Decreased thin filament density and length in human atrophic soleus muscle fibers after spaceflight

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Riley, Danny A., James L. W. Bain, Joyce L. Thompson, Robert H. Fitts, Jeffrey J. Widrick, Scott W. Trappe, Todd A. Trappe, and David L. Costill. Decreased thin filament density and length in human atrophic soleus muscle fibers after spaceflight. J. Appl. Physiol. 88: 567–572, 2000.—Soleus muscle fibers were examined electron microscopically from pre- and postflight biopsies of four astronauts orbited for 17 days during the Life and Microgravity Sciences Spacelab Mission (June 1996). Myofilament density and spacing were normalized to a 2.4-µm sarcomere length. Thick filament density (~1,062 filaments/µm²) and spacing (~32.5 nm) were unchanged by spaceflight. Preflight thin filament density (2,976/µm²) decreased significantly (P < 0.01) to 2,215/µm² in the overlap A band region as a result of a 17% filament loss and a 9% increase in short filaments. Normal fibers had 13% short thin filaments. The 26% decrease in thin filaments is consistent with preliminary findings of a 14% increase in the myosin-to-actin ratio. Lower thin filament density was calculated to increase thick-to-thin filament spacing in vivo from 17 to 23 nm. Decreased density is postulated to promote earlier cross-bridge detachment and faster contraction velocity. Atrophic fibers may be more susceptible to sarcomere reloading damage, because force per thin filament is estimated to increase by 23%.

skeletal muscle; electron microscopy; myofilaments; unloading; atrophy

Astronauts returning to Earth after spaceflight show skeletal muscle weakness, fatigue, and lack of coordination and delayed-onset muscle soreness (3, 6, 23). The muscle soreness, which occurs without strenuous exercise, indicates increased susceptibility of atrophic muscle fibers to weight-bearing-induced damage. The antigavity soleus and adductor longus muscles of rats either flown in space or subjected to hindlimb suspension unloading for 2 wk exhibited extensive segmental necrosis of muscle fibers 1–2 days after return to a weight-bearing condition (12, 13, 18–21, 23). Possible factors contributing to damage susceptibility may be weakened structural proteins, increased Ca²⁺-activated protease activity, and compromised microcirculation (15, 18, 19, 21, 23, 26). After 17 days of bed rest, soleus muscle fibers exhibited a 23% decrease in thin filament concentration in the overlap A band region and no reduction in muscle fiber specific tension (force/cross sectional area) (17). This raised the force per remaining thin filaments an estimated 30%. The higher load per filament may have weakened the sarcomere structure. The present study examined myofilament concentrations and thin filament lengths in the soleus muscles of astronauts participating in the 17-day Life and Microgravity Sciences Spacelab Mission, which had been simulated earlier by bed rest (17). On the basis of bed-rest findings, spaceflight unloading is expected to generate a disproportionate loss of thin filaments, and this decrease is anticipated to correlate directly with the elevated shortening velocity demonstrated physiologically for soleus fibers from the same astronauts (17, 31).

Methods

Test subjects. Four male astronauts, averaging 43 ± 4 yr old, 183 ± 8 cm tall, and 86 ± 6 kg body wt, were biopsied before spaceflight and after landing subsequent to a 17-day exposure to microgravity during the Life and Microgravity Sciences Spacelab Mission (STS-78, June 1996). Subjects were designated A–D for anonymity. An open-incision, needle biopsy was obtained 45 days before launch from the left soleus muscle of each individual as a preflight control. Preflight muscle adaptations were minimized by requesting the crew to maintain a constant daily activity pattern from 90 days before and up to the day before launch. Within 3 h after landing, a postflight biopsy was removed from the right soleus. Physical activity was minimized postflight by restricting the subjects to wheelchairs until biopsy, because return to weight bearing can induce secondary degenerative changes in atrophic muscles (13, 18, 19, 21, 23). Before (90, 60, 30, and 15 days before launch), during (day 2 or 3, day 8 or 9, and day 12 or 13), and after flight (2 and 8 days after landing), subjects underwent physiological testing with use of an isokinetic dynamometer and cycle ergometry. Each testing session consisted of determination of isometric and isokinetic torque performance at work rates up to 85% of preflight maximum O₂ consumption (31). These sessions were identical to those described in detail for an earlier 17-day bed-rest simulation of this mission (17, 30). Individuals performed ad libitum aerobic exercise inflight, which was not possible to document. Edgerton et al. (7) reported no effect of this type of exercise on muscle atrophy. Heavy resistance exercise is necessary for preventing atrophy (1). The human use protocol was approved by the Institutional Review Boards of the

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Tissue processing. With dissecting microscope magnification, individual biopsies were subdivided on a saline-soaked gauze into portions for single-fiber contractile physiology and biochemistry, histochemistry, and electron microscopy (17, 27, 30, 31). For the present study, bundles of muscle fibers (~1 mm diameter, ~4 mm long) were pinned out straight to flat plastic sticks and immersion fixed in 20 ml of 4% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.2) with 5 mM CaCl2 for 2 h at room temperature and then for 24–48 h at 4°C. The samples were shipped overnight at 4°C to the Medical College of Wisconsin for rinsing in 0.1 M cacodylate buffer with 5 mM CaCl2 for 1 h and postfixing for 2 h at room temperature in 1.3% OsO4 in 0.1 M cacodylate buffer with CaCl2. After each bundle was rinsed in buffer without CaCl2, it was dehydrated in graded ethanol to 100%, cleared in propylene oxide, infiltrated with epoxy resin, and cut into four equal pieces before polymerization at 60°C (17). Longitudinal and cross thin sections (70 nm) were cut and poststained with uranyl acetate and lead citrate before they were examined and photographed in a JEOL 100 CXII electron microscope. Some sections were poststained with 0.5% tannic acid to accentuate glycogen. Morphological changes induced by spaceflight were identified by comparing the ultrastructural features of the postflight test samples with those of the preflight control tissue from the same individual. Fibers damaged by dissection were excluded. The means of the pre- and postflight morphometrics were analyzed by a paired-sample t-test, with each subject serving as his own control. Bed-rest data supported an a priori prediction of decreased thin filament densities in the postflight condition and permitted a one-tailed t-test.

Quantification of thick and thin filament density. The packing densities of thick and thin filaments (filament number per unit area) were directly altered by sarcomere length; i.e., higher density and closer spacing (interfilament distance) resulted as sarcomeres were lengthened, and the opposite occurred in shortened sarcomeres (2, 17). In the present study the sarcomere lengths across all aldehyde-preserved fibers ranged from 1.8 to 2.6 µm [2.3 ± 0.2 (SD) µm]. This made it necessary to analyze each fiber first in cross section for filament density and then to recut it longitudinally for sarcomere length determination, as performed previously (17). Longitudinal semithin sections (0.5 µm) from the reoriented blocks were sectioned and stained with toluidine blue for determination of sarcomere length, as defined by the average of the four adjacent sarcomeres in series with the sarcomeres sampled in cross section. To compare density and spacing values from fibers at different lengths, raw measurements were normalized to a standard sarcomere length of 2.4 µm, as conducted previously for the companion bed-rest study (17). Density is directly proportional to sarcomere length, and spacing is inversely proportional to the square root of the sarcomere length (2, 17). To detect short thin filaments, thin filament concentration was determined for three regions of the sarcomere in cross section at a final magnification of ×201,000: region 1, in the I band within ~150 nm of thin filament origin at the Z band; region 2, about mid-I band; and region 3, where thin filaments first overlap with thick filaments in the A band (Fig. 1, A–C). If all thin filaments were long enough to originate from the Z band (region 1) and penetrate the A band (region 3), then the thin filament densities would be the same at the three measurement sites. The presence of short filaments manifests as a lower density in the A band region than near the Z band. Thick filament concentration was assessed in the overlap A band region. Micrographs were overlain with a transparency of grid squares of 0.0056 µm² at ×201,000. The number of filaments counted per 0.0056 µm² was multiplied by 178.57 to compute filament density as number per micrometer squared. The grid squares were positioned at random in the sampling regions of centrally located myofibrils. Peripheral myofibrils were avoided, because sarcomere structure was normally variable and sometimes incomplete, especially near myonuclei. To avoid biasing filament counts downward, grid placement was shifted when the myofilaments were not oriented in cross section or the predominant feature was an aggregation of glycogen. Five fibers per subject were assayed morphologically for each of two conditions (pre- and postflight). For thin filaments, three grid squares were chosen that contained filaments in a square-array pattern near the Z band. Three grid squares were also used in the mid-I band and overlap A band regions. Myofilament numbers per grid square were counted following Gunderson’s rules for sampling (10). Within the A band sampling region, center-to-center spacing distances for the nearest neighbors of thick-to-thick and thick-to-thin filaments were measured using computer-assisted digitizing morphometry. Unless otherwise indicated, values are means ± SE.

RESULTS

Myofibrils of soleus fibers in the preflight controls were large (wide) and minimally separated by sarcoplasm, which contained glycogen-like particles and occasional lipid droplets (Fig. 2A). Mitochondria were numerous and resided at the level of the I band (Fig. 2A). After 17 days of spaceflight, muscle fibers decreased in cross-sectional area an average of 15% (31). Myofibrils were noticeably thinner, as evidenced by the...
shorter transverse lengths of the Z bands (Fig. 2B). Shorter Z bands indicated atrophy (19). Sarcomeres looked remarkably intact, with no patchy loss of myofilaments. Postflight fibers had more lipid droplets than controls (Fig. 2B). The mitochondria and glycogen-like particles between myofibrils appeared similar in amounts to controls.

As expected from the bed-rest study, myofilament density was directly modulated by sarcomere length (Fig. 3) (17). After normalization for the sarcomere length differences of each muscle fiber to a standard length of 2.4 µm, thick filament density was found unchanged by spaceflight: 1,071 ± 9 and 1,054 ± 7 filaments/µm² pre- and postflight, respectively. Thick filament spacing was also unaltered: 33.8 ± 1.4 and 31.1 ± 1.9 nm pre- and postflight, respectively. The mean thick-to-thin filament spacing was not significantly different in these aldehyde-fixed fibers: 17.3 ± 0.7 and 18.4 ± 0.7 nm pre- and postflight, respectively. After spaceflight the density of thin filaments was lower in each of the three regions measured (Fig. 1, B-D). The average density near the Z band was significantly (P < 0.05, t = 3.007, df = 3) decreased 17% compared with the preflight control, the mid-I band value was lower by 21%, and the density in the A bands was significantly (P < 0.01, t = 5.508, df = 3) decreased by 26% (Table 1).

To assess whether thin filaments arising from the Z band were long enough to project into the A band, the thin filament density near the Z band was compared with that in the overlap A band region for each fiber. The occurrence of short filaments in preflight control fibers manifested as a 13% mean lower density in the overlap A band region than near the Z band. In postflight fibers, thin filament density was 22% lower in the overlap A region than near the Z band, indicating that after spaceflight the percentage of short thin filaments increased from 13 to 22%, an additional 9%. The thin filament concentration in the postflight overlap A band region was decreased by 26% compared with the preflight overlap A band (Table 1). The 26% decrease was the sum of 17% missing thin filaments plus 9% short thin filaments (Table 1).

DISCUSSION

The present results demonstrate that 17 days of spaceflight unloading decrease myofibrillar proteins, as evidenced by thinner myofibrils in the soleus muscle fibers of astronauts. This loss is consistent with previous reports of selective diminution of contractile pro-
Table 1. Thin filament density before and after spaceflight

<table>
<thead>
<tr>
<th>Subject</th>
<th>Pre</th>
<th>Post</th>
<th>%Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3,043 ± 63</td>
<td>2,989 ± 153</td>
<td>-2</td>
</tr>
<tr>
<td>B</td>
<td>3,036 ± 37</td>
<td>2,464 ± 54</td>
<td>-19</td>
</tr>
<tr>
<td>C</td>
<td>3,914 ± 169</td>
<td>2,946 ± 125</td>
<td>-25</td>
</tr>
<tr>
<td>D</td>
<td>3,711 ± 140</td>
<td>2,932 ± 51</td>
<td>-21</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>3,426 ± 227</td>
<td>2,833 ± 124*</td>
<td>-17</td>
</tr>
</tbody>
</table>

Thin filament number was counted in I band within ~150 nm near Z line and in A band near A-I border. Filament density values were normalized to a sarcomere length of 2.4 µm. Values for each subject represent averages for 5 fibers/condition. Pre, before spaceflight; Post, after spaceflight. Post mean density values for I and A bands are significantly different from their Pre values: *P < 0.05; †P < 0.01, respectively (paired-samples t-test). Subject C exhibited largest decrease in thin filament density and increase (57%) in average velocity of shortening, whereas subject A showed smallest decrease in thin filament density and increase (16%) in shortening velocity (31).
muscle cell length, because the plateau of the length-tension curve is broadened (24). It is of interest to determine whether unloading atrophy alters the levels of thin filament regulatory proteins, consistent with a higher percentage of short filaments.

Density and interfilament spacing of thick filaments were unchanged by spaceflight. Thick-to-thin filament spacing was also unchanged. This latter result was, at first, puzzling, because the 26% reduction in thin filament density in the overlap A band region was calculated to increase average spacing between thick and thin filaments by 35%. A similar lack of change in thick-to-thin filament spacing was obtained for 17 days of bed rest (17). In both studies, spacing was measured for chemically fixed fibers, in which glutaraldehyde fixation cross-linked myosin cross bridges to actin. Therefore, with the assumption of no change in cross-bridge length, the nearest-neighbor thick-to-thin filament distance should be similar in pre- and postflight aldehyde-fixed fibers (17). The lack of increased distance between thick and thin filaments in aldehyde-fixed fibers does not rule out a wider separation in vivo with functional consequences. Fewer thin filaments means a greater distance from the thick filament to the nearest thin filament in vivo. This is depicted schematically in Fig. 4. Comparison of our morphological data with physiological results from the same astronaut biopsies indicates that the increased average velocity (32%) and decreased thin filament density (26%) may be causally related (31). The spaceflight and bed-rest studies together support the hypothesis that decreased thin filament density increases the velocity of shortening of slow-twitch fibers, which do not express fast myosin heavy chains (17, 30, 31).

After spaceflight, the force per cross-sectional area (specific tension) of atrophic soleus fibers was mildly decreased 7–9%, and specific tension remained at control levels after 17 days of bed rest (30, 31). This suggests that the normal thick-to-thin filament ratio of 6:1 supplies an excess of actin-binding sites for myosin cross bridges, and reducing the ratio to ~5:1 during atrophy continues to provide sufficient cross-bridge binding to maintain specific tension. In the soleus muscle fibers of rat hindlimb unloaded for 1 wk, thick filaments were disproportionately lost, which decreased the number of cross bridges, and specific tension was reduced (17%)(16, 22). Alterations in thick-to-thin filament ratios would be expected to change myosin-to-actin protein ratios. The myosin-to-actin ratio decreased in rat soleus muscles immobilized at a shortened length, which produced a disproportionate loss of thick filaments (11). Slow-twitch fibers from the same biopsy studied in the present study showed a 14% increase in the myosin-to-actin ratio, consistent with a greater loss of actin thin filaments during spaceflight-induced atrophy (unpublished observations).

Reduced thin filament density and near-normal force generation exist in atrophic fibers after spaceflight and bed rest (17, 30, 31). This means that the average stress per remaining thin filament is 23–30% higher than normal. Elevated stress per filament may increase susceptibility of atrophic muscles to reloading damage (20, 23). The adaptation of unloaded slow-twitch fibers to reduce thin filaments may increase susceptibility to sarcromere damage during reloading. The present findings suggest that inflight countermeasures to prevent the accelerated loss of thin filaments may be necessary to avert delayed-onset muscle soreness experienced by astronauts.

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