Skeletal muscle fiber atrophy: altered thin filament density changes slow fiber force and shortening velocity

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Riley, D. A., J. L. W. Bain, J. G. Romatowski, and R. H. Fitts. Skeletal muscle fiber atrophy: altered thin filament density changes slow fiber force and shortening velocity. Am J Physiol Cell Physiol 288:C360–C365, 2005. First published October 6, 2004; doi:10.1152/ajpcell.00386.2004.—Single skinned fibers from soleus and adductor longus (AL) muscles of weight-bearing control rats and rats after 14-day hindlimb suspension unloading (HSU) were studied physiologically and ultrastructurally to investigate how slow fibers increase shortening velocity ($V_0$) without fast myosin. We hypothesized that unloading and shortening of soleus during HSU increases densities of thin filaments, generating wider myofilament separations that increase $V_0$ and decrease specific tension ($k$N/m$^2$). During HSU, plantarflexion shortened soleus working length 23%. AL length was unchanged. Both muscles atrophied as shown by reductions in fiber cross-sectional area. For AL, the 60% atrophy accounted fully for the 58% decrease in absolute tension (mN). In the soleus, the 67% decline in absolute tension resulted from 58% atrophy plus a 17% reduction in tension and levels and provided evidence for myofilament spacing modulating tension and $V_0$.

WE OBSERVED PREVIOUSLY (17–19, 26, 28, 29) that 17-day spaceflight and bed rest produced disproportionate losses (~25%) of thin filaments and commensurate increases in the shortening velocities of slow fibers in human soleus muscles. Unexpectedly, the elevated velocity of shortening ($V_0$) was not explained by fast myosin heavy chain expression. We concluded that decreased thin filament densities increase the average spacing distance between thick and thin fibers, resulting in an increased rate of cross-bridge cycling, although changes in other myofibrillar proteins may have been involved in generating faster shortening. The present study was undertaken to determine whether similar changes in $V_0$ and myofilament densities occur in the fibers of unloaded rat soleus and adductor longus (AL) muscles, to establish a model and examine potential mechanism(s). In rats, atrophy of soleus and AL during hindlimb suspension unloading (HSU) is similar in degree, but soleus exhibits decreased myofilament density with a moth-eaten appearance of myofibrils on day 12 (20). The foci of disrupted myofibrils were called central corelike lesions (CCLs). CCLs did not occur in the AL muscles. Foot drop posture and the resulting shortening of the soleus in the pendant hindlimb were postulated to cause CCL formation because similar lesions occurred in muscles abruptly shortened by tenotomy or casting immobilization (2, 4, 10, 11). Judging from the position of the hip joint during standing and HSU, the AL did not appear shortened during HSU. Humans floating freely in microgravity develop a plantarflexion posture, with the ankle joint opening from 90° to 100° during the first week and persisting during long-duration spaceflight (5, 21). The plantarflexion postural adaptation in microgravity indicates that the disproportionate loss of thin filaments in humans may result from shortening and unloading of the soleus. The present study tested the hypothesis that, compared with unloading without muscle length shortening, unloading plus shortening decreases thin filament density and increases fiber velocity. In the HSU rat, the soleus appears both unloaded and shortened, whereas AL is unloaded but not shortened. Comparing the responses of these two muscles in HSU rats can potentially distinguish the effects of unloading and shortening on fiber atrophy, myofilament density, and fiber velocity.

METHODS

Animal Groups

The single-fiber physiology study utilized seven male Sprague-Dawley rats (275–300 g). Four rats served as singly housed controls, and three were subjected to HSU for 14 days by tail harnessing as performed previously (1, 20). After 14 days, the HSU rats were deeply anesthetized with pentobarbital sodium (35–50 mg/kg ip) while suspended to avoid hindlimb reloading, and the soleus and AL muscles were excised. After muscles were removed from HSU and control rats, the anesthetized animals were killed by pneumothorax. Protocols were approved by the Animal Care Committees of the Medical College of Wisconsin and Marquette University to comply with National Institutes of Health guidelines.

Soleus and AL Muscle Fiber Lengths During Simulated Standing and HSU Unloading Postures

Previously, we (20) videotaped rats and observed that the anterior ankle angle is ~30° during quiet, quadrupedal standing. When the rats were hindlimb suspension unloaded, resting quietly, and not moving their hindlimbs, the feet were plantarflexed with the anterior ankle angle opening to 90° or greater. In contrast, the position of the hip joint changed little during the transition from standing to the head-
down tail suspension posture. During HSU, dorsiflexion to 30° was infrequent compared with ambulating rats (20). These observations suggested that tail suspension unloading caused shortening of the soleus working range, whereas the length of the adductor longus was minimally altered. To assess quantitatively the consequence of the hindlimb suspension posture on muscle length, soleus and AL muscle fiber lengths were measured in four deeply anesthetized male Sprague-Dawley rats (~595 g). The skin was removed from the left hindlimb of the anesthetized rat to expose the muscles. The hindlimb was immobilized by skeletal clamps to approximate both the standing and suspension postures in quiescent animals. The full lengths of the superficial fibers in the midposterior region of the soleus were determined with calipers marking the proximal and distal myotendinous junctions. The lengths of the caudal-most fibers of the AL muscles were measured from the medial attachment on the pubic bone to the distal myotendinous junction. To obtain an accurate measurement of length, slack was removed from soleus fibers by electrical stimulation (100 Hz, 1-ms pulses, supramaximal) of the soleus nerve to evoke tetanic isometric contraction during the measurement. Because of the occult location of the AL muscle, it was not possible to electrically stimulate the AL nerve, hold the hip joint fixed, and clamp the lumbar spine and femur. Slack was removed by reaching in and grasping the middle of the AL with a forceps and pulling the distal tendon tight and straight. The myotendinous junctions were brought into a position comparable to a tetanic contraction. Caliper measurements were repeated three times for each muscle at each position, and the average lengths were calculated. On completion of measurements, the anesthetized rats were killed by pneumothorax.

Single-Fiber Physiology

Preparation of fiber bundles and individual fiber segments. Excised soleus and AL muscles were dissected into small bundles (~1 mm in width) and tied to glass capillary tubes. Bundles were stored in skimming solution at 4°C for 24 h, after which the solution was replaced by fresh skimming solution. The bundles were stored at ~20°C until used but not longer than 4 wk, when physiological properties deteriorate (8).

On the day of the experiment, fiber bundles were removed from the skimming solution and placed in a 4°C relaxing solution. A single fiber segment (~3 mm in length) was isolated from a bundle and transferred to the experimental chamber. The fiber ends were secured between a force transducer (model 400A; Cambridge Technology, Watertown, MA) and a servomotor (Cambridge model 300B) as previously described (17). The experimental chamber was secured to the stage of an inverted microscope. Under ~800 magnification, sarcomere length was set at 2.5 μm. Fiber segment length was determined by measuring the distance between the points of attachment. Fiber diameter was measured by taking a Polaroid photograph while the fiber was briefly suspended in air. The width of the fiber was measured at three points along the length of thephotographed fiber segment, and the average value was used to calculate the diameter, assuming a circular fiber (26). One-half the diameter was used for calculating fiber cross-sectional area (πd/2)².

Single-fiber function and fixation for electron microscopy. Peak force (P₀) and maximal V₀ of each fiber were determined at 15°C by slack test analysis exactly as described previously (26). Briefly, the fiber segment was moved from a well containing relaxing solution (pCa 9.0) to a second well containing activating solution (pCa 4.5) and, after the obtainment of peak force, slack a predetermined distance. After redevelopment of force, the segment was transferred back to relaxing solution. The procedure was repeated for a total of five slack distances between 100 and 400 μm (but not greater than 20% of fiber length). The time required for the redevelopment of force was determined and plotted vs. the imposed slack step distance. V₀ (normalized for fiber segment length) was determined from the slope of the least-squares regression line.

After V₀ determination, fibers were removed from the transducers and a small (~1 mm) piece was cut from one end for SDS-polyacrylamide gel analysis of myosin heavy chain content (26). The remaining segment (~2 mm) was prep-f obra electron microscopy (EM) fixation by placing it in a small dish (20 × 13 mm) containing relaxing solution. The dish contained a polymerized silicone elastomer-covered bottom with 12 individual 5-mm-diameter wells. A small dab of polyurethane foam sealant was placed on the bottom of the well at one side. The fiber end was carefully placed into the foam sealant, which solidified within 15 min, holding the fiber end in place. The other end of the fiber was secured to a second dab of foam ~2 mm away from the first. After a 5-min wait for the foam to start setting up, the dish was moved to the inverted microscope, where each fiber was viewed. In preparation for EM fixation, the fiber segment was straightened by stretching to a sarcomere length of 2.5 μm. The fiber length was adjusted by moving the unset foam with forceps. The dishes were placed in a fume hood, and the relaxing solution was exchanged for fixative (4% glutaraldehyde and 2% paraformaldehyde in relaxing solution). A piece of Parafilm was placed over the dish, and the fiber segment was fixed for 30 min at room temperature. The dish was then stored for 1–4 days in an airtight plastic container at 4°C until being transferred to D. A. Riley’s laboratory for postfixation of the fibers with 1.3% OsO₄ and embedding in epoxy resin (19). Fibers were coded by dish number and well position in the R. H. Fitts lab. This ensured that the EM analysis was performed blinded to group identity and physiologic results. When the physiological and ultrastructural measurements were completed, the data sets were combined to permit correlation of structure and function in an unbiased manner. Fibers determined by gel electrophoresis to contain fast myosin were eliminated from the data sets.

In a second set of fibers, after the determination of P₀ and V₀ in normal activating solution, fibers were not removed from the transducers but rather transferred to relaxing solution containing 5% (wt/vol) dextran. P₀ and V₀ were remeasured as described above except the activating solution contained 5% dextran. At the completion of these measures, fibers were prepared for fixation as described above except that the primary fixative, rinses, and secondary fixative contained 5% dextran to maintain the hyperosmotic condition.

Analysis of Thick and Thin Filament Number and Spacing Distance

Single muscle fibers were thin sectioned (~70 nm) in cross section for EM. A series of images were taken of myofibrils cut through the A bands in the region of thick and thin filament overlap at a magnification of ×67,000 with a JEOL 100CX electron microscope. The EM negatives were scanned into a computer and analyzed morphometrically at a final magnification of ×201,000 with Meta- morph 4.5 Imaging software. In each image, regions were identified that contained well-defined thick and thin filaments oriented in cross section. Sampling of myofilament densities was accomplished by placing a standard square, 150 nm per side, over five sites per fiber. The sampling square (0.0056 μm² at ×201,000) was positioned so as not to include mitochondria or internal membranes, which would artificially lower myofilament counts. Orientation of the square was otherwise placed randomly, except that the ordered arrangement of myofilaments necessitated positioning at a 45° angle to the direction of the myofilament lattice (row of thick filaments), with a thick filament occupying the upper left corner. The number of thick and thin filaments inside the box were counted, following the rules of Gundersen (9). Myofilaments touching the left and top sides of the square were included, whereas those on the bottom or right side were excluded. Interfilament spacing of thick to thin filaments inside the sampling square was also determined. The thick-to-thin distances were measured from the central thick filament to each of the thin filaments encircling it. The number of filaments counted per 0.0056 μm² was multiplied by 178.57 to compute filament density as number of filaments per square micrometer as performed previously (18, 19).
Determination of Sarcomere Length on Fixed Muscle Fibers

Mean sarcomere length was determined for each fiber in a region adjacent to that sampled ultrastructurally because myofilament spacing can change with sarcomere length (19). The fiber blocks previously cut in cross section were reoriented and sectioned longitudinally at 0.5 μm. The sections were dried on glass slides and stained with toluidine blue to enhance sarcomere cross striations. Images of the sarcomeres near the cross-sectioned ends of the fibers were captured digitally with a Spot II charge-coupled device camera through a ×20 objective on a Nikon Eclipse E600 light microscope. Sarcomere length was determined by using the Metamorph distance tool to measure the longitudinal distance covered by 10 sarcomeres and dividing the result by 10. The measurement process was repeated at five sites on each fiber, and the mean distance between Z bands was calculated to define average sarcomere length.

Statistical Testing of Data

For single-fiber physiology, a paired t-test was used to determine the effect of 5% dextran on fiber P0 and V0. Analyses were performed with StatSoft STATISTICA for Windows, Release 5.1 (Statsoft, Tulsa, OK). For the EM analyses, unpaired t-tests and ANOVA were used to compare group means. Values are given as means ± SE. When ANOVA indicated significant difference within groups, a post hoc Tukey analysis was performed. In all cases, P < 0.05 was considered significant.

RESULTS

Soleus and AL Muscle Fiber Lengths During Tail Suspension

During quadrupedal standing on the floor, the smallest angle reached at the front of the ankle was ~30° dorsiflexion. This was consistent with the 30–90° range reported by Kawano et al. (12). In the pendant hindlimbs of anesthetized rats simulating tail suspension, the mean anterior angle of the ankle was 105 ± 6°. This angle fell within the 90–160° dynamic range reported by Kawano et al. (12). Soleus fibers were 17.6 ± 1.0 mm long during simulated standing and significantly (P < 0.05) shorter (13.5 ± 1.0 mm) during suspension plantarflexion. The AL fiber lengths were 24.8 ± 2.8 mm during simulated standing and essentially unchanged (23.3 ± 2.6 mm) in the suspension posture. These measurements revealed that when rats were transitioned from quadrupedal standing to HSU, the soleus muscle length was shortened by 23%.

Muscle Fiber Atrophy

On the average, similar reductions in cross-sectional area (calculated from fiber diameters) were observed for soleus and AL muscle fibers after 14 days of HSU (Table 1). The absolute loss of fiber area in soleus and AL fibers was not similar, because the 0% dextran control AL fibers were larger than the 0% dextran control soleus fibers (Table 1).

Changes in Myofilament Densities and Spacing

Fibers tested physiologically and exhibiting no detectable fast myosin were analyzed ultrastructurally (Table 2). Myofilament densities and spacing were not adjusted mathematically because sarcomere lengths were tightly clustered at 1.78 ± 0.06 μm for both the soleus and AL muscle fibers fixed for EM. If normalized to the length (2.5 μm) at which fibers were tested physiologically, density values obtained at 1.78 μm would be multiplied by 1.4 (2.5 μm/1.78 μm) and spacing would be multiplied by 0.85 (square root of 2.5 μm minus 1.78 μm) (17). Soleus fibers of HSU rats exhibited a reduction in thin filament density and an increase in velocity of shortening compared with controls (Table 2). The loss of myofilaments in 0% dextran HSU soleus fibers is evident in cross sections through the A bands (Fig. 1, top). Consistent with the reduc-

Table 1. Slow fibers studied physiologically

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Dia, μm</th>
<th>Area, μm²</th>
<th>P0, mN</th>
<th>P0, kN/m²</th>
<th>V0, 0%, FL/s</th>
<th>V0, 5%, FL/s</th>
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<tr>
<td>Soleus</td>
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<tr>
<td>0% Control</td>
<td>16</td>
<td>78 ± 2.86</td>
<td>4,883 ± 374</td>
<td>0.61 ± 0.04</td>
<td>126 ± 3.6</td>
<td>1.131 ± 0.05</td>
<td>1.133 ± 0.02</td>
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<tr>
<td>0% HSU</td>
<td>11</td>
<td>50 ± 3.15</td>
<td>2,059 ± 259</td>
<td>0.20 ± 0.02</td>
<td>105 ± 2.7</td>
<td>1.331 ± 0.03</td>
<td>1.332 ± 0.03</td>
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<td>5% Control</td>
<td>10</td>
<td>72 ± 1.85</td>
<td>4,096 ± 226</td>
<td>0.56 ± 0.02</td>
<td>137 ± 3.7</td>
<td>1.099 ± 0.04</td>
<td>0.914 ± 0.04</td>
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<tr>
<td>5% HSU</td>
<td>11</td>
<td>54 ± 2.00</td>
<td>2,361 ± 184</td>
<td>0.29 ± 0.02</td>
<td>124 ± 6.0</td>
<td>1.370 ± 0.04</td>
<td>0.959 ± 0.04</td>
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<td>Adductor</td>
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<tr>
<td>0% Control</td>
<td>8</td>
<td>90 ± 2.3</td>
<td>6,370 ± 329</td>
<td>0.69 ± 0.03</td>
<td>109 ± 3.3</td>
<td>1.133 ± 0.02</td>
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<tr>
<td>0% HSU</td>
<td>5</td>
<td>57 ± 3.3</td>
<td>2,544 ± 665</td>
<td>0.29 ± 0.04</td>
<td>115 ± 8.4</td>
<td>1.216 ± 0.04</td>
<td>1.216 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE for n fibers. Thin, thin filament; Thick, thick filament; Thick–Thin, average thick-to-thin filament distance. *P < 0.05, †P < 0.01 HSU vs. matching control; ‡P < 0.01 5% dextran HSU soleus vs. 0% dextran HSU soleus; §P < 0.01 V0 (FL/s) 5% dextran vs. V0 (FL/s) 0% dextran.

Table 2. Slow fibers studied physiologically and electron microscopically

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Dia, μm</th>
<th>P0, mN</th>
<th>P0, kN/m²</th>
<th>V0, 0%, FL/s</th>
<th>V0, 5%, FL/s</th>
<th>Thin, no./μm²</th>
<th>Thick, no./μm²</th>
<th>Thick–Thin, nm</th>
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<tr>
<td>Soleus</td>
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<tr>
<td>0% Control</td>
<td>7</td>
<td>80 ± 3.1</td>
<td>133 ± 5.9</td>
<td>1.08 ± 0.03</td>
<td>2,428 ± 66</td>
<td>1,138 ± 52</td>
<td>18.3 ± 0.2</td>
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<tr>
<td>0% HSU</td>
<td>7</td>
<td>49 ± 4.1</td>
<td>105 ± 5.4</td>
<td>1.37 ± 0.03</td>
<td>1,821 ± 106</td>
<td>1,031 ± 46</td>
<td>19.6 ± 0.3*</td>
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<tr>
<td>5% Control</td>
<td>6</td>
<td>70 ± 1.6</td>
<td>139 ± 3.7</td>
<td>1.06 ± 0.02</td>
<td>3,554 ± 140</td>
<td>1,482 ± 92</td>
<td>15.5 ± 0.2</td>
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<tr>
<td>5% HSU</td>
<td>7</td>
<td>52 ± 1.8</td>
<td>122 ± 8.8</td>
<td>1.40 ± 0.06</td>
<td>3,240 ± 145</td>
<td>1,505 ± 78</td>
<td>15.7 ± 0.4†</td>
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<tr>
<td>0% Control</td>
<td>6</td>
<td>90 ± 2.2</td>
<td>107 ± 2.0</td>
<td>1.152 ± 0.03</td>
<td>2,312 ± 101</td>
<td>1,098 ± 38</td>
<td>18.7 ± 0.2</td>
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<tr>
<td>0% HSU</td>
<td>5</td>
<td>57 ± 3.7</td>
<td>115 ± 8.4</td>
<td>1.216 ± 0.04</td>
<td>2,321 ± 108</td>
<td>1,021 ± 48</td>
<td>18.8 ± 0.3</td>
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</table>

Values are means ± SE for n fibers. Thin, thin filament; Thick, thick filament; Thick–Thin, average thick-to-thin filament distance. *P < 0.05, †P < 0.01 HSU vs. matching control; ‡P < 0.01 5% dextran HSU soleus vs. 0% dextran HSU soleus; §P < 0.01 V0 (FL/s) 5% dextran vs. V0 (FL/s) 0% dextran.
tions in thin filament densities, the thick-to-thin filament average distance was significantly increased in soleus after HSU (Table 2). In the same HSU rats, AL fibers had no significant changes in thin filament density or $V_0$ compared with controls. For AL HSU, the thick-to-thin spacing distance was not different from control, and the myofilament lattices of HSU and control fibers appeared indistinguishable (Table 2; Fig. 1, bottom).

Treating HSU soleus fibers with 5% dextran dramatically elevated thin and thick filament densities and significantly slowed the velocity of shortening compared with the fibers tested without added dextran (Table 2). After dextran osmotic compaction of thick and thin filaments, spacing distances were similar in the 5% control and 5% HSU soleus fibers, and both exhibited closer spacing than that in the 0% controls (Table 2; Fig. 1, middle). The shortening velocities of the 5% control soleus and 5% HSU soleus fibers were measured before and after adding dextran. The addition of 5% dextran significantly reduced the $V_0$ of control and HSU soleus muscle fibers (Tables 1 and 2).

Fig. 2. Shortening velocity is inversely related to average thin filament density in a nonlinear fashion (polynomial correlation). Average thin filament densities of single fibers from the 0% soleus control and HSU, 5% soleus control and HSU, and 0% AL control and HSU groups are plotted against their shortening velocities. At high thin filament density, velocity of shortening approaches a minimum. As density decreases, velocity increases steeply. FL, fiber segment length.

Myofilament Density and Spacing and Shortening Velocity

For the total population of soleus and AL muscle fibers studied physiologically, HSU significantly increased $V_0$ in the 0% soleus groups, but not in the 0% AL groups, compared with control (Table 1). Osmotically compacting myofilaments with 5% dextran markedly reduced $V_0$ of the control and HSU soleus fibers. The 5% control and HSU fiber $V_0$ were lowered to similar levels (Table 1). For the slow fibers examined by physiology and EM, average shortening velocity was inversely related to thin filament density (Fig. 2). Average thick-to-thin filament spacing was directly correlated with $V_0$ (Fig. 3).

Alterations in Force and Specific Tension

Absolute reductions in $P_0$ (mN) occurred in soleus and AL fibers after HSU (Table 1). The specific tension of the 0% HSU soleus fibers was reduced compared with the 0% control fibers, and that of the 0% HSU AL fibers was not different from control fibers (Table 1). Compacting the soleus fibers with 5% dextran increased, albeit not significantly, specific tension for control and HSU fibers (Table 1).

DISCUSSION

After 14-day HSU, the soleus and AL muscle fibers both atrophied in cross-sectional area. For AL fibers, the 60% atrophy fully accounted for the 58% decrease in absolute tension (mN). However, in soleus fibers, the 67% tension fall
was greater than the 58% atrophy, and a 17% reduction in specific tension (kN/m²) made up the difference. Thin filament density was reduced 25% in soleus but unchanged in AL fibers. It is important to note that the decrease in thin filament density would not be resolved in histological sections at the light microscopic level. Thick filament densities tended to be lower than control, but not significantly. In the simulated pendant posture of the hindlimb in an HSU rat, muscle fiber lengths were significantly shortened by 23% in soleus but not significantly changed in AL. During HSU, the soleus and AL were both unloaded from weight bearing, and, in addition, the soleus experienced a reduction in working length. We conclude that unloading caused a similar percent atrophy of both muscles, whereas the shortening of soleus was responsible for the disproportionate loss of thin filaments in this muscle. The reduction in thin filament density in soleus is consistent with reduced cross-bridge formation, explaining the fall in specific tension after HSU observed in the present study and earlier reports (3, 24, 25). Furthermore, the decreased myofilament density also explains the reduction in peak stiffness observed in soleus fibers after 14-day HSU (25). During muscle unloading atrophy, selective loss of myofibrillar proteins has been demonstrated by numerous investigators. Our findings imply that, although both thick and thin filaments are removed during atrophy, the process does not operate stoichiometrically. A disproportionateate loss of thin filaments is induced in unloaded-shorteden muscles. Further studies are needed to elucidate the molecular mechanisms underlying targeted thin filament elimination.

Starting in the first week of spaceflight, humans assume a plantarflexion foot drop posture whenever floating freely in microgravity (5, 21). The soleus is shortened in this position. Assuming insufficient lengthening movements to stretch soleus during spaceflight, the muscle would be expected to adapt to the reduced working length (1). The present findings strengthen our previous conclusion (18) that shortening was responsible for the enhanced loss of actin thin filaments in human soleus muscles after 17-day spaceflight. A similar reduction occurred after a 17-day bed rest (17). During bed rest, the feet tend to plantarflex (16). Physical therapists apply foot boards daily to chronically bedridden patients to prevent continuous plantarflexion, which unabated leads to shortening contracture of the plantarflexors. Our results suggest that countermeasures for maintaining muscle health require both loading and lengthening activity across the normal working range.

The shortening velocity studied physiologically and electron microscopically was significantly increased by 26.9% after HSU for soleus fibers and unchanged for AL fibers. These data indicate that reduction in thin filament density contributes to an increased velocity of shortening. We previously reported (26, 28) that, after bed rest or spaceflight for 17 days, velocity of shortening increased in slow fibers, which did not express detectable fast myosin. In the present and previous single-fiber studies of HSU rats, elevated velocity also occurred in slow fibers lacking fast myosin (3, 24). In the rat and the two human unloading models, increased $V_0$ directly correlated with decreased thin filament density (17, 18). As myofilament density decreases and thick-to-thin filament spacing increases, $V_0$ rises rapidly. With tighter packing induced with 5% dextran, velocity falls. Thus thin filament density and $V_0$ are tightly correlated in the slow fibers of normal muscles, unloading-nonshortened muscles, and unloading-shortened muscles.

When thin filament density is decreased in slow fibers, the resultant increased velocity is hypothesized to result from faster cycling of the slow myosin cross bridges. One explanation is that the greater distance between the thick and thin filaments causes the myosin cross bridge to detach sooner from its actin binding site. This reduces the internal drag associated with attached cross bridges near the end of their power stroke, which in turn increases the average cross-bridge cycle velocity. In support of these concepts, the average thick-to-thin filament spacing distance was 1.3 nm greater after HSU in soleus slow fibers exhibiting increased $V_0$. Myofilament spacing has profound effects on $V_0$ and many other physiological properties, such as cross-bridge binding strength, sensitivity to pCa, thin filament protein interactions, and cross-bridge cooperativity (7, 13, 23, 27).

When myofilaments were osmotically compacted with 5% dextran solution, $V_0$ was reduced in both HSU and control soleus slow fibers. The compaction and velocities of the HSU soleus fibers reached the same level as normal fibers in 5% dextran. Repeated measures of control and HSU soleus fibers at 0% and 5% dextran demonstrated that osmotic compaction significantly diminished $V_0$ in control and HSU soleus fibers. EM quantitation revealed that myofilament density was increased by hyperosmotic treatment and thick-to-thin filament spacing was reduced in the dextran-treated fibers. Thus physical packing of thick and thin filaments imparts a major influence on $V_0$. These findings agree with the results from earlier studies (15). Preliminary observations on soleus muscle fibers from humans who spent 6 mo on the International Space Station indicate that velocity and myofilament packing have returned to normal (Fitts RH and Riley DA, unpublished observations). If this observation is substantiated for long-term spaceflight, the increased velocity and decreased thin filament density detected after 17-day spaceflight and bed rest and 14-day HSU represent transient changes as the soleus adapts to unloading at a shortened length. Even if velocity and myofilament density return to normal by 6 mo, a countermeasure is needed to prevent this transition because the adaptation is predicted to generate muscle fibers shorter than normal for a 1-g terrestrial environment. Reduction in muscle fiber length is thought to occur by sarcomere elimination at the myotendinous junction, and chronically shortened muscles, when adapted to the shortened working range, have fewer sarcomeres in series.
(10, 30). On return to Earth and reloading, the short plantar-plexors would be overstretched and working on the descending phase of the length-tension curve. This would weaken force output of the already atrophied muscle, decrease motor performance, and increase the probability of muscle injury. After 17-day spaceflight and bed rest, length-tension properties of the plantarflexors appeared unaltered because maximal isometric torques measured at anterior ankle angles of 80°, 90°, and 100° were not different before and after unloading (22). Length-tension properties may not change in 17 days but may adapt over several months during spaceflight.

Baever et al. (1) recently reported for rat HSU that a 10 min/day stretch with voluntary isometric contraction profoundly reduced soles atrophy and increased myofibril stability. Partial reversals of atrophy, lowered specific tension, decreased pCa sensitivity, and increased V\(_{\text{max}}\) were achieved by allowing HSU rats to stand for 10 min four times daily during suspension (3). Soleus atrophy induced by 7-day HSU was significantly reduced by continuous dorsiflexion of the foot for 30 min/day and fully maintained by four 15-min periods of returning to ground support (6, 14). In view of our findings, standing is effective because the feet are dorsiflexed during weight bearing, and this motor activity loads and stretches the actively contracting soleus muscle. The result is amelioration of the loss of thick and thin filaments, which is consistent with the benefits to atrophy, specific tension, pCa sensitivity, and V\(_{\text{max}}\) (3, 24). Daily stretching of muscles through the full working range by physical therapists is an important prophylactic means of reducing muscle deterioration. Including stretch in countermeasure designs may improve muscle health in astronauts in space as well as in humans on Earth who are chronically bedridden by illness.

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