Desmin integrates the three-dimensional mechanical properties of muscles

ALADIN M. BORIEK, Y. CAPETANAKI, WILLY HWANG, TODD OFFICER, MUFFASIR BADSHAH, JOE RODARTE, and JAMES G. TIDBALL

Departments of Medicine and Cell Biology, Baylor College of Medicine, Houston, Texas 77030; and Department of Physiological Science, University of California, Los Angeles, California 90095

Received 23 March 2000; accepted in final form 14 August 2000

Skeletal muscle functions in a complex, three-dimensional, mechanical environment in which forces can be transmitted across the lateral surfaces of the muscle cell in the transverse plane of the cell, as well as at the ends of the muscle cell, parallel to the longitudinal axis of the cell (2, 18). Biaxial loading is most obviously a feature of the mechanical environment of the diaphragm, in which muscle fibers experience both transverse and longitudinal loads during each respiratory cycle (13). Any elastic sheet that is loaded uniaxially along one direction would have a different length-tension relationship than when the sheet is also loaded in the perpendicular direction. This is true for muscles, and therefore, this complicates the use of uniaxial in vitro properties to analyze in vivo biaxial behavior of the diaphragm muscle. Conducting in vitro physiological experiments under biaxial loading of the diaphragm muscle is therefore critical to the understanding of diaphragm function. Furthermore, the pathways of force transmissions in the diaphragm may be different from those in other skeletal muscles; therefore, specific cytoskeletal proteins and transmembrane proteins may play a unique role in mediating transverse loading of the diaphragm. Our previous findings showed that diaphragmatic muscle is stiffer in the transverse plane (1, 3, 4), which suggests that there are distinct structural components in diaphragm that determine the mechanical characteristics of muscle in the transverse plane. However, the identity of those structures, their mechanical properties, and whether they are functionally coupled to longitudinal structural elements is unknown.

Previous investigations of the structure of active and passive components of the muscle cytoskeleton have led to the thorough description of two functionally and molecularly distinct populations of structural proteins. The first group consists of sarcomeric proteins that are assembled into well-ordered macromolecular assemblies that dominate the active force production by muscle (i.e., sarcomeric actin and myosin) and also others that contribute importantly to the longitudinal passive properties of muscle (e.g., titin and nebulin; see Ref. 22). The second group consists of membrane-associated structural proteins that function in part to transmit forces generated by sarcomeric proteins across the cell membrane (e.g., the integrins and associated structural proteins and the dystrophin complex; see Ref. 19). This latter group is highly concentrated at the ends of the muscle fibers where longitudinally transmitted active and passive forces would be transmitted across the cell membrane. However, these proteins are also enriched in periodic structures, called costameres, at the lateral surface of muscle fibers, which suggests...
that they function in the transmission or dissipation of forces applied in the transverse plane of the cell. Although the presence of costameres implies that loading of muscle cells in the transverse plane may be a significant feature of muscle physiology and previous investigations (1, 3, 13) showed that the diaphragm muscle fibers experience transverse loading during normal function, the identity of the transverse structural elements has not been explored. Desmin intermediate filaments provide a prominent candidate for the transverse structural element in muscle in that they are cytoskeletal structures located in the transverse plane of muscle fibers and appear to connect adjacent Z disks in parallel (21). However, desmin intermediate filaments differ from other prominent cytoskeletal structures in muscle because they are oriented in both the transverse and longitudinal planes of the cell (15, 21). This dual orientation suggests the possibility that desmin may not only contribute to the mechanical properties in both the transverse and longitudinal planes, but desmin may also integrate the transverse and longitudinal mechanical systems.

In the present investigation, we used desmin-null mice developed by Milner et al. (14) to test the hypothesis that desmin intermediate filaments integrate the three-dimensional active and passive mechanical properties of the muscle. We tested the contribution of desmin to mechanical properties in muscle that experiences exclusively uniaxial loading along the length of the muscle fibers and in muscle that undergoes biaxial loading. Our findings show that desmin plays a significant role in modulating both the active and passive mechanical functions of muscle, and therefore it contributes to muscle force transmission. The mechanism by which desmin modulates these properties involves coupling the transverse and longitudinal structural systems in muscle.

METHODS

Measurements of passive mechanical properties. Costal hemidiaphragms from eight 129SV wild-type mice (weight: 20–24 g; age: 8–12 wk old) and eight 129SV desmin-null mice (weight: 18–24 g; age: 8–10 wk old) were used in these experiments. After anesthetizing the mice and excising the left hemidiaphragm, muscle was submerged quickly in Krebs-Ringer solution bubbled with 95% O2-5% CO2 at 25°C. Two pairs of surgical silk thread markers were sutured along the muscle fibers along both axes, and force data were collected at a strain rate of 0.4 or 1 N cm−2 s−1. Two-dimensional coordinates were obtained for each marker, and displacement was computed using MATLAB (version 5.2) software. To compute the length-tension relationship, muscles were passively lengthened and shortened along the muscle fibers as well as transverse to the muscle fibers. Muscles were passively lengthened from un-stressed length (~0.7 optimal length) to ~1.25–1.35 optimal muscle length (or the length at which twitch force is maximal). Muscles were then passively shortened until passive force was negligible. Tension in Newtons per centimeter was computed by dividing muscle force in Newtons by the clamp width in centimeters. With the use of MATLAB (version 5.2) software, the extensibility of diaphragm muscle during passive lengthening was assessed by computing the extension ratio λ, the ratio of muscle length at the deformed state to muscle length at the un-stressed state.

Measurements of contractile properties. Muscles from eight wild-type 129SV mice (weight: 19.5 ± 4.1 g; age: 67.3 ± 8.8 days) and nine desmin-null 129SV mice (weight: 20.3 ± 2.2 g; age: 80.3 ± 8.5 days) were used. Upon anesthetizing the animals, biceps femoris muscle was excised and placed in a muscle bath with continuously circulating 25°C oxygenated 95% O2-5% CO2 Krebs-Ringer solution. Muscle positioned between two stainless steel mesh electrodes, and optimal length was determined by twitch responses (0.1-ms stimulus duration, supramaximal voltage). At optimal length, we tetanically stimulated the muscle at 100 and 10 Hz with 120 s recovery (supramaximal voltage, 0.5-ms pulses; and tetanic train duration of 500 ms). Tetanic stimulations were repeated in the presence of a transverse load of ~0.01 N. Subsequently, the diaphragm was excised and stimulated at 100 and 10 Hz preceded by twitches. Muscle stimulations were executed in the presence of a transverse to fiber load of 0, 0.01, and 0.02 N with 120 s recovery, and the sequence was repeated two more times. All data were acquired at 300 Hz.

Thickness measurements. Frozen sections were cut at 10-μm thickness through diaphragm and biceps femoris muscles along the axis of loading. Sections were then observed by Nomarski optics, and the clamp-to-clamp distance for the tissue in each section and the total area of tissue in each section was measured using a digital imaging system (Bioquant, Nashville, TN). Mean thickness of the muscle along the axis of loading was then calculated by dividing the tissue area by the clamp-to-clamp distance.

Stress-relaxation assays. With the use of the same muscles from the passive length-tension protocol, stress-relaxation data were obtained by holding the muscle length constant after passively loading the muscle to ~0.25 of peak active stress. Muscle tension was allowed to relax asymptotically until it essentially reached a plateau. Three passive stretching maneuvers were executed: uniaxial along the fibers, uniaxial transverse to the fibers, and a biaxial loading. In the biaxial protocol, a 0.01 N was applied in the transverse fiber direction, and then muscle was stretched to a length that is equivalent to muscle optimal length. We then fit exponential equations based on Kelvin’s mechanical model of viscoelastic material properties to the four sets of stretch-relaxation curves. Kelvin’s model, also commonly called the standard linear model, describes the muscle fiber as a parallel combination of a dashpot with coefficient of viscosity, η1, and a linear spring with spring constant, μ1, with a second linear spring with spring constant μ2 (9). The relaxation function based on this model has the form F = E0 [(1 − (1 − τ1/τ2) e−τ2t)], where F is the relaxation force, τ is time, E0 is the relaxed elastic modulus, τ1 is the relaxation time constant for strain, and τ2 is the relaxation time for constant strain. After obtaining the three constants E0, τ1, and τ2, we calculated the viscoelastic coefficients η1, μ1, and μ2, based on the force-
displacement relationships of the model. The ratio \( \eta / \mu \) is a relaxation time, and it characterizes the rate of relaxation of the dashpot.

**Electron microscopy.** At the end of experimental treatments, muscles were fixed in 1.4% glutaraldehyde in 0.20 M sodium cacodylate buffer, pH 7.2, on ice. After 30 min, muscles were rinsed in cacodylate buffer and dissected into blocks containing the muscle. Samples were postfixed in 1% OsO4 and rinsed in buffer. Samples were then dehydrated in graded ethanol concentrations and embedded in epoxy resin. Sections of 0.5-μm thickness were cut in the longitudinal or transverse midbelly plane of each muscle. Samples free of preparation artifacts and detectable by light microscopy were then thin sectioned for electron microscopy. Thin sections at 60-nm thickness were stained with uranyl acetate and lead citrate and were observed using a Zeiss EM10AG transmission electron microscope.

**Western analysis.** Entire diaphragms or biceps femoris muscles from control mice were prepared by homogenizing samples in 80 mM Tris-HCl, pH 6.8, 0.1 M dithiothreitol, 70 mM SDS, and 1.0 mM glycerol. The homogenates were then heated to 100°C for 1 min and centrifuged to remove insoluble material. Protein concentration in the supernatant was determined by measuring absorbance at 280 nm. Samples containing 50 μg of total protein were separated on 12% acrylamide gels according to Laemmli (12). Proteins were then electrophoretically transferred to nitrocellulose membranes (5). After transfer, the uniformity of protein loading and efficiency of transfer were assessed by staining membranes with Ponceau S (Sigma). Nonspecific binding to proteins in the nitrocellulose membranes was blocked by immersioning the membranes in buffer containing 0.5% Tween 20, 0.2% gelatin, and 3.0% dry milk (blocking buffer) for at least 1 h at room temperature. Membranes were probed with polyclonal anti-desmin (Sigma, St. Louis, MO) for 90 min at room temperature. Membranes were overlaid with alkaline phosphatase-conjugated anti-rabbit IgG (Sigma) for 1 h at room temperature. The membranes were washed six times for 10 min in 0.5% Tween 20, 0.2% gelatin, and 0.5% dry milk and then were developed using nitroblue tetrazolium and bromochloroindolyl phosphate. The relative concentration of desmin in each sample was determined by scanning densitometry (Alpha Innotech, San Leandro, CA).

**Statistical analysis.** Statistical differences between groups were assessed by ANOVA with the use of the SAS Procedure “Mixed” Program. The model was a two-factor fixed- or random-effects model for two groups (desmin-null vs. controls) and two treatments (uniaxial vs. biaxial). A 0.05 level of significance was chosen a priori.

**RESULTS**

**Desmin increases stiffness in the transverse plane of diaphragm muscle.** We first tested the hypothesis that the diaphragm that experiences transverse loading in vivo would display structural, mechanical, and molecular specialization that reflects its specialized mechanical environment. Data in Fig. 1 show that the length-tension relationship of normal diaphragm is shifted to the right compared with the length-tension curve in the transverse plane, that is, the normal diaphragm muscle is anisotropic with a greater extensibility in the direction of the muscle fibers than transverse to the fibers. In contrast, in the desmin-null diaphragm, the length-tension curve along the fibers is essentially superimposed on that in the transverse plane. Using all diaphragms from desmin-null and control mice, we computed the extension ratio, \( \lambda \), at tension of ∼0.1 N/cm along the fiber direction and found \( \lambda \) to be statistically similar in the desmin-null and normal mice diaphragms (Des\(^{−/−}\)/λ = 1.18 ± 0.05; Des\(^{+/+}\)/λ = 1.17 ± 0.03; \( P < 0.74 \)). Data in Fig. 1 show that the length-tension transverse to the fibers is shifted to the right in the desmin-null diaphragm compared with that of the normal diaphragm. Using all diaphragms from desmin-null and control mice, we computed the extension ratio at ∼0.1 N/cm applied in the direction transverse to muscle fibers and found that \( \lambda \) was significantly greater in the desmin-null mice than in control mice (Des\(^{−/−}\)/λ = 1.23 ± 0.04, Des\(^{+/+}\)/λ = 1.06 ± 0.03; \( P < 0.001 \)). These data demonstrate that the diaphragm in the transverse plane has significantly more extensible muscle in the desmin-null mouse than in the normal mouse.

**Desmin couples the transverse and longitudinal mechanical properties of diaphragm muscle.** Further experimentation provided evidence that structures contributing to transverse stiffness in normal diaphragm muscle are mechanically coupled to longitudinal structural elements. Transverse loads increased the twitch stress in the diaphragm by ∼28% (Des\(^{−/−}\)/17.6 ± 1.3
showed hypercontraction of the sarcomeres adjacent to normal diaphragm muscle but not biceps femoris. However, loaded muscle fibers protruded laterally, reflecting the transverse structural elements. Both normal and desmin-null mouse diaphragms placed under transverse loads showed sites where the sarcolemma of the loaded muscle fibers protruded laterally, reflecting the effect of a transverse load placed on the fiber. However, normal diaphragm muscle but not biceps femoris showed hypercontraction of the sarcomeres adjacent to these sites, while the lengths of sarcomeres of desmin-null muscle at sites of transverse loading did not differ from sarcomere lengths elsewhere in the muscle fiber (Fig. 3). In the diaphragm muscle, the maximal longitudinal compressive sarcomere strain in normal muscles was ~75% in the zones of shortened myofibrils in the transversely loaded controls. In an identically transversely loaded sample of desmin-null muscle, however, there was ~0% longitudinal sarcomere strain, that is, sarcomere length in the loaded muscle was about the same as relaxed sarcomere length. These measurements were calibrated relative to the thick filament length in fixed, embedded, and sectioned tissue. Thick filament length was set at 1.0 μm. For example, for normal muscle, the length of the fixed shortened sarcomeres was 25% of the length of the fixed relaxed sarcomeres; therefore, no further adjustment for differential shrinkage should be necessary. The absence of desmin reduced the mechanical coupling between transverse loading and either tetanic or twitch stress production. Our data do not exclude residual coupling in the absence of desmin, suggesting the presence of additional, unknown structural elements that link longitudinal and transverse mechanical properties. However, no sarcomeric shortening was observed in desmin-null diaphragm muscles subjected to transverse loading, which provides further support for a major role of desmin in coupling longitudinal and transverse structural elements.

As stated earlier, no sarcomeric shortening in either wild-type or desmin-null biceps femoris muscles placed under transverse loads was observed. Similarly, no increase in either maximal tetanic stress as shown in Fig. 2. It is noteworthy that tetanic stresses were reproducible after recovery. Electron microscopic observations provided structural correlation for the mechanical evidence of coupling between transverse and longitudinal mechanical elements. Both normal and desmin-null mouse diaphragms placed under transverse loads showed sites where the sarcolemma of the loaded muscle fibers protruded laterally, reflecting the effect of a transverse load placed on the fiber. However, normal diaphragm muscle but not biceps femoris showed hypercontraction of the sarcomeres adjacent to

vs. 13.8 ± 1.3 N/cm²; Des⁻/⁻: 17.9 ± 0.6 vs. 22.1 ± 0.8 N/cm²). Furthermore, transverse passive loads increased maximal tetanic stress by ~45% as shown in Fig. 2. It is noteworthy that tetanic stresses were reproducible after recovery. Electron microscopic observations provided structural correlation for the mechanical evidence of coupling between transverse and longitudinal mechanical elements. Both normal and desmin-null mouse diaphragms placed under transverse loads showed sites where the sarcolemma of the loaded muscle fibers protruded laterally, reflecting the effect of a transverse load placed on the fiber. However, normal diaphragm muscle but not biceps femoris showed hypercontraction of the sarcomeres adjacent to

As stated earlier, no sarcomeric shortening in either wild-type or desmin-null biceps femoris muscles placed under transverse loads was observed. Similarly, no increase in either maximal tetanic stress as shown in Fig. 2. It is noteworthy that tetanic stresses were reproducible after recovery. Electron microscopic observations provided structural correlation for the mechanical evidence of coupling between transverse and longitudinal mechanical elements. Both normal and desmin-null mouse diaphragms placed under transverse loads showed sites where the sarcolemma of the loaded muscle fibers protruded laterally, reflecting the effect of a transverse load placed on the fiber. However, normal diaphragm muscle but not biceps femoris showed hypercontraction of the sarcomeres adjacent to

As stated earlier, no sarcomeric shortening in either wild-type or desmin-null biceps femoris muscles placed under transverse loads was observed. Similarly, no increase in either maximal tetanic stress as shown in Fig. 2. It is noteworthy that tetanic stresses were reproducible after recovery. Electron microscopic observations provided structural correlation for the mechanical evidence of coupling between transverse and longitudinal mechanical elements. Both normal and desmin-null mouse diaphragms placed under transverse loads showed sites where the sarcolemma of the loaded muscle fibers protruded laterally, reflecting the effect of a transverse load placed on the fiber. However, normal diaphragm muscle but not biceps femoris showed hypercontraction of the sarcomeres adjacent to

As stated earlier, no sarcomeric shortening in either wild-type or desmin-null biceps femoris muscles placed under transverse loads was observed. Similarly, no increase in either maximal tetanic stress as shown in Fig. 2. It is noteworthy that tetanic stresses were reproducible after recovery. Electron microscopic observations provided structural correlation for the mechanical evidence of coupling between transverse and longitudinal mechanical elements. Both normal and desmin-null mouse diaphragms placed under transverse loads showed sites where the sarcolemma of the loaded muscle fibers protruded laterally, reflecting the effect of a transverse load placed on the fiber. However, normal diaphragm muscle but not biceps femoris showed hypercontraction of the sarcomeres adjacent to

As stated earlier, no sarcomeric shortening in either wild-type or desmin-null biceps femoris muscles placed under transverse loads was observed. Similarly, no increase in either maximal tetanic stress as shown in Fig. 2. It is noteworthy that tetanic stresses were reproducible after recovery. Electron microscopic observations provided structural correlation for the mechanical evidence of coupling between transverse and longitudinal mechanical elements. Both normal and desmin-null mouse diaphragms placed under transverse loads showed sites where the sarcolemma of the loaded muscle fibers protruded laterally, reflecting the effect of a transverse load placed on the fiber. However, normal diaphragm muscle but not biceps femoris showed hypercontraction of the sarcomeres adjacent to

As stated earlier, no sarcomeric shortening in either wild-type or desmin-null biceps femoris muscles placed under transverse loads was observed. Similarly, no increase in either maximal tetanic stress as shown in Fig. 2. It is noteworthy that tetanic stresses were reproducible after recovery. Electron microscopic observations provided structural correlation for the mechanical evidence of coupling between transverse and longitudinal mechanical elements. Both normal and desmin-null mouse diaphragms placed under transverse loads showed sites where the sarcolemma of the loaded muscle fibers protruded laterally, reflecting the effect of a transverse load placed on the fiber. However, normal diaphragm muscle but not biceps femoris showed hypercontraction of the sarcomeres adjacent to

As stated earlier, no sarcomeric shortening in either wild-type or desmin-null biceps femoris muscles placed under transverse loads was observed. Similarly, no increase in either maximal tetanic stress as shown in Fig. 2. It is noteworthy that tetanic stresses were reproducible after recovery. Electron microscopic observations provided structural correlation for the mechanical evidence of coupling between transverse and longitudinal mechanical elements. Both normal and desmin-null mouse diaphragms placed under transverse loads showed sites where the sarcolemma of the loaded muscle fibers protruded laterally, reflecting the effect of a transverse load placed on the fiber. However, normal diaphragm muscle but not biceps femoris showed hypercontraction of the sarcomeres adjacent to

As stated earlier, no sarcomeric shortening in either wild-type or desmin-null biceps femoris muscles placed under transverse loads was observed. Similarly, no increase in either maximal tetanic stress as shown in Fig. 2. It is noteworthy that tetanic stresses were reproducible after recovery. Electron microscopic observations provided structural correlation for the mechanical evidence of coupling between transverse and longitudinal mechanical elements. Both normal and desmin-null mouse diaphragms placed under transverse loads showed sites where the sarcolemma of the loaded muscle fibers protruded laterally, reflecting the effect of a transverse load placed on the fiber. However, normal diaphragm muscle but not biceps femoris showed hypercontraction of the sarcomeres adjacent to

As stated earlier, no sarcomeric shortening in either wild-type or desmin-null biceps femoris muscles placed under transverse loads was observed. Similarly, no increase in either maximal tetanic stress as shown in Fig. 2. It is noteworthy that tetanic stresses were reproducible after recovery. Electron microscopic observations provided structural correlation for the mechanical evidence of coupling between transverse and longitudinal mechanical elements. Both normal and desmin-null mouse diaphragms placed under transverse loads showed sites where the sarcolemma of the loaded muscle fibers protruded laterally, reflecting the effect of a transverse load placed on the fiber. However, normal diaphragm muscle but not biceps femoris showed hypercontraction of the sarcomeres adjacent to

As stated earlier, no sarcomeric shortening in either wild-type or desmin-null biceps femoris muscles placed under transverse loads was observed. Similarly, no increase in either maximal tetanic stress as shown in Fig. 2. It is noteworthy that tetanic stresses were reproducible after recovery. Electron microscopic observations provided structural correlation for the mechanical evidence of coupling between transverse and longitudinal mechanical elements. Both normal and desmin-null mouse diaphragms placed under transverse loads showed sites where the sarcolemma of the loaded muscle fibers protruded laterally, reflecting the effect of a transverse load placed on the fiber. However, normal diaphragm muscle but not biceps femoris showed hypercontraction of the sarcomeres adjacent to

As stated earlier, no sarcomeric shortening in either wild-type or desmin-null biceps femoris muscles placed under transverse loads was observed. Similarly, no increase in either maximal tetanic stress as shown in Fig. 2. It is noteworthy that tetanic stresses were reproducible after recovery. Electron microscopic observations provided structural correlation for the mechanical evidence of coupling between transverse and longitudinal mechanical elements. Both normal and desmin-null mouse diaphragms placed under transverse loads showed sites where the sarcolemma of the loaded muscle fibers protruded laterally, reflecting the effect of a transverse load placed on the fiber. However, normal diaphragm muscle but not biceps femoris showed hypercontraction of the sarcomeres adjacent to

As stated earlier, no sarcomeric shortening in either wild-type or desmin-null biceps femoris muscles placed under transverse loads was observed. Similarly, no increase in either maximal tetanic stress as shown in Fig. 2. It is noteworthy that tetanic stresses were reproducible after recovery. Electron microscopic observations provided structural correlation for the mechanical evidence of coupling between transverse and longitudinal mechanical elements. Both normal and desmin-null mouse diaphragms placed under transverse loads showed sites where the sarcolemma of the loaded muscle fibers protruded laterally, reflecting the effect of a transverse load placed on the fiber. However, normal diaphragm muscle but not biceps femoris showed hypercontraction of the sarcomeres adjacent to

As stated earlier, no sarcomeric shortening in either wild-type or desmin-null biceps femoris muscles placed under transverse loads was observed. Similarly, no increase in either maximal tetanic stress as shown in Fig. 2. It is noteworthy that tetanic stresses were reproducible after recovery. Electron microscopic observations provided structural correlation for the mechanical evidence of coupling between transverse and longitudinal mechanical elements. Both normal and desmin-null mouse diaphragms placed under transverse loads showed sites where the sarcolemma of the loaded muscle fibers protruded laterally, reflecting the effect of a transverse load placed on the fiber. However, normal diaphragm muscle but not biceps femoris showed hypercontraction of the sarcomeres adjacent to

As stated earlier, no sarcomeric shortening in either wild-type or desmin-null biceps femoris muscles placed under transverse loads was observed. Similarly, no increase in either maximal tetanic stress as shown in Fig. 2. It is noteworthy that tetanic stresses were reproducible after recovery. Electron microscopic observations provided structural correlation for the mechanical evidence of coupling between transverse and longitudinal mechanical elements. Both normal and desmin-null mouse diaphragms placed under transverse loads showed sites where the sarcolemma of the loaded muscle fibers protruded laterally, reflecting the effect of a transverse load placed on the fiber. However, normal diaphragm muscle but not biceps femoris showed hypercontraction of the sarcomeres adjacent to

As stated earlier, no sarcomeric shortening in either wild-type or desmin-null biceps femoris muscles placed under transverse loads was observed. Similarly, no increase in either maximal tetanic stress as shown in Fig. 2. It is noteworthy that tetanic stresses were reproducible after recovery. Electron microscopic observations provided structural correlation for the mechanical evidence of coupling between transverse and longitudinal mechanical elements. Both normal and desmin-null mouse diaphragms placed under transverse loads showed sites where the sarcolemma of the loaded muscle fibers protruded laterally, reflecting the effect of a transverse load placed on the fiber. However, normal diaphragm muscle but not biceps femoris showed hypercontraction of the sarcomeres adjacent to
Desmin concentration is greater in the diaphragm than hindlimb muscles. Western analysis followed by densitometry of immunoblots shows that diaphragm muscle contains 38% more desmin than the biceps femoris muscle (Fig. 5; biceps femoris desmin normalized at 1.00 arbitrary unit, SE = 0.01; diaphragm desmin concentration = 1.38 units; SE = 0.01; n = 3; P < 0.05; Mann-Whitney) and suggests that this difference in desmin concentration may relate to the difference in detectable coupling between transverse and longitudinal mechanical properties of diaphragm and hindlimb muscle.

Desmin increases diaphragm stress relaxation and decreases diaphragm muscle force production. Stress-relaxation assays showed that, when a static load was applied to diaphragm muscle, the decrease in muscle stress over time was reduced significantly in desmin-null diaphragms (Fig. 6). Stress relaxation in the normal diaphragm is greater than in the desmin-null diaphragm. After uniaxial loading in the direction along the fibers, the values of the relaxed elastic modulus, $E_R$, for the desmin-null was greater than the control diaphragm ($E_R = 0.75$; Des$^{-/-}$: $E_R = 0.67$). After uniaxial loading in the direction transverse to fibers, these values were Des$^{-/-}$: $E_R = 0.84$ and Des$^{+/+}$, $E_R = 0.68$. The effect of desmin deficiency on the stress-relaxation curve is pronounced in both longitudinal and transverse planes. Furthermore, the effect of desmin deficiency was also pronounced after a biaxial load (biaxial: Des$^{-/-}$: $E_R = 0.82$ and Des$^{+/+}$, $E_R = 0.60$). Stress relaxation appears to be smaller in the absence of desmin, and the effect seems more pronounced after biaxial loading than after uniaxial loading. Differences in stress relaxation curves as a result of different loading conditions appear to be smaller in the desmin-null mouse than in controls. (P < 0.012). The effect of desmin deficiency on the stress relaxation curve is pronounced in both longitudinal and transverse planes. Furthermore, the effect of desmin deficiency was also pronounced after a biaxial load (biaxial: Des$^{-/-}$: $E_R = 0.81 \pm 0.05$ and Des$^{+/+}$, $E_R = 0.64 \pm 0.07$; P < 0.001). Thus desmin contributes to muscle viscoelasticity in diaphragms experiencing loading along the muscle longitudinal axis, loaded only in the transverse plane, or subjected to biaxial loading, but the effect is most prominent during biaxial loading. These findings indicate that desmin intermediate filaments may contribute to the dissipation of mechanical energy during passive loading of diaphragms during normal breathing.

Measurements of mean ± SE twitch stress (uniaxial Des$^{+/+}$: 13.8 ± 1.3 N/cm² vs. Des$^{-/-}$: 17.9 ± 0.6 N/cm²; biaxial Des$^{+/+}$: 17.6 ± 1.3 N/cm² vs. Des$^{-/-}$: 22.1 ± 0.8 N/cm²) and mean ± SE maximal twitch stress as shown in Fig. 2 in diaphragmatic muscle showed that Des$^{+/+}$ muscle produced significantly more stress than wild-type diaphragms. This may indicate that desmin plays an energy-dissipating role in active and
passive muscle mechanics. A similar increase in tetanic stress production was observed in desmin-null biceps femoris muscles (Fig. 4), which indicates a force-dissipating function for desmin in nondiaphragm muscle. However, desmin deficiency did not increase twitch stress production in biceps femoris muscle.

DISCUSSION

A previous discovery demonstrated that desmin intermediate filaments were oriented in both the transverse and longitudinal planes of the muscle cell and appeared to join Z disks in series in a single myofibril and in parallel in adjacent myofibrils (15, 21). This discovery led to speculations that desmin could function as a template for sarcomere assembly (10) or as a structural element conferring stiffness to muscle fibers in both the transverse and longitudinal planes (15, 20, 21). However, the generation of desmin-null mutant mice capable of forming apparently normal sarcomeres and myofibrils (14) disproved the view that desmin was essential for sarcomeric organization. Our data substantiate the expectation that desmin increases passive stiffness of diaphragm muscle but show that this contribution to stiffness exists primarily in the transverse plane of the diaphragm muscle. More importantly, the results support two distinct, prominent roles of desmin in skeletal muscle. First, desmin couples transverse and longitudinal structural elements in the muscle cytoskeleton, and second, it acts as a viscous element that dissipates mechanical energy in both the longitudinal and transverse planes of the muscle.

The contribution of desmin to muscle viscoelasticity in stress relaxation experiments and to active force dissipation in contracting muscle may reflect a common energy-dissipating feature of the desmin cytoskeleton. Although the present findings do not allow identification of the energy-dissipative structure, knowledge of the structure and organization of intermediate filaments (11, 17) indicates several sites as potential viscous, force-dissipating elements. First, it is feasible that elongation of the coiled-coil domain of desmin could contribute to muscle viscosity and that the intermediate filaments would increase muscle stiffness after elongation of the coiled-coil domains was complete. Alternatively, electron microscopic observations of desmin intermediate filament organization in muscle show that the filaments are not aligned perfectly in the transverse or longitudinal planes. Thus the initial viscous response of the desmin cytoskeletal system may reflect intermediate filament reorientation, after which the aligned filaments would increase muscle stiffness.

Identification of a structural protein involved in coupling the mechanical behavior of muscle cells in the transverse and longitudinal planes is unprecedented and may have substantial significance in the understanding of the mechanisms of force transmission in skeletal muscle in vivo. For example, the finding that biaxial loading of muscle can increase twitch and tetanic stress production indicates that the determinants of muscle contractility in vivo are more complex than predicted by excised muscle in vitro preparations in which the muscle is subjected exclusively to longitudinal loading (6, 7, 16). Although at first view it may appear contradictory that the presence of desmin reduces twitch and tetanic stresses in the diaphragm, the increase in twitch and tetanic stresses that result from biaxial loading of muscle requires the presence of desmin. However, the two sets of findings are compatible in more than one hypothetical model. For example, if the viscous element in the desmin cytoskeletal system resides primarily in the coiled-coil domain of the molecule, the application of biaxial loading could pre-load the viscous element so that it would not dissipate force during muscle contraction. Alternatively, if the viscosity is attributable to energy dissipation during realignment of the desmin intermediate filaments, application of transverse loads could cause realignment before contraction and thereby reduce energy losses.

The ability to amplify twitch stress production by 28% through the application of transverse loads to muscle indicates a new level of molecular specialization of muscle that can have important implications for muscle function. By comparison, differences in muscle fiber type permit variability in maximum twitch force production by ~80% and yield no significant change in tetanic stress production (8). In addition, changes in muscle fiber type result from muscle adaptions controlled at the transcriptional and translational levels. The ability of muscle to modulate maximum stress production by the application of transverse passive stress is a more flexible system for the modulation of twitch stress production. Such a modulation does not require regulation through the relatively slow processes that regulate protein synthesis, and it is a regulatory mechanism that is under voluntary control. The extent to which this newly identified mechanism for amplifying muscle stress is exploited through physiologically distinct muscles and various species remains to be explored.

In summary, our data demonstrate that passive transverse stress alters the production of both maximal and submaximal contractile properties in normal diaphragm, and this effect is absent in the desmin-null muscles. Furthermore, the diaphragm in the transverse plane is significantly more extensible in the desmin-null mouse than in the normal mouse. In addition, desmin intermediate filaments may contribute to the dissipation of mechanical energy of the diaphragm during normal breathing. We conclude that both physiological and structural data suggest that the mechanism of force transmission is altered by desmin. In particular, desmin contributes to force transmission as an integrator of the three-dimensional mechanical properties of the diaphragm.

The authors thank Drs. D. Milner and Deshen Zhu for technical assistance. They also thank Drs. G. Cooper, M. P. Sheetz, and G. Gundersen for insightful and valuable comments.

This investigation was supported by National Institutes of Health Grants HL-63134, AR-39617, and AR-40343.
REFERENCES