Maximal and submaximal forces of slow fibers in human soleus after bed rest

KATSUMASA YAMASHITA-GOTO,1 RYOKO OKUYAMA,1 MASANORI HONDA,1 KENSUKE KAWASAKI,1 KAZUHIKO FUJITA,1 TAKAHIRO YAMADA,2 IKUYA NONAKA,3 YOSHINOBU OHIRA,4 AND TOSHITADA YOSHIOKA1,5

1Department of Physiology, St. Marianna University School of Medicine, Kawasaki City, Kanagawa 216-8511; 2Department of Orthopaedic Surgery, University of Tokai, Isehara City, Kanagawa 259-1193; 3National Center for Neurology and Psychiatry, Kodaira City, Tokyo 187-8551; 4Department of Physiology and Biomechanics, National Institute of Fitness and Sports, Kanoya City, Kagoshima 891-2393; and 5Aomori University of Health and Welfare, Aomori City, Aomori 030-8505, Japan

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Yamashita-Goto, Katsumasa, Ryoko Okuyama, Masanori Honda, Kensuke Kawasaki, Kazuhiro Fujita, Takahiro Yamada, Ikuya Nonaka, Yoshinobu Ohira, and Toshitada Yoshioka. Maximal and submaximal forces of slow fibers in human soleus after bed rest. J Appl Physiol 91: 417–424, 2001.—The effects of 2 and 4 mo of bed rest, with or without exercise countermeasures, on the contractile properties of slow fibers in the human soleus muscle were examined. Mean fiber diameters were 8 and 36% smaller after 2 and 4 mo of bed rest, respectively, than the pre-bed rest level. Maximum tetanic force (P0), maximum activated force (Fmax) per cross-sectional area (CSA), and the common-logarithm value of free Ca2+ concentration required for half-maximal activation (pCa50) also decreased after 2 and 4 mo of bed rest. In contrast, maximum unloaded shortening velocity (V0) was increased after 2 and 4 mo of bed rest. After 1 mo of recovery, fiber diameters, P0, Fmax per CSA (P > 0.05), and pCa50 were increased and V0 decreased toward pre-bed rest levels. Effects of knee extension/flexion exercise by wearing an anti-G Penguin suit, and the effects of loading or unloading of the plantar flexors with (Penguin-1) or without (Penguin-2) placing the elastic loading elements of the suit, respectively, were investigated during 2–4 mo of bed rest. In the Penguin-1 group, mean fiber diameter, P0, Fmax per CSA, V0, and pCa50 were similar before and after bed rest. However, the responses of fiber size and contractile properties to bed rest were not prevented in the Penguin-2 group, although the degree of the changes was less than those induced by bed rest without any countermeasure. These results indicate that long-term bed rest results in reductions of fiber size, force-generation capacity, and Ca2+ sensitivity, and enhancement of shortening velocity in slow fibers of the soleus. The data indicate that continuous mechanical loading on muscle, such as stretching of muscle, is an effective countermeasure for the prevention of muscular adaptations to gravitational unloading.

human soleus fibers; contractile properties; countermeasure

MECHANICAL LOADING IS ONE of the primary factors regulating the synthesis and degradation of proteins in skeletal muscles (16, 26, 36, 42). Increased loading of skeletal muscles, such as occurs with exercise training or mechanical stretch, stimulates protein synthesis and often results in hypertrophy. In contrast, decreased loading, such as occurs during exposure to actual microgravity or a simulation model such as bed rest, results in a loss of muscular protein and/or atrophy, especially in primary antigravity muscles such as the soleus (27, 28). Animal studies have shown that chronic periods of unloading result in a shift from oxidative to glycolytic metabolic profile (12, 17, 49), transition of fiber types from slow to fast (3, 4, 11, 38), increase in shortening velocity (9, 15, 47), decrease in force-generation capacity (9, 47, 48), and alteration in excitation-contraction coupling (7, 20, 25, 31, 37, 50), such as increased rate of Ca2+ uptake and leakage to and from sarcoplasmic reticulum (50), in hindlimb muscles, e.g., the soleus, tibialis anterior, and vastus lateralis.

There are a few reports regarding the effects of decreased activity on the contractile properties of human soleus muscle fibers (3, 28, 33, 44–46). For example, an increase in unloaded shortening velocity (V0) and/or decrease in force production associated with fiber atrophy were observed after 17 days of bed rest (44, 45). However, the contractile responses to long-term unloading are still unclear. Furthermore, the development of countermeasures to prevent muscle atrophy during long-term unloading is important for clinical medicine, as well as space medicine. Muscle deconditioning, such as an atrophy and decrease in maximum force, of knee extensors and/or plantar flexors during 20 or 14 days of bed rest was prevented by daily isometric leg-press exercise (3 s × 30 repetitions with 30-s interval; Ref. 1) or by concentric/eccentric plantar flexion (5 sets of 6–10 repetitions every other

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day; Ref. 5), respectively. However, the duration of the bed rest period was relatively short in these studies. Therefore, the present study was performed to examine the effects of long-term bed rest on the contractile properties of fibers from the human soleus muscle and to determine whether a specific loading paradigm could prevent any bed rest-associated adaptations.

**MATERIALS AND METHODS**

*Subjects and experimental design.* The Institute of Biomedical Problems (IBMP) in Moscow, Russia, performed this study for the National Space Development Agency of Japan (NASDA) (Contract no. 8H0-046, The Performance of NASDA’s Bed Rest Study at IBMP Facility). Thirteen healthy Russian male volunteers participated in this study. 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 subject. Physical characteristics of subjects in each group are shown in Table 1. The Human Use Committees at IBMP, NASDA, and St. Marianna University School of Medicine approved this study.

Detailed experimental protocols have been described in our group’s previous papers (27, 28). The study consisted of two separate experiments. The first experiment was performed to study the effects of 4 mo of bed rest without any exercise countermeasure and 1 mo of recovery on the contractile properties of slow-type fibers in soleus muscle. Six volunteers (“control” group) were subjected to 4 mo of bed rest at a 6° head-down-tilt position. Muscle biopsies were taken from soleus muscles four times, i.e., ~3 wk before bed rest, after ~2 and ~4 mo of bed rest, and after ~1 mo of normal ambulatory recovery. The subjects were fed three times per day, and daily total energy intake was ~3,000 kcal for each subject. 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.

The second experiment was designed to determine the effects of exercise countermeasures performed during the bed rest period on the same muscle properties as described above. Seven subjects were assigned to one of two Penguin suit groups. They were subjected to a 2-mo bed rest at a 6° head-down-tilt position. All subjects in the second experiment wore an anti-G Penguin suit for 10 h/day (11 AM to 9 PM) and additionally performed knee extension and flexion exercise in a supine position in bed against a resistance of 100 N for the last 15 min of each hour. 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. Thus this exercise emphasized knee, not ankle, movement. The legs were relaxed, keeping the knee at an extended position during the remaining 45 min.

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.* During bed rest, the subjects were carried to the medical treatment room for performance of the muscle biopsies. Biopsies were taken from the left soleus with the subjects in a prone position by using the procedures described by Bergstrom (8). 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 muscle. The biopsy sample was divided into several portions. One of the sample portions was longitudinally tied to a glass bar by use of surgery thread and was placed in a cold 50% (vol/vol) glycercin solution (4°C) for 24 h. The glycercin solution was exchanged with freshly prepared glycercin solution, and the muscle tissues in the solution were kept at ~20°C for 8–10 wk before the contractile analyses were performed.

Because the samples were not treated for skinning immediately after biopsy by using Triton X-100, for example, the muscle fibers were kept in glycercin solution to skin for 5 wk at ~20°C before the analyses of contractile properties, and all groups were treated in the same way. The analyses were completed within 2 wk, and the order of analysis was random across the groups.

**Analytical procedures.** On the day of the experiment, first a bundle was placed in relaxing solution for 15 min and then individual fibers were carefully dissected from the bundle. A single muscle fiber (~5 mm long) was transferred to an experimental chamber (0.3 ml volume) filled with the relaxing solution. The relaxing solution (47, 48) consisted of 10 mM EGTA, 3.5 mM MgATP, 1.5 mM Mg2+, and 20 mM PIPES at pH 7.0 (20°C). The ionic strength was adjusted to 0.2 M with potassium methane sulfonate. One end of the fiber was secured with T-shaped aluminum clips to a semiconductor transducer element (AE801, Aksjeselskapet Mikro-Elektronikk), and the other end was attached to a hook connected to a servomotor (G120D, General Scanning, Watertown, MA).

All experiments were performed at 20 ± 1°C. The Ca2+ concentration of the activating solution was adjusted between pCa 7.0 and pCa 4.0 (47, 48). Before the mechanical experiments, the fibers were secured in the analytical apparatus and treated with a skinning solution containing 1% (vol/vol) Triton X-100 for 3 min. Isometric tension was recorded digitally at 12 kHz by an analog-to-digital converter (AD216, Nittobo Acoustic Engineering) and stored on a hard disk connected to a personal computer (PC-9821Ap, NEC) for later analysis.

Sarcomere length was adjusted to 2.5 ± 0.2 μm by use of a laser diffraction pattern and was monitored using a microscope and charge-coupled device camera connected to the microscope. If any irregularity in the sarcomere was observed, the fiber was discarded. The fiber diameter, absolute

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**Table 1. Physical characteristics of subjects**

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Body Weight, kg</th>
<th>Height, cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (<em>n</em> = 6)</td>
<td>30.8 ± 3.1</td>
<td>80.0 ± 7.6</td>
</tr>
<tr>
<td>Penguin-1 (<em>n</em> = 4)</td>
<td>30.5 ± 1.9</td>
<td>72.0 ± 3.3</td>
</tr>
<tr>
<td>Penguin-2 (<em>n</em> = 3)</td>
<td>33.3 ± 2.8</td>
<td>70.0 ± 6.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. Control, bed rest for 4 mo without any exercise countermeasure. See MATERIALS AND METHODS for description of the Penguin-1 and Penguin-2 groups.
maximally activated force \( (P_o) \), relative maximally activated force \( (F_{max}) \) per fiber cross-sectional area \( (CSA) \), \( V_o \), and \( Ca^{2+} \) sensitivity of the myofilaments were determined for each fiber. The CSA of a fiber was calculated from the two diameters in two perpendicular projections. Assuming that the shape of muscle fiber is a cylinder, we measured the width and then thickness of fiber by shifting the stage of the microscope. Fiber CSA then was calculated by using width and thickness as reported by Frontera and Larsson (13).

The pCa-tension relationships were determined by activating the fibers in a series of solutions containing free \( Ca^{2+} \) concentrations \( (pCa; 7.0, 6.5, 6.0, 5.5, 5.0, \text{ and } 4.0) \). After washing the muscle fiber in a preactivating solution containing 2 mM EGTA, pCa solution was applied. Then, the fiber was relaxed by washing in the relaxation solution after the tension reached a plateau level. The \( F_{max} \) was determined in pCa 4.0 solution first. Then the fiber was relaxed and \( Ca^{2+} \) solution was applied from a lower concentration \( (pCa 7.0) \) to a higher concentration. After application of a pCa solution, the sample was washed in relaxing solution containing 10 mM EGTA and in preactivating solution containing 2 mM EGTA, and then the next pCa solution was applied. The experiment was terminated when the \( F_{max} \) decreased more than 10% in pCa 4.0 solution, the resting tension did not return to zero, or irregularity in the sarcomeres was observed.

Hill plot analysis was used to determine the \( Ca^{2+} \) sensitivity of individual fibers. The \( Ca^{2+} \) sensitivity of fibers was evaluated by the concentration of \( Ca^{2+} \) for half-maximal activation \( (pC_{a_{0}}) \). The \( V_o \) was determined by the slack test procedure. The times required for the redevelopment of force after five to six imposed slack steps \( \text{[each step <20% of fiber length \( (FL) \)]} \) were plotted against the corresponding slack length, and the points were fitted with a linear regression line. The slope of this line, which was \( V_o \), was normalized to the FL and expressed as \( FL/o \) per second \( (FL/s) \).

After the mechanical measurements, the fiber segments were removed from the apparatus; solubilized in 0.03 ml of an SDS buffer solution consisting of 0.1 M Tris·HCl (pH 8.8), 5 mM EDTA, 10% SDS, and 50 mM DTT; boiled for 3 min; and stored at −80°C until subsequent gel electrophoresis analyses. The fiber sample solutions were run on 12% acrylamide gels and stained by Bio-Rad Silver Stain Plus (Bio-Rad, Hercules, CA). The expression of myosin heavy chain (MHC) isoforms in the same fibers was determined (27). NIH Image was used to quantify the relative content of each isoform. In the present study, all data were determined from fibers expressing only type I MHC with the total number of fibers used equal to 25 per group. Some of the isolated fibers contained either I+IIa, I+IIa+IIX, IIa, or IIa+IIX MHC expression (Table 2). However, the data from these fibers were not used because the distribution of fibers having these MHC profiles was low and not always detected in some samples. The number of fibers analyzed in each muscle was seven to eight because of the small sample size.

**Statistical analysis.** All values are expressed as means ± SE. For the first experiment, the values were analyzed by using ANOVA followed by Scheffé’s post hoc test. For the second experiment, one-tailed paired t-tests were used for the comparison between pre- and post-bed rest. ANOVA followed by Scheffé’s post hoc test was used for comparisons of the percent change after 2 mo of bed rest, relative to the pre-bed rest levels, between the control and Penguin suit groups. Statistical significance was set at \( P < 0.05 \).

### RESULTS

**Effects of bed rest without countermeasure.** Figure 1 shows the responses of the fiber diameter (A), \( P_o \) (B), \( F_{max} \) per CSA (C), and \( V_o \) (D) to 4 mo of bed rest without any countermeasure. The patterns of response for fiber diameter, \( P_o \), and \( F_{max} \) per CSA were similar. Mean fiber diameters were ~8 and 36% smaller after 2 and 4 mo of bed rest than before bed rest, respectively.

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>I+IIa</th>
<th>I+IIa+IIX</th>
<th>IIa</th>
<th>I+IIa+IIX</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-bed rest</td>
<td>91.6±3.3</td>
<td>2.5±1.2</td>
<td>0</td>
<td>5.4±2.0</td>
<td>0</td>
</tr>
<tr>
<td>2 Mo bed rest</td>
<td>83.3±5.0</td>
<td>2.5±1.4</td>
<td>1.3±0.5</td>
<td>8.3±2.2</td>
<td>4.6±1.5</td>
</tr>
<tr>
<td>4 Mo bed rest</td>
<td>75.8±4.4</td>
<td>4.2±2.2</td>
<td>4.6±2.4</td>
<td>12.5±3.0</td>
<td>5.0±2.0</td>
</tr>
<tr>
<td>1 Mo after bed rest</td>
<td>86.6±5.2</td>
<td>4.6±2.3</td>
<td>3.3±1.6</td>
<td>2.9±1.2</td>
<td>4.2±1.8</td>
</tr>
<tr>
<td><strong>Penguin-1 group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-bed rest</td>
<td>91.2±4.1</td>
<td>2.2±0.5</td>
<td>0</td>
<td>6.0±2.0</td>
<td>0</td>
</tr>
<tr>
<td>2 Mo bed rest</td>
<td>82.3±5.2</td>
<td>1.9±0.6</td>
<td>6.5±2.2</td>
<td>9.0±1.9</td>
<td>4.4±1.7</td>
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<td><strong>Penguin-2 group</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pre-bed rest</td>
<td>92.1±3.2</td>
<td>2.2±0.5</td>
<td>0</td>
<td>5.8±1.6</td>
<td>0</td>
</tr>
<tr>
<td>1.5 Mo bed rest</td>
<td>83.1±5.0</td>
<td>2.7±0.8</td>
<td>1.5±0.4</td>
<td>8.0±2.2</td>
<td>5.0±2.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. Number of fibers analyzed: 7–8 per muscle.

Table 2. Percent fiber-type distribution based on myosin heavy chain expression

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Ca<sup>2+</sup> concentration required for pCa<sub>50</sub> in the control group was 5.92 (1.2 ± 0.1 μmol). The values of pCa<sub>50</sub> were lower after 2 and 4 mo of bed rest, i.e., 5.79 (1.6 ± 0.2 μmol) and 5.68 (1.7 ± 0.2 μmol), respectively, than the pre-bed rest level. There was no significant difference in pCa<sub>50</sub> between 2 and 4 mo of bed rest. After 1 mo of recovery, the pCa<sub>50</sub> level was increased (5.89, 1.3 ± 0.1 μmol) relative to the level obtained at the end of the 4 mo of bed rest period and was similar to the pre-bed rest level.

Effects of loading and/or knee exercise during bed rest. The countermeasures performed during bed rest in the Penguin-1 and -2 groups had different effects on the contractile properties (Figs. 2 and 3). Before bed rest, there were no significant differences in fiber diameter (Fig. 3A), P<sub>o</sub> (Fig. 3B), F<sub>max</sub> per CSA (Fig. 3C), and V<sub>o</sub> (Fig. 3D) between Penguin-1 and -2 groups (Fig. 3). The countermeasures performed by the Penguin-2 group ameliorated but did not prevent the adaptations to bed rest observed in the control group (Fig. 1). For example, the percent changes from pre-bed rest levels in fiber diameter, P<sub>o</sub>, F<sub>max</sub> per CSA, and V<sub>o</sub> after 2 mo of bed rest in the Penguin-2 group were −8, −38, −27, and +83%, respectively. These adaptations in the control group were −8, −38, −27, and +83%, respectively. These parameters were similar before and after bed rest in the Penguin-1 group.

The pCa<sub>50</sub> level was lower in the Penguin-1 (5.93, 1.2 ± 0.1 μmol) than the Penguin-2 group (5.77, 1.7 ± 0.2 μmol) after 2 mo of bed rest (Fig. 2). There also was a significant difference in the pre- and post-bed rest pCa<sub>50</sub> values for the Penguin-2 group but not for the Penguin-1 group.

**DISCUSSION**

Effects of bed rest without countermeasures. To our knowledge, this is the first report of the effects of 2 and 4 mo of bed rest on the contractile properties of single muscle fibers in the human soleus muscle. In the present study, the F<sub>max</sub> per CSA and P<sub>o</sub>, as well as fiber diameter, were significantly decreased and the V<sub>o</sub> significantly increased after 2 and 4 mo of bed rest. These contractile responses to long-term bed rest are similar to the results obtained after 17 days of spaceflight and bed rest in human subjects (44–46).

The F<sub>max</sub> is thought to be dependent on fiber size, because larger fibers have relatively more cross bridges...
and can generate higher forces than smaller fibers. The relative $F_{\text{max}}$ per square meter of fiber CSA was 27 and 42% lower after 2 and 4 mo, respectively, of bed rest than before bed rest. Although an $\sim 8$ and $36\%$ decrement in fiber diameter was observed after 2 and 4 mo of bed rest (Fig. 1A), respectively, the $P_o$ was 38 and 76% lower for the same time periods (Fig. 1B). Thus factors other than fiber atrophy influenced the decrease in $P_o$. This observation is consistent with the report of Widrick et al. (44) of an $\sim 10\%$ decrease in $F_{\text{max}}$ per CSA after 17 days of bed rest. The larger decrease in $F_{\text{max}}$ per CSA in the present study most likely reflects the longer experimental period.

The mechanism(s) for the reduction in $F_{\text{max}}$ per CSA is (are) unknown. One possibility is a reduction in the number of cross bridges per CSA in atrophied muscle fibers. Because the cross bridge is formed between thick and thin filaments and is the origin of force, $F_{\text{max}}$ is dependent on the number of cross bridges per CSA of a maximally activated muscle. A decrement in thin filament density after 17 days of spaceflight or bed rest has been reported (33, 34, 46). Therefore, the decrease in $F_{\text{max}}$ per CSA after bed rest may reflect a decrement in thin filament density.

Other factors, such as the transition from weakly to strongly bound cross bridges, may be related to the decrement in $F_{\text{max}}$ after bed rest. Active force in muscle fibers may be generated by the transition of cross bridges from a weakly to a strongly bound state (21). In addition, the chemomechanical efficiency of cross bridges may affect the $F_{\text{max}}$. Chemomechanical efficiency is the energy-transferring efficiency when chemical energy released in association with one molecule ATP breakdown is converted into physical energy by one cross bridge. A decrease in this efficiency may cause a large reduction of force development per CSA. However, whether changes in cross-bridge transition patterns or in chemomechanical efficiency of cross bridges are induced after bed rest is unknown.

The mean $V_o$ of type I fibers in the soleus muscle has been reported to increase by $30\%$ over control values after 17 days of bed rest (44). In the present study, the $V_o$ of type I fibers was more than $70\%$ faster after 2 and 4 mo of bed rest than before bed rest. $V_o$ is thought to be dependent on the MHC and myosin light chain isoform profiles and the myosin ATPase activity of the fiber (22, 32, 39). In hindlimb-unloaded rats, the relative composition of the fast MHC isoforms increases significantly in the predominantly slow soleus muscle (9–11), and this appears to be closely related to an increase in $V_o$ (11). Previously, our laboratory reported that 2 and 4 mo of bed rest resulted in a small percentage ($\sim 10–20\%$) of soleus muscle fibers transforming from slow to a fast phenotype in the same subjects (27, 28). An $\sim 40\%$ decrease of slow-twitch fibers was also observed after hindlimb suspension of rats (10). In the present study, however, all fibers used expressed only the slow MHC isoform. Therefore, fiber type transformation cannot explain the observed increase in $V_o$ after bed rest. One possibility for the increase in $V_o$ may be related to the kinetic transitions of cross-bridge states. For example, Widrick et al. (46) proposed the possibility that changes in lattice spacing induced by decreased activity results in a reduction in the internal drag, which, in turn, increases the velocity of shortening. However, the precise mechanism(s) for the elevation of $V_o$ in slow-twitch fibers after bed rest remain undefined.

It is generally accepted that $Ca^{2+}$ sensitivity is related to $Ca^{2+}$ affinity of troponin C (18, 30, 41) and/or the cooperativity between thin and thick filaments (20, 24). Slow-type troponin C isoform is expressed in slow fibers and has two high- and low-affinity binding sites for $Ca^{2+}$. One of the low-affinity sites is usually inactive (40). Modulations of $Ca^{2+}$ sensitivity during periods of decreased activity have been reported (23, 25,
Recently, a depression in Ca\(^{2+}\) sensitivity after bed rest (45) and spaceflight (46) has been reported. The right shift in pCa-tension relationship could be related to an increased filament spacing due to a selective loss of actin filaments (45). It has been reported that the cooperativity between filaments is affected by troponin T isoforms (14, 19, 29). An elevation in the intracellular free Ca\(^{2+}\) concentration in atrophied soleus muscles in a resting state has been reported (20). Intracellular Ca\(^{2+}\) concentration may affect the affinity of troponin C for Ca\(^{2+}\). Experiments using glycerinated fibers cannot evaluate Ca\(^{2+}\) movement via the sarcoplasmic reticulum, which is related to Ca\(^{2+}\) sensitivity. It has been reported, however, that sarcoplasmic reticulum function is altered by periods of decreased activity (7, 20, 25, 31, 37, 50). The protein and mRNA levels of the “fast” isoform of the sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase are markedly increased in the rat soleus muscle during 28 days of hindlimb unloading (37). The sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase pre-mRNA and mRNA transcript levels in soleus have been shown to increase by two-, three-, and sevenfold after 2, 4, and 10 days of hindlimb unloading, respectively (31).

**Effects of loading and/or exercise during bed rest.** The countermeasure effects of two types of resistive knee extension/flexion exercise induced by wearing Penguin suits were determined. In the Penguin-1 group, continuous resistance was provided at the knee and ankle. In the Penguin-2 group, the loading on the ankle was removed. Therefore, although there were no differences in the duration and type of knee extension/flexion exercise between the two groups, loading at the ankle induced passive stretch of soleus muscle in the Penguin-1 but not the Penguin-2 group.

The resistive exercise with loading in the Penguin-1 group prevented the adaptations in the contractile properties observed in the control group after 2 mo of bed rest. However, the same type of exercise performed by the Penguin-2 group, in which no resistive forces on the plantar flexors were imposed, was less effective. It is clear that the passive stretch of soleus for 10 h/day during bed rest in Penguin-1 group had a beneficial effect. The stretching of the muscles, not necessarily the knee extension/flexion exercise, during bed rest was effective to prevent the alteration of contractile properties.

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**Fig. 3.** Effects of knee extension/flexion exercise with or without loading on ankle plantar flexors by wearing a Penguin suit on the contractile properties of slow soleus muscle fibers during 2 mo of bed rest. **A:** fiber diameter. **B:** P. **C:** F\(_{\text{max}}\) per cross-sectional area. **D:** unloaded shortening velocity. Values are means ± SE. *Significantly different from pre-bed rest level (P < 0.05). Number of fibers: 25 per group.

50).
In the Penguin-2 group, the changes in fiber diameter, F_max per CSA, Ca^{2+} sensitivity, and V_o after 2 mo of bed rest were smaller than those observed after bed rest alone (control group). It has been reported that intermittent exercise could partially prevent the alteration of the contractile properties during periods of decreased activity (2, 6, 35, 43). Therefore, the type of exercise, for example, tonic or phasic, is an important factor as the countermeasure for prevention of unloading-related changes in muscle properties.

The present data suggest that mechanical loading by stretching of muscle, not necessarily the contraction of muscle reported by Akima et al. (1) and Bamman et al. (5), is a useful countermeasure for prevention of deconditioning in slow muscle fibers with antigavity function. This effect may be induced by a stimulation of tonic activity due to stretching. It is also indicated that stretching has to be considered as a possible countermeasure for prevention of deconditioning in antigavity muscles. However, the effective or minimum workload, duration, and/or exercise frequency required as the countermeasure is still unclear.

In conclusion, effects of 2 and 4 mo of bed rest, with or without exercise countermeasures, on the contractile properties of slow fibers in the human soleus muscle were examined. The fiber diameter, P_o, F_max per CSA, and pCa_{50} were reduced after 2 and 4 mo of bed rest. The V_o was increased in response to 2 and 4 mo of bed rest. After 1 mo of reambulation period, however, these changes were generally recovered. These results indicate that long-term inactivity causes the reduction of fiber size, force generation, and Ca^{2+} sensitivity, and the elevation of V_o in single muscle fibers of human soleus. As a result of daily muscle stretching during 2 mo of bed rest, fiber diameter, P_o, F_max per CSA, pCa_{50}, and V_o remained unchanged. Thus it is suggested that continuous mechanical loading on muscle, such as stretching of muscles, is effective as the countermeasure for prevention of muscular adaptation to gravitational unloading.

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