Resistance Training during Unweighting Maintains Muscle Size and Function in Human Calf

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ABSTRACT

AKIMA, H., J.-I. USHIYAMA, J. KUBO, S.-I. TONOSAKI, M. ITOH, Y. KAWAKAMI, H. FUKUOKA, H. KANEHISA, and T. FUKUNAGA. Resistance Training during Unweighting Maintains Muscle Size and Function in Human Calf. Med. Sci. Sports Exerc., Vol. 35, No. 4, pp. 655–662, 2003. Purpose: A 20-d 6° head-down tilt bed rest project was conducted to evaluate the effect of dynamic leg press and plantar flexion resistance training on muscle size and function in human plantar flexors (PF) throughout the prolonged bed rest. Methods: Twelve healthy men participated in this study and were divided two groups: resistance training (BR-Tr group: N = 6, age: 23 ± 2 yr, height: 170 ± 3 cm, weight: 66 ± 7 kg) and nontraining (BR-Cont group: N = 6, age: 23 ± 1 yr, height: 170 ± 3 cm, weight: 67 ± 6 kg) during the bed rest. Physiological cross-sectional area (PCSA) and peak torque of the PF muscle group was determined. Spin-spin relaxation times (T2) of the medial (MG) and lateral gastrocnemius (LG) and soleus (Sol) muscle was measured at rest and immediately after unilateral calf-raising exercise (5 sets of 10 reps). Results: PCSA of the PF muscle group did not show any significant change in BR-Tr group; however, for the BR-Cont group, PCSA decreased by 13% after bed rest (P < 0.05). There was no significant change in exercise-induced T2 change of the MG, LG, or Sol muscles between before and after the bed rest in BR-Tr group; however, in the BR-Cont group, significant increases in T2 were found in these three muscles after the bed rest (P < 0.05 to 0.01). Conclusion: We conclude that dynamic leg press and plantar flexion resistance training during bed rest maintains muscle size and function (torque and T2), and that this training could be useful for prevention of progressive muscle deconditioning during spaceflight. Key Words: HEAD-DOWN TILT BED REST, MUSCLE FUNCTION, T2, MAGNETIC RESONANCE IMAGING, HUMAN

It is well known that deconditioning of the plantar flexor (PF) muscle group in humans is induced by unweighting such as bed rest (6–8,19,27,36), unilateral lower limb suspension (ULLS) (18,31), and spaceflight (5,20). Bamman et al. (12) showed that resistance training during 14 d of bed rest prevented the decline of muscle strength without any neural activation change. Moreover, they showed that muscle strength decreased in the nonresistance training group during bed rest without showing any changes in electromyogram (EMG) activity and suggested that the loss of strength in the nonresistance training group was due primary to muscle atrophy (11). This result was different to those of Ploutz-Snyder et al. (31) and Deschenes et al. (17), who demonstrated that ULLS-induced deconditioning was mainly associated with neuromuscular adaptation. This discrepancy may be partly due to the technical difficulty in making repeat surface EMG measurements over a prolonged time (30,40).

Although magnetic resonance imaging (MRI) has been used to acquire anatomical information (5–7,10), use of human skeletal muscle has also been assessed with exercise-induced changes in spin-spin relaxation times (T2) of functional MRI (1,2,9,10,15,21,24,28). It has been demonstrated that T2 change correlates with integrated EMG activity of an active muscle region during concentric and eccentric exercise (1), which is related to isometric torque induced by electromyostimulation (EMS) (2) and which increases with exercise intensity (1,2,30,43). Moreover, the metabolic state of skeletal muscle can be evaluated noninvasively using this technique, because there is a close relationship between exercise-induced T2 change and intramuscular metabolic state, such as intracellular pH and ratio of inorganic phosphate to phosphocreatine (Pi/PCr) by 31-phosphorus MR spectroscopy (14,39). Thus, exercise-induced changes in T2 in muscle functional MRI seem an ideal way to assess muscle activity during exercise. As far as we know, only one study has been carried out as to the effect of unweighting on muscle T2 with functional MRI (31). Ploutz-Snyder et al. (31) showed that more muscle (fibers) was engaged in the
Values are mean $\pm$ SE. *P < 0.01 vs BR-Tr.
PCSA, physiological cross-sectional area; TQ, peak torque during isometric maximal plantar flexion.
* P < 0.05 vs Pre.

Materials and Methods

Approach to the problem and experimental design. This study was designed to determine a resistance-training program for prevention of the PF muscle group deconditioning during 20 d of bed rest. We have reported that static and dynamic leg press training during bed rest did not preserve atrophy of the PF muscle group (6,7), suggesting that these type of training stimuli were inadequate to maintain the size of the PF muscle group. Thus, in this study, a plantar flexor exercise with which the PF muscle group was primarily engaged was added to the training regimen.

To evaluate the effects of training on the function and morphology (i.e., atrophy) of the PF muscle group, we employed muscle functional MRI and anatomic MRI for the quadriceps, because of atrophy and strength loss, to perform a knee extension exercise against the same absolute load after the ULLS as compared with before. In this study, we assume that an exercise-induced T2 change is a function of muscle and therefore, a greater T2 change in exercised muscle would demonstrate greater muscle contractile activity, as has been reported by Prior et al. (32).

The goal of this study was to study the effect of resistance training (dynamic leg press and plantar flexion exercise) during 6° head-down tilt bed rest on functional MRI for the PF muscle group. We hypothesize that dynamic resistance training during bed rest may prevent deconditioning of the PF muscle group.

Table 1. Physical characteristics, muscle function, and morphology of the subjects for resistance training (BR-Tr) and control (BR-Cont) groups before and after bed rest.

<table>
<thead>
<tr>
<th></th>
<th>BR-Tr</th>
<th>BR-Cont</th>
<th>% Change</th>
<th>Range of % Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>23.3</td>
<td>22.7</td>
<td>1.2</td>
<td>9.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.8</td>
<td>169.6</td>
<td>0.2</td>
<td>21.9</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>65.5</td>
<td>67.3</td>
<td>-1.2</td>
<td>-0.5</td>
</tr>
<tr>
<td>PCSA (cm²)</td>
<td>117.9</td>
<td>138.5</td>
<td>18.0</td>
<td>27.2</td>
</tr>
<tr>
<td>TQ (Nm)</td>
<td>140.1</td>
<td>127.1</td>
<td>-9.5</td>
<td>-21.9</td>
</tr>
</tbody>
</table>

Values are mean $\pm$ SE; *P < 0.01 vs BR-Tr.
PCSA, physiological cross-sectional area; TQ, peak torque during isometric maximal plantar flexion.
* P < 0.05 vs Pre.
for 1 s, followed by a 2-s flexion. In the afternoon bilateral plantar flexion training session, subjects performed five sets of 10 repetitions with a load of 70% MIF during plantar flexion and with a 1-min rest between sets. This training was performed using a horizontal leg-press training device, being the same device as was used for the bilateral leg press training. The exercise was done with a range of motion from the fully dorsiflexed position to the plantar flexed position with the subjects’ knee joints were completely extended. The primary reason why we used this resistance-training regimen was that it has been demonstrated the resistance training prevents muscle deconditioning of the quadriceps during bed rest (6).

For both leg press and plantar flexion resistance training, the MIF of the bilateral leg press and plantar flexion was measured four times in the 20 d of bed rest period. Based on the MIF tests we determined the load for the appropriate load for the training sessions but relative training load, i.e., 70% MIF for both the leg press and plantar flexion exercises, did not change for the entire bed rest period.

**Torque measurement.** Maximal voluntary isometric torque (TQ) of the left PF muscle group was determined using a specially designed dynamometer (7,26). The subject sat in a chair with the dynamometer with their knee fully extended and the ankle joint angle at 90° (the anatomical position), and secured at the waist and thigh with the hip joint flexed to approximately 110°. Care was taken to fix the subjects’ trunk and lower limb with identical hip and ankle joint angles before and after bed rest. After a brief warm-up by performing several submaximal and maximal contractions, the subjects were required to exert maximal plantar flexion force for 3–4 s. Strong verbal encouragement was used to motivate the subjects during the test. The signal from the load cell was stored on a computer (Macintosh Performa, Apple Corp., Cupertino, CA).

**Functional MRI.** Standard spin-echo MR images of the left leg were taken using a 0.2-T magnet (AIRIS II, HITACHI Medical Corp., Tokyo, Japan) essentially as done previously (10,15,31). Five 10-mm-thick transaxial images (repetition time = 1500 ms; echo time = 20 and 75 ms) of the right leg spaced 10 mm apart were collected using 1.0 NEX with an extremity coil. A 256 × 256 matrix was acquired with one excitation and a 25-cm field of view. Total MR image collection time was 3 min and 30 s. Ink marks on the left leg aligned with cross-hairs of the imager allowed for similar positioning in the magnet bore over repeat scans.

Figure 1 shows that an illustration of unilateral calf-raising exercise to induce signal changes in muscle functional MRI. At the beginning of the exercise, the subjects stood on their left leg on a wooden box (height: 15 cm, width: 20 cm, length: 100 cm), then they extend their ankle joint for 2 s and flexion for 2 s against their own body weight. The range of motion of the exercise was from anatomical position (i.e. ankle joint angle ≠ 90°) to fully plantar flexed position. This exercise was consisted of five sets of 10 repetitions with 1-min rest between sets.
from two to three approximately 2- to 3-cm² region of interests (ROI) for each of the medial gastrocnemius (MG), lateral gastrocnemius (LG), and soleus (Sol) muscles to give a single value for each muscle. Exercise-induced T2 changes to the resting value were also determined. The T2 values of inactive muscle for the calf-raising exercise, i.e. the tibialis anterior (TA) muscle, and bone marrow (Marrow) of the tibia, were calculated to check the validity of the T2 calculation for repeated scans. We paid an attention to exclude visible aponeurosis, vessels, fat, and nerves from the ROI.

To evaluate reproducibility of resting T2 of individual muscles of the PF muscle group, functional MRI of the right leg for four subjects (not subjects in this study) were taken on two occasions separated by about 3 wk. Mean resting T2 values of the MG, LG, Sol, TA muscles, and Marrow at the first measurement were 32.9, 33.3, 33.1, 31.6, and 67.1 ms, and at the second measurement were 33.5, 32.3, 34.1, 31.8, and 66.4 ms, respectively. We found no significant difference in the resting T2 values between the first and second measurement, and a strong correlation between the measurements (r = 0.996, P < 0.0001).

Anatomic MRI. MR images were collected pre and post bed rest. Premeasurement were performed 3 d before bed rest, and postmeasurement before reambulation. All images were taken after 15- to 30-min rest to avoid fluid shifts that might induce interstitial and/or intracellular volume changes. T1-weighted spin echo, transaxial-plane imaging was performed with the following variables: TR 450 ms, TE 20 ms, matrix 256 × 172, field of view 300 mm, slice thickness 10 mm, and interislice gap 7 mm. The subjects were imaged in a prone position with the ankle kept at ~120° with 180° being full extension of the joint. Coronal plane images were taken to identify the origin of the MG or LG muscle. Consecutive axial images were obtained from the origin of the MG and/or LG muscles to extermis distalis of tibia. The number of transaxial images obtained for each subject was 43–48. The muscles investigated were as follows: MG, LG, and Sol muscles. From the series transaxial images, outlines of each muscle were traced, and the traced images were transferred to a Macintosh computer (Power Macintosh G4, Apple Computer) for calculation of the anatomical cross-sectional area (ACSA), which represents the end-on view of the muscle area at each level that the section has been made, using a public domain National Institute of Health (NIH) image software package (written by Wayne Rasband at the NIH and available from the Internet by anonymous ftp from zippy.nimh.nih.gov or on floppy disk from NITS, 5285 Port Royal Rd., Springfield, VA 22161). The muscle volume was determined by summing the ACSA of each image times the thickness (10 mm) and interislice gap (7 mm) of each section. Test-retest reliability of muscle volume measurement was 1.6%. Muscle fiber length was calculated as muscle length times the ratio of fiber length to muscle length reported by Wickiewicz et al. (42). It was reported that the physiological cross-sectional area (PCSA) of each muscle was determined from the following equation (6,7,42):

\[ \text{PCSA} = \text{muscle volume} \times \cos \theta \times (\text{fiber length})^{-1} \]

where \( \theta \) is muscle fiber pennation angle derived from Wickiewicz et al. (42).

Statistics. Resting and exercised T2 was analyzed by using four-way (2 × 2 × 2 × 6; exercise × time × group × muscle) repeated measures ANOVA and change in T2 was analyzed by using two-way (2 × 6; time × muscle) repeated measures ANOVA. Significant main effects and interactions were compared using the least squares difference (LSD) post hoc test. Physical characteristics, PCSA, and TQ were analyzed by using paired Student’s t-test. All analysis was performed using the SuperANOVA and the StatView for Macintosh statistical package. The level of significance was set at \( P < 0.05 \). Data are presented as mean ± SE.

RESULTS

Table 1 shows body weight, PCSA, and TQ of the PF muscle group of the BR-Tr group and BR-Cont group before and after bed rest. There was no change in the body weight in either group. The PCSA of the PF muscle group did not show any significant changes in regard to BR-Tr group; however, in regard to BR-Cont group, it was found to be significantly decreased after bed rest. The TQ during isometric plantar flexion did not show significant changes in regards to the BR-Tr nor BR-Cont groups.

Table 2 shows the resting and exercised T2 before and after bed rest in the BR-Tr group and BR-Cont groups. There was a significant four-way interaction between the exercise (calf-raising exercise), the time (bed rest), and the group and muscle (\( P < 0.0001 \)). There was no significant difference in the resting T2 between the BR-Tr and BR-Cont groups in any region both before and after bed rest, but the resting marrow in the BR-Cont group is lower than that in the BR-Tr group (\( P < 0.05 \)). Exercise induces significant increases in the T2 of the MG, LG, and Sol muscles in both groups (\( P < 0.01 \) to 0.0001). Before bed rest, the exercised T2 of the MG and LG muscles in the BR-Cont group were lower than those in the BR-Tr group (\( P < 0.05 \), 0.01,
respectively) and after bed rest, the exercised T2 of the Sol muscle in the BR-Cont group was higher than that in the BR-Tr group ($P < 0.001$). In the BR-Tr group, the resting T2 of the TA muscle after the bed rest was significantly higher than that before ($P < 0.05$), and in the BR-Cont group, the resting T2 of the Sol muscle after the bed rest was also significantly higher than before bed rest ($P < 0.05$).

Figure 2 shows the change in the T2 of the MG, LG, and Sol muscles before and after bed rest. There was a significant two-way interaction between time (bed rest) and muscle ($P < 0.01$). In the BR-Tr group, there was no significant difference in the change in the T2 of the MG, LG, and Sol muscles before and after bed rest. The change in the T2 of the MG muscle was higher than that in either the LG and Sol muscles, both before and after bed rest ($P < 0.05–0.01$).

In the BR-Cont group, the change in the T2 of the MG, LG, and Sol muscles after bed rest was significantly higher than that before bed rest, and there were significant differences between the change in the T2 of the MG muscle and that in the Sol muscles after bed rest ($P < 0.05$).

**DISCUSSION**

To our knowledge, this is the first paper describing the effects of resistance training during unweighting on muscle functional MRI. The novel finding with this study was that bilateral dynamic leg press and plantar flexion training during unweighting did not evoke a further increase in an exercise-induced T2 change of the MG, LG, and Sol muscles without atrophy of the PF muscle group. In our previous study, we demonstrated that bilateral dynamic leg press training during 20 d of bed rest preserved muscle size of the quadriceps and hamstring muscles; however, these two types of training programs were not effective for maintaining the PF muscle size (6). Therefore, plantar flexion training was added to the regimen for this study. To assess the function of the PF muscle group as a result of resistance training during bed rest, we measured the muscle T2 after a unilateral calf-raising exercise (5 sets $\times$ 10 reps with 1-min rest between sets). In this study, we considered the change in the T2 was able to be considered the index of muscle activation if no significant change in body weight was observed because it has been demonstrated that T2 changes are closely correlated with iEMG activity and exercise intensity. Thus, this would be a good index for estimation of muscle function as suggested by many previous studies (1–4,34,35,38).

We have demonstrated that bed rest induces atrophy in the PF muscle group in our previous studies (5,7), and overall the degree of reduction in the PCSA of PF muscle group was about 12% (7), comparable to the findings in the current study for the BR-Cont group (i.e., 12.7%). One of the most important findings in this study is that atrophy of the PF muscle group was prevented by resistance training. In our previous studies (6,7), isometric and concentric leg press training during 20 d of bed rest did not maintain the size of the PF muscle group, suggesting that mechanical stress by these types of training was insufficient to prevent reduction of the PF muscle group. In this study, the plantar flexion exercise has been added to the resistance training program to prevent atrophy of the PF muscle group; as a result, muscle size was maintained.

The TQ of the PF muscle group in BR-Tr group did not show any significant change. The resistance training programs used in this study were effective for the prevention of strength loss during prolonged bed rest, supporting the findings of our previous studies (6,7,26). An unexpected finding with our study was that the TQ of the PF muscle group did not significantly decrease for the BR-Cont group. Bamman et al. (12) reported that MIF and peak torque decreased by 9% and 13%, respectively, in the PF muscle group after 14 d of bed rest. The current study was 6 d longer than that of Bamman et al. (12), but a similar reduction was noted for the TQ. The mechanisms of nonsignificant change in the TQ for the BR-Cont was unclear; however, one possible explanation may partly be that it is due to the number of subjects ($N = 6$), for the BR-Cont group was insufficient for statistically significant results; because one of the six subjects in the BR-Cont group did not show a decline in regard of the TQ of the PF muscle group (2.5% increase), this has a major influence on the statistical results.

We found no significant change in body weight in either group, demonstrating that individual subjects performed the calf-raising exercise against the same absolute load (i.e., body weight). For the BR-Cont group, more significant increases in change in the T2 of the MG, LG, and Sol muscles were observed after bed rest than before, suggesting a greater number of muscle fibers were involved in per-
forming the exercises under the same absolute load (31). A similar finding was shown by Ploutz-Snyder et al. (31), who demonstrated that a greater volume of muscle in the quadriceps femoris was needed to lift the same absolute loads (25, 40, 55, and 70% of 5 sets × 10 reps of knee extension exercise) of a repetitive knee-extension exercise after 5 wk of ULLS compared with before ULLS. Thus, the results found in this study support Ploutz-Snyder and coworkers’ study (31). On the other, for the BR-Tr group, the change in the T2 of the MG, LG, and Sol muscles shows a similar response before and after bed rest. To our knowledge, only three studies not related to unweighting have demonstrated an exercise-induced T2 change after resistance training (10,15,30), they being investigations of neuromuscular adaptation resulting from resistance training, and they showed recruitment plasticity in a given muscle region (30) and individual muscles (10,15). Ploutz et al. (30) and Conley et al. (15) demonstrated that derecruitment occurred when performing exercise with the same absolute loads after resistance training but that there was no change in the oxidative metabolic demand of muscle fibers (succinate dehydrogenase activity) (30), suggesting that fewer motor units (MU) were being activated when carrying out the exercise. Taken together, we suggested that the reason for maintaining an exercise-induced T2 change for the BR-Tr group in this study was the resistance training effect on the exercise-induced T2, as shown by Ploutz et al. (30) and Conley et al. (15), would be offset by the unweighting effect, as demonstrated by Ploutz-Snyder et al. (31).

The possible explanation for the greater increase in change in T2 of the MG, LG, and Sol muscles for BR-Cont group after bed rest would be partly due to the change of neural factors, i.e., recruitment and derecruitment of specific muscle fibers, during the unilateral calf-raising exercise. We have already demonstrated that progressive resistance training during bed rest is effective for maintaining the neural drive of the knee extensors using the twitch interpolation technique (26). Furthermore, changes in neural activation can partly account for changes in strength loss due to weightlessness, demonstrating that neural activation is one key factor to maintaining muscle function. A recent study by Deschenes et al. (17) also shows that unloading-induced strength loss of the quadriceps accounts for neural factors, using the EMG technique.

It is well known that there are two main strategies as to the controlling force output in MU: recruitment and rate coding (firing rates) (13). It is well accepted that an exercise-induced T2 change reflects recruitment of the muscle not provided by the firing rates of MU (1,2). According to Conwit et al. (16), the firing rate of active motor neurons in the knee extensors (vastus medialis muscle: VM) increases with force increments above 30% of MVC, thereby augmenting force. However, MU recruitment makes a greater contribution than the firing rates modulation for force production in large muscles such as the VM (27). Recent studies have shown that increases in MVC (25%) of the fifth finger abduction (abductor digiti minimi: ADM) occurred without any changes in the mean MU discharge rates after 7 wk of resistance training (29). Furthermore, 8 wk of knee-extension resistance training evokes a 36% improvement in MVC without any change in firing rate of the vastus lateralis (VL) muscle with a submaximal contraction. These studies suggest that the firing rates of both small (ADM) and large muscle (VL) of the MU did not show further increases as a result of resistance training, and that increasing the firing rates appears not to act as a force facilitation strategy for these muscles after resistance training. If so, recruitment would be the main factor in increasing the force output after resistance training, as has been reported in previous muscle functional MRI studies (10,15,30). A recent study by Seki et al. (37) has shown the effect of 3-wk joint immobilization on the firing rates of single MU. They report a decrease in the force-mean firing rates relationship and estimated maximal mean firing rates of the first dorsal interosseous (FDI). Although the force output in small muscle such as the FDI is chiefly controlled by firing rates modulation, the PF muscle group appears not to have the same strategy as the FDI after decreased activity (16,27).

The metabolic state of the exercising muscle is also a key factor affecting changes in the T2. Exercising muscle T2 has been shown to closely correlate with muscle energetics by 31-phosphorus MR spectroscopy (39,41). Moreover, it has been shown that exercise induced-T2 changes can occur in healthy individuals, but not in those afflicted with McArdle’s disease, suggesting that increases in the T2 in previously active muscle are related to glycogenolysis and/or lactate production because of the inability of these patients to catabolize glycogen (22). Considering the previous studies, the metabolic state would also be important to understand the result. In a previous unweighting study, Hikida et al. (23) showed that a 30-d period of bed rest induces a decline in the skeletal muscles’ capacity for aerobic energy supply (as measured by citrate synthase activity), but anaerobic energy supply (as measured by lactate dehydrogenase and phosphofructokinase activity) is unaffected. Human spaceflight studies by Edgerton et al. (20) also showed similar aerobic and anaerobic enzyme responses after 5 or 11 d of flight. If a similar muscle metabolic response were to occur in this study, due to reduced aerobic capacity and fatigability after bed rest, the PF muscles for BR-Cont group would induce greater fatigue, e.g., higher Pi and lower pH, thereby the greater T2 change of the MG, LG, and Sol muscles was induced for BR-Cont group.

A significant exercise-induced T2 response was observed among individual muscles of the PF muscle group (Fig. 2). This response was mainly found in the BR-Tr group. The difference in the metabolic capacity (PCr breakdown and anaerobic ATP turnover) between the gastrocnemius and soleus muscles during the plantar flexion exercise has been clearly demonstrated using 31-phosphorus MR spectroscopy (33). In this study, however, a difference in the T2
change was found between the MG and LG muscles (Fig. 2). The fiber types of the MG and LG muscles were 44% and 51% Type I fibers, respectively, suggested that their metabolic state would be similar during exercise (25). Vandenboume et al. (39) have, in fact, demonstrated that the Pi/PCr ratio, pH, and the T2 of the MG and LG muscles during plantar flexion exercise are similar. In this study, we used a calf-raising exercise in the standing position, as shown in Figure 1. For the BR-Tr group, the higher exercised T2 values in the MG muscle than those in the LG and SOL muscles were found both before and after bed rest. Based on these findings, we suggest that the MG muscle contributes more in performing the exercise than the LG and SOL muscles. Similar recruitment plasticity has also been reported by other research groups, even during simple exercises such as knee-extension and ankle dorsiflexion (10,15). In other words, the neuromuscular system shows plasticity with respect to recruitment of muscles and/or muscle regions (i.e., neuromuscular compartments) depending on behavior.

In summary, the effect of resistance training during 20 d of 6° head down tilt bed rest on muscle function MRI was evaluated. The PCSA and TQ of the PF muscle group were higher in performing the exercise than the LG and SOL muscles. Changes in muscle proximal transverse relaxation times and acidity during exercise and recovery. J. Appl. Physiol. 79:1370–1378, 1995.

REFERENCES


