Fiber Type Differences in the Ca^{2+} Activation of Force and Actomyosin ATPase Activity

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Abstract

It is well established that many of the mechanical and energetic properties of skeletal muscle fibers differ between fiber types. Slow fibers typically exhibit greater calcium (Ca^{2+}) sensitivity and economy of force production than do fast fibers. These differences could be due, in part, to differences in cross-bridge (XB) cycling kinetics between fibers. We used simultaneous measurements of force and myosin ATPase activity in skinned fibers to investigate whether the apparent rate constant for the transition of XBs from the strong binding to the weak binding states (g_{app}) differs between fast and slow fibers. We found that under conditions of maximal Ca^{2+} activation, ATPase was significantly greater in fast fibers than in slow. We also found that g_{app}, which decreases with increasing free [Ca^{2+}] is much larger in fast fibers than in slow. Reducing [MgATP] from 2.0 to 0.5mM slowed g_{app} of the fast fibers resulting in an increase in both Ca^{2+} sensitivity and economy of force production to levels that were not significantly different from those of slow fibers. Based on these results, we conclude that g_{app} is slower in slow fibers than in fast and that the reduced g_{app} of slow fibers appears to account for a portion of the difference in Ca^{2+} sensitivity and economy for force production.

Key words: ATPase activity, contractile apparatus, cross-bridge kinetics, muscle energetics, skinned fibers.

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It is well accepted that many of the mechanical and energetic properties of skinned fibers taken from fast skeletal muscle differ from those of fibers obtained from slow muscles. For example, slow fibers typically exhibit greater calcium (Ca^{2+}) sensitivity as well as increased economy of force production [1, 10, 13, 14]. On the other hand, fast fibers show increased velocity of unloaded shortening [17]. To date, the exact mechanisms which account for these differences are not completely understood. In both relaxed and contracting muscle fibers, actin and myosin bind to form cross-bridges (XB) [9]. During Ca^{2+} activation, XBs cycle between groups of weak binding (WB), non-force generating states and groups of strong binding (SB) force generating states [2-4, 9]. Transitions between these states are characterized by f_{app} and g_{app}, the apparent rate constants for the transition from WB to SB and from SB to WB, respectively. Using these parameters, [3,4] describes isometric force production as:

$$F = F_{av} \$ [M] \$ A \$ L_2 \$ f_{app} / (f_{app} + g_{app}) \quad [1]$$

where [M] is the concentration of myosin per liter, A is the fiber cross-sectional area, L_2 is the length of one-half sarcomere and F_{av} is the average force per XB. Assuming that one ATP molecule is hydrolyzed per-XB cycle, the rate of myosin ATPase activity can be expressed as:

$$ATPase = [M] \$ A \$ a \$ L_2 \$ g_{app} \$ (f_{app} / (f_{app} + g_{app})) \quad [2]$$

where a is the number of half-sarcomeres within the fiber.

To account for differences in the mechanical and energetic properties between fiber types, one or more of the above parameters should differ between fast and slow fibers. Most agree that differences in [M], F_{av} and A between fast and slow fibers are small [7]. In addition, a and L_2 can be held constant under experimental conditions. Thus, f_{app} and/or g_{app} must differ between fiber types. At submaximal levels of Ca^{2+} activation, force output and economy are greater in slow fibers than in fast. This can easily be accounted for by a lower value of g_{app} in slow fibers. From equation [1], it is apparent that reducing g_{app}, results in increased force production (i.e. increased Ca^{2+} sensitivity). In addition, equation [2] predicts that reducing

g_{app} would reduce ATPase. This coupled with increased force output would result in elevated economy. In the companion manuscript of this issue [20], we demonstrate that g_{app} is Ca^{2+} sensitive and influences the economy of force production by the contractile apparatus. In addition we showed that experimental manipulations which slow g_{app} increase both the Ca^{2+} sensitivity and economy of force production as predicted by equations [1] and [2]. Based on this, it seems reasonable to hypothesize that g_{app} differs between fiber types and that slowed g_{app} in slow fibers accounts for differences in Ca^{2+} sensitivity and economy. If such is the case then two conditions must be met. First, the magnitude and Ca^{2+} sensitivity of g_{app} must differ between fast and slow fibers. Second, reducing g_{app} in fast fibers must increase Ca^{2+} sensitivity and the economy of force production to levels similar to those of slow fibers. Therefore, the purpose of this investigation was to use simultaneous measurements of isometric force and ATPase activity to examine g_{app} in fast and slow skeletal muscle fibers.

Methods

Animals

Muscle fibers were harvested from adult, male Sprague-Dawley rats (body mass 250-300g). Slow fibers were obtained from the soleus muscle whereas fast fibers were acquired from the extensor digitorum longus muscle (EDL). Following pentobarbital anesthesia (50 mg\$kg^{-1}, IP), both muscles were removed and individual fibers dissected as described in the companion manuscript in this issue [20]. Animals were then euthanized by pentobarbital overdose (>70 mg\$kg^{-1}, IP).

Solutions

Two stock solutions, which differed in free Ca^{2+} concentration, contained 85 mM K^+ and Na^+, 1 mM Mg^{2+}, 7 mM ethylene glycol-bis(B-aminoethyl ether)-N,N,N,',N',-tetra acetic acid (EGTA), 2.0 or 0.5mM MgATP, and propionate as the major anion. The standard relaxing solution contained no added Ca^{2+} resulting in a free [Ca^{2+}] of pCa 9.0 and the activating solution contained 6.6 mM added calcium to achieve [$10^{-4.5}$]M $Ca^{2+}{}_{free}$ (pCa 4.5). Ionic strength of all solutions was adjusted to 0.18M and pH maintained at 7.0 with imidazole propionate.

For measurements of ATPase activity, the solutions contained 0.4mM NADH, 5mM phosphoenol pyruvate (PEP), 100 U\$ml^{-1} pyruvate kinase (PK), 140 U\$ml^{-1} lactate dehydrogenase (LDH) and 0.2mM P_1,P_5-di(adenosine-5)pentaphosphate (AP_5A) [8, 12]. Complete details are provided in the companion manuscript in this issue [20]. Temperature was maintained at 20°C.

Measurement of Isometric force and ATPase activity

The skinned fiber procedures and simultaneous measurements of isometric force and ATPase activity were identical to that described in provided in the companion manuscript in this issue [20].

Results

Soleus and EDL fibers did not differ in maximal force (F_{max}) or in the Ca^{2+} sensitivity of ATPase (i.e. $[Ca^{2+}]_{50}$) (Table 1). They did however, differ in maximal ATPase (A_{max}) and in the force $[Ca^{2+}]_{50}$ with soleus fibers exhibiting lower A_{max} and a greater Ca^{2+} sensitivity to force. Comparison of force and ATPase $[Ca^{2+}]_{50}$ values within each fiber type indicate that these parameters were significantly different in the EDL fibers but not in the soleus fibers. Specifically, the difference between force and ATPase $[Ca^{2+}]_{50}$ was 1.41∀0.06μM in EDL fibers and 0.28∀0.03μM in soleus fibers (mean∀SE, p<.05). The relationships between force, ATPase and free [Ca^{2+}] in both the EDL and soleus fibers are shown in Figure 1. As can be seen, there is a much larger difference in the Ca^{2+} sensitivity between force and ATPase in the EDL fibers than in the soleus fibers.

As previously described [3, 12]. the ratio of ATPase to force (ATPase / F) is proportional to g_{app} and decreases with increasing free [Ca^{2+}]. The large difference in $[Ca^{2+}]_{50}$ between force and ATPase of the EDL fibers compared to soleus fibers results in ATPase to force ratios (i.e., g_{app}) that are greater than those of soleus fibers. These differences are particularly evident at submaximal free [Ca^{2+}]. In Figure 2, g_{app} values were calculated for free [Ca^{2+}] values where force was clearly resolvable, that is where force was greater than or equal to 10% of F_{max}. As described in detail in the companion manuscript [20], g_{app} values were computed using ATPase/F and A_{max} values. As can be seen, g_{app} of the EDL fibers varies considerably at force output values of <50% of F_{max} while at higher levels of force, g_{app} remains relatively constant. In the soleus fibers, g_{app} shows considerably less variation with increasing force output than does g_{app} of EDL fibers. Under conditions of maximal Ca^{2+} activation (pCa 4.5;

Table 1. Comparison of force- and ATPase-free Ca^{2+} relationships.

	Force			ATPase		
Muscle	F_{max} (kN\$m^{-2})	$[Ca^{2+}]_{50}$ (μM)	N	A_{max} (μM\$s^{-1})	$[Ca^{2+}]_{50}$ (μM)	N
Soleus	96.03∀8.67	1.48∀0.17^{H}	3.03∀0.19	219.36∀6.23^{H}	1.20∀0.16	2.33∀0.13^{H}
EDL	92.91∀3.18	3.02∀0.27*	3.97∀0.26	457.64∀51.33	1.61∀0.24	3.28∀0.42

Rat soleus (n=9) and EDL (n=7) fibers (2.0 mM [MgATP], 20°C), * p<.05 versus ATPase value within muscle, ^{H}p<.05 versus EDL fibers.

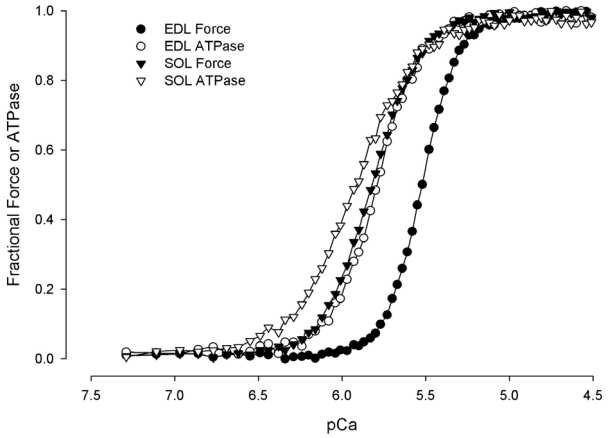

Figure 1. Relationships between force, ATPase activity and pCa in EDL and soleus fibers (2.0mM MgATP). In this figure, as in the others, curves were constructed from mean $[Ca^{2+}]_{50}$ and N values. The symbols indicate free $[Ca^{2+}]$ levels at which force and ATPase were recorded.

fractional force ~ 1), g_{app} of the EDL fibers was 3.13∀0.35 s^{-1} compared to 1.50∀0.04 s^{-1} for soleus fibers (p<.05) (Figure 2).

As expected, reduced [MgATP] from 2.0 to 0.5 mM lowered A_{max} by 71 and 54% in EDL and soleus fibers, respectively. Reducing [MgATP] also significantly decreased the $[Ca^{2+}]_{50}$ of force in the EDL fibers but did not significantly alter that of F_{max} nor ATPase (Figure 3). In contrast, lowering [MgATP] did not significantly alter the $[Ca^{2+}]_{50}$ of either force or ATPase in the soleus fibers. This resulted in a significant reduction in the $[Ca^{2+}]_{50}$ difference between force and ATPase in the EDL fibers. As can be seen in Figure 3, the $[Ca^{2+}]_{50}$ of force and ATPase in the EDL fibers at 0.5mM MgATP was not significantly different from those of the soleus fibers at either 2.0 or 0.5mM MgATP.

Figure 4 shows the relationships between g_{app} and free

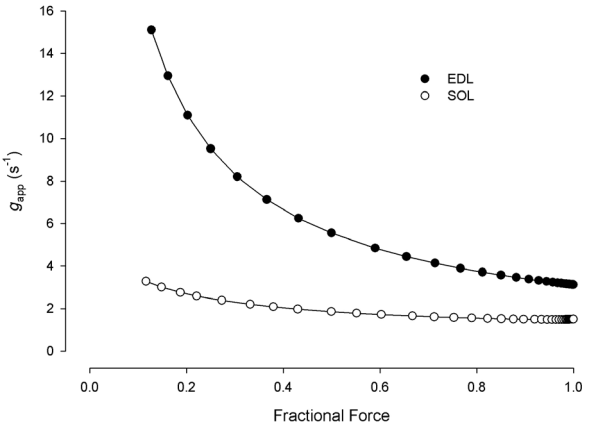

Figure 2. The relationship between g_{app} and fractional force output in fast and slow fibers. Values for g_{app} were calculated as described in the companion manuscript (Ward, Williams Kerrick, 2004).

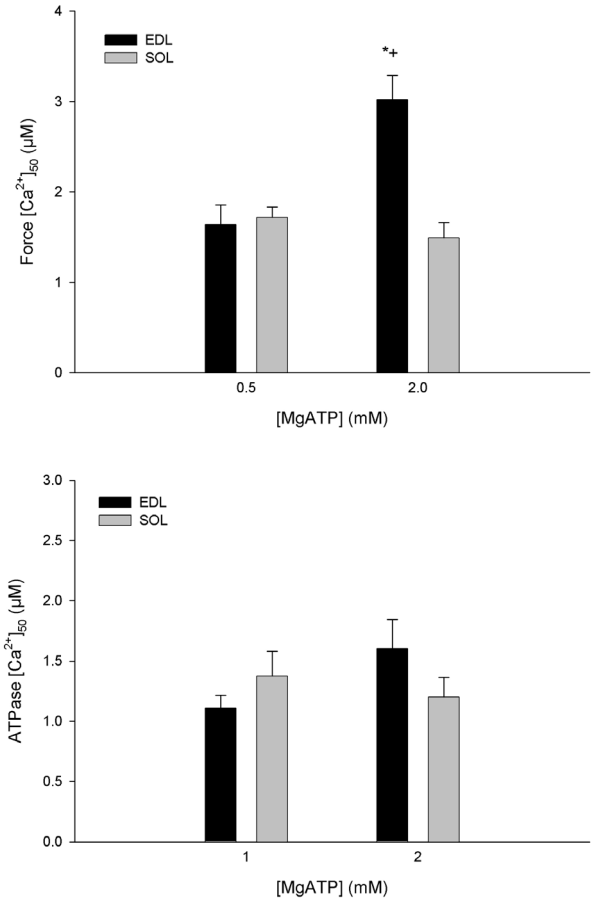

Figure 3. Changes in the $[Ca^{2+}]_{50}$ of force and ATPase activity in EDL and soleus fibers as a result of reduced [MgATP] (p<.05 versus 2.0mM, H p<.05 versus soleus fibers at 2.0mM, n=8 for each fiber and [MgATP]).*

$[Ca^{2+}]$ in soleus fibers at 2.0mM MgATP and EDL fibers at 2.0 and 0.5mM MgATP. When [MgATP] is reduced in the EDL fibers, the magnitude and Ca^{2+} sensitivity of g_{app} is nearly identical to that of the soleus fibers at control [MgATP]. Since reduced [MgATP] did not markedly alter g_{app} of slow fibers, these data were omitted for clarity of presentation..

Economy values for both EDL and soleus fibers are depicted in Figure 5. Economy was calculated as the energy cost (ATP hydrolyzed · sec^{-1}) at peak tension (F_{max}). At 2.0mM MgATP, economy was significantly greater in the soleus fibers than in the EDL fibers. Also, reducing [MgATP] significantly increased economy in both fibers. This was the result of large decreases in A_{max} coupled with slight increases in F_{max} at 0.5mM MgATP. As shown in Figure 5, reducing [MgATP] in the EDL fibers resulted in economy that was not significantly different from that of the soleus fibers at 2.0mM MgATP.

Discussion

In this investigation, we show that the difference in Ca^{2+} sensitivity between force and ATPase is much larger in

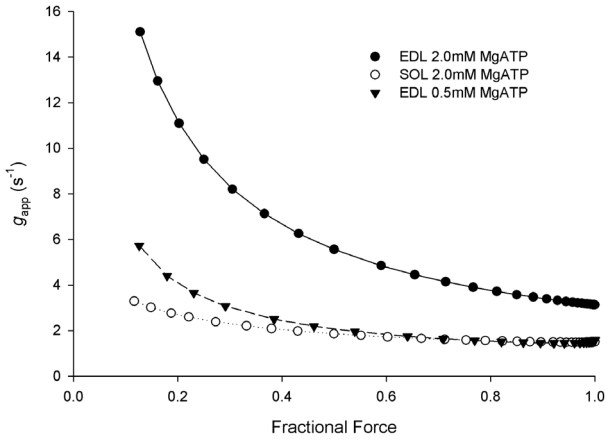

Figure 4. Comparison of g_{app} measured in soleus fibers in the presence of 2.0 mM MgATP and in EDL fibers at 2.0 and 0.5 mM MgATP. g_{app} of the soleus fibers was not altered by 0.5 mM MgATP. For this reason and for clarity, this curve has been omitted.

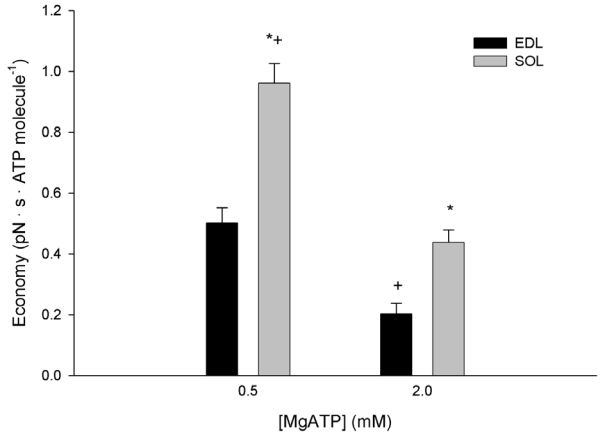

Figure 5. Changes in fiber economy under maximal Ca^{2+} activation in EDL and soleus fibers as a result of reduced [MgATP] ($^{*}p<.05$ versus 2.0mM, $^{H}p<.05$ versus soleus fibers at 2.0mM, n=8 for each fiber and [MgATP]). Economy was calculated as the F_{max} divided by the rate of ATP hydrolyzed per second.

fast, EDL fibers than in slow, soleus fibers and that A_{max} is more than two-fold greater in EDL fibers than in slow fibers. Based on these results, we estimate that under conditions of maximal Ca^{2+} activation, g_{app} of the EDL fibers is approximately $3.1s^{-1}$ compared to $1.5s^{-1}$ in the soleus fibers. With increasing free $[Ca^{2+}]$, g_{app} decreases by nearly six-fold in the EDL fibers versus a two-fold decrease in the soleus fibers. We also show that reducing [MgATP] slows g_{app} of the EDL fibers and results in force $[Ca^{2+}]_{50}$ and economy values that are not significantly different from those of the soleus fibers. These results lead us to conclude that the transition of XBs from the SB to WB states is slower in slow fibers compared to fast and that this accounts for a portion of the difference in Ca^{2+} sensitivity and economy of force production between fiber types.

Metzger and Moss [13, 14] have provided strong evidence indicating that the kinetics of XB cycling (i.e. transitions to and from the SB states) differ between fast and slow fibers. They showed that the rate constant of tension redevelopment after a rapid release and subsequent restretch (k_{tr}) is greater in fast, rat superficious vastus lateralis (SVL) fibers than in soleus fibers. In addition, the Ca^{2+} sensitivity of k_{tr} is somewhat greater in the soleus fibers than in the SVL. In Brenner's model [3], k_{tr} is defined as the sum of f_{app} and g_{app}. Thus, differences in k_{tr} between fiber types must be due to differences in f_{app}, g_{app} or both. Our data suggest that a portion of the k_{tr} dissimilarity between fiber types found by Metzger and Moss [13, 14] is due to reduced g_{app} in slow fibers. Under maximal Ca^{2+} activation, where force is nearly 100% of F_{max}, k_{tr} is eight-fold greater in fast *vs.* slow fibers and the influence of g_{app} on k_{tr} is likely to be small. At pCa 4.5, we estimate that f_{app} is six- to eight-fold greater than g_{app} [20]. In addition, at this level of free Ca^{2+}, g_{app} of fast fibers is only about twice that of slow fibers (Figure 2). Thus, the large difference in k_{tr} at maximal Ca^{2+} activation is most likely due to a higher f_{app} in fast fibers.

When free $[Ca^{2+}]$ increases from pCa 7 to 4.5, f_{app} increases in sigmoidal manner [3, 4]. whereas g_{app} decreases [20]. Thus, low and intermediate free $[Ca^{2+}]$, values of f_{app} are smaller and g_{app} larger than those at pCa 4.5. This suggests that g_{app} may have a greater influence on k_{tr} at these intermediate levels of free $[Ca^{2+}]$. When force is less than 50% of F_{max} the difference in k_{tr} between SVL and soleus fibers is approximately three-fold [13]. At these low levels of force output, g_{app} varies considerably and is substantially larger in fast fibers than in slow (Figure 2). Thus, at submaximal free $[Ca^{2+}]$ and low force output, g_{app} may account for a larger portion of the difference in k_{tr} between fast and slow fibers observed by Metzger and Moss [13, 14].

Under conditions of maximal Ca^{2+} activation, we found that g_{app} is approximately two-fold larger in EDL fibers than in soleus fibers. While our data do not identify a mechanism which explains why g_{app} differs between fast and slow fibers, several possibilities exist. It is important to point out that g_{app} characterizes several steps in the XB cycle which include: ADP release from the actin-myosin complex, ATP binding to this complex, isomerization of ATP, and detachment of the rigor bond [11, 19]. Thus, one or more of these intermediate steps must be slower in slow fibers than in fast. Using sinusoidal analyses and altered [MgATP] and [MgADP], Wang and Kawai [19] have demonstrated that release of ADP is slower in slow fibers as ADP binds more tightly in rabbit soleus fibers than in psoas fibers. Siemanowski et al. [18] have argued that ADP dissociation is sufficiently slow to limit unloaded shortening velocity and may be the rate limiting step in the SB to WB transition. It is therefore possible that the kinetics of ADP dissociation determines g_{app} and accounts for lowered g_{app} in slow fibers. In addition, Wang and Kawai [19] showed that the rate constants for ATP isomerization

and XB detachment in psoas fibers are ten to 13 times those of soleus fibers. In addition, Cooke and Pate [6] used force velocity experiments to show that the XB detachment step is three times faster in slow fibers than in fast. While differences in the magnitude of these parameters may be explained by experimental differences, the data of Wang and Kawai [19] and Cooke and Pate [6] suggest that dissociation of the actin-myosin rigor bond may account for slower g_{app} in slow fibers.

Several groups have shown that both the magnitude and Ca^{2+} sensitivity of k_{tr} is altered by extraction of myosin light chain 2 (MLC_2) and troponin C (TnC). When these proteins are extracted in skinned fibers, k_{tr} is increased at submaximal free [Ca^{2+}] [5, 13-16]. Metzger and Moss [15] have concluded that MLC_2 represses the WB to SB transition (i.e. f_{app}). However, it seems reasonable to suggest that since k_{tr} represents the sum of f_{app} and g_{app}, g_{app} may also be influenced by MLC_2. Since fast and slow fibers differ in their MLC_2 and TnC isoform (see [7]), it is possible that reduced g_{app} in slow fibers is due, in part to isoform differences in thick and/or thin filament regulatory proteins. Clearly, additional work is needed to identify the mechanisms which influence the magnitude and Ca^{2+} sensitivity of g_{app} as well as those which account for differences in g_{app} between fiber types.

It is well accepted that Ca^{2+} sensitivity and economy of force production is greater in slow fibers than in fast. In the previous investigation [20] we proposed that both of these parameters are influenced by g_{app}. Slowing g_{app} by reducing [MgATP] increased the Ca^{2+} sensitivity of force and elevated economy [20]. In the present study, reducing [MgATP] from 2.0 to 0.5mM reduced g_{app} and significantly decreased the force $[Ca^{2+}]_{50}$ of fast fibers (i.e., EDL) but did not affect that of slow fibers (i.e., soleus). Also, this treatment increased economy of both EDL and soleus fibers. The smaller effect of reduced [MgATP] on g_{app} and Ca^{2+} sensitivity of force in slow fibers is not surprising given that g_{app} of these fibers is relatively small and shows little Ca^{2+} sensitivity compared to that fast fibers. It is also possible that the greater affinity of ATP in slow fibers [11, 19] accounted for the lack of effect that reduced [MgATP] had on slow fibers. When [MgATP] was reduced in fast fibers, g_{app} values approximated those of slow fibers examined at control [MgATP] (Figure 4). The reduction in g_{app} in the EDL fibers to the level of slow fibers resulted in force $[Ca^{2+}]_{50}$ values that were not significantly different than those of slow fibers at 2.0mM MgATP (Figure 4). In addition, economy of fast fibers at lowered [MgATP] was not different from that of slow fibers (Figure 5). Thus, slowing g_{app} in fast fibers resulted in Ca^{2+} sensitivity and economy values that were similar to those of slow fibers. Based on this, we conclude that differences in the Ca^{2+} sensitivity and economy of force production between fast and slow fibers are due, in part, to differences in g_{app}.

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