Influence of fixed muscle length and contractile properties on atrophy and subsequent recovery in the rat soleus and plantaris muscles

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Summary. This study examined muscular atrophy and the recovery process induced by hindlimb unloading and joint immobilization in the rat soleus and plantaris muscles. Rats were divided into control, hindlimb unloading (HU), hindlimb unloading with ankle joint immobilization at the maximum dorsiflexion (HUD), and maximum plantarflexion (HUP) groups. The hindlimb was reloaded after fourteen days of unloading, and muscle atrophy and walking ability were assessed at 0, 3, and 7 days of reloading. A cross sectional area of muscle fibers in the soleus muscle on day 0 of reloading revealed sizes in order from the control, HUD, HUP down to the HU group, indicating that the HU group was the most atrophied among the four groups. These values in the plantaris muscle ranged in order from the control, HU, HUD, to HUP groups, the HUP group being the most atrophied among the four groups. These muscles recovered from atrophy in the same descending order, and the values in the HUD and HUP groups slowly recovered during the reloading periods. The HUD and HUP groups showed a central core lesion and reloading-induced lesions in some type I muscle fibers after the immobilization and reloading, one possible reason for the delayed recovery in these groups. The muscle atrophy in the HU, HUD, and HUP groups remained at day 7 although the walking ability appeared to be normal. Accordingly, further rehabilitation therapy might be necessary even if the functional ability appears to be normal.

Introduction

Joint immobilization with a cast, is a common treatment for fractures and post surgical operations, induces skeletal muscle atrophy and reduces work capacity (Yue et al., 1997; Yasuda et al., 2005; Stevens et al., 2006). Muscle atrophy due to joint immobilization is a significant problem that has not yet been resolved. The immobilization of a joint with a cast at any position fixes the muscles taking part in the motion of the joint at shortened or stretched positions. The influence of joint immobilization on muscle atrophy is well documented, and muscle atrophy is milder when the muscle is fixed at a stretched position rather than at a shortened position (Williams and Goldspink, 1973; Baker and Mastumoto, 1988; Ohira et al., 1997).

It is important to minimize muscle atrophy in order to allow optimal recovery. Therefore, various preventive interventions can be implemented before exposure to the atrophic environment (Fujino et al., 2009) and during immobilization (De-Doncker et al., 2000; Kyparos et al., 2005). A previous study in the mouse immobilized the ankle joint at the maximum dorsiflexion and the maximum plantarflexion positions with a cast and assessed muscle atrophy in the tibialis anterior and soleus muscles (Fujita et al., 2009). The results showed that muscle atrophy depends not only on fixed muscle length but also on joint immobilization, especially in the soleus...
muscle (Fujita et al., 2009). These findings suggest that, besides the fixed muscle length, the fiber type and muscle contractile property might be also important factors associated with muscle atrophy (Fujita et al., 2009). However, the influence of fixed muscle length on the recovery from muscle atrophy is still undetermined.

Skeletal muscle atrophy is induced by various factors, such as joint immobilization, hindlimb unloading, spaceflight, denervation, tenotomy, and ageing (Ohira, 2000; Edgerton et al., 2002). Therefore, there might be some differences among these factors in the mechanism of muscle atrophy; when the mechanism of the muscle atrophy is different, the manner of recovery might also differ. The present study examined the recovery process after muscle atrophy induced by hindlimb unloading and joint immobilization in the rat soleus and plantaris muscles.

Materials and Methods

Materials

This study was approved by the Institution Animal Care and Use Committee (Permission number: P090613) and carried out according to the Kobe University Animal Experimentation Regulations. The present study used 48 adult male Wistar rats (Japan SLC, Shizuoka) weighing in 227–272 g. The animals were randomly divided into four groups: 1) control, 2) hindlimb unloading without bilateral ankle joints immobilization (HU), 3) hindlimb unloading with bilateral ankle joints immobilization at the maximum dorsiflexion (HUD), and 4) hindlimb unloading with bilateral ankle joints immobilization at the maximum plantarflexion (HUP; Fig. 1). The soleus and plantaris muscles were fixed at a stretched position in the HUD group and at a shortened position in the HUP group, respectively. An isotonic contraction occurred in the soleus and plantaris muscles in the HU group, while isometric contraction occurred in the HUD and HUP groups (Baewer et al., 2004).

Hindlimb unloading

Hindlimb unloading was applied to animals by suspending their tails for 14 days, according to the method described by Morey (1979). Briefly, each animal in the HU, HUD, and HUP groups was fitted with a tail harness and was suspended by a string just high enough to prevent the hindlimbs from bearing weight on the floor or sides of the cage. The forelimbs were allowed to maintain contact with the floor of the cage and the animals had full access to food and water. The animals in each group were housed in an isolated and environmentally controlled room at 25°C with a 12:12-h light-dark cycle.

Ankle joint immobilization method

The animals in the HUD and HUP groups were lightly anesthetized by an intraperitoneal injection of pentobarbital sodium of 50 mg/kg body weight during the joint immobilization procedure. The plaster casts covered both sides, from the upper part of the knee joint to the forefoot. The animal's toes were not wrapped; instead, they were exposed to confirm the occurrence of edema. The plaster cast was immediately and properly rewound and replaced when loosening of the plaster cast or edema occurred. The animals in the control and HU groups were both anesthetized at the same level as in the HUD and HUP groups to exclude any influence of the anesthetic.

Experimental design

The casts were removed after 14 days of hindlimb unloading, and the animals reloaded their own weights to the hindlimb to investigate its recovery. The animals were weighed after 14 days of hindlimb unloading (day 0) and after 3 (day 3) and 7 (day 7) days of the reloading, and their ability to walk was assessed by using the beam walking test described below. Subsequently,
the animals were deeply anesthetized by pentobarbital sodium, and the range of motion on the ankle joint was assessed. The muscles from the right side leg were used to examine isometric twitch and tetanus contractile tension, and the ones from the left side, to measure the wet weight. Thereafter, muscles were immediately frozen in acetone-cooled dry ice and stored at \(-80^\circ C\) until use for morphological analysis.

**Beam walking test**

The animal's ability to walk was assessed using a slightly modified beam walking test prior to measuring the range of motion (Carter et al., 2001). This task requires the animals to walk on a wooden beam with a length of 100 cm and width of 3 cm. The beam was elevated above the floor on an incline of 15° leading to the goal of the darkened home cage. This test involved 10 trials. The animals started from the end point at the first three trials, the following three starting at the medium end, and the last four starting at the start point. Between the trials, each animal remained in its home cage with the lights in the room turned off for 2 min. Each of the last three trials was videotaped (Sanyo, Osaka), and measured the latency to traverse the beam. The 30-sec test trial began when the animal could stay balanced on the beam without assistance. The trial ended when one forelimb passed the end point of the test distance, or when 30 sec had elapsed or when the animal had fallen from the beam. If the animal fell off the beam or could not traverse the test distance within 30 sec, the score was designated to be 30 sec.

**Measurement of the range of motion on ankle joint**

The range of motion on dorsiflexion and plantarflexion of the ankle joint of each animal was measured after performing the beam walking test. Measurement of the range of motion was monitored with the force transducer (Aikoh Engineering, Osaka). The probe of the force transducer to measure the range of motion was fitted to the distal part of the fifth metatarsal bone, with dorsiflexion and plantarflexion of the ankle joint obtained at 400 \(\pm 20\) mN and 200 \(\pm 20\) mN, respectively. The range of motion on dorsiflexion and plantarflexion was recorded 20 cm from the upper side by digital camera. These digital images were analyzed using ImageJ software package (National Institutes of Health, Maryland, USA). According to the previous study (Okita et al., 2004, 2009), measurement of the range of motion was defined as the angle (0 to 180°) between a straight line connecting the fifth metatarsal bone and lateral malleolus to a line connecting the lateral malleolus and the caput fibulae when the knee joint was flexed 90°.

**In situ isometric contractile measurements**

The right soleus and plantaris muscles and sciatic nerve were used for contractile measurements. These muscles were isolated with the blood and nerve supply intact. The muscles were kept moist by Krebs-Ringer solution at 41°C during the experiment. The distal tendon of the muscle was secured by a silk thread (4-0) and then was attached to a small hook on the end of the arm of a force transducer (Aikoh Engineering) for measurement of isometric contraction. All contractile measurements were made at a muscle temperature of 35°C, with the core temperature held at 37°C. Contraction of the muscles was induced by electrical stimulation of the sciatic nerve using a stimulator (Nihon Kohden, Tokyo). The leg flexor muscles including the gastrocnemius and long digital flexors were denervated except for the soleus and plantaris muscles, and a bipolar silver electrode was placed on the sciatic nerve to apply stimulation. The muscle was stimulated by using supramaximal, square-wave pulses of 0.5 ms duration. Initially, the optimal length was determined for each muscle by eliciting twitches. The length of the muscle was adjusted to produce the peak twitch tension (Pt). A voltage approximately two to three times greater than the minimal voltage was required to produce the Pt. Second, the peak tetanic tension (Po) was measured at frequencies of 10, 20, 40, 60, 80, 100, 120, 140, and 160 Hz for the duration (>500 ms for the soleus muscle and >300 ms for the plantaris muscle). The highest value of measurement was considered the Po regardless of stimulation frequencies. The interval between contractions was 2 min. After the isometric contraction of the soleus muscle was measured, these experimental protocols were then repeated in the plantaris muscle.

**Morphological analysis**

The left soleus and plantaris muscles were used for morphological analysis. Serial cross sections of 10 \(\mu m\) were made with a cryostat from the middle part of the muscle belly in the soleus and plantaris muscles (Shiraimatsu, Osaka) at \(-20^\circ C\), mounted on glass slides, and stained with hematoxylin and eosin (H-E) for histological observation. For myofibrillar adenosine triphosphatase (ATPase) histochemistry, these sections were pretreated at pH 4.1, 4.25, and 10.8 to categorize the muscle fiber as type I, IIA, or IIB (Lind and Kernell, 1991). The sections for the ATP histochemistry pretreated
at pH 4.25 were used to determine the composition of muscle fiber types and to measure cross sectional areas of each muscle fiber type. Classification of the muscle fibers was according to a previous study (Staron et al., 1999). Photographs (× 200) were taken from each section with a digital camera (Olympus, Tokyo) attached to a light microscope (Olympus). A measuring field was set over the entire muscle cross section for the muscle fiber type composition. At least 100 randomly-selected cross sectional areas of each muscle fiber type were investigated. All sections were statistically calculated by using the ImageJ software program.

**Statistical analysis**

Significant differences between the four experimental groups or within groups over time were analyzed by using a two-way analysis of variance followed by Tukey’s post hoc test. The statistically significant level was set at \( P < 0.05 \).

**Results**

**Muscle wet weight**

The mean values of the wet weight of the soleus muscle on day 0 were 122.7 ± 3.6 mg in the control group, 61.5 ± 2.5 mg in the HU group, 112.3 ± 5.5 mg in the HUD group, and 75.0 ± 5.8 mg in the HUP group (Fig. 2A). This value in the HUD group was significantly larger than that in the HUP group (\( P = 0.01 \)), while this value in the HU group was significantly less than that in the HUD group (\( P < 0.001 \)) and appeared to be less than that in the HUP group. The order of these values in the soleus muscle (in descending order of the control, HUD, HUP and HU groups) was similar on recovery days 3 and 7.

The mean values of the wet weight in the plantaris muscle at day 0 were 311.0 ± 8.8 mg in the control group, 206.5 ± 16.6 mg in the HU group, 192.0 ± 6.5 mg in the HUD group, and 187.2 ± 5.3 mg in the HUP group (Fig. 2B). This value in the HUD group was similar to that in the HUP group. These values in the HUD and HUP groups appeared to be slightly less than those in the HU group, though statistically insignificant. The order of these values (in descending order from the control, HU, HUD to the HUP group) was still observed on days 3 and 7.

The muscle weight in the HU group generally increased during the 7 day reloading period in both the soleus and plantaris muscles. However, the recovery of the muscle wet weights of these muscles in the HUD and HUP groups occurred slowly during the reloading periods.

**Morphological appearance and cross sectional area of muscle fibers**

The cross section of the soleus muscle in the control group on day 0 showed that the muscle fibers were polygonal in shape, and the spaces among the muscle fibers were very narrow (Fig. 3). The ATPase histochemistry revealed that the soleus muscle was chiefly composed of type I and IIA fibers, with very few intermediate fibers (Fig. 4). Accordingly, the cross sectional areas of type I and IIA fibers were respectively...
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The soleus muscle in the HUP group at day 0 (arrowheads in Fig. 3D). These foci, known as the central core lesion, gradually decreased in number after removal of the immobilization. The central core lesion was extremely rare in the HU or HUD groups. Muscle fibers showing degenerating features were noted in the soleus muscle in both the HUD and HUP groups at 3 days of reloading (Fig. 6A, B, E, F). These fibers contained cellular elements throughout the sarcoplasm. The morphological appearance of these fibers was very similar to that described as the "reloading-induced lesion" (Frimel et al., 2005; McClung et al., 2007), meaning that the cellular elements in the sarcoplasm might be macrophages (St Pierre and Tidball, 1994). The fibers showing a reloading-induced lesion were type I fibers; these decreased in

compared among the four groups (Fig. 5). At day 0 of reloading, the mean values of the cross sectional area of the type I fibers in the control, HUD, HUP, and HU groups were 3352 ± 33, 2762 ± 49, 2282 ± 33, and 1471 ± 17 μm², and those of type IIA, 2535 ± 25, 2014 ± 41, 1680 ± 19, and 1413 ± 15 μm², respectively. The mean values of the areas of both the type I and IIA fibers in the HUD group were significantly larger than those in the HU group (P < 0.001). The mean values of the areas of both the type I and IIA fibers in the HUP group were significantly less than those in the HUD and HUP groups (P < 0.001). At days 3 and 7, the order of the mean values of both the type I and IIA fibers was the same as that at day 0 (Fig. 5). Several type I fibers exhibiting a faintly stained foci at the center of sarcoplasm were observed in the soleus muscle in the HUP group at day 0 (arrowheads in Fig. 3D). These foci, known as the central core lesion, gradually decreased in number after removal of the immobilization. The central core lesion was extremely rare in the HU or HUD groups. Muscle fibers showing degenerating features were noted in the soleus muscle in both the HUD and HUP groups at 3 days of reloading (Fig. 6A, B, E, F). These fibers contained cellular elements throughout the sarcoplasm. The morphological appearance of these fibers was very similar to that described as the "reloading-induced lesion" (Frimel et al., 2005; McClung et al., 2007), meaning that the cellular elements in the sarcoplasm might be macrophages (St Pierre and Tidball, 1994). The fibers showing a reloading-induced lesion were type I fibers; these decreased in

Fig. 3. ATPase staining (pH 4.25) of the cross section of the soleus muscle on day 0. A: control group; B: HU group; C: HUD group; D: HUP group. Type I fibers (I) were deeply stained, while type IIA fibers (IIA) were lightly stained. Muscle fibers in the control group were polygonal in shape, and inter-muscle fibers spaces were hardly noticeable. Although the morphological appearance in the control and HUD groups was similar, the inter-muscle fibers spaces in the HUD group were more evident than those in the control group. There is a possibility that this observation with wide inter-muscular fiber spaces shows fibrosis and edema. Note the central core lesions (arrowheads), particularly evident in the HUP group. Bar = 50 μm
Fig. 4. Distribution (%) of muscle fiber types in the soleus (A) and plantaris (B) muscles among the four groups examined on days 0, 3, and 7 of reloading. Almost 80% of the soleus muscle consists of the type I fiber in all four groups. This composition continues to days 3 and 7. The plantaris muscle was chiefly composed of type IIB fibers in all groups during the reloading periods. I, type I fiber; INT, intermediate fiber; IIA, type IIA fiber; IIB, type IIB fiber.

Fig. 5. Cross sectional area (μm²) of type I fibers (A) and type IIA fibers (B) in the soleus muscle on days 0, 3, and 7 of reloading. In both the type I and IIA fibers, the mean values of the cross sectional area ranged in order from the control, HUD, HUP to the HU group during the reloading periods. These values in the HUD and HUP groups recovered more slowly than those in the HU group. a, b and c: significantly different from control, HU, and HUD, respectively; e and f: significantly different from days 0 and 3, respectively, at P < 0.03. Values are the means ± standard error.

number by day 7 of reloading. The central core lesion and degenerating features were still observed in the HUD and HUP groups on day 7 (Fig. 6C, D, G, H).

The cross section of the plantaris muscle in the control group on day 0 showed the muscle fibers to be polygonal in shape, with few spaces among the muscle fibers (Fig. 7). The HU, HUD, and HUP groups showed the spaces among the muscle fibers on day 0. The plantaris muscle was composed of type I, IIA, and IIB fibers, and the type IIB fiber was the dominant fiber type in this muscle in all groups at 7 days of reloading (Fig. 4). At day 0, the mean values of the cross sectional area of the type IIB fibers in the plantaris muscle ranged in size from the control, HUD to the HUP group (3411 ± 39 μm², 2723 ± 42 μm², 1839 ± 26 μm², and 1719 ± 26 μm², respectively; Fig. 8C). The cross sectional area of the type IIB fibers showed the same pattern (in descending order of the control, HU, HUD, and HUP groups) during all reloading periods (Fig. 8C). The areas of the type IIB fibers in the HUD and HUP groups were significantly less than those in the HU group at all periods of reloading (P < 0.001). There were no significant differences in the type IIB fibers between the HUD and HUP groups during all reloading periods. Few muscle fibers in the plantaris muscle showed either a central core lesion or reloading-induced lesion in any group or on any day of reloading.

The cross sectional areas of both the soleus and plantaris muscles in the HUD and HUP groups slowly recovered during reloading periods (Fig. 5, 8). Although the values of the HUD and HUP groups in type IIB fiber in the plantaris muscle were the lowest on day 0, the recovery of these values progressed slowly.
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Fig. 6. Serial cross sections of the soleus muscle on days 3 and 7 of reloading. Sections were assayed for H-E staining (A–D) and ATPase staining (E–H) pretreated at pH 4.25. In both the HUD and HUP groups, central core lesions (arrowheads) in muscle fibers and degenerating features (arrows) were observed after reloading. A, E: HUD group at day 3; B, F: HUP group at day 3; C, G: HUD group at day 7; D, H: HUP group at day 7. Bar = 50 μm
Muscle contractile tension

The mean values of the twitch and tetanus contractile tension in the soleus muscle on day 0 ranged in size in order from the control, HUD, HUP down to the HU group (twitch: 230 ± 11 mN, 190 ± 7 mN, 132 ± 10 mN, and 102 ± 4 mN; tetanus: 1093 ± 1 mN, 808 ± 2 mN, 560 ± 10 mN, and 456 ± 11 mN, respectively; Fig. 9). The twitch and tetanus tensions of the HUD group on day 0 were significantly larger than those of the HUP group ($P = 0.01$). This value in the HU group was significantly less than that in the HUD group ($P = 0.01$) and appeared to be less than that in the HUP group, though statistically insignificant. The order of these values (in descending order of the control, HUD, HUP, and HU groups) was observed during all periods of reloading.

The mean values of the twitch and tetanus tensions in the plantaris muscle at day 0 ranged in order from the control, HU, HUD down to the HUP group (twitch: 789 ± 27 mN, 585 ± 21 mN, 545 ± 58 mN, and 511 ± 19 mN; tetanus: 2988 ± 209 mN, 2058 ± 36 mN, 1965 ± 81 mN, and 1837 ± 15 mN, respectively; Fig. 10). The mean values of the twitch and tetanus tensions of the HU, HUD, and HUP groups were significantly smaller than those of the control group at day 0 ($P < 0.03$), but the values in the HU, HUD, and HUP groups appeared to be almost the same. However, the twitch and tetanus tensions of the HU group gradually increased over time, while those values showed little change on days 3 and 7 in the HUD and HUP groups.

Beam walking test

The scores of the beam walking test in the HU, HUD and
HUP groups were significantly lower than those in the control group at days 0 and 3 of reloading ($P = 0.01$; Fig. 11A). The scores of the HUD and HUP groups were significantly lower than those of the HU group at days 0 and 3 ($P < 0.001$). There were no significant differences in these scores between the HUD and HUP groups at any time. However, at day 7 of reloading, the scores of the HU, HUD, and HUP groups were identical values to those in the control group.

**Range of the motion on ankle joint**

The range of motion on plantarflexion in the HUD group on days 0 and 3 was significantly larger than that in the control, HU and HUD groups ($P = 0.02$; Fig. 11B). The range of motion on dorsiflexion in the HUP group was significantly smaller than that in the control, HU, and HUD groups during the same periods ($P < 0.001$). The range of motion of both plantarflexion and dorsiflexion were similar among the four groups on day 7 of reloading.

The recovery of the range of motions was also similar to the recovery pattern in the beam walking test. There was a significant positive correlation between the scores of the beam walking test and the range of motion on plantarflexion ($P = 0.01, r = 0.41$). In addition, the scores of the beam walking test and range of motion on dorsiflexion had a significant negative correlation ($P = 0.01, r = −0.50$).

**Discussion**

The present study demonstrated that the muscle wet weight, cross sectional area of muscle fibers, and muscle contractile tension in the soleus muscle on day 0 of reloading all decreased in order from the control, HUD, HUP to the HU group, indicating that the HU group was the most atrophied among the four groups. On the other hand, those values in the plantaris muscle on day 0 decreased in order from the control, HU, HUD to the HUP group, which indicates that the HUP group was most atrophied among the groups. Therefore, fixing muscles at a stretched position might be effective to reduce muscle atrophy, since muscle atrophy in both the soleus and plantaris muscles was severer in the HUP group than in the HUD group. This is also consistent with the findings in previous studies (Williams and Goldspink, 1973; Baker and Mastumoto, 1988; Ohira et al., 1997). However, we showed that muscle atrophy in the soleus muscle was severer in the HU group than in the HUP group. The muscle length in the HU group was slightly longer than in the HUP group, and the ankle joint was kept at the lightly plantarflexion position because hindlimb unloading was applied without any ankle joint immobilization (Loughna et al., 1986; Riley et al., 1990). These findings suggested that muscle atrophy might depend not only on the static muscle length, but also on some other factors.
A previous study showed that isometric contractile activity can occur in the soleus and plantaris muscles when the ankle joint is fixed with a cast during hindlimb unloading (Baewer et al., 2004), and isometric contraction with ankle joint immobilization results in greater protection from muscle atrophy of the slow muscle than the fast muscle (Fitts, 2003; Hurst and Fitts, 2003). On the other hand, the present study showed that the soleus muscle was chiefly composed of type I (slow) fibers and the plantaris muscle, type IIB (fast) fibers. Therefore, the soleus muscle can be regarded as a slow muscle, and the plantaris muscle, as a fast muscle, respectively. The present results on day 0 of reloading were also very similar to a previous study in which the tibialis anterior and soleus muscles of the mice were observed (Fujita et al., 2009). All these findings taken together suggest that muscle fiber type and contractile properties are also essential factors that affect muscle atrophy.

The descending order of the values of the muscle wet weight, cross sectional area of muscle fibers, and muscle contractile tension during the recovery from muscle atrophy in the soleus and plantaris muscles among four groups on days 3 and 7 of reloading was the same on day 0. This suggests that the recovery from the muscle atrophy after reloading largely depends on the degree of the atrophy that occurs during the unloading. However, the values of muscle wet weight, cross sectional area of muscle fibers, and muscle contractile tension in both the soleus and plantaris muscles in the HUD and HUP groups recovered more slowly than in the HU group during the reloading period. Why did this difference in the recovering pattern occur between the groups with and without immobilization? Our findings showed that several muscle fibers in the soleus muscle in the HUP group exhibited a central core lesion at day 0 of reloading, and a reloading-induced lesion was observed both in

Fig. 9. Twitch (A) and tetanus (B) contractile tensions (mN) of the soleus muscle on days 0, 3, and 7 of reloading. The mean values of the twitch and tetanus contractile tension ranged in order from the control, HUD, HUP to the HU group during reloading periods. These values in the HUD and HUP groups recovered more slowly than those in the HU group. a, b and c: significantly different from control, HU, and HUD, respectively; e: significantly different from day 0, at $P < 0.05$. Values are the means ± standard error.

Fig. 10. Twitch (A) and tetanus (B) contractile tensions (mN) of the plantaris muscle on days 0, 3, and 7 of reloading. The mean values of the twitch and tetanus contractile tension ranged in order from the control, HUD, HUP to the HU group during reloading periods. These values in the HUD and HUP groups recovered more slowly than those in the HU group. a, b and c: significantly different from control, HU, and HUD, respectively; e: significantly different from day 0, at $P < 0.05$. Values are the means ± standard error.
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the soleus muscle in the HUD and HUP groups. Thus, the appearance of these lesions might be related to the difference in the recovery pattern between the groups with and without immobilization.

Previous studies demonstrated that a central core lesion due to missing myofibrils is produced by a continuously shortened state (Abou Salem et al., 2001; Baewer et al., 2008) and is inhibited by a stretched state (Baewer et al., 2004, 2008). The central core lesion also occurs mainly in slow muscle fibers (Murakami et al., 2008). In this study, although the plantaris and soleus muscles in the HU and HUP groups were kept at a shortened position, the central core lesion was extremely rare in the plantaris muscle and soleus muscle in the HU group. The ATPase histochemistry also showed that the central core was composed of type I (slow) fibers. We also showed that isometric contraction occurred in the soleus muscle in the HUP group because of the ankle joint immobilization with a cast, while isotonic contraction occurred in the muscle in the HU group. It has been established that increased calcium in chronically shortened muscle induces the central core lesion (Baewer et al., 2008). Thus, the amount of intracellular calcium released from the sarcoplasmic reticulum might be larger in the HUP group than in the HU group because contractile activity of the soleus muscle by isometric contraction was greater than that by isotonic contraction (Hurst and Fitts, 2003). Removing the contractile activities by denervation inhibits the formation of a central core lesion (Talesara and Jasra, 1986), suggesting that the elevation of intracellular calcium by muscle contractile activities might be one reason for the formation of the central core lesion in the soleus muscle in the HUP group. However, it is difficult to decide whether the central core lesion is due to protective adaptation or degeneration from the present findings although electron microscopic observations show that aggregates of mitochondria predominantly occur at the intact peripheral zone of the central core lesion (Abou Salem et al., 2001).

In this study, muscle fibers exhibiting degenerating features were also observed in the soleus muscle in the HUD and HUP groups after reloading. The mechanism that induces the reloading-induced lesion might be different from that of the central core lesion because the morphological appearance of the reloading-induced lesion was quite different from that of the central core lesion. The sarcomere length shortens, and muscle elasticity decreases due to immobilization for 1 week (Okita et al., 2004). Therefore, the elasticity of the soleus and plantaris muscles in the HUD and HUP groups may have decreased during the immobilization of the ankle joint for 14 days. These muscles in the HUD and HUP groups were more susceptible to reloading than in the HU group because the ankle joint was not immobilized in the HU group. Vijayan et al. (1998) reported that reloading-induced muscle lesions occur preferentially in type I fibers. In the present study, serial sections showed that the reloading-induced lesion was mainly in type I fibers in the soleus muscle in

Fig. 11. Beam walking test scores (A) and range of motion (°) on ankle joint (B) on days 0, 3, and 7 of reloading. At days 0 and 3, the scores of beam walking test in the HUD and HUP groups were very low and ranges of motion on plantarflexion and dorsiflexion were restricted in the HU and HUP groups, respectively. Both the scores of beam walking test and ankle motion in the HU, HUD, and HUP groups were similar to those of the control group at day 7, so there was a clear correlation between the recovery of walking ability and ankle motion. a, b, c and d: significantly different from control, HU, HSD, and HUP, respectively, at \( P < 0.03 \). Values are the means ± standard error.
the HUD and HUP groups.

The scores of the beam walking test in the HUD and HUP groups on days 0 and 3 were significantly lower than those in the control and HU groups, but, they recovered to those in the control group on day 7. The range of motion on plantarflexion and dorsiflexion at days 0 and 3 in the HUD and HUP groups was severely restricted, but, this recovered to the level in the control group at day 7. There was a close relationship between the recovery of the score of the beam walking test and that of the ankle motion. On the other hand, although the twitch and tetanus contractile tension of the soleus and plantaris muscles in the HU, HUD, and HUP groups gradually recovered after the reloading, they did not fully recover to that in the control group by 7 day of reloading. This recovery pattern paralleled the fact that the muscle wet weight and cross sectional area of muscle fibers and the muscle atrophy in the HU, HUD, and HUP groups be remained at day 7. These findings suggest that the recovery from the muscle atrophy might not yet be complete even if the dysfunction assessed by walking ability and range of motion appears to have become normal. Riley (1998) indicated that rapid recovery of the ability to walk is a function of the central nervous system undergoing relearning rather than a recovery from the muscle atrophy. In the present study, the improvement of the walking ability in the HU, HUD, and HUP groups on day 7 suggests the possibility of compensatory neuromuscular recruitment. In addition, the susceptibility of muscles to lesions was increased at the early phase after reloading (Warren et al., 1994; Ploutz-Snyder et al., 1996). Rehabilitation therapy should be carried out until there is sufficient recovery of muscular contractile tension to provide an optimal recovery from muscular atrophy.

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