

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

IZUMI TABATA,¹ YOUJI SUZUKI,² TETSUO FUKUNAGA,³ TOSHIKO YOKOZEKI,¹ HIROSHI AKIMA,³ AND KAZUO FUNATO³

¹Laboratory of Exercise Physiology, Division of Health Promotion, National Institute of Health and Nutrition, Shinjuku City, Tokyo 162-8636; ²Department of Health Administration, School of Health Science and Nursing, Faculty of Medicine, University of Tokyo, Bunkyo City, Tokyo 113; ³Department of Life Sciences, Graduate School of Arts and Sciences, University of Tokyo, Meguro City, Tokyo 153-8902, Japan

Tabata, Izumi, Youji Suzuki, Tetsuo Fukunaga, Toshiko Yokozeki, Hiroshi Akima, and Kazuo Funato.

Resistance training affects GLUT-4 content in skeletal muscle of humans after 19 days of head-down bed rest. *J. Appl. Physiol.* 86(3): 909–914, 1999.—This study assessed the effects of inactivity on GLUT-4 content of human skeletal muscle and evaluated resistance training as a countermeasure to inactivity-related changes in GLUT-4 content in skeletal muscle. Nine young men participated in the study. For 19 days, four control subjects remained in a -6° head-down tilt at all times throughout bed rest, except for showering every other day. Five training group subjects also remained at bed rest, except during resistance training once in the morning. The resistance training consisted of 30 isometric maximal voluntary contractions for 3 s each; leg-press exercise was used to recruit the extensor muscles of the ankle, knee, and hip. Pauses (3 s) were allowed between bouts of maximal contraction. Muscle biopsy samples were obtained from the lateral aspect of vastus lateralis (VL) muscle before and after the bed rest. GLUT-4 content in VL muscle of the control group was significantly decreased after bed rest (473 ± 48 vs. 398 ± 66 counts \cdot min⁻¹ \cdot μ g membrane protein⁻¹, before and after bed rest, respectively), whereas GLUT-4 significantly increased in the training group with bed rest (510 ± 158 vs. 663 ± 189 counts \cdot min⁻¹ \cdot μ g membrane protein⁻¹, before and after bed rest, respectively). The present study demonstrated that GLUT-4 in VL muscle decreased by $\sim 16\%$ after 19 days of bed rest, and isometric resistance training during bed rest induced a 30% increase above the value of GLUT-4 before bed rest.

inactivity; glucose metabolism; isometric training; vastus lateralis muscle

SKELETAL MUSCLE is responsible for at least 80% of insulin-stimulated glucose uptake. Glucose transport is the rate-limiting step in skeletal muscle glucose metabolism under most physiological conditions (19). Furthermore, maximal insulin- and contraction-stimulated glucose transport activity is reported to be linearly related to the content of GLUT-4 isoform of the glucose transporter in muscle (12, 14). Therefore, the level of GLUT-4 in skeletal muscle may be an important determinant of whole body glucose disposal.

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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 exercise 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 60 min. The pellet (the membrane fraction) was suspended in 450 μ l of buffer, containing 1 mM EDTA and 10 mM Tris·HCl, pH 7.5, and was blended vigorously in a Vortex mixer until the pellets were completely dispersed. Then this solution was solubilized by adding 50 μ l of 0.35 M (10% wt/vol) SDS, mixed well in a Vortex mixer, and kept for 10 min at room temperature. This solution was transferred to a microcentrifuge tube and centrifuged at 10,000 *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.

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) glycerol, 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 for 20 h and ¹²⁵I-labeled protein A for 24 h at 4°C. Autoradiography was performed with Kodak X-AR film at -70°C for 4–12 h. To quantify the glucose transporters, we cut out pieces of sheet containing the GLUT-4 protein and counted the radioactivity in a gamma counter.

Measurement of maximal voluntary isometric force. Maximal voluntary isometric contraction torque of the knee extensors was measured 4 days before bed rest and 2 days after the end of the bed-rest period. The angle of the knee was fixed at 90°. Each subject was seated at a test bench and was secured by placing restraining straps about the hip and thigh to minimize extraneous movement. The best performance of three trials was recorded as the maximal value.

Determination of cross-sectional area (CSA). CSA of thigh muscles, including VL muscle, was determined by using magnetic resonance imaging (1) 2 days before the start of bed rest and 1 day after the end of bed rest. The subjects were imaged in a prone position, with the knee and ankle kept at 180 and 120°, respectively, with 180° being full extension of each joint. Magnetic resonance imaging was performed with a 1.0-T GYROSCAN T10-NT Powertrak 1000 (Philips Medical Systems). T1-weighted, spin-echo, axial-plane imaging was performed with the following variables: 450-ms TR, 20-ms TE, 256 × 172 matrix, 300-mm field of view, 10-mm slice thickness, and 7-mm interslice gap. First, coronal plane images were taken to identify the spina iliaca anterior

superior, which is the origin of the sartorius. Consecutive axial images were obtained from the anterior superior iliac spine to the end of the tibia. From the serial cross-sectional images, outlines of quadriceps femoris muscle, including VL muscle, were traced on the axial images at 50% of the femur length. Traced images were transferred to a personal computer (Power Macintosh 8100/80 AV, Apple Computer) for calculation of the CSA with the use of the National Institutes of Health public-domain image software package.

Statistical procedure. Unless otherwise stated, the differences between physiological variables obtained before and after bed rest were evaluated by two-tailed Student's *t*-test for paired observations. When data are not distributed normally, nonparametric analysis with the Mann-Whitney ranked sum test was used. The AUCs for plasma glucose and insulin were analyzed with a one-tailed *t*-test (11). Values are expressed as means \pm SD. Differences were considered statistically significant if *P* < 0.05.

RESULTS

Physical characteristics of subjects. In the period after bed rest, body mass of the subjects in the control group (66.9 \pm 11.1 vs. 65.9 \pm 10.1 kg, before and after bed rest, respectively) and training group (69.7 \pm 9.8 vs. 68.3 \pm 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 \pm 4.6 vs. 172.4 \pm 4.3 cm before and after bed rest, respectively, *P* < 0.05).

After the bed-rest period, CSA of VL muscle of the control group was smaller (-7.3 \pm 4.0%; *P* = 0.05), whereas bed rest did not significantly affect CSA of the VL muscle of the training group (Table 1). 1. After the bed-rest period, total CSA of thigh muscles decreased in both control and training group by -8.1 \pm 1.9% (*P* < 0.01) and -4.3 \pm 3.6% (*P* < 0.05), respectively (Table 2).

Twenty days of bed rest did not affect maximal voluntary isometric torque of knee extension in the training group (Table 3). Although muscle strength of the control group tended to decrease after bed rest, it was not statistically significant.

GLUT-4 protein concentration. GLUT-4 content in VL muscle of the control group significantly decreased after 19 days of bed rest (*P* < 0.05), whereas it significantly increased during 19 days of bed rest plus isometric training (*P* < 0.01) (Fig. 1).

OGTTs. Plasma glucose concentration during an OGTT after 20 days of bed rest was not significantly different in the control group (Fig. 2A), whereas the

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

Condition	Cross-Sectional Area of VL Muscle, cm ²	
	Control group	Training group
Before bed rest	27.0 \pm 5.6	29.4 \pm 1.3
After bed rest	25.0 \pm 5.3*	28.2 \pm 3.3

Values are means \pm 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.

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

Condition	Total Cross-Sectional Area of Thigh Muscle, cm ²	
	Control group	Training group
Before bed rest	104.5 ± 12.2	112.1 ± 12.0
After bed rest	96.0 ± 10.6†	105.0 ± 6.7*

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.

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

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

Condition	Maximal Voluntary Isometric Knee Extension Torque, Nm	
	Control group	Training group
Before bed rest	185 ± 49	154 ± 41
After bed rest	191 ± 16	189 ± 11

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

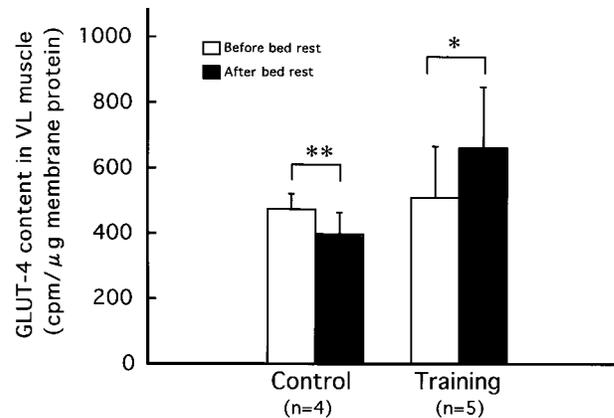


Fig. 1. Response of GLUT-4 concentration in vastus lateralis (VL) muscle to 19 days of bed rest in control and resistance-training (Training) subjects. Values are means ± SD; *n* = no. of men in each group; cpm, counts/min. **P* < 0.05, ***P* < 0.01.

activity, are the most potent stimuli to increase in 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

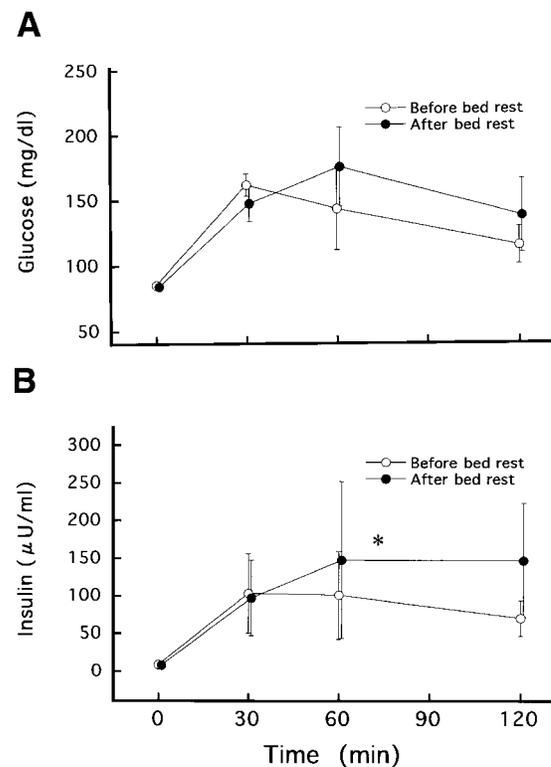


Fig. 2. A: response of plasma glucose to 75-g oral glucose challenge before (○) and after (●) 20 days of bed rest in control subjects. B: response of plasma insulin to 75-g oral glucose challenge before (○) and after (●) 20 days of bed rest in control subjects. Values are means ± SD; *n* = 4 men. *Area under the curve for plasma insulin level was significantly higher in test after bed rest than in test before bed rest; *P* < 0.05.

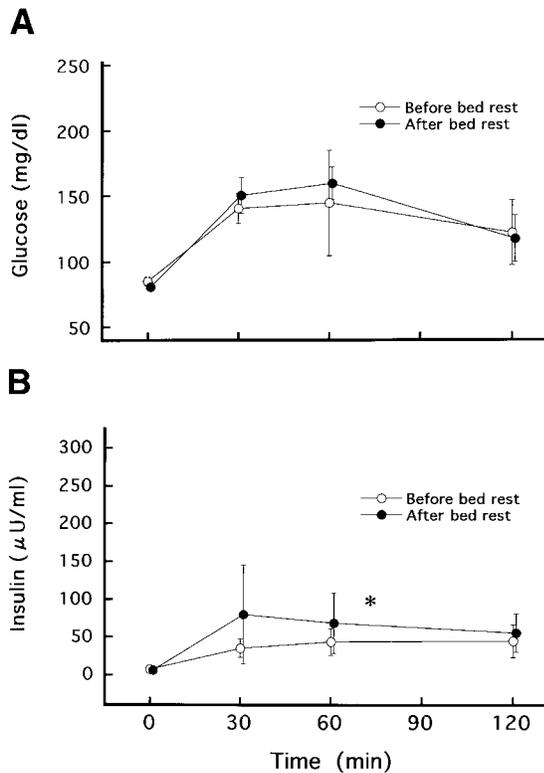


Fig. 3. *A*: response of plasma glucose to 75-g oral glucose challenge before (○) and after (●) 20 days of bed rest in trained subjects. *B*: response of plasma insulin to 75-g oral glucose challenge before (○) and after (●) 20 days of bed rest in trained subjects. Values are means \pm SD; $n = 5$ men. *Area under the curve for plasma insulin levels during test after bed rest was significantly higher than during test before bed rest; $P < 0.05$.

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 be-

lieve 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 musculature (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 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

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 inconsistent relationship between CSA and maximal isometric torque of the knee extensors in the control group may be explained by the statistical limitation of the present study, especially the small number of the control subjects. On the other hand, it is reasonable to speculate that the trained subjects could preserve their knee extension torque during bed rest through compensating for decreased muscle CSA with increased specific tension (force per muscle CSA in N/cm²) as a result of the resistance training (9, 29).

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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Address for reprint requests and correspondence: I. Tabata, Laboratory of Exercise Physiology, Div. of Health Promotion, National Institute of Health and Nutrition, 1–23–1 Toyama, Shinjuku City, Tokyo 162-8636, Japan (E-mail: tabata@nih.go.jp).

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