

# Impact of resistance exercise during bed rest on skeletal muscle sarcopenia and myosin isoform distribution

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<sup>1</sup>Department of Human Studies, University of Alabama at Birmingham, Birmingham, Alabama 35294; <sup>2</sup>Life Sciences Research Laboratories, National Aeronautics and Space Administration Johnson Space Center, Houston, Texas 77058; <sup>3</sup>Department of Physiological Sciences, University of California, Los Angeles, California 90095; <sup>4</sup>Department of Physiology, University of Florida, Gainesville, Florida 32611; and <sup>5</sup>Department of Internal Medicine, University of Texas Medical Branch, Galveston, Texas 77550

**Bamman, Marcos M., Mark S. F. Clarke, Daniel L. Feedback, Robert J. Talmadge, Bruce R. Stevens, Steven A. Lieberman, and Michael C. Greenisen.** Impact of resistance exercise during bed rest on skeletal muscle sarcopenia and myosin isoform distribution. *J. Appl. Physiol.* 84(1): 157–163, 1998.—Because resistance exercise (REx) and bed-rest unloading (BRU) are associated with opposing adaptations, our purpose was to test the efficacy of REx against the effects of 14 days of BRU on the knee-extensor muscle group. Sixteen healthy men were randomly assigned to no exercise (NoEx;  $n = 8$ ) or REx ( $n = 8$ ). REx performed five sets of leg press exercise with 80–85% of one repetition maximum (1 RM) every other day during BRU. Muscle samples were removed from the vastus lateralis muscle by percutaneous needle biopsy. Myofiber distribution was determined immunohistochemically with three monoclonal antibodies against myosin heavy chain (MHC) isoforms (I, IIa, IIx). MHC distribution was further assessed by quantitative gel electrophoresis. Dynamic 1-RM leg press and unilateral maximum voluntary isometric contraction (MVC) were determined. Maximal neural activation (root mean squared electromyogram) and rate of torque development (RTD) were measured during MVC. Reductions ( $P < 0.05$ ) in type I (15%) and type II (17%) myofiber cross-sectional areas were found in NoEx but not in REx. Electrophoresis revealed no changes in MHC isoform distribution. The percentage of type IIx myofibers decreased ( $P < 0.05$ ) in REx from 9 to 2% and did not change in NoEx. 1 RM was reduced ( $P < 0.05$ ) by 9% in NoEx but was unchanged in REx. MVC fell by 15 and 13% in NoEx and REx, respectively. The agonist-to-antagonist root mean squared electromyogram ratio decreased ( $P < 0.05$ ) 19% in REx. RTD slowed ( $P < 0.05$ ) by 54% in NoEx only. Results indicate that REx prevented BRU-induced myofiber atrophy and also maintained training-specific strength. Unlike spaceflight, BRU did not induce shifts in myosin phenotype. The reported benefits of REx may prove useful in prescribing exercise for astronauts in microgravity.

muscle atrophy; spaceflight; immunohistochemistry; strength; neural activation; unloading

SKELETAL MUSCLE SARCOPENIA (i.e., reduced muscle mass and strength) after a period of sustained unloading is preferential to weight-bearing muscles. Both spaceflight and bed-rest unloading (BRU) lead to atrophy in slow and fast muscle fibers (12, 18) and marked loss of strength (9, 36). In rodents, sarcopenia is accompanied by an increased distribution of fast myosin heavy chain (MHC) isoforms after both spaceflight (5) and ground-based (35) unloading. This shift in myosin phenotype

has been associated with increased maximum velocity of shortening and increased fatigability (5). In humans, spaceflight has been shown to result in a similar slow-to-fast myosin shift (37) and an increase in fatigability (7).

Prolonged resistance training during normal weight bearing enhances muscle mass and strength (10, 17) and reduces fast type IIx MHC distribution (1). On the basis of these adaptations, increasing mechanical load via resistance exercise is an attractive countermeasure against unloading-induced sarcopenia and has been recommended for spaceflight (3, 6, 10). Despite its implicit benefit, however, resistance training has received little attention in spaceflight or BRU. To date, except for resistance exercise provided by the Mini-Gym in later Skylab missions, astronauts have performed almost exclusively endurance exercise (e.g., cycle ergometry or low-impact treadmill exercise) in orbit in an effort to combat cardiovascular deconditioning and orthostatic intolerance (6). However, endurance training does not effectively counter sarcopenia during spaceflight (12, 36). This is not surprising because endurance training during normal weight bearing does not enhance either muscle mass (24) or strength (25).

It is generally accepted that changes in muscular strength are influenced by both neurogenic and myogenic factors. Improved strength after resistance training results from increases in both neural drive and muscle fiber size (23), whereas decreased strength after unloading has been linked to reductions in both neural drive and muscle fiber size (11). Taken together, these results suggest that an effective countermeasure against unloading-induced sarcopenia should protect both muscle mass and neural drive. Use of resistance exercise during BRU has been limited to two studies (14, 16). In both studies, frequent (5–6 days/wk) concentric isokinetic (16) or concentric isokinetic combined with isometric (14) exercise attenuated loss of strength specific to the mode of training, but muscle fiber size and neural activation were not evaluated.

Critical to the understanding of the effects of resistance exercise is a characterization of the training stimulus employed. Volume, load, frequency, and rest intervals are important determinants of training adaptations. In ambulatory subjects, improvements in strength, muscle mass, and neural drive and downregulation

lation of MHC type IIX distribution are consistently found after training 2–3 days/wk at 75–85% of concentric one repetition maximum (RM) or 6–12 repetitions (1, 10, 17, 23). We hypothesized that a similar resistance training regimen would protect load-bearing muscles from the deleterious adaptations to BRU. Thus the purpose of this study was to test the efficacy of a concentric-eccentric constant resistance exercise countermeasure against the effects of BRU on knee-extensor maximum voluntary strength, myofiber size, MHC distribution, and neural drive.

It is important to note that the MHC transcript expressed in human muscle fibers histochemically classified as type IIB fibers has been shown to be similar in sequence to the rat IIX MHC gene (30). As a result, in this paper we identify the slowest migrating human MHC and its associated fiber type as IIX rather than IIB. However, the traditional nomenclature (IIA, IIAB, IIB) is used in discussing histochemical results from other papers.

## METHODS

**Subjects.** Sixteen healthy men were recruited from the greater Houston (TX) metropolitan area and were randomly assigned to no exercise (NoEx;  $n = 8$ ) or resistance exercise (REx;  $n = 8$ ). Subjects were 23–41 yr of age, normotensive, nonsmoking, and nonobese (body mass index <28) and passed a comprehensive physical examination including a diagnostic stress test. Each subject gave written informed consent after being briefed on all procedures.

To ensure that the effects of BRU were studied exclusive of the effects of resistance detraining, only individuals who had not participated in a resistance training program for at least 1 yr before the start of the study were allowed to participate. Subjects were not excluded for recent endurance training. A weekly activity history indicated that some subjects did not exercise on a regular basis, whereas others were highly active: two subjects had recently completed military training (primarily run training), three were trained cyclists, one was a former collegiate soccer player and current club soccer player, four were recreational athletes (e.g., basketball 2–3 days/wk), two were laborers, and four subjects reported no regular exercise history. Subjects were housed in the General Clinical Research Center at the University of Texas Medical Branch at Galveston (UTMB) for 16 days (1 ambulatory, 14 bed rest, 1 recovery). The protocol was approved by the Institutional Review Boards of National Aeronautics and Space Administration Johnson Space Center and UTMB.

**Bed-rest procedures.** Subjects were maintained on an isocaloric diet with protein consumption held near 1.1 g/kg. Body weight was assessed daily via a bed scale. Subjects remained in a 6° head-down position at all times throughout BRU except during defecation (a commode chair was placed next to the bed) and for 30 min every other day during either supine resistance training (REx) or supine out-of-room rest (NoEx). At no time were subjects allowed to stand during the 14 days.

**Resistance training.** Training consisted of concentric-eccentric constant-resistance leg-press exercise performed for five sets every other day during BRU. Subjects remained recumbent during exercise on a horizontal leg-training device (Cybex Strength Systems, Ronkonkoma, NY). Resistance was adjusted throughout training to consistently induce volitional failure near eight repetitions (i.e., 80–85% of 1 RM). Resistance training was performed at this volume and intensity to mimic paradigms shown previously to induce gains in strength

and muscle mass during weight bearing (10, 17). Sets were separated by a 90-s rest period. The load during a single warm-up set of 10–12 repetitions was ~67% of the previous training session's 8 RM. Total (concentric + eccentric) work performed during each training session was calculated from average load (kg), displacement (m), and total repetitions. REx subjects averaged 7.9 repetitions per set and 56.7 kJ of work per training session.

**Muscle biopsy procedure.** Samples were removed from the vastus lateralis muscle of the dominant leg by percutaneous needle biopsy as previously described (13). Samples were immediately mounted on cork with Tissue-Tek OCT mounting medium (Miles, Elkhart, IN), oriented cross-sectionally by using a dissecting microscope, quickly frozen in liquid nitrogen-cooled isopentane, and stored at  $-80^{\circ}\text{C}$  until analysis. Pre- and post-BRU muscle samples were prepared and analyzed simultaneously. Muscle blocks were sectioned (10  $\mu\text{m}$  thick) in a cryostat (Zeiss Microm HM 500 OM) cooled to  $-25^{\circ}\text{C}$ . Twenty sections were stored in precooled microcentrifuge tubes for gel electrophoresis. Serial sections for histochemistry and immunohistochemistry were picked up on precooled Superfrost Plus adhesion slides (Erie Scientific, Portsmouth, NH).

**Myofiber cross-sectional area (CSA).** Myofibers were classified as type I or II by metachromatic dye-adenosinetriphosphatase (ATPase) histochemistry by using methods previously described (26). Metachromasia was revealed by 0.1% toluidine blue after acid preincubation (pH 4.5) and incubation in 0.15% ATP disodium salt (pH 9.4). Myofiber types I and II were colored turquoise and violet, respectively. CSA by type was assessed using Global Lab Image software. An average of 339 myofibers (range 150–662) per sample were analyzed.

**MHC sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).** Preparation of washed myofibrils was performed according to the method of Talmadge and Roy (33) modified for cryostat sections. Briefly, 50  $\mu\text{l}$  of refrigerated homogenization buffer [250 mM sucrose, 100 mM KCl, 5 mM EDTA, and 20 mM tris(hydroxymethyl)aminomethane (Tris), pH 6.8] were added to 20 frozen 10- $\mu\text{m}$ -thick sections. Sections were homogenized with a micropestle and centrifuged in a cold temperature ( $5^{\circ}\text{C}$ ) at 10,000  $g$  for 10 min. Supernatant was discarded, and the myofibril pellet was resuspended in 50  $\mu\text{l}$  of wash buffer (175 mM KCl, 2 mM EDTA, 0.5% Triton X-100, and 20 mM Tris, pH 6.8). Centrifugation was repeated, supernatant was again discarded, and the myofibril pellet was resuspended in 50  $\mu\text{l}$  of final resuspension buffer (150 mM KCl and 20 mM Tris, pH 7.0). Total protein was assayed according to the bicinchoninic acid method. Myofibril isolation and protein assay were performed on all 32 samples simultaneously.

Myofibrillar protein was diluted in sample buffer [1.0%  $\beta$ -mercaptoethanol, 4.0% SDS, 16.0% 1.0 M Tris (pH 6.8), 20% glycerol, and 0.2% bromophenol blue] to a final concentration of 0.2  $\mu\text{g}/\mu\text{l}$ , reduced (boiled 2 min at  $100^{\circ}\text{C}$ ), and stored at  $-80^{\circ}\text{C}$  until gel run. Separating (8%) and stacking (4%) polyacrylamide gels were cast for the Bio-Rad Mini-PROTEAN II cell (Bio-Rad Laboratories, Hercules, CA) as previously described (33). Ten microliters of re-reduced sample were loaded in each lane (10 per gel). Upper (3.6% Tris, 3.4% glycine, and 0.3% SDS) and lower (1:6 dilution of upper) running buffers were added. Run conditions were 150-V constant voltage for 20 h in an ice bath. Within subjects, pre- and post-BRU samples were run in adjacent lanes to ensure identical run conditions. Gels were stained with rapid Coomassie blue (Research Products International) for 90 min and destained with  $^2\text{H}_2\text{O}$  overnight. Gels were captured by video, and each lane was analyzed for optical density by using an

IS-1000 digital imaging system (Alpha Innotech). MHC isoforms were identified according to their apparent molecular masses as indicated by migration rate (I > IIa > IIx).

**Western blot analysis.** MHC antibody specificity was confirmed by immunoblotting before immunohistochemical staining. A single sample was loaded across the width of a minigel. After SDS-PAGE, proteins were blotted to 0.2- $\mu$ m nitrocellulose membranes over 90 min at 24-V constant voltage by using a Bio-Rad transblot semidry transfer cell. The gel was then stained with rapid Coomassie blue as described above. The nitrocellulose membrane was cut into strips, which were blocked for 1 h with 4% milk and 0.1% Tween-20 in Dulbecco's phosphate-buffered saline (DPBS). Three primary immunoglobulin G (IgG) monoclonal antibodies (MAbs) against type I [Novacastra Laboratories-MHC (NCL-MHCs), Novacastra], type II (SC-71, Dr. Stefano Schiaffino, University of Padova, Padova, Italy), and types I+IIa (BF-35, Dr. Stefano Schiaffino)MHC were diluted in blocking buffer to final concentrations of 1:100, 1:50,000, and 1:200,000, respectively. Adjacent nitrocellulose strips in primary MAb were shaken for 1 h. Strips were rinsed  $2 \times 1$  min and  $3 \times 6$  min in rinse buffer (0.1% Tween-20 in DPBS). Secondary antibody (anti-mouse IgG peroxidase conjugate; Sigma Chemical A6782) was diluted 1:1,000 in rinse buffer and applied for 1 h. The rinse protocol was then repeated. Antigen recognition was detected by chemiluminescence (enhanced chemiluminescence kit, Amersham Life Science). Results are shown in Fig. 1.

**MHC immunohistochemistry.** Myofiber distribution was assessed by MHC immunoperoxidase staining performed with a Vector ABC Kit [horseradish peroxidase (HRP) IgG elite kit]. Blocking solution, biotinylated secondary antibody, and ABC (avidin and biotinylated HRP complex) reagent were prepared according to manufacturer's instructions. Final working dilutions of NCL-MHCs, SC-71, and BF-35 were, 1:100, 1:100,000 and 1:5,000, respectively. Sections [10  $\mu$ m thick] were air-dried for 60 min and rehydrated with DPBS (pH 7.4) for 15 min. Blocking solution was then applied for 20 min. For sections receiving BF-35, it was found that a "preblock" of 4% milk in DPBS was required for fibers containing primarily IIx MHC to stain negative. Primary MAbs were applied on humidified serial sections overnight at 4°C. Secondary antibody control sections in each series received blocking buffer in lieu of primary MAb. Sections were washed with DPBS ( $2 \times 2$  min,  $1 \times 10$  min). Secondary antibody was applied for 60 min at room temperature followed by washes with DPBS. ABC reagent was applied for 60 min followed by the same wash protocol. Immunoreactivity was revealed with peroxidase substrate [diaminobenzidine (DAB), Vector HRP-DAB kit]. An average of 348 myofibers (range 132–741) per sample were classified as type I, IIa, or IIx. The sum of myofibers staining positive with NCL-MHCs and SC-71 defined the entire population. Myofibers staining positive for type II MHC with SC-71 but negative with BF-35

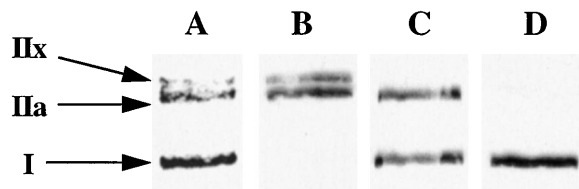


Fig. 1. Western blot analysis of anti-myosin monoclonal antibodies. Lane A: SDS-polyacrylamide gel electrophoresis gel stained with Coomassie blue. Lane B: SC-71 showing immunoreactivity to types IIa (IIa) and IIx (IIx) myosin heavy chain (MHC). Lane C: BF-35 showing immunoreactivity to types I (I) and IIa. Lane D: NCL-MHCs showing immunoreactivity to type I myosin heavy chain.

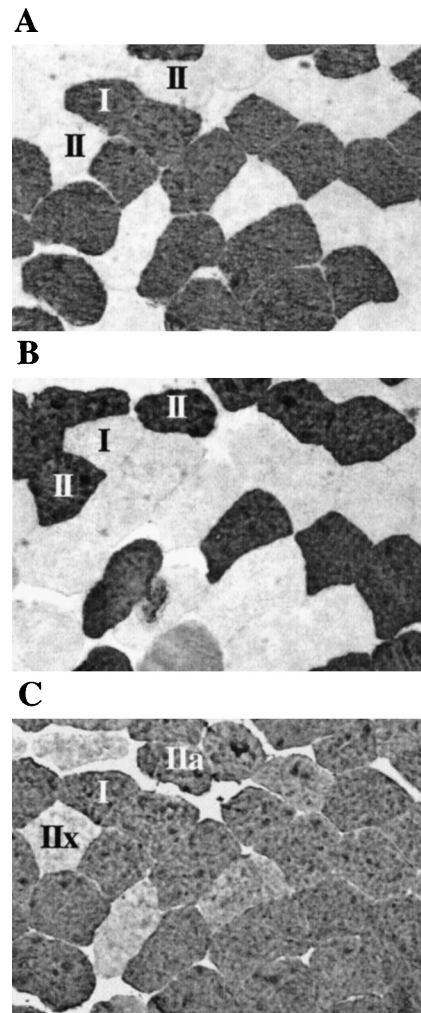


Fig. 2. Serial sections immunostained with anti-myosin monoclonal antibodies. A: positive staining of type I myofibers with NCL-MHCs. B: positive staining of type II (II) myofibers with SC-71. C: positive staining of type I and type IIa myofibers with BF-35.

were classified as IIx fibers. Myofibers staining positive with both SC-71 and BF-35 defined the IIa population. Immunoreactivity on serial sections is shown in Fig. 2.

**1-RM strength.** 1-RM leg-press strength was tested by using the horizontal training device. After sufficient warm-up, sets of one repetition were executed with increasing resistance up to two failed attempts at a given load. The greatest load lifted successfully was the 1 RM. Trials were separated by 2–3 min. The range of motion was standardized for each subject and repeated post-BRU. One familiarization session preceded 1-RM testing. 1-RM strength tests occurred on the same day as muscle biopsy and in the same sequence pre- and post-BRU.

**Maximal voluntary isometric strength and neural activation.** Angle-specific isometric torque was determined with the dominant leg during knee extension and flexion on an isokinetic dynamometer (LIDO, Loredan Biomedical). Tests were conducted at 1.05 rad of knee flexion. After several dynamic warm-up contractions performed at 1.05 rad/s, three 4-s maximal voluntary isometric contractions (MVCs) were completed. Subjects were instructed to contract with maximal effort and as rapidly as possible. Trials were separated by 2–3 min to prevent fatigue (29). The rate of torque development (RTD) was determined from 10 to 60% MVC as previ-

Table 1. *Subject characteristics by group*

Group	Age, yr	Height, cm	Weight Pre-Bed Rest, kg	Weight Post-Bed Rest, kg
NoEx	29.9 ± 2.4	177.9 ± 2.1	71.5 ± 4.0	72.4 ± 3.8
REx	30.4 ± 2.4	178.0 ± 1.4	78.2 ± 5.6	78.4 ± 5.4

Values are means ± SE for 8 subjects in each group. NoEx, no exercise; REx, resistance exercise.

ously described (29). Electromyographic (EMG) activity of vastus lateralis, vastus medialis, rectus femoris, semitendinosus, and biceps femoris was detected with preamplified bipolar skin-surface electrodes by using methods we have described in detail previously (4). Both agonist activity and coactivation of antagonists were measured. To ensure the same electrode positions after BRU, sites were marked during pretesting and maintained with ink. All electrodes were labeled, and care was taken to apply the same electrode to each muscle before and after BRU. Raw EMG was full-wave rectified, and root mean squared (RMS) amplitude was determined by using standard calculations. RMS for each muscle was calculated over 1,000 ms centered about peak torque. RMS was then averaged across agonists and across antagonists.

**Data analysis.** All data are reported as means ± SE. Percent data from SDS-PAGE and immunohistochemistry were analyzed by using a 2 (group) × 3 (MHC type) × 2 (time) multivariate analysis of variance. All other dependent variables were analyzed by using 2 (group) × 2 (time) analysis of variance. Effects of time within groups were tested by means comparisons. Differences were considered significant at  $P < 0.05$ .

## RESULTS

**Subjects.** Subject characteristics by group are presented in Table 1. No group differences were noted in age, height, or weight. Furthermore, body weight did not change during BRU.

**Myofiber size and MHC distribution.** Myofiber CSA and MHC distribution data are presented in Table 2. Interactions were found for mean and type II myofiber CSAs ( $P < 0.05$ ). Reductions ( $P < 0.05$ ) in type I (14.6%), type II (17.4%), and mean (16.8%) myofiber CSAs were found in NoEx after BRU. Type I, type II, and mean myofiber CSAs were maintained in REx.

Results from SDS-PAGE showed no differences in the distributions of MHC I, IIa, or IIx within groups after 14 days of BRU or between groups. A group × type × time interaction was noted for myofiber distribution determined by MHC immunohistochemistry ( $P < 0.05$ ). In REx, the percentage of myofibers staining negative with BF-35 (type IIx myofibers) decreased from 9 to 2% ( $P < 0.05$ ). Type IIx myofiber distribution did not change in NoEx pre- (4%) to post- (5%) BRU. Neither group showed an alteration in type I or type IIa myofiber distributions after BRU.

**Strength performance.** Strength-performance data are presented in Table 3. An interaction ( $P < 0.05$ ) was found in leg-press 1 RM, which decreased by 9% in NoEx ( $P < 0.05$ ) and did not change in REx. Main effects were found for knee-extensor and knee-flexor MVCs ( $P < 0.05$ ). Knee-extensor MVC was reduced after BRU in both NoEx (15%) and REx (13%) ( $P < 0.05$ ). Knee-flexor MVC was reduced 12% in REx ( $P < 0.05$ ) while a similar but nonsignificant ( $P = 0.08$ ) trend was noted in NoEx. A main effect was noted ( $P < 0.05$ ) for RTD during knee extension, which slowed by 54% in NoEx. Knee-flexion RTD was slowed (26%) in REx ( $P < 0.05$ ). During maximal knee extension, a main effect was found for the agonist-to-antagonist RMS ratio, which was reduced by 19% in REx ( $P < 0.05$ ). During maximal knee flexion, a main effect was noted for antagonist coactivation ( $P < 0.05$ ), indicating that knee-extensor coactivation decreased after BRU; however, no changes were detected within groups.

## DISCUSSION

We have shown for the first time prevention of myofiber atrophy during unloading with resistance exercise. Our resistance exercise protocol incorporated both concentric and eccentric contractions against a constant resistance, ~80–85% of concentric 1 RM. We utilized this specific protocol design based on important findings from ambulatory resistance training studies. First, a training intensity of 75–85% 1 RM (6–12 repetitions) has been repeatedly shown to enhance both

Table 2. *Histochemical, immunohistochemical, and SDS-PAGE results*

Variable	No Exercise			Resistance Exercise		
	Pre-bed rest	Post-bed rest	<i>P</i> value	Pre-bed rest	Post-bed rest	<i>P</i> value
Myofiber CSA, $\mu\text{m}^2$						
Type I	3,952 ± 538	3,373 ± 300	0.04	4,189 ± 324	4,094 ± 444	0.72
Type II*	4,334 ± 630	3,581 ± 459	0.01	4,027 ± 353	4,356 ± 407	0.19
Mean*	4,159 ± 541	3,461 ± 360	0.01	4,075 ± 332	4,194 ± 399	0.63
Myofiber distribution, %†						
Type I	36.7 ± 4.5	39.5 ± 3.0	0.32	38.0 ± 3.9	41.4 ± 3.9	0.24
Type IIa	59.1 ± 3.8	55.4 ± 2.3	0.20	52.8 ± 4.7	57.1 ± 4.4	0.14
Type IIx	4.2 ± 1.2	5.1 ± 1.9	0.77	9.2 ± 2.3	1.5 ± 1.1	0.01
MHC distribution, %						
Type I	39.5 ± 3.5	38.5 ± 2.1	0.80	37.1 ± 1.7	40.3 ± 2.9	0.42
Type IIa	42.7 ± 2.1	37.2 ± 2.1	0.16	36.9 ± 3.3	35.2 ± 1.5	0.65
Type IIx	17.8 ± 3.6	24.3 ± 2.6	0.10	26.0 ± 3.9	24.5 ± 2.5	0.71

Values are means ± SE for 8 subjects. CSA, cross-sectional area; myofiber distribution, immunohistochemical results from 3 monoclonal myosin heavy chain (MHC) antibodies (NCL-MHCs, SC-71, BF-35); MHC distribution, results from MHC sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). *P* values are from within-group means comparisons. \* Group by time interaction,  $P < 0.05$ . † Group by time interaction,  $P < 0.05$ .

Table 3. *Dynamic and isometric strength-performance results*

Variable	No Exercise				Resistance Exercise			
	<i>n</i>	Pre-bed rest	Post-bed rest	<i>P</i> value	<i>n</i>	Pre-bed rest	Post-bed rest	<i>P</i> value
1-RM leg press, kg*†	7	229 ± 7	208 ± 8	<0.01	8	208 ± 11	209 ± 10	0.77
Isometric MVC, N·m								
Knee extension†	8	248 ± 10	212 ± 7	<0.01	8	253 ± 15	220 ± 19	<0.01
Knee flexion†	7	119 ± 7	106 ± 5	0.08	7	129 ± 12	114 ± 13	0.03
RTD, N·m·s <sup>-1</sup>								
Knee extension†	8	948 ± 198	432 ± 83	<0.01	8	1,136 ± 156	869 ± 135	0.09
Knee flexion	7	355 ± 88	308 ± 53	0.46	7	510 ± 38	377 ± 39	0.05
Knee extension RMS, μV								
Extensors (VM, RF, VL)	8	194.7 ± 21.9	184.0 ± 18.4	0.43	8	215.9 ± 30.4	191.6 ± 31.5	0.08
Flexors (BF, ST)	8	19.7 ± 2.0	18.8 ± 1.2	0.57	8	17.9 ± 1.7	18.6 ± 2.2	0.67
Agonist-to-antagonist ratio†	8	10.12 ± 0.95	9.86 ± 0.94	0.76	8	12.60 ± 2.04	10.27 ± 1.62	0.02
Knee flexion RMS, μV								
Flexors (BF, ST)	7	204.1 ± 40.3	185.0 ± 43.4	0.32	7	198.4 ± 26.9	163.6 ± 16.1	0.08
Extensors (VM, RF, VL)†	7	13.0 ± 1.5	9.6 ± 1.0	0.17	7	16.6 ± 4.8	12.5 ± 2.1	0.11
Agonist-to-antagonist ratio	7	15.65 ± 1.79	18.90 ± 2.79	0.15	7	13.88 ± 1.19	14.18 ± 1.33	0.89

Values are means ± SE; *n*, no. of subjects. 1 RM, one repetition maximum; MVC, maximal voluntary isometric contraction; RTD, rate of torque development from 10 to 60% MVC; RMS, root mean squared electromyogram; VM, vastus medialis; RF, rectus femoris; VL, vastus lateralis; BF, biceps femoris; ST, semitendinosus. *P* values are from within-group means comparisons. \* Group by time interaction, *P* < 0.05. † Main effect of time, *P* < 0.05.

muscle hypertrophy and strength gain (10, 17, 24, 25). Second, compared with exclusively concentric work, strength gain and myofiber hypertrophy are enhanced when at least 50% of the work is eccentric (10, 17, 22). It is generally thought that a countermeasure to spaceflight atrophy should include eccentric contractions (3, 10). We have previously reported that BRU substantially decreases the rate of muscle protein synthesis and that our resistance exercise protocol maintained muscle protein synthesis during BRU (13). Because there is a lack of comparative data, it is unknown whether contraction intensity and/or the inclusion of eccentric loading was key in maintaining muscle protein synthesis and thus preventing atrophy, but, based on ambulatory studies of myofiber hypertrophy, it is likely that both components were important (10, 17).

Before the present study, limited existing data suggested the atrophic response during BRU followed a longer time course than did atrophy during actual spaceflight. Vastus lateralis mean myofiber atrophy is ~20% after short-duration (11 days) spaceflight (12) and 15% after longer duration (30 days) BRU (18). However, without muscle samples after 30 days of spaceflight and/or 11 days of BRU, these results are difficult to compare. We found mean myofiber atrophy of similar magnitude (17%) after only 14 days of BRU (without exercise). Our data combined with the 30-day BRU results of Hikida et al. (18) suggest the time course of myofiber atrophy during unloading follows a rapid onset, but with continued unloading a plateau occurs. Extended-duration unloading with frequent muscle biopsies would be required to better characterize what appears to be an alinear atrophy response across time; however, it is interesting to note this same trend in rodents as indicated by 30% soleus myofiber atrophy after both 4 days (21) and 14 days (27) of spaceflight.

In contrast to spaceflight (37), BRU did not significantly affect MHC isoform distribution. Myosin isoform expression is known to be influenced by neural, hor-

monal, and mechanical factors, and alterations in any one of these may result in shifts of myosin phenotype (34). The slow-to-fast myosin shift reported after 11 days of spaceflight (37) was most likely of neuromechanical origin (i.e., decreased loading and/or firing) but also may have been influenced by altered thyroid status because elevated serum levels of thyroxine and thyroid-stimulating hormone have been reported after spaceflight (19). We hypothesized that a similar slow-to-fast myosin shift would occur during BRU (without exercise) and that this would be associated with an increased RTD. However, because we found no important shifts in MHC distribution and RTD was slowed, i.e., not increased, it appears that the stimulus for altering myosin isoform expression in microgravity cannot be reproduced during a similar duration of human unloading in 1 G.

Lack of a shift in MHC distribution in NoEx (as determined by SDS-PAGE) was supported by immunohistochemical results on serial sections. Although no myosin shift was revealed by either technique in NoEx, it is interesting to note that the MHC Iix isoform was ~20% of total MHC by SDS-PAGE but that only 5% of fibers were identified as pure Iix fibers on immunostained sections. This observation indicates that a large portion of myofibers that expressed MHC Iix also expressed MHC Iia (i.e., Iiax hybrids). Others have put forth a similar explanation (1). For antibody BF-35, Western blot analysis (Fig. 1) revealed no immunoreactivity against MHC Iix but strong reactivity against MHC Iia. Type II myofibers that stained positive with BF-35 were classified as type Iia fibers, although some of these fibers were likely type Iiax hybrids. Because we did not identify the Iiax subtype, we probably overestimated the percentage of type Iia fibers. Interestingly, it has been suggested that, when myofibers are classified by ATPase histochemistry and this hybrid fiber is not identified, the IIB (i.e., Iix) population rather than IIA may be overestimated because of classifying IIAB (i.e., Iiax) hybrids as IIB (23). For

example, in human vastus lateralis after spaceflight, 20% of myofibers were classified as IIB by ATPase histochemistry (12) but only 4% were IIX by immunohistochemistry (37). The important type IIAB hybrid fiber can be differentiated from primary types by ATPase histochemistry (28, 31, 32). There is evidence for a continuum of fiber subtypes within the type II family, however, that stain at varying intensities based on their relative proportions of MHC IIa and MHC IIX (31). This could lead to error in fiber classification, particularly for those type IIAB hybrids in which the dominant MHC is IIX (these fibers are misclassified as type IIB) (31).

Some cross-reactivity of antibody BF-35 with both MHC IIa and MHC IIX on sections could explain our relatively high type IIa and low type IIX myofiber percents, but only if antigen recognition by BF-35 differs for myosin proteins in the reduced (Western blot) vs. native states. Assuming BF-35 specificity for antigen is the same in both states, a more likely explanation is a substantial number of hybrid type IIax fibers in our samples. Although variable, the exercise history of most of the subjects would support a low type IIX fiber number (see *Subjects* in METHODS). Most subjects in NoEx (6 of 8) and one-half of the subjects in REx participated in some form of regular nonresistance exercise before entering bed rest. Both endurance and resistance training reduce the distribution of fast type IIX myofibers (1, 24, 32). Clearly, the type IIax hybrid fiber population is substantially larger than the type IIX population in moderately trained (28) subjects, including astronauts (37). The diverse training state of our subjects closely paralleled that of the astronaut corps whose physical conditioning is largely unstructured and typically consists of endurance running, competitive sports (e.g., basketball), or no regular exercise (20).

Based on negative staining with BF-35, the percentage of pure type IIX fibers was reduced after 2 wk of resistance training during BRU, suggesting that most of these myofibers probably coexpressed MHC IIX and MHC IIa after training. Because the percentage of IIX fibers was quite small (9%) before training, however, increased expression of MHC IIa in this small number of fibers did not alter MHC band densities on SDS-PAGE gels. A reduction in the percentage of IIX fibers after 2 wk of resistance training is consistent with previous findings (32). This is the first report, however, of a similar training effect during a period of unloading. The apparent shift may have been influenced by a lack of regular exercise in four of eight REx subjects before this study. In these four subjects, a rapid downregulation of MHC IIX with resistance training would be expected.

Resistance training prevented strength loss after BRU as measured by 1 RM in the training exercise but did not protect isometric strength. Others have shown a specificity effect of ambulatory resistance training by which strength gain is maximized when tests are conducted by using the same mode or exercise used in training (8, 25). When dynamic constant-resistance training is used, 1-RM constant-resistance tests show marked strength enhancement, whereas isokinetic (25)

and isometric (8) tests show little to no improvement. In the present unloading model, the aim of resistance training was to prevent reductions in strength, not to induce gains, but the same trends applied. After BRU, mean 1-RM and isometric-strength losses in NoEx were 9 and 15%, respectively. In REx, 1 RM did not change and isometric strength fell by 13%. These data clearly indicate an effect of training specificity because consistent decrements in NoEx were found in both test modes but changes in REx in the two test modes did not display similar consistency.

Changes in strength are due to both muscular and neural adaptations (11, 23). Because myofiber size was maintained in REx, the reduced MVC may have resulted from neural deconditioning. The agonist-to-antagonist neural activation ratio was reduced 19% during MVC in REx, indicating less relative agonist muscle activation during this specific test. The leg press performed in training was a bilateral, closed-chain movement, and the isometric knee-extension test was a unilateral, open-chain movement. The EMG and strength testing results suggest that preservation of maximal voluntary motor unit activation during unloading is highly specific to the countermeasure performed. Future resistance training countermeasures in unloading studies should probably consist of combined dynamic and maximal isometric contractions for more universal strength preservation.

Because the majority of existing data indicates that knee flexors are not compromised to the same degree as are knee extensors during unloading (2, 9, 15), we aimed our countermeasure at preventing knee-extensor reductions with little activation of the antagonistic flexors. A main effect of time, however, was found for knee-flexor MVC. Within groups, only REx showed a reduction (12%). With no assessment of knee-flexor muscle size and no significant decrease in neural activation ( $P = 0.08$ ), it is difficult to speculate as to why this adaptation occurred. Perhaps this strength-test result is further evidence in support of training specificity. After 2 wk of almost exclusively knee-extensor training, the REx subjects may have been tentative and/or unable to maximally contract the antagonistic flexor group.

In summary, high-intensity, concentric-eccentric resistance exercise prevented myofiber atrophy during BRU. Maintenance of strength, however, was specific to the mode of training. A combination of dynamic and isometric contractions should be considered in future investigations. The atrophic response to BRU was similar in magnitude and time course to data reported previously from spaceflight (12); however, 14 days of bed rest did not induce a slow-to-fast shift in myosin phenotype as previously seen after spaceflight (37). Although the latter may identify a limitation of the bed-rest model, the practical benefits of resistance training found in this study should prove useful in the design of exercise programs for astronauts in microgravity.

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