Unilateral lower limb suspension does not mimic bed rest or spaceflight effects on human muscle fiber function


METHODS


1Department of Biology, Marquette University, Milwaukee, Wisconsin 53201; 2Human Performance Laboratory, Ball State University, Muncie, Indiana 47306; and 3Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Received 21 December 2001; accepted in final form 26 March 2002

HUMANS EXPOSED TO SPACEFLIGHT experience skeletal muscle atrophy, particularly of the antigravity, postural muscles of the lower limbs and back (8). Efforts to understand the mechanisms underlying the adaptations of skeletal muscle to microgravity have been hindered by the limited number of human spaceflights and by the expense and logistical problems associated with studying humans in space. An alternative approach has been to use ground-based models of non-weight bearing that mimic the effects of spaceflight. The most commonly used ground-based model of spaceflight has been bed rest. This model appears to induce adaptations in muscle cells that are similar to those experienced during spaceflight (25–27). However, although ground-based bed rest eliminates or reduces many of the problems associated with human spaceflight, the procedure is still relatively expensive and logistically complex.

An alternative ground-based model of non-weight bearing is unilateral lower limb suspension (ULLS). In this model, as originally described by Berg et al. (2), the treatment limb is flexed and suspended above the ground by the use of a shoulder harness. Subjects ambulate using crutches and are thus able to participate in most of their routine daily activities. The procedure reduces many of the expenses and logistical issues associated with bed rest studies, induces atrophy of the extensor muscles that comprise the treatment limb, and results in a decline in the voluntary neuromuscular strength of these muscle groups (1–3, 5, 11). Consequently, the procedure has been proposed as an alternative ground-based model of spaceflight (2, 5).

In previous work, our laboratory reported that the functional properties of individual muscle cells are altered during various models of non-weight bearing (9, 12, 25–28). If ULLS is a valid model of spaceflight, or an effective substitute for ground-based bed rest, then one would expect the procedure to affect the contractility of human muscle fibers in a manner consistent with these other models. Therefore, the primary purpose of this study was to test the hypothesis that 12 days of ULLS induce alterations in cross-bridge mechanisms of skeletal muscle contraction consistent with spaceflight and bed rest. A secondary objective was to assess whether reloading after 12 days of ULLS affected single-fiber contractile function. The result, that reloading depressed fiber force, has important ramifications for spaceflight experiments.

METHODS

This study was approved by the Institutional Review Boards at Marquette University and Ball State University. All subjects were fully informed of the purpose, procedures, and risks of the study and provided written consent before participation. Four women [age, height, and weight of 26 ± 1
(SE) yr, 169 ± 1 cm, and 60.3 ± 2.0 kg, respectively] and four men (30 ± 4 yr, 181 ± 1 cm, and 81.5 ± 7.1 kg) served as subjects.

Muscle samples of the soleus and medial gastrocnemius were obtained by using the percutaneous needle-biopsy technique. Pre-ULLS muscle samples were obtained from one limb, and within 72 h, the opposite limb was subjected to the ULLS procedure (the opposite leg was suspended to ensure that the post-ULLS treatment muscle samples were not affected by the recent pre-ULLS biopsy procedure). The ULLS procedure was similar to the one described by Berg et al. (2). After subjects had familiarized themselves with the use of crutches, a nylon strap was fastened around the ankle and foot of the treatment limb. The strap was extended to a shoulder harness. Adjustment of the length of the strap flexed the knee and raised the foot of the treatment limb so that it did not contact the floor or other supporting surfaces. We did not utilize a thick-soled shoe on the supporting limb as originally described by Berg et al. because it was possible to completely suspend the lower limb by use of the shoulder strap. Instructions were given not to bear weight on the suspended limb at any time. Thus subjects physically lifted, moved, and supported the treatment limb so that it did not bear weight when they were getting into and out of chairs and bed, while they were bathing, etc. When seated, subjects rested the extended treatment limb on another chair or stool so that the ankle extensors were not able to contract against an external resistance.

After 12 days of ULLS, but before resumption of weight-bearing activity, soleus and gastrocnemius biopsies were obtained from the treatment leg (post-0 h sample). Subjects were then allowed to resume normal weight-bearing activities but were required to remain in the laboratory for the next 6 h. During this time, subjects walked at 4.8 m/h on a motorized treadmill for a 15-min period every hour. Six hours after the resumption of weight-bearing activity, a second soleus and gastrocnemius biopsy were obtained from the treatment leg (post-6 h sample).

All muscle samples were divided into several portions, with one portion immediately placed in a skinning solution containing 125 mM K-propionate, 20 mM imidazole (pH 7.0), 2 mM EGTA, 4 mM ATP, 1 mM MgCl₂, and 50% glycerol (vol/vol). This portion was shipped overnight at 4°C to Marquette University where, on arrival, the sample was placed in fresh skinning solution and stored at −20°C. Over the next 4 wk, muscle bundles were placed in relaxing solution (for composition, see below) where short segments of individuals muscle fibers were isolated for study. Individual fiber segments were transferred to an experimental chamber where they were submerged under relaxing solution and attached to an isometric force transducer (model 400, Cambridge Technology, Watertown MA) and high-speed position motor (model 300B, Cambridge Technology) as previously described (9, 25). Sarcomere spacing was set at 2.5 μm by using an eye-piece micrometer. Fiber length (FL) was measured as the length of fiber suspended between the two attachment points. Fiber diameter was calculated as the mean of three diameter measurements obtained directly from a photograph taken of the fiber while it was briefly suspended in air (14).

Fibers were activated by a rapid transfer from the relaxing solution into an adjacent chamber containing a Ca²⁺-activating solution. The relaxing and activating solutions contained 7 mM EGTA, 20 mM imidazole, 14.5 mM creatine phosphate, 1 mM free Mg²⁺, and 4 mM MgATP. Ionic strength and pH were adjusted to 180 mM and 7.0, respectively, by addition of KCl and KOH. The free Ca²⁺ concentrations ([Ca²⁺]) of the relaxing and activating solutions were pCa 9 and pCa 4.5, respectively (where pCa = −log free [Ca²⁺]). The computer program of Fabiato and Fabiato (7) was used to determine the concentration of the metals, ligands, and metal-ligand complexes of each solution based on the stability constants (adjusted for temperature, ionic strength, and pH) reported by Godt and Lindley (10).

Peak Ca²⁺-activated force and unloaded shortening velocity (V₀) were evaluated during slack test measurements. Once the fiber had attained peak force, the investigator triggered a servo-controller, causing the position motor to impose a slack length step on the fiber (always ≤20% of FL). Force momentarily dropped to zero but redeveloped once the activated fiber had shortened sufficiently to take up the imposed slack. The fiber was then transferred back into relaxing solution and reextended to its original length. The entire procedure was repeated so that each fiber was subjected to approximately five different slack steps. The time required for the redevelopment of tension was plotted against the slack length steps. The slope of a least squares line fit to the points was taken as V₀. All velocity measurements were normalized to FL and expressed as FL per second.

Most fibers were also subjected to a series of brief (50–120 ms) isotonic contractions. After the fiber attained peak force, the servo-controller imposed a rate of shortening that caused fiber force to plateau at a predetermined level. After 50–120 ms of shortening, the servo-controller stepped the fiber to a second isotonic force and finally to a third force. The fiber was then slacked, transferred back into relaxing solution, and reextended to its original length. Force and shortening velocity were determined from the force and position records obtained over the final half of each isotonic step. Approximately 15 force-velocity data pairs were obtained for each fiber. For each individual fiber, force and shortening velocity data points were fit by the hyperbolic Hill equation, (P + a/V + b) − (P₀ + a/b), where P is force, V is shortening velocity, P₀ is force when V = 0, and a and b are constants having dimensions of force and velocity, respectively. Peak power for each fiber was determined from the parameters describing the fitted hyperbola (30).

After the physiological measurements, the fiber was removed from the apparatus, solubilized in a SDS sample buffer, and stored at −80°C. Later, a portion of each fiber was run on 5% PAGE to determine myosin heavy chain (MHC) isoform composition (25).

Fibers were grouped according to their MHC isoform composition for analysis. To determine the effect of ULLS unloading, pre- and post-ULLS means were compared with a two-way factorial ANOVA with main effects of subject and ULLS treatment. To assess the impact of reloading, we used a two-way ANOVA (subject × treatment) to compare post-0 h with post-6 h means. Statistical significance was accepted at P < 0.05.

RESULTS

Fiber diameter and peak Ca²⁺-activated force: pre vs. post-0 h ULLS fibers. Type I fibers obtained from the soleus immediately after ULLS (post-0 h) were significantly smaller in diameter than corresponding pre ULLS fibers (Table 1). The 7% reduction in the mean diameter of these fibers is equivalent to a 14% reduction in their cross-sectional area (CSA). The peak Ca²⁺-activated force of the fibers was significantly reduced immediately after ULLS, but the relative change, 18%, was greater than the reduction in the CSA of the fibers (Table 2). The remaining force deficit
was attributed to a 5% reduction in specific tension (kN/m²). The peak absolute force of the post-0 h gastrocnemius type I fibers was 14% less than the pre-ULLS mean value. This was primarily attributed to a drop in specific force. The diameter and peak Ca²⁺-activated force of all post-0 h type II fibers (soleus IIa, gastrocnemius IIa, and gastrocnemius IIx) were unaffected by the ULLS treatment.

\( V_o: \) pre vs. post-0 h ULLS fibers. The \( V_o \) of the post-0 h type I soleus fibers was reduced by 10% after 12 days of ULLS (Table 3). In contrast, \( V_o \) of the type IIa gastrocnemius fibers rose by 12% over the same period. There were no changes in the \( V_o \) of any other group of fibers.

**Peak power: pre vs. post-0 h ULLS fibers.** Figure 1 illustrates composite force-velocity and force-power relationships for soleus (A) and gastrocnemius (B) fibers expressing type I MHC (too few fast fibers were studied for statistical analysis). Consistent with the slack test results, the maximal shortening velocity (determined by extrapolation of the force-velocity relationship) and the peak Ca²⁺-activated force of the soleus type I fibers were reduced by 14% (from 0.37 ± 0.02 to 0.32 ± 0.02 FL/s; \( P < 0.05 \)) and 17% (from 0.70 ± 0.02 to 0.58 ± 0.02 mN; \( P < 0.05 \)), respectively, immediately after ULLS. Type I soleus fibers obtained immediately after ULLS also showed a significant increase in \( a/P_o \) (from 0.048 ± 0.002 to 0.063 ± 0.008; \( P < 0.05 \)), indicating that the force-velocity relationships were less curved compared with before ULLS. Consequently, peak power occurred at significantly greater relative forces and velocities after ULLS. Nevertheless, the forces (mN) and shortening velocities that elicited peak power still averaged 11 and 15% less immediately after ULLS. As illustrated in Fig. 1, this resulted in a significant 27% reduction in the peak power of the post-0 h type I soleus fibers (pre: 7.36 ± 0.44 \( \mu \)N·FL·s⁻¹, post-0 h: 5.39 ± 0.29 \( \mu \)N·FL·s⁻¹; \( P < 0.05 \)).

Again, consistent with the slack test results, post-0 h type I gastrocnemius fibers displayed a 17% reduction in peak Ca²⁺-activated force (from 0.54 ± 0.03 to 0.45 ± 0.03 mN; \( P < 0.05 \)) and no change in maximal shortening velocity (pre: 0.39 ± 0.03 FL/s, post-0 h: 0.37 ± 0.02 FL/s; \( P > 0.05 \)). In contrast to the type I soleus fibers, \( a/P_o \) was not altered (pre: 0.056 ± 0.008, post-0 h: 0.044 ± 0.003; \( P > 0.05 \)). Thus the reduction in force at peak power output (−18%) dropped in proportion to the change in peak Ca²⁺-activated force. Because shortening velocity at peak power was unaltered, post-0 h peak power dropped by 16%, in direct proportion to the reduction in force production (pre: 5.42 ± 0.35 \( \mu \)N·FL·s⁻¹, post-0 h: 4.53 ± 0.38 \( \mu \)N·FL·s⁻¹; \( P < 0.05 \)).

Although both the soleus type I and the gastrocnemius type I fibers showed similar reductions in peak normalized force, normalized power was significantly reduced for the soleus fibers (pre: 1.33 ± 0.06 kN·m⁻²·FL⁻¹·s⁻¹, post-0 h: 1.12 ± 0.05 kN·m⁻²·FL⁻¹·s⁻¹; \( P < 0.05 \)) but not the gastrocnemius fibers (pre: 1.31 ± 0.07 kN·m⁻²·FL⁻¹·s⁻¹, post-0 h: 1.26 ± 0.07 kN·m⁻²·FL⁻¹·s⁻¹; \( P > 0.05 \)). Normalized power fell for the soleus fibers because these fibers displayed reductions in both specific force and shortening velocity. In comparison, only specific force was reduced for the post-0 h gastrocnemius fibers.

**Properties of post-6 h ULLS fibers.** Well over 50% of the post-6 h fibers isolated for study broke during their initial activation. This greatly exceeded the failure rate for the pre and post-0 h fibers (<10%). The average fiber diameter and \( V_o \) of the post-6 h soleus type I, soleus IIa, gastrocnemius type I, and gastrocnemius IIa fibers were similar to the values of the corresponding post-0 h fibers (Tables 1 and 2). The post-6 h soleus IIa, gastrocnemius I, and gastrocnemius IIa fibers also

Table 1. Muscle fiber diameter before and after 12 days of unilateral lower limb suspension

<table>
<thead>
<tr>
<th>Muscle</th>
<th>MHC</th>
<th>Pre</th>
<th>Post-0 h</th>
<th>Post-6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sol</td>
<td>I</td>
<td>85 ± 2(110)</td>
<td>79 ± 1(94)†</td>
<td>76 ± 1(289)</td>
</tr>
<tr>
<td></td>
<td>IIa</td>
<td>83 ± 3(40)</td>
<td>75 ± 2(24)</td>
<td>73 ± 3(13)</td>
</tr>
<tr>
<td>Gast</td>
<td>I</td>
<td>69 ± 2(77)</td>
<td>68 ± 5(57)</td>
<td>72 ± 3(33)</td>
</tr>
<tr>
<td></td>
<td>IIa</td>
<td>65 ± 3(32)</td>
<td>66 ± 2(21)</td>
<td>67 ± 6(8)</td>
</tr>
<tr>
<td></td>
<td>IIx</td>
<td>72 ± 3(22)</td>
<td>72 ± 3(13)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE with no. of fibers in parentheses. Pre fibers were obtained before limb suspension, post-0 h fibers were obtained immediately after 12 days of limb suspension but before the resumption of weight-bearing activity, and post-6 h fibers were obtained 6 h after the resumption of weight-bearing activity. MHC, myosin heavy chain; Sol, soleus; Gast, gastrocnemius. The primary purpose of the study was to evaluate the effects of unilateral lower limb suspension on muscle fiber function: *significant difference between pre and post-0 h means, \( P < 0.05 \). A secondary aim was to examine whether reloading further altered muscle fiber function: †significant difference between the post-0 h and post-6 h means, \( P < 0.05 \).

Table 2. Muscle fiber peak Ca²⁺-activated force before and after 12 days of unilateral lower limb suspension

<table>
<thead>
<tr>
<th>Muscle</th>
<th>MHC</th>
<th>Peak Force, mN</th>
<th>Peak Force, kN/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post-0 h</td>
<td>Post-6 h</td>
</tr>
<tr>
<td>Sol</td>
<td>I</td>
<td>0.68 ± 0.02</td>
<td>0.56 ± 0.02†</td>
</tr>
<tr>
<td></td>
<td>IIa</td>
<td>0.66 ± 0.03</td>
<td>0.54 ± 0.03</td>
</tr>
<tr>
<td>Gast</td>
<td>I</td>
<td>0.49 ± 0.02</td>
<td>0.42 ± 0.02*</td>
</tr>
<tr>
<td></td>
<td>IIa</td>
<td>0.44 ± 0.03</td>
<td>0.43 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>IIx</td>
<td>0.51 ± 0.03</td>
<td>0.48 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE. No. of fibers per mean is same as in Table 1. The primary purpose of the study was to evaluate the effects of unilateral lower limb suspension on muscle fiber function: *significant difference between pre and post-0 h means, \( P < 0.05 \). A secondary aim was to examine whether reloading further altered muscle fiber function: †significant difference between the post-0 h and post-6 h means, \( P < 0.05 \).
had specific forces that were similar to corresponding post-0 h fibers. However, the post-6 h type I soleus fibers produced 17% less specific force than did the post-0 h fibers. Figure 2 shows that many of the post-6 h fibers had specific forces similar to those of pre and post-0 h ULLS fibers. However, 38% of the post-6 h fibers produced <90 kN/m². In contrast, only 2% of the pre and 6% of the post-0 h ULLS fibers had specific forces below 90 kN/m².

**DISCUSSION**

The effects of ULLS on voluntary neuromuscular function have been extensively investigated (1–3, 5, 11). To investigate the cellular mechanisms underlying these functional changes, we examined the contractile properties of Ca²⁺-activated skinned fiber segments obtained before and after 12 days of ULLS. The primary findings are that ULLS primarily affects slow muscle fibers, particularly those making up the soleus, with little effect on fibers expressing fast MHC isoforms. Slow fibers displayed reductions in absolute and specific peak Ca²⁺-activated force (soleus and gastrocnemius fibers), Vₑ (soleus fibers), and peak power (soleus and gastrocnemius fibers). In addition, there was evidence that reamingulation after ULLS elicited cell damage.

Because ULLS has been proposed to be a substitute model for bed rest or spaceflight, an important question is how the cellular changes induced by ULLS compare with what is currently known about the responses of human muscle fibers to these other models of non-weight bearing. The finding that ULLS caused preferential atrophy of soleus fibers is consistent with our laboratory’s earlier results obtained after a 17-day spaceflight (27, 28). Thus, at least for the plantar flexors, ULLS primarily affects the same muscle fibers that are most susceptible to spaceflight. However, 12 days of ULLS did not completely replicate the changes in muscle fiber function that have been observed after spaceflight or bed rest.

The 18% reduction in peak Ca²⁺-activated force of the post-ULLS type I soleus fibers compares favorably with 13–21% reductions observed after bed rest or spaceflight (25, 27, 28). However, ULLS induced a greater drop in fiber-specific tension than either of these other models. ULLS also appears to cause greater reductions in the absolute and specific force of slow gastrocnemius fibers than does spaceflight. Neither ULLS or spaceflight altered the peak Ca²⁺-activated force of fast gastrocnemius fibers. Thus, although ULLS and spaceflight reduce peak Ca²⁺-activated force in similar fiber populations (soleus fibers and slow gastrocnemius fibers), the magnitude of these reductions is greater in the ULLS model.

ULLS reduced the Vₑ of slow soleus fibers by 10% without affecting the Vₑ of the slow gastrocnemius fibers. This stands in contrast to short-term bed rest or spaceflight, as well as the ground-based rodent hindlimb-suspension model, which all increase slow fiber Vₑ by ~30% (9, 13, 17, 21, 25, 27). Thus short-term ULLS is unique in that type I fibers experience reductions in both force and shortening velocity. The elevated shortening velocities observed after bed rest, spaceflight, or hindlimb suspension serve to partially compensate for the reduced force generated by these fibers and modulate losses in peak power (12, 25). Because this velocity response was absent in slow fibers obtained after ULLS, fiber peak power declines to a greater extent than that noted for either bed rest or spaceflight. The 27% reduction in peak power of the type I fibers of the present study is considerably greater than the reductions observed after 17 days of bed rest or spaceflight (25, 26).

One limitation of these comparisons is that the duration of ULLS was 5 days less than the duration of the

**Table 3. Muscle fiber unloaded shortening velocity before and after 12 days of unilateral lower limb suspension**

<table>
<thead>
<tr>
<th>Muscle</th>
<th>MHC</th>
<th>Pre</th>
<th>Post-0 h</th>
<th>Post-6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sol</td>
<td>I</td>
<td>0.48 ± 0.02</td>
<td>0.43 ± 0.02</td>
<td>0.43 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>IIa</td>
<td>2.27 ± 0.14</td>
<td>2.24 ± 0.19</td>
<td>2.36 ± 0.66</td>
</tr>
<tr>
<td>Gast</td>
<td>I</td>
<td>0.46 ± 0.03</td>
<td>0.42 ± 0.02</td>
<td>0.43 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>IIa</td>
<td>2.05 ± 0.11</td>
<td>2.29 ± 0.12</td>
<td>2.28 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>IIx</td>
<td>5.43 ± 0.38</td>
<td>5.14 ± 0.42</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE given in fiber lengths/s. No. of fibers per mean is same as in Table 1. The primary purpose of the study was to evaluate the effects of unilateral lower limb suspension on muscle fiber function. *Significant difference between pre and post-0 h means, P < 0.05. A secondary aim was to examine whether reloading further altered muscle fiber function: there were no differences between post-0 h and post-6 h means.
spaceflight and bed rest studies. The ULLS study was completed over a year in advance of the spaceflight and bed rest studies. Therefore, the exact duration of the upcoming spaceflight and the duration of the corresponding bed rest study were not known during the design and completion of the ULLS study. However, McDonald et al. (12, 13) found significant alterations in rat soleus fiber function after 7 days of hindlimb suspension, and the magnitude of these alterations either remained constant, or increased, through 21 days of suspension. Thus it seems likely that extending ULLS several more days would have either maintained or increased, not reversed or diminished, the functional differences observed between ULLS and these other models of non-weight bearing.

Bed rest- and spaceflight-induced changes in muscle fiber function are related to alterations in cell ultrastructure (18, 19). Preliminary observations reveal that more severe cellular degradation, distinct from bed rest and spaceflight, occurs in fibers exposed to the ULLS procedure (D. A. Riley and R. H. Fitts, unpublished observations). Post-ULLS fibers obtained from the present subjects displayed areas of central corelike degeneration that were devoid of myosin immunostaining, particularly in slow fibers. In contrast, central corelike lesions were not observed in fibers obtained after bed rest or spaceflight (18, 19). Thus the greater myofibrillar disorganization after ULLS may be responsible for the greater reductions in force and shortening velocity noted for this model. Alternatively, changes to the thin filament regulatory proteins during ULLS could result in incomplete activation of the fiber, even in the presence of the normally saturating intracellular [Ca$^{2+}$] used in the present preparation. For instance, partial extraction of troponin C reduces force and causes a biphasic response to induced slack with a slowing of shortening velocity at longer slack steps (15). We did not observe any evidence of biphasic slack test plots in this study. However, our slack tests were based on five data points, and it is possible that more points are required to detect a break point in shortening velocity. Thus a partial loss of troponin C could explain both the lower specific force and the reduced $V_o$ of the slow soleus fibers.

Why does ULLS induce distinct changes in cell ultrastructure and function? A unique aspect of human bed rest or spaceflight is that the unloaded limbs are allowed a free range of motion. In contrast, all ULLS procedures use a restraint looped about the ankle or foot to partially flex the knee and keep the unloaded limb suspended above the ground. Central corelike lesions are a characteristic of muscle confined to contracting over a shortened working range (20). Thus one possibility is that the restraint systems used in ULLS, by maintaining the soleus at a shorter working length, may induce central corelike lesions. Because central corelike lesions are not characteristic of bed rest or spaceflight, ULLS does not completely replicate the ultrastructural and functional changes brought about by these other models.

Muscle length changes during ULLS may be different for the gastrocnemius and soleus. Although the soleus is most likely shortened by the restraint system, the length of the gastrocnemius will be dependent in part on the angle of the knee joint. One possibility is that the gastrocnemius fibers displayed smaller functional changes because the length of this muscle was constantly being altered as a result of knee flexion and extension during sitting, sleeping, etc. Because we did not control or evaluate soleus or gastrocnemius muscle length in the present study, these possibilities are speculative, but they may be important variables to consider in the design of future studies. Additionally, gastrocnemius fibers are smaller in CSA than soleus fibers under weight-bearing conditions (25, 28), and fibers with smaller CSA appear to be less responsive to the effects of non-weight bearing (6, 25).

There is evidence that ULLS may also alter blood flow in the treatment limb. Berg and Tesch (3) reported calf swelling, lower calf skin temperature, and inci-

---

![Graph](image-url)
dents of calf venous thrombosis during 10 days of ULLS. We observed that the treatment calf felt cool to the touch after 12 days of ULLS, something that was not observed for bed rest or spaceflight subjects. Models of reduced blood flow or oxygen availability, such as ischemia and hypoxia, depress force in isolated muscles and skinned muscle fibers (4, 29). Importantly, hypoxia during muscle stimulation is associated with the degradation of troponin C (4). As discussed above, reductions in fiber-specific force and \( V_0 \) are consistent with a partial loss of troponin C from the thin filament. Thus compromised blood flow could be an additional factor contributing to the differences noted between bed rest or spaceflight and the ULLS model.

Effect of resumption of weight bearing after ULLS.

We experienced a high breakage rate for experiments performed on fibers obtained several hours after the ULLS subjects were allowed to resume weight-bearing activity. Those fibers that eventually broke during maximal Ca\(^{2+}\)- activation showed no visible signs of damage or disruption when examined at \( \times 800 \) in relaxing solution before activation.

The rodent adductor longus shows an increased susceptibility to muscle damage after hindlimb suspension (22, 23). Human subjects show reductions in ankle extensor torque as they resume normal weight-bearing activity after bed rest or spaceflight, presumably as a result of activity-induced muscle damage (16). The high breakage rate of the post-6 h fibers is evidence that muscles are more susceptible to activity-induced damage after ULLS. Because we observed excessive fiber breakage only in those fibers obtained after reanimation, the damage appears to be associated with resumption of weight-bearing activity and not the ULLS procedure per se. We hypothesize that 1) some sarcomeres are weakened during ULLS, 2) these weakened sarcomeres are more susceptible to damage during reanimation, and 3) when fibers are isolated and activated, the damaged sarcomeres fail. In our preparation, sarcomere failure causes the maximally activated skinned fiber segment to break.

Fast soleus and slow and fast gastrocnemius fibers obtained after reloading fell into two groups: a population functionally similar to atrophied fibers obtained before the resumption of weight-bearing activity and a population presumably damaged to the point where they were unable to survive activation. For the post-6 h type I soleus fibers, three populations were evident: a group of fibers functionally similar to the post-0 h fibers, a group with lower specific force, and a group that was damaged to the point where fibers did not survive activation. A reduction in specific force is characteristic of fibers damaged by contractile activity (24). Thus a population of type I soleus fibers may have experienced less damage than other groups of fibers. A number of factors determine a fiber’s susceptibility to damage during reloading, including fiber-type composition and recruitment pattern (22, 23). How the interaction of these factors leads to a group of soleus fibers less vulnerable to damage is unclear.

Summary and recommendations. The ULLS model appears to preferentially affect the soleus vs. the gastrocnemius, consistent with other human models of non-weight bearing, including bed rest and spaceflight. However, ULLS induces greater losses in peak Ca\(^{2+}\)- activated specific force than either spaceflight or bed rest and results in a reduction in the \( V_0 \) of the slow soleus fibers, in direct opposition to the effects of spaceflight or bed rest. Because both force and shortening velocity are reduced by ULLS, slow fiber peak power is depressed up to twofold more by ULLS. Although ULLS is relatively simple to perform, the model does not reproduce the cellular changes that occur during other models of non-weight bearing. This may limit the generalizability of the results obtained by using the ULLS procedure.

The ULLS model is very complex because the calf muscles are subjected to reduced loading, restricted mobility, and, possibly, reduced blood flow. Future studies should explore the differences between ULLS and other models of non-weight bearing, with particular attention focused on ways of modifying ULLS to induce changes in muscle cell function similar to those observed after bed rest or spaceflight. To isolate the effects of microgravity vs. reloading, future spaceflight missions should evaluate muscle function immediately postflight before subjects are allowed to reanimate.

The authors thank Jennifer Sherwood for assistance during this project.

This work was supported by National Aeronautics and Space Administration Grant NAS9-18768 (to R. H. Fitts).

REFERENCES

10. Godt RE and Lindley BD. Influence of temperature upon contractile activation and isometric force production in mechan-


