Myonuclear domain and myosin phenotype in human soleus after bed rest with or without loading

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1Department of Physiotherapy and Biomechanics, and 2Research Center for Sports Training and Education, National Institute of Fitness and Sports, Kanoya City, Kagoshima Prefecture 891–2393; 3National Center for Neurology and Psychiatry, Tokyo 187-8551; and 4Department of Physiology II, St. Marianna University School of Medicine, Kawasaki City 216–8511, Japan; 5Department of Neuropsychology, Institute of Biomedical Problems, Moscow 123007, Russia; and 6Brain Research Institute and 7Department of Physiological Science, University of California, Los Angeles, California 90095-1527

Ohira, Y., T. Yoshinaga, M. Ohara, I. Nonaka, T. Yoshioka, K. Yamashita-Goto, B. S. Shenkman, I. B. Kozlovskaia, R. R. Roy, and V. R. Edgerton. Myonuclear domain and myosin phenotype in human soleus after bed rest with or without loading. J. Appl. Physiol. 87(5): 1776–1785, 1999.—After 2 or 4 mo of bed rest (6° head-down tilt) and 1 mo of ambulation, there was a tendency toward a higher percentage of fibers expressing fast myosin heavy chain (MHC) isoforms and a de novo appearance of fibers coexpressing type I+IIa+Iix and IIa+IIX MHC in human soleus fibers. After 2 and 4 mo of bed rest, the mean size of type I fibers decreased by 12 (P > 0.05) and 39%, respectively. Because myonuclear number/mm of fiber length was unchanged, myonuclear domain was smaller after bed rest than before. The mean size and myonuclear domain of type I fibers were largest after 1 mo of recovery. The effects of wearing an antigavity device (Penguin suit), which had a modest but continuous resistance at the knee and ankle (Penguin-1) or knee resistance without loading on the ankle (Penguin-2), for 10 consecutive h/day were determined during 2 mo of bed rest. Mean fiber sizes in Penguin-1, but not Penguin-2, group were maintained at or above pre-bed-rest levels, whereas neither group showed phenotype changes. Myonuclear domain in type I fibers was larger in Penguin-1 and smaller in Penguin-2 group post-compared with pre-bed-rest, indicating that a single daily 10-h bout of modest muscle loading can prevent bed-rest-induced soleus fiber atrophy but has minimal effect on myosin phenotype. The specific adaptive cellular strategies involved may be a function of the duration and magnitude of the adaptive stimulus as well as the immediate activity history of the fiber before the newly changed functional demands.

bed rest; microgravity; exercise countermeasures; muscle atrophy; fiber type; myonuclear number

MULTIPLE MOLECULAR AND CELLULAR strategies are used in the adaptive processes, resulting in skeletal muscle atrophy and hypertrophy. To understand the atrophic and hypertrophic responses at the single-fiber level, considerable efforts have focused on the molecular control of proteins by individual nuclei in these multinucleated cells (12). More recently, however, it has become evident that skeletal muscle tissue can use another cellular strategy to accommodate increased or decreased demands for cellular maintenance. For example, after fiber atrophy, there are fewer nuclei available to regulate the muscle proteins, whereas when hypertrophy is induced, there is an increase in the amount of DNA available to the muscle fibers. More specifically, in rats and cats muscle atrophy is associated with as much as a 30% decrease and hypertrophy with as much as a threefold increase in the number of myonuclei per fiber (1, 2, 23). This dynamic modulation of myonuclear number suggests that a prolonged exposure to altered demands on muscle stimulates a more long-term adaptive strategy than does the more immediate up- or downregulation of mRNAs and translational events (8). Based on these observations, our working hypothesis is that a sustained reduction in muscle activity and loading will reduce the number of myonuclei, thus enabling the remaining myonuclei to function within a more normal range of regulation of mRNA expression. Similarly, a normal range of mRNA-regulating events can occur when a muscle hypertrophies by increasing the number of myonuclei that are available for regulation of the cytoplasm.

The present paper addresses the following issues regarding the dynamics of the myonuclear population in human skeletal muscle fibers: Is there modulation of myonuclear number in atrophying skeletal muscle fibers of healthy human subjects? Is the regulation of myonuclear number a function of the fiber myosin phenotype? Can the myonuclear number be modulated by the level of neuromuscular activity, i.e., activation and/or loading? To address these questions, we studied muscle fibers in the soleus of humans after prolonged bed rest, recovery from bed rest, and bed rest accompanied by two different levels of neuromuscular activity.

MATERIALS AND METHODS

Subjects and Experimental Design

Thirteen healthy male volunteers participated in these studies. All subjects were evaluated clinically and were considered to be in good physical condition. All subjects were informed about the possible risks in taking muscle biopsies, and a signed informed consent was obtained from each
heel, which slid on a smooth surface of a platform with the bed. The subject's shoes were equipped with rollers on the extension and flexion movements against a resistance of 100 head-down-tilt position for 2 mo. All subjects wore a Penguin Penguin suit groups were subjected to bed rest at a 6° assigned to one of two Penguin suit groups. All subjects in the day-night cycle was regulated at 8 AM and 6 PM. The temperature and humidity in the room were maintained within a normal range, and the kcal for all subjects. The temperature and humidity in the room were maintained within a normal range, and the day-night cycle was regulated at 8 AM and 6 PM.

In the second study, seven subjects (see Table 2) were assigned to one of two Penguin suit groups. All subjects in the Penguin suit groups were subjected to bed rest at a 6° head-down-tilt position for 2 mo. All subjects wore a Penguin suit for 10 h/day (11 AM to 9 PM) and performed knee-extension and flexion movements against a resistance of 100 N for the last 15 min of each hour while in a supine position in bed. The subject's shoes were equipped with rollers on the heels, which slid on a smooth surface of a platform with the ankle maintained at a relatively constant position, i.e., at ~10° dorsiflexed relative to a 90° ankle position. Thus this exercise emphasized knee, not ankle, movement.

The Penguin suit group was divided into two subgroups: Penguin-1 (n = 4) and Penguin-2 (n = 3). Subjects in the Penguin-1 group wore the full assembly of the Penguin suit, which included all of the elastic loading elements, during the 10-h period. Subjects in the Penguin-2 group also wore the full assembly of the Penguin suit, except that the elastic loading elements at the ball of the foot were disconnected. Therefore, in Penguin-1 subjects ~60–70 N of force were applied to the foot, i.e., the distal tarsal bones at the ball of the foot, when the subject was plantar flexing the ankle or when the plantar flexor muscles were relaxed. No such resistive forces on the plantar flexors were imposed by the suit in the Penguin-2 group. For the Penguin suit groups, the soleus muscle was biopsied twice, i.e., ~2 wk before bed rest and immediately after ~2 mo (Penguin-1) or ~1.5 mo (Penguin-2) of bed rest.

Muscle Biopsy Procedures

During bed rest, the subjects were carried to the medical treatment room where the biopsy was performed. Biopsies were taken from the left soleus with the subjects in a prone position by using the procedures described by Bergstrom (6). Local anesthesia was induced via subcutaneous injection of 4 ml of a 2% lidocaine hydrochloride solution. A skin incision (5 mm) was made, and a Bergstrom sterile needle was inserted toward the center of the muscle (2–3 cm in depth). The muscle samples were quick frozen in liquid nitrogen and stored at −10°C until analysis.

Isolation of Single-Fiber Segments

The muscle samples were prepared for single-fiber isolation as described by Allen et al. (1, 2). Briefly, frozen soleus samples were put in vials containing 50% glycerol-50% low-calcium relaxing solution (11) and placed in a −20°C cryostat for several hours to equilibrate to this temperature. These samples were then stored in a −5°C freezer overnight. The following morning, the vials were transferred to a refrigerator for ~2 h, and then the muscle samples were pinned to a Sylgard-coated culture dish in chilled 100% relaxing solution for 20–30 min at room temperature. Segments of ~40–46 single muscle fibers were mechanically isolated from each muscle sample by using micromanipulation instruments under a dissecting microscope. These fiber segments, ~2–4 mm in length, were then placed on gelatin-coated slides and stored at −20°C until use.

Table 1. Anthropometric characteristics of subjects and experimental schedule in control group

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age, yr</th>
<th>Body Weight, kg</th>
<th>Height, cm</th>
<th>Days Muscle Samples Taken</th>
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<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>114</td>
<td>190</td>
<td>Baseline: 19, Bed rest: 58, Recovery: 119 (+34)</td>
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<tr>
<td>2</td>
<td>38</td>
<td>77</td>
<td>186</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>77</td>
<td>178</td>
<td></td>
</tr>
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<td>4</td>
<td>27</td>
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<td>5</td>
<td>30</td>
<td>64</td>
<td>175</td>
<td></td>
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<td>6</td>
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<td>SE</td>
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<td>7.6</td>
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Table 2. Anthropometric characteristics of subjects and experimental schedule in Penguin suit group

<table>
<thead>
<tr>
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<th>Body Weight, kg</th>
<th>Height, cm</th>
<th>Days Muscle Samples Taken</th>
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<tr>
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<td>Baseline: 13, Bed rest: 60</td>
</tr>
<tr>
<td>2</td>
<td>33</td>
<td>65</td>
<td>176</td>
<td></td>
</tr>
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<td>3.3</td>
<td>3</td>
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<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age, yr</th>
<th>Body Weight, kg</th>
<th>Height, cm</th>
<th>Days Muscle Samples Taken</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>38</td>
<td>67</td>
<td>173</td>
<td>Baseline: 18, Bed rest: 47</td>
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<tr>
<td>2</td>
<td>34</td>
<td>83</td>
<td>180</td>
<td></td>
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Analysis of Single-Fiber Myonuclear Number, Cross-Sectional Area (CSA), and Myonuclear Domain

Single-fiber segments to be analyzed were removed from the −20°C freezer, thawed, and air dried for 5 min. Each fiber was circled with a hydrophobic PAP pen barrier (Kiyota International, Elk Grove Village, IL) and rinsed briefly in PBS for 5 min. Fibers were stained for 5 min with 54 µM acridine orange in PBS, then for 5 min with 1.5 × 10^-7 M propidium iodide in PBS. This combination was found to produce the best staining of, and contrast between, the cytoplasm and myonuclei. After staining, the fibers were rinsed with PBS and mounted in 100% glycerol with coverslips with “struts” of hardened nail polish on the corners to prevent fiber compression. A Sarastro 2000 confocal microscope with an argon laser (Molecular Dynamics, Sunnyvale, CA) was used to analyze fiber CSA, myonuclear number, and cytoplasmic volume per myonucleus (myonuclear domain).

A series scan was taken through the entire Z thickness of a fiber by using the proper filter sets for acridine orange fluorescence. Myonuclear number was determined by counting all the myonuclei in the stack for this region and converting them into myonuclei per millimeter by dividing by the length of the fiber (~173 µm) and multiplying by 1,000. A maximum-intensity projection rotated orthogonally to the long axis of the fiber was produced from the stack, and fiber CSA was measured by using calibrated measurement software (Silicon Graphics, Salt Lake City, UT).

To correct for possible effects of different states of fiber stretch, the average length of 10 consecutive sarcomeres was determined. Both myonuclei per millimeter and CSA were corrected for differences in sarcomere length by multiplying by the observed sarcomere length and dividing by 2.5 to normalize to a 2.5-µm sarcomere length. Mean cytoplasmic volume per myonucleus was calculated by multiplying fiber CSA by the length of the fiber region (173 µm) and dividing by the number of myonuclei counted in that region. For each fiber, three nonoverlapping regions were chosen randomly along the fiber length and analyzed. Data from all three regions were pooled and averaged to produce a single value for the fiber. Fiber ends, damaged regions, and excessively stretched (sarcomere lengths >3.5 µm) regions were omitted from analysis.

Single-Fiber Gel Electrophoresis

After confocal analysis, gel electrophoresis was carried out in the same single-fiber segments. Briefly, fiber segments were unmounted, rinsed free of glycerol with PBS, dehydrated, and destained in 50% ethanol for 5 min. Fibers were air dried for 5 min, scraped off the slide with a clean razor blade, and placed in a microcentrifuge tube containing 10–15 µl of 1× electrophoresis sample buffer (21). Fibers in sample buffer were stored at −5°C until electrophoresis was performed. 

Electrophoresis was carried out by using a Bio-Rad Mini-Protean II Dual Slab electrophoresis unit (Bio-Rad, Richmond, CA) as described by Talmadge and Roy (29). Briefly, to enhance the migration and separation of myosin heavy chain (MHC) isoforms, the separating gel contained 8% acrylamide and 30% glycerol. Gels were run at 80 V overnight in an ice-packed cooler and were then stained with Rapid Coomassie (Diversified Biotech, Boston, MA) as per the supplier’s instructions. On each gel, ~7 µl of each single-fiber sample were run in a single lane. To facilitate an accurate determination of MHC isofrom expression, two “standard” lanes were loaded with ~10 µl of a homogenate of human biceps brachii muscle. The MHC expression was determined by visual examination of the gels.

Statistical Analyses

Values are reported for each type of fiber as means ± SE. For the study in which 4-mo bed rest was performed, a repeated-measures univariate ANOVA was performed followed by Scheffe’s post hoc test to determine individual group differences. For the other study in which 2-mo bed rest was performed, pre- to post-bed-rest comparisons were determined by using paired t-tests. Significant differences were established at P < 0.05.

RESULTS

Effects of Bed Rest

Nonphenotype-specific responses. Body weight increased slightly in all subjects, except one subject who did not perform the leg exercise during the 4-mo bed-rest period (Table 1). Compared with before bed rest, the overall fiber CSA was decreased by 12 (P < 0.05) and 35% after 2 and 4 mo of bed rest, respectively (Fig. 1A). The mean fiber size was significantly smaller after 4 compared with after 2 mo of bed rest and was increased by 89% relative to the 4-mo group after 1 mo of recovery. The number of myonuclei per millimeter was not significantly different from the pre-bed-rest condition after either 2 or 4 mo of bed rest or 1 mo after recovery (Fig. 1B). However, the mean myonuclear number per millimeter was significantly larger after 1 mo of recovery compared with that observed after 2 mo of bed rest. Because the changes in the number of myonuclei after 2 and 4 mo of bed rest were small compared with pre-bed-rest values, the cytoplasmic volume/myonucleus ratios were changed similar to fiber CSA (Fig. 1C).

Phenotype-specific responses. Before the initiation of bed rest, the soleus comprised ~92% pure type I MHC fibers, ~5% pure type IIa MHC fibers, and ~3% of fibers coexpressing both type I and IIa MHC (Fig. 2). There were no significant changes in the fiber type composition of the soleus after bed rest or recovery. There was a tendency (P > 0.05), however, for the percentage of pure type I MHC fibers to decrease after 2 (8%) and 4 mo (16%) of bed rest and to return closer to pre-bed-rest values after 1 mo of recovery (6% lower). In addition, some fibers coexpressed types I+IIa+IIx or IIa+IIx MHC after bed rest and after 1 mo of ambulation. No fibers from the pre-bed-rest biopsies contained fibers with these two combinations of MHCs. 

Compared with pre-bed rest, the mean CSA of the pure type I MHC fibers was smaller by 12 (P > 0.05) and 39% after 2 and 4 mo of bed rest, respectively (Fig. 3). In addition, the mean CSA was significantly smaller at 4 compared with at 2 mo of bed rest. After 1 mo of recovery, the mean CSA of pure type I MHC fibers was significantly larger than at all other time points. No atrophy was observed in pure type IIa or type I+IIa MHC fibers.

Bed rest had no significant effect on the number of myonuclei per millimeter for any fiber type (Fig. 4). In contrast, the myonuclear number per millimeter in
pure type I MHC fibers after 1 mo of recovery was significantly higher than that observed after 2 (15%) or 4 mo of bed rest (13%). The only other significant change was an increase in the myonuclear number per millimeter in type IIa fibers after 4 compared with 2 mo of bed rest.

Because the number of myonuclei in pure type I fibers was unaffected by bed rest, the changes in the myonuclear domain (the cytoplasmic volume/myonucleus ratio) were similar to those for fiber CSA, i.e., a significant decrease at 4 mo of bed rest compared with pre-bed rest and 2 mo of bed rest (Fig. 5). Similarly, because the fiber CSA increased relatively more than the myonuclear number per millimeter in the pure type I MHC fibers after recovery, the myonuclear domain was larger after recovery than at all other time points. The myonuclear domain in fibers expressing type IIa MHC also was lower after 4 compared with after 2 mo of bed rest. In addition, the myonuclear domain of fibers expressing type I+IIa MHC was higher after recovery than after 4 mo of bed rest.

Relationship between fiber size and myonuclear number, and fiber size and myonuclear domain. A significant positive correlation was observed between fiber CSA and myonuclear number in pure type I fibers at all time points. The highest correlation \( r = 0.62 \) was observed before bed rest. The correlation coefficients at 2 and 4 mo of bed rest and 1 mo after bed rest were 0.46, 0.36, and 0.32, respectively. There also was a significant positive correlation between fiber CSA and myonuclear domain for the type I MHC fibers at all time points (Fig. 6).
The levels for the other fiber types were lower (not significant), most likely reflecting the relatively small number of fibers in these groups.

Effects of Exercise Countermeasures During Bed Rest

Nonphenotype-specific responses. The resistive exercise during bed rest in Penguin-1 and Penguin-2 groups produced different overall responses in the muscle fibers. In the Penguin-1 group at 2 mo of bed rest, mean fiber CSA was slightly larger (1.9%, \(P < 0.05\)) and mean number of myonuclei was slightly lower (6.4%, \(P < 0.05\)) compared with pre-bed-rest values, resulting in a larger myonuclear domain (Fig. 7). In contrast, in the Penguin-2 group at 2 mo of bed rest, mean fiber CSA was slightly lower (7.5%, \(P < 0.05\)) and the mean number of myonuclei was slightly higher (6.5%, \(P < 0.05\)) compared with pre-bed rest, resulting in a smaller myonuclear domain.

Phenotype-specific responses. Before bed rest, the soleus of both Penguin-1 and -2 groups was composed of ~96, ~2, and ~2% pure type I, pure IIa, and I+IIa MHC fibers. There was a slight shift of fibers toward fast types expressing IIa or I+IIa MHC isoforms in both Penguin-1 and Penguin-2 groups. The percentage of pure type I MHC fibers decreased by ~8% \((P > 0.05)\), and there was a de novo appearance of type IIa+IIx MHC fibers in both groups.

There was no fiber type-specific atrophy in the Penguin-1 group: the mean fiber size of each fiber type either was maintained or was slightly larger at 2 mo of bed rest than at pre-bed rest (Fig. 8). In the Penguin-2 group, the mean size of each fiber type was somewhat smaller at 2 mo of bed rest than at pre-bed rest, with the difference being significant for the type I+IIa fibers. There were no significant differences in mean myonuclear number per millimeter between pre-bed rest and after 2 mo of bed rest in either group. There was a tendency \((P > 0.05)\), however, for the myonuclear number per millimeter to be lower in the Penguin-1 group and higher in the Penguin-2 group for all fiber types after bed rest. Therefore, the mean cytoplasmic volume/myonucleus ratio tended to be higher at 2 mo of bed rest compared with pre-bed rest in the Penguin-1 group and lower in the Penguin-2 group for all fiber types (Fig. 9). These differences were significant for the pure type I fibers in both groups.

**DISCUSSION**

Level and Pattern of Atrophy

Both the level and pattern of muscle fiber atrophy observed in the present bed-rest study were somewhat surprising. First, the overall 35% decrease in fiber size was greater than that previously reported for human muscle fibers or muscles after prolonged periods (4–17 wk) of bed rest (4, 5, 9, 17, 19, 22) or 4–6 wk of unilateral limb unloading (4, 18, 26). Second, based on numerous animal studies, the rate of atrophy would be expected to be greater in the first 2-mo period of bed rest compared with the period between 2 and 4 mo of bed rest. The observation that at least as much atrophy occurred in the last 2 mo compared with the first 2 mo of bed rest suggests that there may be multiple and temporally specific mechanisms of adaptation associated with long-term compared with short-term unloading. For example, the soleus muscle of rats atrophies as much as 25% after 4 days of spaceflight (20), but this value reaches only 35% after 2 wk of spaceflight (27). Rapid rates of atrophy during the early phases, i.e., the initial 7–10 days, of hindlimb suspension in rats fol-
lowed by a modest rate of atrophy also have been reported (13, 14, 27, 31).

The third unexpected observation related to the atrophic process was the supercompensation effect during the 1-mo recovery period, i.e., the mean fiber sizes were larger after 1 mo of normal ambulation after bed rest than before bed rest. This finding suggests that the responsiveness of muscle fibers to a given level of activity, e.g., normal daily ambulation, is dependent on the prior pattern and level of activity. An alternative interpretation is that, after bed rest, the subjects became more active during post-bed-rest ambulation than they were before bed rest. However, this issue cannot be addressed because the activity patterns of these subjects were not quantified before bed rest or during the recovery period.

Phenotype Changes

The phenotypic adaptations observed in the present study are consistent with those of a previous study (19) of unloaded human skeletal muscle: 1) there was an increase in the number of muscle fibers expressing fast MHC isoforms; and 2) this change occurred in only a small proportion of the muscle fibers sampled. The most notable effects of bed rest on fiber phenotype were the modest increases in the percentages of fibers expressing multiple MHC isoforms and of pure type IIa MHC fibers.

Furthermore, it should be noted that I+IIa+IIx and IIa+IIx MHC fibers were observed after 2 or 4 mo of bed rest, but not before bed rest, and that some of these fibers remained even after 1 mo of recovery. It is noteworthy that the percentage of fibers that changed in the types of MHC expressed were small, and that this small percentage seems to have been resistant to readaptation in response to normal reambulation. Similar changes in fiber phenotype have been reported after spaceflight (2, 7, 15, 25, 33), with adaptations being observed in the vastus lateralis in astronauts after flights of 5 or 11 days (15, 33).

Phenotype-Specific Changes in Muscle Fiber Size and Myonuclear Number

The pattern of change in the pure type I MHC fibers, associated with bed rest, differed somewhat from that observed in the other fiber phenotypes (Fig. 3). For example, only the pure type I MHC fibers atrophied significantly after 4 mo of bed rest in the present study. Similar atrophy in type I MHC fibers of soleus muscle was induced by 17 days of bed rest (32), although significant atrophy was also observed in type II, as well as type I, MHC fibers of human vastus lateralis after only 14 days of bed rest (3). A consistent pattern, however, was that each fiber phenotype category had a larger mean CSA after 1 mo of recovery than after either 2 or 4 mo of bed rest. Thus it appears that "supercompensation" in fiber size was a general phenomenon associated with normal ambulation after prolonged bed rest.

In the present study, the pure type I MHC fibers seem to be the most affected by chronic changes in neuromus-
cular activity level, perhaps because these fibers are the most active normally. In addition, it should be pointed out that the other fiber types represented such a small fraction (a maximum of ~10% for any individual type) of the total fiber population in the soleus that these adaptations at the whole muscle level seem rather insignificant physiologically as well as statistically.

Modulation of Myonuclear Number Relative to Myonuclear Domain and Muscle Fiber Size

The significant correlation between fiber size and myonuclear number per millimeter in the pre-bed-rest muscles is consistent with previous observations in cats and rats (1). This relationship, however, was more variable after 2 or 4 mo of bed rest. The lower correla-

Fig. 7. Mean (± SE) overall cross-sectional area (A), no. of myonuclei/mm (B), and myonuclear domain of soleus fibers (C) before bed rest and after 2 mo of bed rest in Penguin-1 and Penguin-2 groups. *Significantly different from pre-bed rest; P < 0.05.

Fig. 8. Mean (± SE) cross-sectional area of each type of fiber in soleus muscle before bed rest and after 2 mo of bed rest in Penguin-1 (A) and Penguin-2 groups (B). *Significantly different from pre-bed rest; P < 0.05.

Fig. 8. Mean (± SE) cross-sectional area of each type of fiber in soleus muscle before bed rest and after 2 mo of bed rest in Penguin-1 (A) and Penguin-2 groups (B). *Significantly different from pre-bed rest; P < 0.05.

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The maintenance of the number of myonuclei at pre-bed-rest levels associated with significant fiber atrophy after 4 mo of bed rest (Fig. 1) appears to be inconsistent with the results from other models of decreased use. For example, Allen and co-workers reported a significant decrease in myonuclear number associated with atrophy of type I MHC fibers in the soleus muscle after 14 days of spaceflight in rats (2) and after 6 mo of inactivity in cats (1). In addition, Day et al. (10) reported concomitant decreases in the size and myonuclear number of type II MHC fibers in the vastus lateralis of astronauts after 11 days of spaceflight. The reason(s) for these apparently conflicting results is unclear.

However, because there were no significant changes in fiber size or myonuclear number after 2 mo of bed rest in the present study, it is possible that significant atrophy occurred only after nearly 4 mo of bed rest. If this were the case, then the 4-mo bed-rest data may
reflect a transitional period when atrophy precedes the loss of myonuclei. Alternatively, the myonuclei may be resistant to change under these bed-rest conditions. It is rather clear that the number of myonuclei per muscle fiber can be modulated during the process of atrophy-reambulation. For example, the number of myonuclei per fiber after 1 mo of ambulation is significantly higher than for control or immediately post-bed rest. Perhaps, in human muscle, new myonuclei can be generated more readily and/or more rapidly than they are lost. These data also suggest that the atrophied muscle fibers can be more readily stimulated to generate more myonuclei than can muscle fibers that have a normal level of load-bearing activity.

Effects of the Penguin-1 and Penguin-2 Protocols During Bed Rest

In contrast to the 12% overall fiber atrophy observed after 2 mo of bed rest without exercise, the size of all fiber types was maintained at or somewhat above pre-bed-rest levels in the Penguin-1, but not the Penguin-2, group. Fiber atrophy in vastus lateralis muscle (3) and decrease in muscle protein synthesis (16) during 14 days of bed rest were also prevented by resistive exercise. Similar to what was observed after 2 mo of bed rest without the subject wearing an antigravity suit, there was no change in the number of myonuclei per millimeter in either group. The myonuclear domain, however, was increased in the Penguin-1 and decreased in the Penguin-2 groups. The adaptations in fiber phenotype distribution in either of the Penguin suit groups during bed rest were similar to those observed after bed rest alone, as would be expected, as this condition did not overload the plantar flexor muscles studied. In effect, the Penguin-2 group served as a sham control group for this study.

The knee-extension-flexion exercise performed in the present study was qualitatively similar to that performed by cosmonauts on the Mir Space Station. This type of exercise was designed to sustain a critical level of motor control of dynamic muscular actions and thus contribute to a better control of posture and locomotion after prolonged periods of unloading as occur in spaceflight.

Although the level of recruitment and the absolute work levels were not quantified, it is clear that the Penguin suit protocols required a relatively low percentage of the involved motor pools. It appears, however, that a total of 150 min of lower limb extensor movement in the Penguin-1 group distributed throughout the day completely eliminated the fiber atrophy in the soleus, despite the relatively modest levels of force imposed on the soleus.

Although little is known about the recruitment of motor units of muscle during long-term continuous activation, there is clear evidence that considerable rotation of activation of motor units can occur during low-level contractions lasting for hours (30). In this regard, it should be noted that the subjects in the Penguin-1 group wore the suit for 10 h/day and that the soleus muscle was either maintained under a low passive stretch (relaxed) or worked against a low resistance when activated. Our previous study in rats also suggested that tension production, by keeping the muscle at or close to the optimum length, is an important factor for prevention of atrophy (24).

It was not possible to determine the relative role of each of these factors in counteracting the atrophic response associated with the unloading. Based on many studies addressing the issue of how much and what kind of neuromuscular efforts are needed to maintain or increase muscle mass, it seems reasonable to conclude from the present results that “exercise” requiring substantially less than maximal effort can prevent atrophy in healthy, but mechanically unloaded, muscles.

Phenotype-Dependent Responsiveness to Bed Rest and to Exercise Countermeasures

The human soleus muscle consists predominantly of fibers expressing only type I MHC, with a few fibers expressing type IIa or type I + IIa MHC. The minimal phenotype response to bed rest or bed rest plus the Penguin suit protocols suggests a strong muscle-specific rather than fiber phenotype-specific response, similar to that reported for rats after spaceflight and hindlimb suspension (14, 25, 27, 28). However, we can only conclude that there is similarity in the atrophic response of different fiber phenotypes within the soleus muscle. This lack of phenotype specificity has also been observed in astronauts after spaceflight (15).
This same logic can be applied to the Penguin suit groups. It would be assumed that, even in the Penguin-2 subjects, at least the readily excitable motor units would have shown some amelioration of fiber atrophy, while a larger proportion of fibers would have been maintained in the Penguin-1 subjects. The results, in contrast, showed no evidence of any select groups of fibers being affected by bed rest, bed rest plus Penguin-1, or bed rest plus Penguin-2 protocols. The logical conclusion from these results is that the neuromuscular activity pattern of a given fiber may not be a primary factor in defining the responsiveness of that fiber to chronically imposed changes in function.

Perspective

Compared with previous bed-rest studies, several new factors became evident from the present results, as follows. 1) Fiber atrophy can occur relatively soon after the onset of bed rest, and this can continue for up to at least 4 mo. 2) After prolonged bed rest, the adaptive response of muscle fibers to normal ambulation is greater than normal. 3) MHC phenotypic adaptations occur in relatively few fibers in response to prolonged bed rest. 4) Only very modest levels of recruitment of motor units, i.e., only a small number of motor units recruited, over prolonged periods have a protective effect on a large population of fibers. 5) Among the multiple factors that affect muscle fiber size, the number of myonuclei in a fiber appears to be a more influential factor when the functional patterns of the muscle are more constant and sustained at a low level of activity.

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REFERENCES


