Force generation, but not myosin ATPase activity, declines with age in rat muscle fibers

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Lowe, Dawn A., David D. Thomas, and LaDora V. Thompson. Force generation, but not myosin ATPase activity, declines with age in rat muscle fibers. Am J Physiol Cell Physiol 283: C187–C192, 2002.—We tested the hypothesis that age-associated decline in muscle function is related to a change in myosin ATPase activity. Single, glycerinated semimembranosus fibers from young (8–12 mo) and aged (32–37 mo) Fischer 344 × Brown Norway male rats were analyzed simultaneously for force and myosin ATPase activity over a range of Ca2+ concentrations. Maximal force generation was ~20% lower in fibers from aged animals (P = 0.02), but myosin ATPase activity was not different between fibers from young and aged rats: 686 ± 46 (n = 30) and 697 ± 46 μM/s (n = 33) (P = 0.89). The apparent rate constant for the dissociation of strong-binding myosin from actin was calculated to be ~30% greater in fibers from aged animals (P = 0.03), indicating that the lower force produced by fibers from aged animals is due to a greater flux of myosin heads from the strong-binding state to the weak-binding state during contraction. This is in agreement with previous electron paramagnetic resonance experiments that showed a reduced fraction of myosin heads in the strong-binding state during a maximal isometric contraction in fibers from older rats.

specific tension; actomyosin adenosinetriphosphate; type II fibers; aging

AGE-RELATED CHANGES IN NEURAL and excitation-contraction coupling processes contribute to diminished contractility (7, 11), but recent evidence indicates that contractile proteins are also altered with age. For example, it has long been recognized that shifts in the myosin heavy chain (MHC) isof orm distribution occur with age, but it appears that the function and structure of myosin are altered with age as well (20, 25).

Myosin is a large multisubunit enzyme that functions as the molecular motor in muscle. The hydrolysis of ATP fuels a structural change in the head region of myosin that results in a force-generating interaction with actin. Thus any age-related change in the structure, function, or enzymatic activity of myosin could affect muscle contractility. Several investigators have used the single, permeabilized fiber preparation, which eliminates excitation-contraction coupling processes, to show that the function of the contractile proteins is diminished with age (15, 24, 25, 33). Höök and coworkers (20, 21) showed specifically that myosin’s function is altered with age by demonstrating that myosin extracted from fibers of aged rodents and humans has reduced abilities to move actin relative to myosin from younger counterparts. Those observations are consistent with reports of age-induced reductions in maximal shortening velocity of fibers (10, 24, 33). Therefore, it appears that the function of the contractile proteins, at least of the motor protein myosin, is affected by age.

We recently showed that the structure of myosin was altered in an age-dependent manner (25). Using electron paramagnetic resonance spectroscopy, we found that, during a maximal isometric contraction, 32% of the myosin heads were in the strong-binding (force-generating) structural state in semimembranosus muscle fibers from young rats, but in fibers from old rats, only 22% of the myosin molecules were in that structure. In addition, the magnitude of the age-related fractional reduction in strong-binding myosin was the same as that of the fractional decline in force generation (30%), indicating that age-related changes in myosin structure represent a likely molecular mechanism underlying muscle weakness. Therefore, evidence suggests that the structure and function of myosin are altered with age. It is important to note that these age-related alterations in myosin were independent of changes in fiber MHC isoform.

Hydrolysis of ATP is directly related to contractile function and structural changes in the head region of myosin during a contraction (2, 3, 32), so it is likely that changes in myosin’s enzymatic activity accompany changes in myosin’s structure and function. However, the effect of aging on myosin ATPase activity in skeletal muscle fibers has not been thoroughly investigated. We hypothesized that myosin ATPase activity is attenuated in muscle fibers from aged animals compared with fibers from younger animals. To test this hypothesis, we simultaneously measured myosin ATPase activity and force generation in single, perme-

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ablized fibers from semimembranosus muscles of aged and young adult rats. The apparent rate constant for myosin detachment from actin ($g_{\text{app}}$) can be calculated from measurements of force and myosin ATPase activity (23). Thus we also were able to determine whether changes in myosin function or enzymatic activity occurred in conjunction with a change in cross-bridge kinetics.

METHODS

Animals. Fischer 344 × Brown Norway F1 hybrid male rats aged 8–12 mo (young adult, $n = 7$) and 32–37 mo (aged, $n = 7$) were obtained from the aging colony maintained by the National Institute on Aging. Three of the young adult and two of the aged rats were used in a previous study (25). All animals were housed locally for ≥1 wk in pathogen-free conditions and received food and water ad libitum. Animals were anesthetized with pentobarbital sodium (55 mg/kg ip), semimembranosus and soleus muscles were carefully dissected, and then animals were euthanized with an overdose of pentobarbital sodium.

Tissue preparation. Immediately after dissection, muscles were submerged in cold relaxing buffer [in mM: 7 EGTA, 0.016 CaCl$_2$, 5.6 MgCl$_2$, 80 KCl, 20 imidazole (pH 7.0), 14.5 creatine phosphate, 4.8 ATP] on ice and pinned to approximately in vivo resting length. Bundles of ~50 muscle fibers were formed, tied onto glass capillary tubes, and then stored at −20°C in glycerinating solution [2 mM EGTA, 1 mM MgCl$_2$, 126 mM potassium propionate, 20 mM imidazole (pH 7.0), 4 mM ATP, 50% glycerol]. Under these conditions, membranes, including the sarcoplasmic reticulum, are permeabilized within 2 days, and fibers were viable for ≥5 wk. The advantage of using permeabilized fibers in this study was that initiation of contraction was controlled by exogenously added Ca$^{2+}$. Therefore, any age-related change in the excitation-contraction coupling process was bypassed, and force generation directly reflected the function of the contractile apparatus.

Force and myosin ATPase activity measurements. Individual fiber segments were studied to determine force- and myosin ATPase-Ca$^{2+}$ relationships on a Scientific Instruments Muscle Research System (Heidelberg, Germany). The system uses an NADH-linked fluorescence method for measuring myosin ATPase activity (1, 18, 23, 30, 34). Briefly, a 2- to 3-mm fiber segment was mounted between two microtweezers, one of which was attached to a force transducer. The fiber and tweezers were placed in a quartz capillary filled with a relaxing solution (1 mM EGTA, 1 mM Mg$^{2+}$, 85 mM K$^+$, 85 mM Na$^+$, 2 mM ATP, 10$^{-9}$ M Ca$^{2+}$, and propionate as the major anion), and the optical equipment was aligned. An activating solution (relaxing solution plus 10$^{-4}$ M Ca$^{2+}$) was then flowed into the cuvette, fiber length was adjusted to elicit peak isometric force, and the fiber was relaxed. Fiber cross-sectional area was determined using a calibrated video system. Simultaneous force and myosin ATPase activity were then measured as fibers were exposed to 10$^{-9}$–10$^{-4}$ M Ca$^{2+}$ in solutions that also contained 0.4 mM NADH, 5 mM phosphoenolpyruvate, 100 U/ml pyruvate kinase, and 140 U/ml lactate dehydrogenase. All experiments were performed at 21°C. Figure 1 depicts typical force- and myosin ATPase-pCa curves. Four to six semimembranosus muscle fibers from each animal were studied.

Hill plots of the force- and myosin ATPase-pCa data were used to calculate pCa$_{50}$ (logarithm of the Ca$^{2+}$ concentration required for half-maximal activation), activation threshold (logarithm of the minimum Ca$^{2+}$ concentration required for activation), and two Hill coefficients [n$_1$ (slope of the fitted line at >50% maximal activation) and n$_2$ (slope of the line at <50% maximal activation)] (16, 27).

Determination of $g_{\text{app}}$. According to Huxley (22), the apparent rate constants for myosin attachment ($f_{\text{app}}$) and detachment ($g_{\text{app}}$) from actin describe the relationship between non-force-generating and force-generating states, i.e., the fraction of myosin heads in weak-binding structural states ($x_w$) and strong-binding structural states ($x_s$), respectively, during contraction (Eq. 1)

$$x_s = f_{\text{app}}/(f_{\text{app}} + g_{\text{app}})$$  (1)

$$\text{ATPase} = [\text{myosin}] * \text{CSA} * a * L_{1/2s} * g_{\text{app}}$$  (2)

$$\text{force} = F_w * [\text{myosin}] * \text{CSA} * L_{1/2s} * f_{\text{app}}/(f_{\text{app}} + g_{\text{app}})$$  (3)

$$\text{ATPase/force} = g_{\text{app}}/(aF_w)$$  (4)

Calculations derived from Huxley’s two-state model of muscle contraction have been explained by others (6, 23, 30), and it has been shown that Eqs. 2 and 3 yield Eq. 4, where CSA is fiber cross-sectional area, $a$ is the number of half-sarcomeres, $L_{1/2s}$ is the length of a half-sarcomere, and $F_w$ is the average force produced per mole of strongly bound myosin. A constant can be determined for $aF_w$, since we assume that these variables do not change under given experimental conditions. In preliminary experiments, the constant was determined by first solving for $g_{\text{app}}$ using Eqs. 1 and 2 and using 220 μM for the concentration of myosin in fibers (16) and 0.32 for $x_s$, as determined previously by electron paramagnetic resonance (25). Then, from Eq. 4, the constant $aF_w$ was calculated to be 0.46 μM myosin-m$^2$·kN$^{-1}$, which was used in all subsequent calculations for determination of $g_{\text{app}}$ (Eq. 5)

$$g_{\text{app}} = \text{ATPase/force} * 0.46$$  (5)

Assessment of MHC isoforms in fiber segments. After each experiment, the fiber segments were solubilized in 20 μl of sample buffer [24 mM EDTA, 60 mM Tris (pH 6.8), 1% SDS, 5% β-mercaptoethanol, 15% glycerol, 2 mg/ml bromphenol blue] and stored at −80°C. MHC isoform contents for each
fiber were determined by gel electrophoresis and silver staining followed by densitometry (31).

Statistical analyses. Values are means ± SE. Student’s t-tests were used to compare results of fibers from the aged animals with those from the young adult animals. Pearson product moment correlations (r) were calculated among fibers within an age group to determine relationships between variables. P ≤ 0.05 was considered significant.

RESULTS

None of the semimembranosus fibers studied expressed type I MHC, and <10% expressed type IIA MHC, as determined by gel electrophoresis. All the type IIA fibers coexpressed types IIX and IIB MHC. Because so few of the fibers expressed type IIA MHC and because of the complexity of those fibers expressing all three MHC isoforms, the data from those fibers were not included in the following results.

We detected an increase in the expression of type IIX MHC with age: 73% of the fibers studied from the aged animals expressed detectable amounts of type IIX MHC, but only 20% of the fibers studied from the young adult animals expressed that isoform. However, no statistical differences were found between fibers expressing 100% IIB, 100% IIX, or IIB/IIX MHC for any of the contractile parameters measured (P ≥ 0.24), except pCa50 for force. Therefore, the following results represent data from all fibers combined, except in the case of the measurement of force vs. pCa50.

Maximal force and myosin ATPase activity. Semimembranosus fibers from aged animals produced ~20% less maximal force than fibers from young adult animals (all force data are presented as force normalized by fiber cross-sectional area; P = 0.02; Table 1). Despite the difference in force, maximal myosin ATPase activity was not different between fibers from young adult and aged animals (P = 0.89). To verify that our technique was sensitive enough to detect differences in myosin ATPase activity between fibers, we examined 13 type I MHC-expressing soleus muscle fibers from two young adult animals. We found that soleus muscle fibers hydrolyzed ATP at a maximal rate that was about half that of the type IIX and IIB MHC-expressing fibers in this study (343 ± 46 μM/s).

The uncoupling of force and ATP utilization in fibers from aged animals is illustrated in Fig. 2. Force and myosin ATPase activity at maximal Ca2+ activation were positively correlated among semimembranosus fibers from young adult animals (r = 0.61, P < 0.01) but not among fibers from aged animals (P = 0.30).

Maximal force and ATPase activity data were used to calculate gapp. These results also indicate an age-related difference (P = 0.01; Table 1).

Submaximal force. The pCa50 for force was less in fibers from aged animals than in fibers from young adult animals (P = 0.02; Table 1). The pCa50 for force was the only parameter measured that was significantly different between fibers expressing different MHC isoforms. Fibers from aged animals that expressed 100% type IIB MHC (n = 7) had a mean pCa50 of 6.32 ± 0.06, but fibers from animals that expressed >50% type IIX MHC required more Ca2+ (pCa50 = 6.23 ± 0.03, n = 16). This resulted in a significant relationship between pCa50 for force and type IIB MHC expression (r = 0.45, P = 0.01) among fibers from aged animals. No other variable measured revealed this relationship with type IIB MHC expression.

No age-related differences were detected in the shape of the force-pCa curves as indicated by Hill coefficients, n1 and n2 (Table 1). Activation threshold for force development was also not affected by age (P = 0.57).

Submaximal myosin ATPase activity. Neither the Ca2+ dependence for myosin ATPase activity between fibers from aged and young adult animals nor the activation threshold was different (P ≥ 0.59; Table 1). However, the shape of the myosin ATPase-pCa curve

Table 1. Force generation and myosin ATPase activity of single, glycerinated semimembranosus fibers from young adult and aged rats

<table>
<thead>
<tr>
<th></th>
<th>Young Adult (n = 30)</th>
<th>Aged (n = 33)</th>
<th>P</th>
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<tbody>
<tr>
<td>Force</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Maximal, kN/m²</td>
<td>162 ± 9</td>
<td>136 ± 7</td>
<td>0.02</td>
</tr>
<tr>
<td>pCa50</td>
<td>6.34 ± 0.02</td>
<td>6.27 ± 0.02</td>
<td>0.02</td>
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<tr>
<td>n1</td>
<td>5.88 ± 0.57</td>
<td>4.80 ± 0.51</td>
<td>0.17</td>
</tr>
<tr>
<td>n2</td>
<td>7.58 ± 0.51</td>
<td>7.30 ± 0.44</td>
<td>0.69</td>
</tr>
<tr>
<td>Activation threshold</td>
<td>6.65 ± 0.02</td>
<td>6.63 ± 0.03</td>
<td>0.57</td>
</tr>
<tr>
<td>Myosin ATPase activity</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Maximal, μM/s</td>
<td>686 ± 55</td>
<td>697 ± 46</td>
<td>0.89</td>
</tr>
<tr>
<td>pCa50</td>
<td>6.33 ± 0.04</td>
<td>6.30 ± 0.03</td>
<td>0.59</td>
</tr>
<tr>
<td>n1</td>
<td>7.39 ± 0.63</td>
<td>5.64 ± 0.67</td>
<td>0.09</td>
</tr>
<tr>
<td>n2</td>
<td>3.85 ± 0.57</td>
<td>2.54 ± 0.32</td>
<td>0.04</td>
</tr>
<tr>
<td>Activation threshold</td>
<td>7.59 ± 0.20</td>
<td>7.66 ± 0.16</td>
<td>0.79</td>
</tr>
<tr>
<td>gapp, m/s</td>
<td>9.52 ± 0.66</td>
<td>12.21 ± 0.98</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE. Experiments were conducted at 21°C. All fibers expressed type IIB and/or IIX myosin heavy chain. pCa50, logarithm of Ca2+ concentration required for half-maximal activation; n1, slope of fitted line at >50% maximal activation; n2, slope of line at <50% maximal activation; activation threshold, logarithm of minimum Ca2+ concentration required for activation; gapp, maximal ATPase activity/(maximal force * 0.46).
was affected by age, as shown by the Hill coefficient, \( n_2 \) 
\( P = 0.04; \) Table 1).

**DISCUSSION**

The major finding in this study, that force but not myosin ATPase activity was diminished in fibers from aged animals, is novel. The only other data of which we are aware on the effects of age on skeletal muscle myosin ATPase activity are from studies in which myofibrils were prepared from whole muscles (14; D. Ferrington, personal communication). In those studies, myosin ATPase activity did not change with age in myofibrils prepared from rat extensor digitorum longus, diaphragm, soleus, or plantaris muscles. There are also reports of no age-related change in myosin ATPase in heart muscle myofibrils, even though some contractile parameters such as twitch duration, which is inversely related to the rate of ATP hydrolysis, are negatively affected by age in cardiac muscle (4). Myosin ATPase activity varies according to the MHC isoform expressed, and shifts in MHC isoform occur with age in skeletal and cardiac muscle. Thus our method of studying myosin ATPase in single functioning fibers is preferable to that in myofibrils prepared from whole muscles, because we are able to identify the MHC composition of each fiber and determine contractile capacities of that fiber. The ATP hydrolysis rate of \( \sim 700 \mu \text{M/s} \) at 21°C that we obtained in semimembranosus fibers is similar to values reported by others, if we consider the various fiber types studied and the temperatures at which the experiments were conducted (5, 19, 34). We observed a sizeable variation in ATPase activity among fibers, even among fibers containing the same MHC isoform, but this variation has also been observed by others (5).

We found an age-related decline in force generation similar to that reported previously; i.e., 10–40% force decrements have been reported in permeabilized muscle fibers from old rodents and humans (10, 15, 24, 25, 33). Despite the age-related force deficits, we found that myosin ATPase activity was not diminished in fibers from the aged animals. This might be considered surprising, in light of reports of an age-related decline in unloaded shortening velocity (10, 24, 33), a parameter that is often correlated with myosin ATPase activity (2). However, the present study focuses on isometric contraction, in which the myosin ATPase rate is much slower and its kinetics are strongly affected by crossbridge strain, probably even changing the rate-limiting step of the ATPase reaction (17). The relationship between force and myosin ATPase rate may be less direct than in shortening muscle but is still significant (5). Myosin ATPase activity is clearly necessary for the generation and maintenance of force in muscle, and so it seems obvious that one mechanism by which force generation could be inhibited would be to inhibit ATPase activity. For example, blocking the reactive thiols in myosin results in declines in force and myosin ATPase (29, 30). In the present study, we raised the hypothesis that the age-related decline in force could be due to (or accompanied by) a decline in myosin ATPase activity.

Our finding that force but not myosin ATPase activity declines with age can be viewed in two ways. First, it is empirically clear that we found a loss of contractile efficiency with age. Fibers from aged animals utilized an equivalent amount of ATP during an isometric contraction but did not generate as much force from that energy as fibers from young adult animals (i.e., force/myosin ATPase was reduced with age). The uncoupling of force generation and myosin ATPase utilization with age in the present study can be visualized in Fig. 2. The rate of ATP hydrolysis by the \( \text{Ca}^{2+} \)-ATPase of the sarcoplasmic reticulum has also been shown to be uncoupled from the function of that protein with age. For example, Narayanan et al. (28) showed that aging caused an impairment of \( \text{Ca}^{2+} \) uptake by the sarcoplasmic reticulum, which resulted in prolonged twitch half-relaxation and contraction times in rat skeletal muscles. However, the \( \text{Ca}^{2+} \)-ATPase activity of the sarcoplasmic reticulum was not affected by aging, demonstrating another age-associated uncoupling of ATP hydrolysis with protein function. The reason for the uncoupling is not known, but we speculate that it involves an age-related alteration in ATPase protein structure. We previously reported an age-related structural change in the ATPase catalytic domain of myosin (25), and others have reported tertiary structural changes around the nucleotide-binding site in the \( \text{Ca}^{2+} \)-ATPase with age (9) and age-related decreases in the conformational stability of the \( \text{Ca}^{2+} \)-ATPase (12).

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**Fig. 3.** Myosin structure and kinetics during a maximal isometric contraction is altered with age in fibers containing type IIB/IIX MHC. Myosin structural data, from electron paramagnetic resonance spectroscopy experiments on semimembranosus fibers, showed that the age-related fractional reduction of myosin in the strong-binding structural state (\( x_s \)) during contraction is proportional to the decline in force in those fibers (i.e., a 16% decline in force generation with age relates to a 16% reduction in \( x_s \)) (25). The present results indicate that the age-related reduction in strong-binding (force-generating) myosin is due to an increase in the apparent rate constant for myosin detachment from actin (\( g_{\text{app}} \)) from 9.52 to 12.21. \( Y \), young adult; \( A \), aged; \( f_{\text{app}} \), apparent rate constant for myosin attachment to actin; \( x_w \), fraction of myosin heads in weak-binding structural state.
The second way to view our data is to explain it mechanistically on the basis of myosin cross-bridge kinetics (22, 23). By measuring ATPase and force, we found that \( g^\text{app} \) was elevated in fibers from aged animals by \( \sim 30\% \) relative to fibers from young adult animals (\( P = 0.03; \text{Fig. 3} \)). That is, in fibers from aged animals, there was a greater flux of myosin heads from the strong-binding structure to the weak-binding structure during a contraction. The \( f^\text{app} \) was calculated from Eq. 1 using \( g^\text{app} \) determined for each fiber and using \( x_s \) values that were determined empirically in our previous study on semimembranosus fibers, i.e., \( x_s = 0.32 \) for young animals (25). Despite the 30% difference in \( g^\text{app} \), we found no difference in \( f^\text{app} \) between fibers from young adult and aged animals (\( P = 0.94 \)), indicating that the flux of myosin heads into the strong-binding structure during a contraction is not altered by age (Fig. 3).

The relationship between force and pCa was only moderately affected by age. In agreement with other reports (8), fibers from aged animals showed a slight rightward shift in the force-pCa curve, requiring 17% more Ca\(^{2+}\) to reach half-maximal force than fibers from young adult animals (537 and 457 nM, respectively). Our absolute force-pCa values are slightly higher than those typically reported for fibers expressing type II MHC (13). This is most likely because we conducted our experiments at 21\(^\circ\)C instead of 15\(^\circ\)C (13). Other parameters such as activation threshold and slopes of the force-pCa curves (Hill coefficients) were not affected, suggesting that minimal changes in Ca\(^{2+}\) sensitivity and cooperativity of the thin filaments occur with age. Myosin ATPase-pCa curves were also minimally affected by age.

In summary, type IIB/IIX MHC-expressing fibers from rats have a reduced capacity to generate force with age, but myosin ATP hydrolysis is not affected. On the basis of these and previous data (25), we propose that age-related declines in muscle contractility are due to a structural alteration of myosin in fibers from aged animals that also results in a change in the kinetics of the cross-bridge cycle.

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REFERENCES


