Resting tension characteristics in differentiating intact rat fast- and slow-twitch muscle fibers

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Mutungi, Gabriel, John Trinick, and K. W. Ranatunga. Resting tension characteristics in differentiating intact rat fast- and slow-twitch muscle fibers. J Appl Physiol 95: 2241–2247, 2003. First published August 22, 2003; 10.1152/japplphysiol.00990.2002.—The postnatal changes in resting muscle tension were investigated at 20°C by using small muscle fiber bundles isolated from either the extensor digitorum longus or the soleus of both neonatal (7–21 days old) and adult rats. The results show that the tension-extension characteristics of the bundles depended on the age of the rats. For example, both the extensor digitorum longus and soleus bundles of rats older than 14 days showed characteristic differences that were absent in bundles from younger rats. Furthermore, the tension-extension relation of the adult slow muscle fiber bundles were similar to those of the two neonatal muscles and were shifted to longer sarcomere lengths relative to those of the adult fast-fiber bundles. Thus, at the extended sarcomere length of 2.9 μm, the adult fast muscle fiber bundles developed higher resting tensions (5.6 ± 0.5 kN/m²) than either the two neonatal (~3 kN/m²) or the adult slow (3.1 ± 0.4 kN/m²) muscle fiber bundles. At all ages examined, the resting tension responses to a ramp stretch were qualitatively similar and consisted of three components: a viscous, a viscoelastic, and an elastic tension. However, in rats older than 14 days, all three tension components showed clear fast- and slow-fiber type differences that were absent in younger rats. Bundles from 7-day-old rats also developed significantly lower resting tensions than the corresponding adult ones. Additionally, the resting tension characteristics of the adult muscles were not affected by chemical skimming. From these results, we conclude that in rats resting muscle tension, like active tension, differentiates within the first 3 wk after birth.

skeletal muscle; rats; passive tension; viscoelasticity

ALTHOUGH THE STEADY-STATE tension-extension behavior of resting adult mammalian muscles has been the subject of numerous previous studies (4, 5, 7, 18, 21, 22), its characteristics in the differentiating sarcomere remain uncertain. In most mammals, including rats, all the limb muscles are slow contracting at birth and progressively differentiate into the two distinct adult muscle types, fast and slow twitch (2, 3). In the rat, this changeover from neonatal to adult muscles occurs within the first 3 wk after birth (3, 24). At birth, all the limb muscles are thought to share the same myosin isoform (neonatal myosin), which is mainly of the slow type. Then, from day 14 onward, this myosin is progressively replaced by the adult myosin isoforms (24). It is also believed that titin acts as a template for thick-filament assembly during myogenesis (Ref. 8 and the references therein) and that the various striated muscles and their fiber types express different titin isoforms (9). The question, therefore, arises as to whether the changes in the myosin isoforms during muscle differentiation are accompanied by corresponding changes in their resting tension behavior.

The aim of this study was to investigate the steady-state resting tension-extension relations and the ramp-stretch-induced resting tension responses in small, intact muscle fiber bundles isolated from both neonatal and adult rats.

Some preliminary data reported here were presented as abstracts to The Physiological Society (16, 17).

MATERIALS AND METHODS

Intact fiber bundles. The experiments were performed at 20 ± 0.1°C on small muscle fiber bundles isolated from both the extensor digitorum longus (EDL; mainly a fast-twitch muscle in adult rats) and the soleus (predominantly a slow-twitch muscle in adult rats) of adult and neonatal (aged between 7 and 21 days) rats. The rats were killed with an overdose of pentobarbitone sodium given intraperitoneally, and small muscle fiber bundles (~2–5 μm in diameter) of rat muscles, mean cross-sectional diameter of ~150 μm; and ~10 muscle fibers in neonatal rats, mean cross-sectional diameter of ~200 μm) were dissected under dark-field illumination. Care was taken to ensure that the bundles were clean and fibers that extended from end to end in each bundle were intact and electrically excitable.

The recording system and general methodology used were basically the same as those described previously by Mutungi and Ranatunga (14, 15) and are only described here briefly. During an experiment, a preparation was mounted horizontally between two stainless steel hooks, one attached to a tension transducer and the other to a servomotor, in a flow-through muscle chamber with a glass bottom. The preparation was then perfused, at a rate of 0.5 ml/min, with Ringer solution, which was continuously bubbled with a mixture of 95% oxygen-5% CO2. The Ringer solution contained (in mM) 109 NaCl, 5.0 KCl, 1 MgCl2, 4 CaCl2, 24 NaHCO3, 1 NaHPO4, 10 sodium pyruvate, and 200 mg/l bovine calf albumin. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
serum. The temperature of the muscle chamber was controlled by using a Peltier device fitted underneath the muscle chamber and monitored with a thermocouple placed inside the muscle chamber.

The fiber bundles were then stimulated with two platinum plate electrodes placed symmetrically ~1-1.5 mm on either side of the preparation. During an experiment, the preparation was continuously stimulated with single supramaximal stimulus once every 90 s. The steady-state resting tension of the preparations was then measured as described by Wang et al. (23). Briefly, the fiber bundles were set at a particular sarcomere length and given time to equilibrate (~5 s) before the resting tension was recorded. Bundles were then manually extended by 0.2 μm/sarcomere and given time to equilibrate, and the resting tension was measured again. This was repeated during lengthening as well as shortening cycles; the two cycles were performed at random and were staggered so that a reading was obtained every 0.1 μm.

In some experiments, small ramp stretches that were 300–400 μm long (~3% initial fiber length ($L_i$) in adult fibers and ~7.5% $L_o$ in the neonatal fibers) were interposed between the twitches, and the resting tension responses to the stretches were recorded. In addition, the sarcomere length change over a 0.5-mm (in the neonatal fibers) and 1- to 2-mm (in the adult fibers) region near the tension transducer end of the bundle was monitored by using a He-Ne laser diffractometer (for details on the diffractometer, see Ref. 15).

Chemically skinned fibers. In another set of experiments, the steady-state tension relation and tension responses to a ramp stretch were recorded in segments of single, chemically skinned muscle fibers isolated from either the EDL or soleus of adult rats. Small bundles (~20–30 muscle fibers) were carefully dissected from the two muscles isolated from freshly killed rats and tied, at in situ length, to short plastic rods. They were then skinned for 2 h at 4°C in a solution containing 0.5% wt/vol Brij 58 and stored at ~20°C in a 50% mixture of skinning solution and glycerol for up to 4 wk.

During an experiment, one of the fiber bundles was removed from the storage solution and placed on a glass petri dish containing liquid paraffin. Segments of single fibers, ~3.0–3.5 mm long, were carefully dissected out under dark-field illumination. They were then mounted horizontally between two hooks: one attached to a servomotor and the other to a transducer system consisting of movable troughs. For details of the trough system see Ranatunga (19). During the mounting process, the muscle fiber ends were carefully wrapped round the hooks and secured with nitro-cellulose glue; care was taken to make sure that only the ends wrapped around the hooks were covered with the glue. The temperature of the solutions in the troughs was kept constant by circulating a mixture of water and coolant, which were held at the experimental temperature, around the trough system.

The fiber was then immersed in a relaxing solution (pCa ~10) with the following composition (in mM): 6 magnesium acetate, 10 glycerol-2-phosphate (as a buffer), 5.5 adenosine triphosphate, 15 EGTA (relaxing solution), 12.5 CP, 10 glutathione (as a reducing agent), and 50 potassium acetate, pH 7.1. Additionally, 1–2 mg/ml creatine phosphokinase (an ATP regenerating system containing 310 units of the enzyme per milligram) was added. Both chemically and mechanically skinned muscle fibers swell on glycerination, and their myofilament lattice can be compressed back to its original dimensions by adding 4% Detran T-500 (11–13). Therefore, to recompress the fibers to their original dimensions, 4% Detran T-500 was added to all solutions.

The preparation was then either manually extended (to record the steady-state resting tension-extension relation) or subjected to a series of ramp stretches (amplitude 2–3% $L_o$) and a variety of stretch speeds (~0.18–34 L/s). The tension and sarcomere length responses were then recorded and stored in a computer for further analysis. To ensure the fiber was stable, the maximum calcium-activated isometric tension of each preparation was recorded at the beginning and end of each experiment. If the maximum calcium-activated isometric tension showed any signs of deterioration, the resting tension data from the fiber was discarded.

Data analysis. The length signal (from the motor), the sarcomere length signal (from the diffractometer), and the force signal (from the force transducer) were then collected via a CED 1401 microlaboratory interface by using Signal 2.0 software (Cambridge Electronic Design, Cambridge, UK) and stored in a Genie-P3–500 computer (Viglen, Middlesex, UK) for further analysis. Up to 10 responses (recorded at least 3 s apart) were averaged at low stretch velocities to increase the signal-to-noise ratio. The initial analyses of the force records, such as half rise and rise times of twitch tension, twitch tension-extension relations, peak tension (P2) after a stretch, the steady tension after a stretch, and the break-point tension, were made by using the Signal 2.0 software. Further analyses of the data, such as curve fitting to P2 data and the calculation of the viscosity coefficient of P1 data, were done by using P software (Biosoft, Cambridge, UK). Additionally, the outputs from the force transducer and thermocouple were continuously displayed on an oscilloscope and a chart recorder and were used to monitor the mechanical stability of each preparation.

The analysis of the tension responses adopted was basically similar to that used by Bagni et al. (1) and Mutungu and Ranatunga (15), and assumed that the tension response to a ramp stretch consists of three tension components: a viscous (P1) tension, viscoelastic (P2) tension, and elastic (P3) tension, which were arranged in parallel. The relaxation time of net P2 tension was obtained by fitting P2 tension plotted against the reciprocal of stretch duration to an equation similar to that used previously by Bagni et al. and Mutungu and Ranatunga. A break in the rate of tension rise was identified by displaying the tension trace at an appropriate time scale and fitting two linear slopes on either side. The break point tension ($P_t$) was taken as the tension at the point of intersection between the two linear slopes.

All the data reported are presented as means ± SE. Comparison of the data was performed with a standard unpaired Student’s t-test.

RESULTS

Steady-state resting tension-extension relations. Figure 1 shows the tension-extension relations recorded from muscle fiber bundles isolated from neonatal and adult rats. As the results illustrate, the tension-extension relations of both the adult and the neonatal bundles are qualitatively similar. However, the tension-extension relations in the adult bundles show clear fast- and slow-fiber type differences (Fig. 1B) that are absent in the neonatal ones (Fig. 1A). For example, the tension-extension relation of the adult slow muscle fibers is shifted to longer sarcomere lengths relative to that of the adult fast fibers. Thus, at the extended sarcomere length of 2.9 μm, the resting tension of 5.6 ± 0.5 kN/m² developed by the adult fast muscle fibers is higher than the 3.1 ± 0.4 kN/m² generated by the adult...
slow muscle fibers. On the other hand, the tension-extension curves obtained from the two neonatal muscles are basically similar and overlap most of the sarcomere lengths (Fig. 1A). Therefore, at the extended sarcomere length of 2.9 μm, the resting tensions of the two neonatal muscles (3.0 ± 0.3 kN/m² in the EDL and 3.3 ± 0.4 kN/m² in the soleus) are basically similar and comparable to those of the adult slow muscle bundles. Furthermore, at long sarcomere lengths, the resting tensions developed by the neonatal bundles are about six times lower than those from the corresponding adult bundles (compare Fig. 1, A and B).

The tension-extension data obtained from bundles isolated from rats that were <14 days old were essentially similar to those recorded from 7-day-old rats, whereas those of rats older than 14 days were qualitatively similar to the adult bundles, except that the fast-/slow-fiber type differences were less marked.

Additionally, the tension-extension data from the adult muscles could be fitted with the sum of two force-extension-relations [one representing the extensibility of Ig domains and the other representing that of the unique titin sequence rich in proline (P), glutamine (E), valine (V), and Lysine (K) (PEVK) region], generated with the equation $F = k_B T/A(z/L + 1/[4(1 - z/L)^2] - 1/4)$, where $k_B$ is Boltzmann’s constant, $T$ is absolute temperature, $A$ is persistence length, $z$ is the end-to-end length, and $L$ is the contour length. The lengths of the Ig domains and PEVK (the main elastic regions of titin) used in the calculations were estimated from the data published by Labeit and Kolmerer (9). The persistence length of the Ig domains, in the I band region, was assumed to be ~15 nm and that of the PEVK segment to be ~2 nm in both fast and slow muscle. The contour length of the tandem Ig domains was assumed to be ~350 nm in fast and ~700 nm in slow muscle, whereas that of the PEVK segment was assumed to be ~400 nm in fast and ~450 nm in slow muscle.

When the steady-state resting tension-extension relations of single, chemically skinned muscle fibers isolated from adult rats were examined, they were found to be qualitatively and quantitatively similar to those of the intact adult muscle fiber bundles (Fig. 5B). Furthermore, the fast-/slow-fiber type differences were still apparent.

**Tension response to a ramp stretch.** To determine the dynamic mechanical properties of resting muscle tension, muscle fiber bundles isolated from both neonatal and adult rats were subjected to small ramp stretches (amplitude of 300–400 μm) at a variety of stretch velocities. The resultant tension and sarcomere length responses were then recorded for further analysis. The tensions developed by the neonatal bundles were low; therefore, to decrease the noise-to-signal ratios, the same amplitude stretches were used for both the adult and neonatal experiments. Additionally, several traces, at a particular stretch speed and recorded at least 90 s apart, were averaged.

Figure 2 shows typical tension and sarcomere length responses to a ramp stretch in a fast muscle fiber bundle isolated from a 7-day-old rat stretched at two velocities (initial sarcomere length, 2.6 μm). Note that during the stretch, in both records, the resting tension rises rapidly to reach a peak at the end of the ramp; thereafter, the tension decays in a complex manner to a plateau at the stretched length when sarcomere length is relatively constant. Moreover, the rising phase of the tension response is not linear but shows at least two rates of rise: an initial rapid increase to a break followed by a second slower increase in tension to the peak. Similarly, the relaxation phase of the tension response is not linear but occurs at two rates: rapidly at first and then slowly to a steady (plateau) level. Additionally, all the components of the tension response increase with stretch speed (compare Fig. 2, A and B) and initial sarcomere length (data not shown). Qualitatively similar tension responses were recorded from older rats, but the amplitudes of all the tension components were larger, and fast-/slow-fiber type differences were apparent in rats older than 14 days.

To characterize the tension response further, its constituent tension components were obtained and plotted against either stretch velocity or the reciprocal of the unique titin sequence rich in proline (P), glutamine (E), valine (V), and Lysine (K) (PEVK) region], generated with the equation $F = k_B T/A(z/L + 1/[4(1 - z/L)^2] - 1/4)$, where $k_B$ is Boltzmann’s constant, $T$ is absolute temperature, $A$ is persistence length, $z$ is the end-to-end length, and $L$ is the contour length. The lengths of the Ig domains and PEVK (the main elastic regions of titin) used in the calculations were estimated from the data published by Labeit and Kolmerer (9). The persistence length of the Ig domains, in the I band region, was assumed to be ~15 nm and that of the PEVK segment to be ~2 nm in both fast and slow muscle. The contour length of the tandem Ig domains was assumed to be ~350 nm in fast and ~700 nm in slow muscle, whereas that of the PEVK segment was assumed to be ~400 nm in fast and ~450 nm in slow muscle.

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To characterize the tension response further, its constituent tension components were obtained and plotted against either stretch velocity or the reciprocal of
stretch duration. The amplitudes of the various components were obtained as previously described by Mutungi and Ranatunga (15). Briefly, the break-point tension (P1), the PT, and the plateau tension (P3) were measured directly from the tension records, whereas the net P2 tension was calculated by subtracting the sum of P1 and P3 tensions from the PT tension.

Figure 3 shows the velocity dependence of the various tension components of the tension response obtained from a single fast muscle fiber bundle isolated from a 7-day-old rat. As the data shows, the amplitude of the plateau tension (P3) is relatively insensitive to stretch velocity (elastic), that of the tension at the break (P1) increased in direct proportion to stretch velocity (viscous), whereas, the amplitude of the third component (net P2 tension, calculated by subtracting the sum of P1 and P3 tensions from PT) increased with stretch velocity to a plateau (viscoelastic, i.e., a viscous element in series with an elastic one). The viscosity coefficient (calculated from the slope of the P1 data plotted against stretch velocity) was \( \sim 0.09 \text{kN} \cdot \text{m}^{-2} \cdot L_0 \text{s}^{-1} \) and the relaxation time of P2 tension (calculated from P2 tension data plotted against the reciprocal of stretch duration) was \( \sim 16 \text{ ms} \). In nine fast and nine slow muscle fiber bundles, P1 tension had a viscosity coefficient of 0.08 \( \pm \) 0.02 in the fast fibers and 0.09 \( \pm \) 0.02 in the slow muscle fibers. In the same preparations P3 tension had a relaxation time of 15.6 \( \pm \) 1.4 ms in the fast and 16.1 \( \pm \) 2 ms in the slow muscle fibers.

All the experiments described above were performed on small muscle fiber bundles and therefore contained a certain amount of connective tissue. Therefore, to determine whether the connective tissue made any contribution to their resting tensions, we examined the tension and sarcomere length responses to a ramp stretch of short segments of single, chemically skinned muscle fibers isolated from the EDL and soleus of adult rats. The tension traces recorded from these fibers were qualitatively similar to those of both the intact

Fig. 2. General features of the resting tension response to a ramp stretch. The tension (top traces) and sarcomere length (bottom traces) responses to a ramp stretch (middle traces) recorded at 2 stretch speeds from a fast-twitch muscle fiber bundle isolated from a 7-day-old rat are shown. Note that the responses are qualitatively similar to those previously reported in adult muscle fiber bundles (14, 15), that the amplitude of the tension response increases with speed, and that a break in the rate of tension rise (arrow) can be seen in both traces. A: average of 10 traces. B: average of 5 traces.

Fig. 3. Velocity dependence of the various tension components in a neonatal rat muscle bundle. Data are from a fast-twitch muscle fiber bundle isolated from a 7-day-old rat. A: peak tension (PT) and the plateau (P3) tension plotted against stretch velocity. B: velocity dependence of P1 tension. C: P2 tension plotted against the reciprocal of stretch duration (proportional to stretch velocity). Note that P2 tension is relatively insensitive to stretch velocity (elastic), P1 tension increases in direct proportion to stretch velocity (viscous), and P2 tension increases with velocity to a plateau (viscoelastic). The curve fitted to the data in C is similar to that used by Mutungi and Ranatunga (15) and gives a relaxation time for P2 of \( \sim 16 \text{ ms} \). $L_0$, initial fiber length.
adult and neonatal muscle fiber bundles. Furthermore, the three tension components seen in the intact bundles could also be easily distinguished and analyzed. The data displayed in Fig. 4 shows that the velocity dependence and amplitudes of these tensions were similar to those of the adult intact bundles, thereby suggesting that the resting tension contributions of connective tissue, under our experimental conditions, were relatively minor.

Postnatal changes in passive muscle viscoelasticity. To investigate the postnatal changes in passive muscle viscoelasticity, the sarcomere length and tension responses to a ramp stretch in muscle bundles isolated from neonatal and adult rats were examined.

![Diagram](https://via.placeholder.com/150)

**Fig. 5.** Comparison between neonatal and adult rat passive muscle viscosity and viscoelasticity. Data in A and B were obtained from muscle fibers isolated from 7-day-old rats, whereas data in C and D were recorded from muscle fibers isolated from adult rats. A and C: P1 data plotted against stretch velocity. B and D: velocity dependency of P2 tension. Note that the data from the adult rats show clear fast/slow fiber-type differences that are absent in the 7-day-old rat. Dotted traces in C and D show the curves fitted to the data obtained from the neonatal fiber bundles (same data as in A and B). Note that the amplitudes of the tensions obtained from the adult rat muscles are 5–10 times larger than those of the 7-day-old rats.
The data shown in Fig. 5 illustrate the velocity dependency of P1 and P2 tension data recorded from fast and slow muscle fiber bundles isolated from a 7-day-old and an adult rat. As the results show in both the adult and neonatal fast and slow bundles, P1 tension shows the characteristics of an apparent viscosity (i.e., its amplitude increases in direct proportion to stretch velocity), whereas P2 tension has the properties of a viscoelastic element (its amplitude increases with stretch speed to a plateau). However, the data obtained from the adult bundles show clear fast/slow fiber-type differences that are absent in the neonatal ones. Furthermore, at velocities >2 L/s, the amplitudes of both P1 and P2 tensions are five to ten times larger in the adult than in the corresponding neonatal fiber bundles. The neonatal fibers also have similar P2 relaxation times (~16 ms), and these are intermediate to those of the adult fast (~8 ms) and slow (~25 ms) muscle fibers. The data recorded from 14-day-old rats showed a similar velocity dependency, but the amplitudes of the various tension components were intermediate to those of fibers isolated from 7-day-old and adult rats. Moreover, the fast/slow fiber-type differences could be seen but these were less marked than in the adult bundles.

Figure 6 shows the summary data collected from both fast and slow muscle fiber bundles isolated from rats of different ages. As the data illustrate, muscle bundles isolated from EDL and soleus muscles of 7-day-old rats have essentially similar characteristics. For example, they have similar viscosity coefficients (~0.09 kN-s^-1-m^-2), P2-relaxation times (~16 ms), and half-twitch rise times (~44 ms in the EDL and 53 ms in soleus). However, as the rats grow older and their muscles differentiate to the adult types, all the parameters change accordingly. For example, the viscosity coefficients of both fiber types increase 10- to 15-fold with age (depending on the fiber type) to attain their adult levels at 21 days of age (Fig. 6A). On the other hand, age has opposing effects on the relaxation time of passive viscoelasticity (P2 tension) in the two fiber types. In the fast bundles, P2 relaxation time decreases with age from ~16 ms in 7-day-old rats to ~8 ms by the time they are 21 days old, whereas in the slow bundles it increases with age from ~16 ms at 7 days to ~23 ms at 21 days (Fig. 6B). In the same preparations, the twitch half-rise time decreases with age in both fiber types to reach its adult values by the time the rats are 21 days old. However, the decline in the twitch development time is greater in the fast than in the slow muscle fibers (Fig. 6C).

DISCUSSION

The results reported here show that at 7 days of age both the steady-state tension-extension relations and the tension responses to a ramp stretch of small muscle fiber bundles isolated from either EDL or soleus are virtually indistinguishable. However, by the time the rats are 14 days old, the resting tension characteristics of the bundles have differentiated sufficiently for the slow/fast fiber-type differences that are apparent in adult rats to show. Of particular interest is the finding that both the steady-state tension-extension relations and the ramp stretch-induced resting tension responses change with age in a similar manner, because it suggests that both tensions have the same underlying structural basis. It is also noteworthy that the passive tension characteristics differentiate over the same period as the contractile properties of the muscles.

From detailed analyses of the tension and sarcomere length responses to a ramp stretch over a wide range of velocities, we have previously suggested that the viscous tension arises from the filamentary resistance to stretch in each half sarcomere. The viscoelasticity arises from the extensible I band region of the
titin molecule (gap filament) behaving more or less like a flexible polymer in a viscous medium, whereas the elastic component arises partly from the gap filament and partly from other elastic elements (15). Therefore, the results reported here provide further evidence in support of this hypothesis. Indeed, the results obtained from short segments of single, chemically skinned muscle fibers suggest that most of the resting tension, under our experimental conditions, is mainly derived from the gap filament, although minor contributions of collagen fibers to the resting tensions cannot be completely ruled out. Moreover, the fast/slow fiber-type differences seen in rats >14 days old probably reflect the differences in the molecular dimensions and structural complexities of the titin molecules that are known to exist in adult rat fast and slow skeletal muscles (5, 9, 22).

Another novel observation in the present study was that neonatal muscle fibers develop only a sixth of the steady-state resting tensions of the corresponding adult fibers. Why this difference exists is not clear. However, most of the steady-state resting tension, in the adult skeletal muscle sarcomere, is known to reside mainly in the I band region of the titin molecule (6). In the A band region of the sarcomere, six titin molecules assemble together to form a gap filament, and each gap filament closely associates with the thick filament in the A band region (10). Furthermore, psoas muscles of neonatal rats have been shown to contain 70% of the myofibrillar density (per fiber) and double the ground substance (i.e., the cell matrix interspersed among myofibrils, sarcotubular elements, and mitochondria) of adult muscles (20). Therefore, it is likely that the lower resting tension responses in neonatal rats may reflect these structural differences.

Therefore, from these results, we conclude that, in rats, passive muscle viscoelasticity and resting muscle tension share the same underlying structural and molecular basis and that both active and resting muscle tension differentiate over the first 3 wk of life.

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DISCLOSURES

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