Skeletal muscle responses to lower limb suspension in humans

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HATHER, BRUCE M., GREGORY R. ADAMS, PER A. TESCH, AND GARY A. DUDLEY. *Skeletal muscle responses to lower limb suspension in humans.* J. Appl. Physiol. 72(4): 1493–1498, 1992.—Eight subjects participated in a 6-wk unilateral lower limb suspension (ULLS) study to determine the influence of reduced weight bearing on human skeletal muscle morphology. The right shoe was outfitted with a platform sole that prevented the left foot from bearing weight while walking with crutches, yet it allowed freedom of movement about the ankle, knee, and hip. Magnetic resonance images pre- and post-ULLS showed that thigh muscle cross-sectional area (CSA) decreased ($P < 0.05$) 12% in the suspended left lower limb, whereas right thigh muscle CSA did not change. Likewise, magnetic resonance images collected post-ULLS showed that muscle CSA was 14% smaller ($P < 0.05$) in the left than in the right leg. The decrease in muscle CSA of the thigh was due to a twofold greater response of the knee extensors (−16%, $P < 0.05$) than knee flexors (−7%, $P < 0.05$). The rectus femoris muscle of the knee extensors showed no change in CSA, whereas the three vastus muscles showed similar decreases of ~16% ($P < 0.05$). The apparent atrophy in the leg was due mainly to reductions in CSA of the soleus (−17%) and gastrocnemius muscles (−26%). Biopsies of the left vastus lateralis pre- and post-ULLS showed a 14% decrease ($P < 0.05$) in average fiber CSA. The decrease was evident in both type I (−12%) and II (−15%) fibers. The number of capillaries surrounding the different fiber types was unchanged after ULLS. Capillary density and the number of capillaries per unit CSA of type I and IIa fibers increased 15, 15, and 14%, respectively ($P < 0.05$). The reductions in muscle and fiber CSA after 6 wk of ULLS indicate that suspension is a viable method for unweighting human skeletal muscle to study atrophy. The results also indicate that the adaptive responses of human skeletal muscle to unweighting are qualitatively, but not quantitatively, similar to those of lower mammals and not necessarily dependent on fiber-type composition.

unweighting; fiber types; magnetic resonance imaging; skeletal muscle biopsy

A RELATIVELY SIMPLE MODEL of muscle unweighting in humans, which employs suspension of one lower limb, recently has been developed (1). Skeletal muscle cross-sectional area (CSA) of the thigh of the suspended lower limb was decreased 7% after 4 wk of this intervention, and the in vivo speed-torque relationship of the knee extensor muscle group was shifted down on average 16%. These responses are similar to the 8% decrease in thigh skeletal muscle CSA after 4 wk of bed rest and the approximate 20% reduction in knee extensor strength after 4 or 5 wk of bed rest or 4 wk of spaceflight (4, 9, 12, 27). Taken together, these results suggest that lower limb suspension in humans induces neuromuscular responses to unweighting, comparable to spaceflight and the more constrained 1-G model, bed rest.

The purpose of this study was to assess more fully the morphological responses of human skeletal muscle to unweighting. This was accomplished by analyzing multiple transaxial magnetic resonance (MR) images of both lower limbs and skeletal muscle biopsies of the unweighted lower limb before and after 6 wk of unilateral lower limb suspension (ULLS). The results suggest that the atrophic response to unweighting in humans is dependent on the anatomic location and functional role of an individual muscle or muscle group and not necessarily fiber type composition. The greater decrease in CSA of extensor than of flexor muscle groups after unweighting may explain the larger reduction in knee extensor strength reported after spaceflight or bed rest (9, 12, 27). The muscle fiber and whole muscle atrophy support the efficacy of ULLS as a simple method of unweighting in humans that imposes moderate constraints on the subjects.

METHODS

Subjects. Three women and five men [30 ± 4 (SE) yr, 173 ± 3 cm, and 73 ± 6 kg] participated in a 6-wk ULLS study approved by the Human Research Review Board at Kennedy Space Center, Florida. All were healthy, and none had musculoskeletal complications. The design and risks associated with participating in the study were explained to each subject, and written consent was provided.

ULLS. Subjects conducted all ambulatory activity on crutches while wearing a shoe with a 10-cm sole on the right foot for 6 wk. This served to remove body weight bearing from the left lower limb. A Velcro strap that was passed from the toe of the shoe of the suspended limb around the ankle prevented the toes from touching the ground while standing or walking with the crutches. Subjects were instructed to manually assist any additional

movements of the suspended limb (e.g., repositioning while sitting). Subjects were familiarized with the use of crutches and the platform shoe once a week during the month before the study. Subjects maintained a diary of their daily activities and were interviewed in person or via telephone by one of the investigators daily during ULLS to encourage compliance.

Imaging techniques. Proton MR images were collected 9 days before and on *days 26* and *40* of ULLS. Images were taken in the morning after a night's sleep, and subjects were encouraged to limit physical activity in an effort to avoid fluid shifts that might induce interstitial and/or intracellular volume changes (13). Subjects put antiembolism stockings on both lower limbs on awakening and wore them during transit to the image unit. Once there, subjects assumed the supine posture, removed the stockings, and rested for 30 min before image collection.

MR images were collected using a 1.5-T superconducting magnet interfaced with a General Electric Signa imaging system (Milwaukee, WI). Single echo transaxial images (repetition time/echo time = 2,000/20) were obtained from both lower limbs with the use of the Signa system whole body coil. Ten-millimeter-thick image slices were collected with a 5-mm slice-to-slice interval. Images were collected from the head of the femur to the knee joint for the thigh and from the knee to the ankle for the leg.

MR image files were ported to a Sun work station (Sun Microsystems, Redwood, CA) for data analysis. Partially automated delineation of fat, bone, and muscle was accomplished by use of proprietary software that outlined anatomic features based on thresholds input by the operator. After manual adjustments, CSA was calculated by integration over the defined region of interest. Image CSA was converted from pixels to centimeters squared with the use of the field-of-view imaging parameter.

For each subject the thigh CSA data set began distally at the first slice containing both vastus lateralis and vastus medialis muscles and ended at the last slice without gluteal muscle. CSA for defined regions of interest was determined for each subject by averaging CSA values for individual slices within these boundaries. Image CSA was calculated for the extensor and flexor muscle groups of the right and left thigh and for the four individual muscles of the quadriceps femoris. A mean of 14.0 ± 0.2 consecutive slices per subject was used to calculate CSA. This represented a region of the thigh $\sim$20 cm in length.

Post-ULLS, leg images were collected at 5-mm intervals from the knee to the ankle. Image CSA was calculated on slices containing contributions from both soleus and gastrocnemius muscles. The leg data for each subject were obtained by averaging CSA of consecutive individual slices fitting this criterion. In the calf, 8.0 ± 0.3 slices representing a region $\sim$11.5 cm in length were used. MR images were not collected for the leg before or on day 26 of ULLS because of limited availability of the imager.

Biopsy technique. Muscle samples were obtained from four males and one female 14 days before and on *day 39* of ULLS from two different sites of the left vastus lateralis muscle. Biopsies were excised at the midthigh level, 16–19 cm proximal to the patella, with use of the percutaneous needle technique (2). Samples were taken in the morning after a night's sleep, and subjects were encouraged to limit physical activity in transit to the laboratory. An antiembolism stocking was put on the left lower limb on awakening and was removed once subjects had assumed the supine posture in the laboratory. The biopsies were taken after 30 min of rest.

Muscle samples were affixed to wood splints with a mixture of OCT embedding medium (Miles Scientific, Naperville, IL) and tragacanth gum and were frozen in isopentane precooled with liquid nitrogen after orientation of the muscle fibers was determined with a dissecting microscope. Samples were stored at -70°C until further processing.

Histochemistry. Serial 12-μm-thick transverse sections of each biopsy were cut with a cryostat (-22°C), picked up on glass coverslips, air-dried at room temperature, and stored at -20°C overnight. A given section was assayed for myofibrillar adenosinetriphosphatase after acid (pH 4.6) preincubation and for periodic acid/Schiff to determine the following: *1*) fiber type composition, *2*) fiber type specific CSA and capillarity, *3*) type II/type I fiber CSA, *4*) capillary density, and *5*) the capillary-to-fiber ratio (14). Adjacent sections were assayed for myofibrillar adenosinetriphosphatase after alkaline (pH 10.4) preincubation for confirmation of fiber type classification (3). Average muscle fiber CSA and type II fiber CSA were calculated from the weighted CSA of each fiber type and the relative CSA of type IIa and IIb fibers, respectively. An average of $1,136 \pm 100$ fibers per subject were used pre- and post-ULLS for fiber-specific measures.

Statistics. Absolute data values are means $\pm$ SE for eight (MR images) or five (biopsies) subjects. Paired Student's *t* tests were used for comparison of data from MR images and for the following muscle variables: *1*) type II/type I fiber CSA, *2*) capillary density, and *3*) capillary-to-fiber ratio. Fiber type-specific CSA and composition data were analyzed using an analysis of variance with repeated measures (time $\times$ fiber type $\times$ subject). The test of a time effect on fiber CSA was done using a one-sided alternative to the null hypothesis. Type IIc fibers were extremely rare and not included in any analysis. Correlation values reported are Pearson correlation coefficients. $P < 0.05$ was considered significant.

RESULTS

MR image data. Image quality was sufficient to distinguish individual muscles within muscle groups in the slices selected for analysis (Fig. 1).

Total muscle CSA in the thigh of the suspended left lower limb was reduced ($P < 0.05$) 12% after 6 wk of ULLS, whereas total thigh CSA did not change ($P > 0.05$) (Table 1). Decreases ($P < 0.05$) in CSA of both the knee flexor and knee extensor muscle groups contributed to the reduction in thigh muscle CSA (Table 1). Rectus femoris muscle of the knee extensors did not change ($P > 0.05$), whereas the vasti muscles experienced similar decreases ($P < 0.05$) (Table 1). No changes ($P > 0.05$) were seen in right thigh muscle CSA (e.g., pre- and post-ULLS knee extensor muscle group CSA 38.2 ± 5.0 and 38.2 ± 4.5 cm^2, respectively).

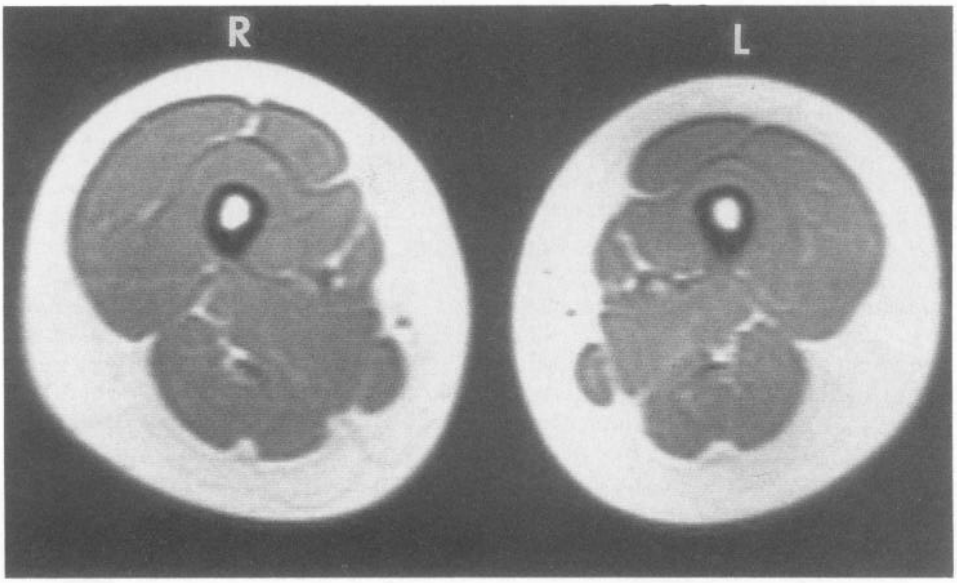

FIG. 1. Representative single-echo transaxial proton magnetic resonance image of both thighs collected in whole body coil after 6 wk of unilateral lower limb suspension (repetition time/echo time 2,000/20, 256 × 256/2.0 number of exitation, field of view 40 cm, and thickness 10 mm). View is horizontal plane through both thighs looking proximal. Substantially smaller muscle cross-sectional area appears in subject's left (L) suspended thigh (appears on viewer's right). R, right.

The relative decreases in muscle CSA of the left thigh were similar whether the post-ULLS left thigh images were compared with the presuspension left or right thigh images or the postsuspension right thigh images. There was, in addition, no change in muscle CSA of the right thigh with ULLS. Accordingly, it seemed reasonable that the post-ULLS right leg MR images could serve as a control for the post-ULLS left leg images. Total muscle and muscle compartment CSA of the left leg, compared with the right leg, were reduced ($P < 0.05$) after 6 wk of ULLS (Table 2).

Muscle fiber data. There were no changes ($P > 0.05$) in the percentage of a given fiber type after suspension (Table 3).

Average muscle fiber CSA was decreased ($P < 0.05$) 14% after 6 wk of ULLS. Type I and II fibers showed decreases of 12 and 15%, respectively (Table 3). Thus the type II/type I fiber CSA was not altered by ULLS (pre 1.19 ± 0.11, post 1.16 ± 0.14; $P > 0.05$). The decrease in type II fiber CSA was due to a reduction ($P < 0.05$) in type IIa CSA. Type IIb CSA did not change ($P > 0.05$).

The capillary-to-fiber ratio and the number of capillaries surrounding the different fiber types were not changed after ULLS (Table 4). Capillary density increased 15% ($P < 0.05$), and the number of capillaries per unit area of type I and IIa fibers increased ($P < 0.05$) 15 and 14%, respectively (Table 4).

TABLE 1. *CSA of the left suspended thigh before and after 6 wk of unilateral lower limb suspension*

Region	Pre-CSA, cm^2	Post-CSA, cm^2	Percent Change
Total thigh	175±14	173±15	−2
Total muscle	93.7±9.7	83.1±9.1*	−12
Knee extensors	38.3±3.9	32.3±3.6*	−16
Knee flexors	27.3±7.3	25.4±7.4*	−7
Vastus lateralis	12.2±0.8	10.2±1.0*	−16
Vastus intermedius	10.4±0.9	9.0±0.9*	−14
Vastus medalis	12.9±0.2	11.0±1.1*	−15
Rectus femoris	5.4±0.4	5.2±0.3	−2

Values are means ± SE or percent change. CSA, cross-sectional area. * Decrease from pre-CSA ($P < 0.05$).

DISCUSSION

The purpose of the present study was to examine the influence of the loss of body weight bearing on skeletal muscle morphology of the lower limb in healthy humans. Unweighting was accomplished by requiring subjects to perform all ambulatory activity on crutches while wearing a shoe with a 10-cm sole on one foot, thereby suspending the contralateral lower limb. Subjects were able to carry on their daily lives with moderate inconvenience in contrast to the confinement required of the bed rest model. The use of ULLS to eliminate body weight bearing in humans was based on the well-accepted hindlimb suspension method of muscle unweighting in rats (22, 26).

The results of the present study confirm and extend the findings reported previously after 4 wk of lower limb suspension in humans (1). The data show that ULLS decreases skeletal muscle CSA of the thigh of the suspended lower limb, whereas muscle CSA of the contralateral limb was not altered (Fig. 2, Table 1). They also indicate that the magnitude of atrophy differs among muscle groups and within individual muscles of a group (Tables 1 and 2) and that decreases in muscle CSA are accompanied by fiber atrophy (Table 3). As a result of the fiber atrophy, capillary density was increased after ULLS (Table 4). We also recently reported that 6 wk of ULLS decreased strength of the knee extensors 21%, on

TABLE 2. *CSA of the leg after 6 wk of unilateral lower limb suspension*

Region	Right CSA, cm^2	Left CSA, cm^2	Percent Change
Total muscle	52.5±2.0	44.9±2.7*	−14
Soleus	15.9±0.7	13.3±0.9*	−17
Gastrocnemius	10.6±0.7	7.9±0.8*	−26

Values are means ± SE or percent change. * Smaller than right ($P < 0.05$).

TABLE 3. *Fiber type percentage and fiber type CSA in biopsies of vastus lateralis muscle of the left suspended lower limb before and after 6 wk of ULLS*

	Percentage			CSA, μm^2				
	Type I	Type IIa	Type IIb	Type I	Type II	Type IIa	Type IIb	Average
Before	33±4	32±4	34±3	3,982±200	4,697±376	5,471±404	3,992±462	4,438±267
After	32±6	29±7	38±3	3,492±134*	4,010±409*	4,661±372*	3,552±376	3,808±277*

Values are means ± SE. ULLS, unilateral lower limb suspension. * Less than pre-ULLS ($P < 0.05$).

average, across speeds of eccentric, isometric, and concentric actions (8). It appears, therefore, that 4–6 wk of suspension induce meaningful changes in the architecture and functional capacity of lower limb skeletal muscle in humans.

Berg et al. (1) recently showed 4 wk of lower limb suspension reduced thigh muscle CSA 7%. This response is similar to the 8% reduction reported after 4 wk of bed rest (4), suggesting that these models impose a similar extent of unweighting. Six weeks of ULLS in the present study caused a 12% decrease in thigh muscle CSA, and CSA of the ankle extensors was 20% smaller in the left than in the right leg after ULLS. The atrophy for the thigh was somewhat larger than that after 4 wk of bed rest or ULLS. The apparent decrease in ankle extensor muscle CSA after 6 wk of ULLS is also larger than the 12% reduction found after 5 wk of bed rest (18). These results, taken together, suggest that the atrophic response of lower limb musculature to unweighting in humans has not reached its nadir after 4 wk. In support of this contention, the MR images collected after 4 wk of ULLS showed a 12% decrease (38.3 ± 3.9 to 33.6 ± 3.6 cm^2, $P < 0.05$) in CSA of the knee extensors of the suspended lower limb. This response was less ($P < 0.05$) than the 16% reduction after 6 wk of ULLS.

The use of multiple cross sections to measure muscle CSA, as done in the present but not past studies, may also have provided a more representative index of the atrophic response (10). The lack of control for fluid shifts could also have confounded the results of previous studies (13). Subjects in the present study donned antiembolism stockings on both lower limbs on awakening and wore them until supine at the image unit. We found in two subjects that 4 h of the upright posture without the stockings resulted in fluid shifts in the leg sufficient to mask the atrophy due to 6 wk of ULLS (data not shown).

The results of the present study show that unweighting due to 6 wk of ULLS caused a greater decrease in CSA of knee extensor than knee flexor muscle group (Table 1). Greater atrophy of extensor than flexor muscle groups has been reported after disuse induced by knee joint injury in humans and by spinal cord transection, hindlimb suspension, and spaceflight in lower mammals (16, 19, 21–24, 28). The greater atrophic response of extensor muscles has been attributed to their serving more of an antigravity role than flexor muscles, whether it be locomotory or postural. Whatever the mechanism(s), the greater atrophy of extensors after unweighting appears to have functional significance, because the knee extensor muscle group shows about twice the loss of strength as the knee flexors after spaceflight or bed rest (9, 12, 27), and the 21% decrease in knee extensor strength found in the present study was highly related to changes in muscle CSA (8). For example, angle-specific torque during concentric actions at 1.73 or 2.99 rad/s was correlated with CSA ($r > 0.96$, $P < 0.05$) both before and after ULLS. Estimation of muscle CSA from multiple cross sections appears, therefore, to provide a reasonable index of force-generating capacity (25).

The individual vasti muscles of the knee extensors showed similar decreases in CSA after 6 wk of ULLS (Table 1). Rectus femoris muscles, in contrast, showed no change in CSA. A lesser atrophic response of rectus femoris than the vasti muscles has also been reported after knee joint immobilization or spinal cord transection in lower mammals (19, 28). The authors argued that the rectus femoris muscle showed less of an atrophic response because it functions across the hip and knee joints, in contrast to the vasti muscles that crossed only the immobilized knee joint (19). Subjects in the present study may have flexed the lower limb about the hip joint while walking with crutches, thereby weighting the rectus femoris muscle to some extent. It is doubtful, however, that this alone was responsible for the maintenance of rectus femoris muscle CSA because spinal cord-transected cats show minimal atrophy of this muscle (28). It appears that the atrophic response of rectus femoris muscle to disuse is more comparable with that of flexor muscles, even though it can function to extend the leg about the knee joint. It follows that disuse, irrespective of the method, must impose a greater relative decrease in weighting history of the vasti than the rectus femoris muscle.

Lieber et al. (19) suggested that the extent of muscle

TABLE 4. *Capillary data from biopsies of vastus lateralis muscle of the left suspended lower limb before and after 6 wk of ULLS*

	Capillaries Surrounding Fiber Type			Capillaries per 1,000 μm^2 Fiber Type CSA			Capillary Density, cap/mm^2	Capillary-to-Fiber Ratio
	I	IIa	IIb	I	IIa	IIb		
Before	4.4±0.2	4.6±0.3	3.8±0.3	1.12±0.09	0.85±0.04	1.00±0.09	376±7.4	1.7±0.1
After	4.4±0.1	4.4±0.3	3.9±0.4	1.28±0.06*	0.96±0.04*	1.11±0.07	432±17.8*	1.7±0.1

Values are means ± SE. * Greater than pre-ULLS ($P < 0.05$).

atrophy after disuse is greater in muscles of a group that function across one than multiple joints. The results found for the knee extensors in the present study support this concept. The three vasti muscles that function about the knee joint demonstrated greater atrophy than their companion, rectus femoris muscle, which also acts as a flexor about the hip. Six weeks of ULLS, however, appeared to cause greater atrophy of the ankle extensor that functions about the ankle and knee joints, the gastrocnemius muscle, than of the one that functions about only the ankle joint, the soleus muscle. Thus factors besides the number of joints a muscle crosses must also determine the magnitude of the atrophic response to unweighting.

The apparent atrophy found in gastrocnemius muscle may appear surprising because of its relatively high composition of fast-twitch fibers (17). Of the ankle extensors, the mainly slow-twitch soleus muscle is generally held to show the greater atrophic response to unweighting (22, 26). We have previously found after 4 wk of bed rest that slow-twitch fibers in biopsies of soleus and vastus lateralis muscles did not atrophy to a greater extent than fast-twitch fibers (15). The results of the present study also show that 6 wk of unweighting by ULLS do not cause preferential slow-twitch fiber atrophy in vastus lateralis muscle (Table 3). Lindboe and Platou (20) found greater fast-twitch fiber atrophy in biopsies of vastus medialis muscles of males than females after knee joint injury and suggested that this reflected the relatively larger fast-twitch fibers in males. The results of several other studies support the concept that fiber size in part influences the atrophic response to unweighting. Lieber et al. (19) reported that the best predictor of fiber atrophy after knee joint immobilization was fast-twitch fiber size. Slow- or fast-twitch fibers in a given muscle may show the greater relative atrophy after space flight or hindlimb suspension of rats and after spinal cord transection in cats depending on which was initially larger (21, 23, 24, 28).

Six weeks of ULLS caused a significant increase in capillary density and the number of capillaries per unit area of type I and IIa fibers (Table 4). The number of capillaries surrounding the different fiber types did not change. These data support the contention that fiber atrophy occurred during ULLS because the relative changes in average fiber CSA and capillary density and in type I and IIa CSA and the number of capillaries per unit CSA of these fiber types were almost identical (Tables 3 and 4). Increases in capillary density after hindlimb suspension or spaceflight, due to fiber atrophy or a greater reduction in fiber CSA than capillaries per fiber, have been reported (5–7, 23). These data suggest that different factors govern changes in fiber CSA and muscle capillarity even though capillarity markedly influences the diffusion characteristics of muscle. The increased capillarity of rat skeletal muscle after unweighting is not accompanied by an increased resistance to fatigue (11, 29). Fatigability was actually increased in fast-twitch muscle. Whether fatigability is increased in humans after unweighting has not been established.

In summary, the results of the present study clearly establish ULLS as a relatively simple model of muscle unweighting in humans. Atrophy was greater in extensor than flexor muscles but not dependent on the number of joints a muscle crossed. The decrease in muscle CSA was accompanied by fiber atrophy, with the response apparently more dependent on fiber size than type. It is suggested that the magnitude of the atrophic response in humans is mainly dependent on the relative reduction in weighting history. When compared with previous studies of hindlimb suspension, the results of the present study suggest that lower mammals show qualitative but not quantitative responses to unweighting similar to those of humans.

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