Resistance training affects GLUT-4 content in skeletal muscle of humans after 19 days of head-down bed rest

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Previous studies demonstrated that exercise training increases GLUT-4 protein content (3, 4, 11, 16), whereas detraining reduces GLUT-4 in skeletal muscle (17, 22, 33). Because several studies demonstrated that inactivity, including bed rest, induces glucose intolerance (13, 18, 21, 24, 33, 34), it is reasonable to speculate that a decrease in muscle activity may play a role in the decrease in glucose tolerance. However, the effect of prolonged bed rest on muscle GLUT-4 in humans has not been investigated (27). We have, therefore, examined the effect of 19 days of bed rest on muscle GLUT-4 in young, healthy men. We also evaluated the effect of brief resistance training as a countermeasure to the effect of bed rest on muscle GLUT-4 and glucose tolerance.

MATERIALS AND METHODS

Subjects. Nine young men [age 22 ± 4 (SD) yr] volunteered to participate in the study after they provided written informed consent. Subjects passed a comprehensive physical examination. The subjects were randomly assigned to control (n = 4) or resistance training groups (n = 5). The protocol was approved by the Institutional Ethics Committee of the National Aero Space Development Agency, Japan.

Bed-rest procedure. After the last blood sampling taken for the oral glucose tolerance test (OGTT) before bed rest, the subjects ate a light breakfast. Then, at approximately 10:30 AM, the subjects started bed rest. For 20 days, the subjects remained in a 6° head-down tilt at all times throughout the study except during resistance training (once in the morning) and showering every other day. The subjects also remained in the supine position during the training and the transfer from head-down tilt to the training device charge. Because this study was done in conjunction with a joint project for studying general adaptations to spaceflight, including cardiovascular system adaptations, the subject remained not in a supine but in a 6° head-down position.

Muscle biopsy. Using Bergstrom biopsy needles (2), we obtained muscle samples from the lateral aspect of vastus lateralis (VL) muscle 4 days before the start of bed rest (before bed rest) and in the evening of the 20th day of bed rest (after bed rest). This time corresponded to 8 h after the last bout of resistance training and 14 h before the end of 20 days of bed rest. Therefore, actual exposure of the sampled muscles to bed rest is 19 days + 6–8 h.

OGTT. Glucose tolerance was determined with a 75-g OGTT that was performed in the morning after an overnight fast just before bed rest and during the last hours of bed rest, as described above. This time corresponded to ~1 day after the last bout of resistance training (in other words, 12–14 h after the biopsy procedure). Blood samples were taken imme-
diately before and 30, 60, and 120 min after the glucose load. Plasma glucose concentration was determined by using an enzymatic method (10). Plasma insulin was determined by using radioimmunoassay (26). The total areas under the curve (AUCs) for glucose and insulin were calculated by using the trapezoidal rule.

Resistance training. Subjects in the training group did resistance training once every morning during the bed-rest period. The resistance training consisted of 30 isometric maximal voluntary contractions for 3 s each; subjects used leg-press exercises to recruit the extensor muscles of the ankle, knee, and hip. The angle of the knee was fixed to be 90°. Pauses of 3 s were allowed between bouts of maximal contractions. The duration of each training session was 3 min.

Measurement of GLUT-4 in muscle. The muscle samples were minced and mixed well. Twenty milligrams of minced muscle were homogenized in 9 ml of buffer containing 0.25 M sucrose, 1 mM EDTA, and 10 mM Tris·HCl, pH 7.5. Homogenate was centrifuged at 175,000 g for 10 min to remove unsolubilized material. The solubilized membranes were used for the assay of protein concentration and the amount of glucose transporters.

Detection of GLUT-4 protein. The solubilized membranes were incubated for 10 min at 37°C in a solution containing 2.5% SDS, 75 mM dithiothreitol, 1.7 M (12.5% vol/vol) glyc- erol, 361 mM (0.025% wt/vol) bromophenol blue, and 12.5 mM Tris·HCl, pH 7.0. SDS-PAGE was performed according to Laemmli (20) with a 0.56 M (4% wt/vol) stacking gel and a 1.4 M (10% wt/vol) resolving gel. Immunoblotting of electrophoresis gels was performed as described previously (5). Proteins in gels were electrophoretically transferred to polyvinylidene difluoride sheets in a transfer buffer. The sheets were incubated successively with antibodies to glucose transporters, rat GLUT-4, 361 mM (0.025% wt/vol) bromophenol blue, and 12.5 mM Tris·HCl, pH 7.5. Proteins in gels were electrophoretically transferred to polyvinylidene difluoride sheets in a transfer buffer. The sheets were incubated successively with antibodies to glucose transporters, rat GLUT-4, and 12.5 mM Tris·HCl, pH 7.5, and was blotted in a Vortex mixer, and kept for 10 min at room temperature. This solution was transferred to a microcentrifuge tube and centrifuged at 175,000 g for 10 min to remove unsolubilized materials. These solubilized membranes were used for the assay of protein concentration and the amount of glucose transporters.

Physical characteristics of subjects. In the period after bed rest, body mass of the subjects in the control group (66.9 ± 11.1 vs. 65.9 ± 10.1 kg, before and after bed rest, respectively) and training group (69.7 ± 9.8 vs. 68.3 ± 8.8 kg, before and after bed rest, respectively) appeared to be less than that observed before bed rest, but the differences were not statistically significant. Body height of the nine subjects increased slightly but significantly during bed rest (172.0 ± 4.6 vs. 172.4 ± 4.3 cm before and after bed rest, respectively, P < 0.05).

Table 1. Changes in cross-sectional area of vastus lateralis (VL) muscle after 20 days of bed rest

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control group</th>
<th>Training group</th>
</tr>
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<tbody>
<tr>
<td>Before bed rest</td>
<td>27.0 ± 5.6</td>
<td>29.4 ± 1.3</td>
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<tr>
<td>After bed rest</td>
<td>25.0 ± 5.3*</td>
<td>28.2 ± 3.3</td>
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Values are means ± SD; n = 4 men in control group, 5 men in training group. *Significant difference between values obtained before and after bed rest, P < 0.05.
AUC for plasma insulin level was higher compared with levels before bed rest (14,038 ± 6,846 vs. 9,832 ± 4,662 µU·ml⁻¹·120 min⁻¹, respectively; *P, 0.05; Fig. 2B). For the training group subjects, 20 days of bed rest did not affect the plasma glucose level during OGTT (Fig. 3A). Furthermore, in the training group, the AUC for plasma insulin levels during the test after bed rest was also significantly higher than the respective AUC during the test before bed rest (7,297 ± 4,088 vs. 4,427 ± 1,652 µU·ml⁻¹·120 min⁻¹, respectively; Fig. 3B).

DISCUSSION

The main findings of this study are that the GLUT-4 content of VL muscle was reduced after 19 days of bed rest, and brief periods of resistance training increased GLUT-4 content in the same muscle during the same duration of bed rest.

This decrease in GLUT-4 content in muscle may reduce insulin-stimulated glucose uptake, resulting in insulin-resistance in skeletal muscle during inactivity (21, 28, 32). The magnitude of the decrement in GLUT-4 was modest (~16%) but is comparable with that reported for insulin-resistant obese subjects (18% lower than that of normal subjects) (5). Furthermore, even in non-insulin-dependent diabetes mellitus patients, GLUT-4 content in skeletal muscle is not different from that in nondiabetic controls (4, 5). Therefore, the inactivity induced by bed rest is a potent physiological stimulus to induce depressed GLUT-4 expression in skeletal muscle.

There has been no previous investigation regarding the effect of resistance training on GLUT-4 content in human skeletal muscle, although endurance training has been shown to increase GLUT-4 content and to improve insulin-stimulated glucose uptake (3, 4, 11). However, because several studies have recently suggested that neurogenic factors, including motor unit activity, are the most potent stimuli to increase GLUT-4 content (8, 23), we believe that it is also possible that intense neuromuscular activity induced during maximal voluntary isometric contractions may have a significant influence on GLUT-4 content in human skeletal muscle recruited by high-intensity short-term isometric contractions. Actually, the present investigation demonstrated that GLUT-4 content in the skeletal muscle was increased by 30% with the short

Table 2. Changes in total cross-sectional area of thigh muscles after 20 days of bed rest

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<tr>
<th>Condition</th>
<th>Total Cross-Sectional Area of Thigh Muscle, cm²</th>
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<tbody>
<tr>
<td>Control group</td>
<td>104.5 ± 12.2</td>
</tr>
<tr>
<td>Training group</td>
<td>112.1 ± 12.0</td>
</tr>
</tbody>
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Values are means ± SD; *n = 4 men in control group, 5 men in training group. Significant difference between values obtained before and after bed rest: *P < 0.05; †P < 0.001.

Table 3. Changes in maximal voluntary isometric contraction torque of knee extensors after 20 days of bed rest

<table>
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<tr>
<th>Condition</th>
<th>Maximal Voluntary Isometric Knee Extension Torque, Nm</th>
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<tbody>
<tr>
<td>Control group</td>
<td>185 ± 49</td>
</tr>
<tr>
<td>Training group</td>
<td>154 ± 41</td>
</tr>
</tbody>
</table>

Values are means ± SD; *n = 4 men in control group, 5 men in training group.

Fig. 1. Response of GLUT-4 concentration in vastus lateralis (VL) muscle to 19 days of bed rest in control and resistance-training (Training) subjects. Values are means ± SD; *n = no. of men in each group; cpm, counts/min. *Area under the curve for plasma insulin level was significantly higher in test after bed rest than in test before bed rest; P, 0.05.

Fig. 2. A: response of plasma glucose to 75-g oral glucose challenge before (●) and after (●) 20 days of bed rest in control subjects. B: response of plasma insulin to 75-g oral glucose challenge before (●) and after (●) 20 days of bed rest in control subjects. Values are means ± SD; *n = 4 men. *Area under the curve for plasma insulin level was significantly higher in test after bed rest than in test before bed rest; P < 0.05.
bouts of resistance exercise training even during 19 days of bed rest that, by itself, depresses GLUT-4 expression. The magnitude of this increase in GLUT-4 was relatively small compared with that reported for moderate-intensity endurance training [98% increase after 7–10 days of cycle ergometer exercise training (11)]. However, because it is probable that the increase in GLUT-4 content in muscle is greater after resistance training without bed rest, it is not known whether the effect of isometric resistance training is less than that of endurance training. Therefore, it will be of interest to determine how much resistance training increases GLUT-4 content in muscle of freely living humans. Furthermore, in terms of developing an effective countermeasure to depressed expression of muscle GLUT-4 during inactivity, it is worthwhile to compare the effect of protocols that differ in intensity, frequency, and duration of isometric resistance training on GLUT-4 content in skeletal muscle.

Expression of GLUT-4 content in brown adipose tissue is enhanced by augmented sympathetic nerve activity by cold exposure (30). Furthermore, it is known that muscle sympathetic nerve activity is inhibited by both acute (25) and prolonged (31) head-down tilt. Therefore, it is possible that head-down tilt might have influenced the change in muscle GLUT-4 content observed in the present investigation. Although we believe that the relative influence of altered muscle sympathetic nerve activity on GLUT-4 content is small, the pure effect of 19 days of inactivity on GLUT-4 content in skeletal muscles will be clarified by a future study that allows subjects to rest on a flat bed for 19 days.

OGTT after bed rest showed that, compared with the OGTT before bed rest, there was no significant change in plasma glucose, and plasma insulin level during the test was marginally increased in both control and training groups. These results demonstrate that 20 days of inactivity induce mild insulin resistance. Furthermore, these results suggest that a small increase in GLUT-4 content in VL muscle of the trained subjects did not succeed in preventing a bed-rest-induced defect of whole body glucose disposal. First, the discrepancy between the change in GLUT-4 and the OGTT of the trained subjects probably results from the fact that GLUT-4 was measured in a discrete muscle bed recruited by the exercise training, whereas the OGTT reflects whole body glucose handling by the total muscleature (trained and untrained) (11). Because GLUT-4 content in the control group was reduced by 16% after bed rest, it is assumable that, in the training group, GLUT-4 content was also decreased, to the same extent, in muscles that were not recruited during the training session. Second, the discrepancy between the GLUT-4 and OGTT response after bed rest in the trained subjects may be explained by the inverse response of muscle mass that affects OGTT scores. Because a further decrease in CSA of the thigh muscle was observed in the control subjects compared with in the trained subjects (Table 2), it is also assumable that, for the training group, more atrophy may have occurred in the untrained muscle than in the trained muscle during bed rest. Therefore, the results of the present investigation suggest that the effect of training-induced increase in GLUT-4 concentration in the trained muscle on whole body glucose disposal might have been overcome by the bed-rest-induced decrease in muscle volume, in both trained and nontrained muscle.

From results obtained from the present investigation, two strategies can be postulated in terms of developing better countermeasures to insulin resistance induced by bed rest. First, the resistance training should recruit as much muscle in the body as possible (for example, back muscle) for the purpose of giving a stimulus for maintaining muscle mass (7). Second, resistance training should be more intense and/or more frequent (for example, two times a day) to induce more increase in GLUT-4 content in trained muscle. The efficacy of these strategies may be proved in future studies.

Because the value of the AUC for plasma insulin of the control group before bed rest was significantly higher than that of the training group (P = 0.04), different characteristics of the two groups may have influenced the result of the present investigation. However, the insulin response of the control group during the OGTT was within the normal range. Furthermore, the content of GLUT-4 in VL muscle of the two groups

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**Fig. 3.** A: response of plasma glucose to 75-g oral glucose challenge before (○) and after (●) 20 days of bed rest in trained subjects. B: response of plasma insulin to 75-g oral glucose challenge before (○) and after (●) 20 days of bed rest in trained subjects. Values are means ± SD; n = 5 men. *Area under the curve for plasma insulin levels during test after bed rest was significantly higher than during test before bed rest; P < 0.05.
was not different before bed rest. Therefore, it seems unlikely that the marginally different characteristics of the two groups affected the essential finding of the present investigation, at least regarding the GLUT-4 response during bed rest.

The CSA of thigh muscles decreased significantly in both the control and training groups after bed rest (Table 2), whereas the maximal voluntary torque of knee extension was not statistically significantly changed in either group (Table 3). This result seems to be in conflict with the general knowledge that these two variables are closely related (15). However, maximal knee extension torque of three of the four control subjects decreased by 5–33%, while one subject in the control group increased his torque marginally (3%) after bed rest. Overall, average isometric knee extension torque decreased by 16%. This value is comparable to the values reported by Funato et al. (9) in a previous bed-rest study. Therefore, we believe that the inconsistence relationship between CSA and maximal isometric torque, proximally denervated rat soleus muscle. Am. J. Physiol. 239 (Regulatory Integrative Comp. Physiol. 41): R429–R432, 1997.

In conclusion, the result of the present study shows that GLUT-4 concentration in VL muscle of humans decreases modestly with 19 days of bed rest and that isometric resistance training not only overcomes this effect but induces a small increase in muscle GLUT-4.

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