiber contraction has been plotted in a slower time scale. Twitches have been elicited by 0.5-msec supramaximal pulse whereas 350-msec or 2-sec trains of pulses have evoked tetani for FDB and EDL or *soleus* fibers, respectively. It is apparent that the specific force in EDL and *soleus* fibers is significantly lower in old than in young mice. Contraction force is expressed as specific force (force normalized to cross sectional area) (*see* Table 1). Dotted lines indicate the baseline (zero force). Numbers near the records are the corresponding filenames.

Contraction Kinetics in EDL and *Soleus* Muscle Fibers from Young, Middle-aged and Old Mice

Time to peak and half relaxation time were analyzed for twitch force recorded in EDL and *soleus* fibers at different ages (Table 2). The time to peak was shorter in EDL than in *soleus* fibers from young, middle-aged and old mice. This parameter became shorter but not significantly in *soleus* fibers from middle-aged mice compared to those from young mice. The time to peak measured in *soleus* fibers from old was not significantly different from that recorded in fibers from young and middle-aged animals. Half relaxation time was shorter in EDL than in *soleus* fibers from young, middle-aged and old mice, however the comparison of the age groups did not show

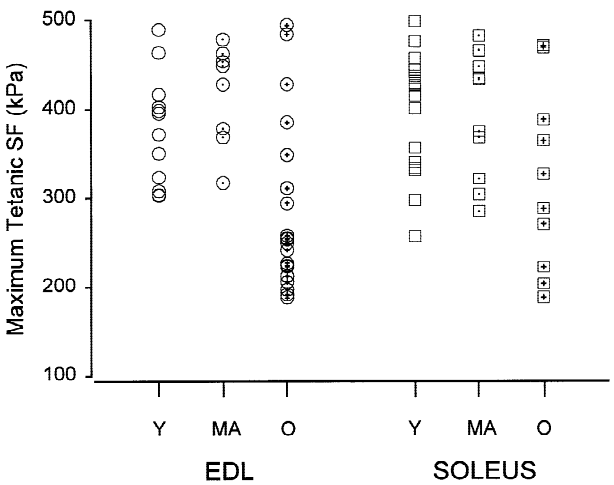

Fig. 3. Maximum tetanic specific force developed by single intact EDL and *soleus* fibers at different ages. All the EDL and *soleus* fibers included in the study are grouped according to the age distribution of the mice into young (Y), middle-aged (MA) and old (O). Each point in the graph represents a single measurement of maximum tetanic specific force (SF).

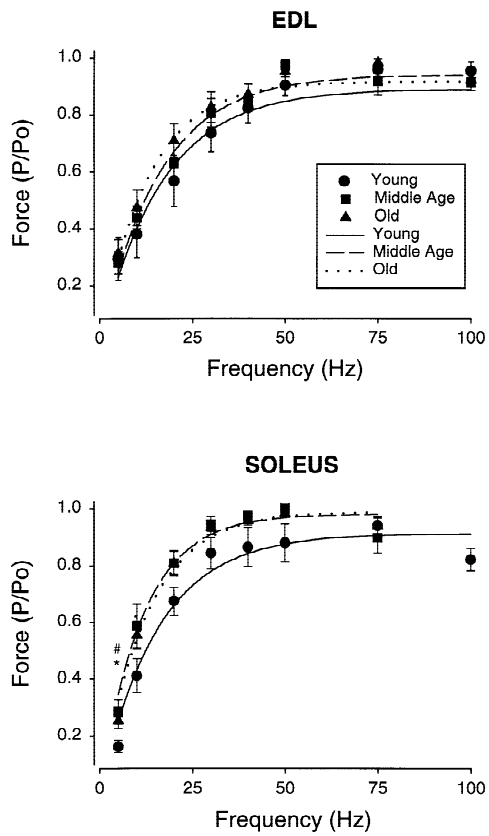

Fig. 4. Frequency-force relationship in single intact *extensor digitorum longus* and *soleus* mouse muscle fibers. Tetanic force (P)/maximum tetanic force (P_o) — frequency relationship for EDL and *soleus* single intact muscle fibers from young, middle-aged and old mice. Slow-twitch muscle fibers (*soleus*) reach maximum force at lower frequencies than fast-twitch fibers (EDL). For the frequency at the maximum tetanic force see Table 1. Data points represent mean ± SEM. Asterisks (*) indicate statistical significant differences between young and old and (#) indicate differences between young and middle-aged mice.

Table 2. Twitch contraction kinetics in intact single muscle fibers

	EDL			*Soleus*		
	Young $n = 11$	Middle age $n = 8$	Old $n = 25$	Young $n = 12$	Middle age $n = 10$	Old $n = 7$
Time to peak (msec)	33 ± 6.1	27 ± 1.3	30 ± 1.5	96 ± 7.5†	73 ± 5.8†	89 ± 18†
Half relaxation time (msec)	62 ± 12	60 ± 6.5	66 ± 8.2	130 ± 14†	170 ± 18†	170 ± 39†

Significant difference between EDL and soleus fibers of mice of the same age (†).
Values are given in mean ± SEM.

statistically significant differences for either EDL or *soleus* muscle fibers. Therefore, no age-dependent changes in time to peak or relaxation time were detected in EDL or *soleus* fibers.

Discussion

In this work we report, for the first time, the specific force generated by single intact EDL and *soleus* fibers and the age-dependent changes in specific force in fibers from EDL and *soleus* muscles. The analysis of maximum isometric contractions allows us to conclude that the maximum specific force recorded in single intact FDB, EDL and *soleus* fibers from mice of the same age does not differ significantly. We also found that the maximum specific force generated by EDL and *soleus* fibers declines significantly with aging and that this phenomenon is not associated with significant changes in contraction kinetics.

Diameter and Cross Sectional Area (CSA) Measured in Single Intact FDB, EDL and *Soleus* Muscle Fibers from Young, Middle-aged and Old Mice

It is uncertain whether the difference in fiber diameter and CSA reported here for *soleus* but not for EDL fibers results from a bias introduced by our dissecting procedure. Because there are no reports in the literature about the values of the diameter and/or CSA in single intact mouse FDB, EDL or *soleus* fibers from different ages, our measurements will be compared with those obtained in whole muscle cross sections. It has been reported that the area corresponding to a specific fiber type does not change significantly in EDL and *soleus* muscles from aged (26–27 months), adult (9–10 months) and young (2–3 months) rats using a nitric acid digestion technique (Brooks & Faulkner, 1988). Another group has reported that the diameter decreased significantly in *soleus* but not in *tibialis anterior* and EDL fibers from 20–24 compared to 6-month-old rats (Larsson & Edström, 1986). The fiber atrophy reported by Larsson et al. (1986) in type-1 fibers from rat *soleus* muscles is consistent with the results reported in the present study.

Specific Force Declines Significantly in Single Intact EDL and *Soleus* Mouse Muscle Fibers with Aging

In the present work we report that the maximum force developed by intact single fibers from EDL and *soleus* muscles decreases with aging. The specific force recorded in fibers from EDL and *soleus* muscles from young and middle-aged mice is similar to that recorded in FDB fibers from young mice in the present work (418 ± 28 kPa) and to that reported for single intact FDB fibers from mouse in the literature (391 ± 59 kPa, range: 300–480 kPa) (Lännergren & Westerblad, 1987). The specific force reported here is greater than that recorded in whole muscle or in bundles of fibers (*see below*). An explanation for this discrepancy can be an overestimation of the cross sectional area or heterogeneous contribution of muscle fibers to force development in multifiber preparations. The lack of homogeneous contribution to force development may result from differences in fiber pennation and/or in optimal fiber length (Sugi & Tsuchiya, 1998).

No studies in isolated fibers or whole FDB muscle or in single intact EDL or *soleus* fibers from aging mammals have been conducted, therefore the discussion will be focused on the comparison of our results with those recorded in multifiber preparations reported in the literature. The specific force recorded here in EDL and *soleus* fibers showed a significant decrease in old compared to young and middle-aged mice. The analysis of the frequency of stimulation–force relationship did not show significant changes when EDL fibers from middle-aged and old mice were compared. Although this difference was significant for *soleus* fibers it should be noticed that there is a trend to develop more force at submaximal frequencies in fibers from middle-aged compared to young mice. These results are similar to those reported for whole muscle by another group (Brooks & Faulkner, 1988). Studies in rat have shown that specific force increases in bundles of *soleus* fibers from old compared to young animals, whereas no significant difference was

observed in EDL bundles between the two age groups (Eddinger et al., 1986). Similar results have been described for skinned fibers in the same publication. These measurements contradict studies in whole mouse muscle in which a significant decrease in EDL specific force but not in *soleus* has been reported (Brooks & Faulkner, 1988). A comparative study in *soleus* and *tibialis* anterior muscles shows a significant decrease in force normalized to muscle weight in old compared to young rats (Larsson & Edström, 1986).

Mechanisms of Age-related Decline in Skeletal Muscle Specific Force with Aging

Neurogenic, myogenic and general factors may contribute to age-dependent decline in muscle force with aging (for a review *see* Loeser & Delbono, 1999). In this work we report age-dependent alterations in the specific force generating capacity of isolated fibers in which contraction has been triggered by sarcolemmal depolarization. The decrease in maximum tetanic specific force is less than that reported for the peak intracellular Ca^{2+} concentration (Delbono, O'Rourke & Ettinger, 1995; Wang et al., 2000). It is feasible that open fibers in the voltage-clamp configuration allow for dialysis of intracellular components existing at a critical level in fibers from older rodents. Some of these components may regulate sarcoplasmic reticulum Ca^{2+} release. We have proposed that alterations in excitation-contraction coupling contribute to the decline in specific muscle fiber force with aging (Delbono et al., 1995; Delbono & Renganathan, 1997). The following evidences support this concept. The number of dihydropyridine receptor and ryanodine receptor decreases with aging in rat (Renganathan, Messi & Delbono, 1997) and mouse (Renganathan, Messi & Delbono, 1998). The reduction in the number of receptors is associated with reductions in charge movement and intracellular peak Ca^{2+} transients in human fast-twitch muscle fibers (Delbono et al., 1995). More recently, we have demonstrated that charge movement and intracellular Ca^{2+} decreases in single FDB fibers from old compared to young and middle-aged mice (Wang et al., 2000). Age-associated decreases in contractile protein density cannot be ruled out as an underlying mechanism for muscle weakness associated with aging. Changes in myosin isoform or fiber type composition have been proposed as a mechanism for muscle weakness in aging rodents (Caccia, Harris & Johnson, 1979) and humans (Lexell, 1995). Some of these studies have been done in volunteers with various nutritional status in inbred rodents that show very low prevalence of age-related pathologies. Studies in Fisher 344 rats and C57BL/6 mice do not show changes in myosin isoform associated with aging (Florini & Ewton, 1989; Phillips et al., 1993). The similar specific force developed by FDB, EDL and *soleus* fibers from young and middle-aged mice reported in the present work does not support the possibility that the age-related decline in specific force reported here derives from changes in myosin isoform. Although an age-related decrease in type II MHC isoform or a switching from type IIB to IIX MHC isoforms has been reported (Larsson et al., 1993; Barton-Davis et al., 1998), this phenomenon may be relevant for contraction kinetics but not in terms of maximum force generation.

The pCa-isometric force relationship measured in skinned fibers does not show significant differences between adult and old mice (Brooks & Faulkner, 1994) suggesting that myofibril sensitivity to Ca^{2+} does not change significantly with aging. These results are in contrast with publications that suggest an age-related decline of muscle strength at the cross-bridge level (Phillips et al., 1991). Studies in humans also show inconsistencies in terms of specific force developed by different muscle groups in the elderly (Hakkinen et al., 1996; Kent-Braun & Ng, 1999; Lynch et al., 1999). Difficulties in measuring muscle cross sectional area *in situ* can account for these discrepancies.

Contraction Kinetics in EDL and *Soleus* Fibers from Young, Middle-aged and Old Mice

Contraction and relaxation times for EDL and *soleus* fibers from young, middle-aged and old mice were analyzed. Twitch kinetics reported here for EDL fibers are similar to that reported for single intact FDB fibers (Lännergren & Westerblad, 1987) after correcting for temperature. In FDB fibers, 14 and 15 msec or 7–8 and 6–7 msec have been reported for contraction and half relaxation time, respectively at 35°C (Lännergren & Westerblad, 1987). Considering Q_{10} values of 3.2 and 4 for contraction and half relaxation time, respectively, the values reported here are within those reported in the literature (Lännergren & Westerblad, 1987).

We did not find age-related changes in twitch relaxation time for EDL and *soleus* fibers despite reported changes in this parameter in whole *soleus* and *gastrocnemius* muscles (Klitgaard et al., 1989; Larsson & Salviati, 1989; Narayanan et al., 1996). Slower relaxation in whole muscle was not recorded in EDL, *tibialis anterior*, *soleus* and superficial *vastus lateralis* muscles by other groups or in a different group of muscles by the same groups (Fitts et al., 1984; Larsson & Edström, 1986; Brooks & Faulkner, 1988). These discrepancies might result from differences among preparations and experimental conditions. We did not find significant changes in time to peak for *soleus* fibers from old compared to young or middle-aged mice despite reported changes in this parameter measured in whole *soleus* and *tibialis anterior* muscles (Larsson & Edström, 1986; Brooks & Faulkner, 1988; Narayanan et al., 1996).

In response to the questions formulated at the beginning of this manuscript: (i) single intact muscle fibers devoid of neural influence develop less force at later ages, (ii) the age-related decline in whole muscle force with aging may result from different factors, one of them being the impairment in the force-generating capacity of single fibers, (iii) alterations in muscle mechanical output results from decreases in the specific force developed by fast- and slow-twitch fibers and (iv) although we did not explore the effects of muscle composition on the age-related decline in muscle force, the results in single fiber conform with most of the studies in whole muscle suggesting that alterations in the fiber itself account for a significant fraction if not for all of the age-related changes recorded in whole muscle.

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