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(Citation)

Bulletin of health sciences Kobe, 27:19-26

(Issue Date)

2011

(Resource Type)

departmental bulletin paper

(Version)

Version of Record

(JaLCD0I)

<https://doi.org/10.24546/81003791>

(URL)

<https://hdl.handle.net/20.500.14094/81003791>



Influence of magnetic stimulation on muscle atrophy in the rat unloading hindlimb muscles

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Abstract

The influence of magnetic stimulation on muscle atrophy and changes in the composition of skeletal muscle fibers were examined in the rat soleus and plantaris muscles. Rats were divided into control (CON), hindlimb unloading (HU), and hindlimb unloading with magnetic stimulation (HUM) groups. Magnetic stimulation (20 s/time, 20 min) was applied to the posterior aspect of the lower thigh for 13 days. The means of the absolute and relative weights and cross-sectional areas of the soleus and plantaris muscles were not significantly different between the HU and HUM groups. The composition of muscle fiber types of the plantaris muscle in the HUM group were not significantly different from those in the HU group, but the values of type I fiber of the soleus muscle was significantly higher in the HUM group than in the HU group. These results indicate that while magnetic stimulation was unable to reduce muscle atrophy induced by hindlimb unloading, it reduced the changes in the properties of the muscle fibers, which suggests that the mechanism to induce muscle atrophy and to control the composition of skeletal muscle fibers may be different.

Key words: muscle atrophy, magnetic stimulation, muscle fiber type

INTRODUCTION

It is well known that weight unloading on skeletal muscle results in severe muscle atrophy¹⁻⁵⁾. Since muscle strength would weaken²⁻⁵⁾ and motor ability decline^{3, 4)} if muscle mass and size were to decrease, the prevention or reduction of muscle atrophy may be very important for patients who are required to receive long periods of bed rest. Besides the loss of muscle weight and size, the composition of muscle fiber types (Type I and Type II) changes as muscle atrophy progresses^{3, 5-7)}. Each skeletal muscle has an individual composition of muscle fiber types⁸⁻¹⁰⁾. Maintaining this composition may be very important for the adequate functioning of each muscle.

It has been reported that electrical stimulation of skeletal muscles is effective in preventing or reducing muscle atrophy¹¹⁻¹⁵⁾. However, when electrical stimulation is applied from the surface of the body, it cannot reach muscles that are deeply located unless a relatively strong current is applied. When this occurs, the patient experiences a very distasteful pain. It has been reported that magnetic stimulation induces an electrical current in the tissues¹⁶⁻¹⁹⁾ that can reach a relatively deeper zone^{18, 19)} than electrical stimulation without producing any distasteful pain^{18, 19)}. Thus, magnetic stimulation may be an effective measure to prevent and reduce muscle atrophy. However, the effects of magnetic stimulation on the prevention of muscle atrophy have not yet been fully examined to date. Therefore, its influence on muscle atrophy was examined morphologically in rat lower leg muscles in this study. The hindlimb unloading method has been widely used to induce muscle atrophy in lower leg muscles^{1-6, 8-12)}. In this study, magnetic stimulation was applied to the posterior aspect of the lower leg every day for 2 weeks during hindlimb unloading, and wet muscle weight, cross-sectional area of muscle fibers, and ratio of muscle fiber types

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in the soleus and plantaris muscles were examined.

MATERIAL AND METHODS

Animals

Twenty-four 8-week-old male Wistar rats (Japan SLC, Shizuoka, Japan) were used in this study. These animals were randomly divided into three groups: control (CON; $n = 5$), hindlimb unloading (HU; $n = 5$), and hindlimb unloading with magnetic stimulation (HUM; $n = 5$) groups. All animal experiments were conducted according to the guidelines for animal experimentation at Kobe University School of Medicine.

Hindlimb unloading

Hindlimb unloading was applied to the animals by suspending them by their tails for 14 days according to the modification of the method described by Morey et al.²⁰⁾. Briefly, each animal belonging to the HU and HUM groups was fitted with a tail harness and suspended by a string high enough to prevent its hindlimbs from bearing weight on the cage floor. The forelimbs were allowed to maintain contact with the floor, and all animals were allowed to move freely to access food and water. The animals in each group were housed in an isolated and environment-controlled room at 25°C with a 12:12 h light-dark cycle.

Magnetic stimulation

In the HUM group, the right posterior lower thigh was stimulated with a magnetic stimulator (Magstim 200, The Magstim Company Limited, UK; maximum magnetic field strength of 2.0 Tesla) during the experimental periods. Forty percent of the maximum output of this stimulator was used, the stimulation duration was approximately 1 ms with a 100 μ s rise time in each pulse, and the repetition rate of the stimuli was 20 s/time. The ankle joint movement in response to the magnetic stimulation was checked visually. The stimulation was carried out for 20 min every day for 13 days.

Morphological analysis

After 14 days of the above-mentioned housing, all the animals were deeply anesthetized, and their soleus and plantaris muscles were removed. Each muscle was cleaned of fats and connective tissues. The wet weight of these muscles was measured, and the relative muscle weight (mg/g) was calculated by dividing the value of the muscle weight (mg) by that of the body weight (g). The animals were then euthanized by intraperitoneal injection of a large dose of anesthesia. Some samples were embedded in optimum cutting temperature compound and traga-canth gum, immediately frozen in acetone cooled by dry ice, and stored at -80°C until used. From the middle part of the muscle belly of the soleus and plantaris muscles, serial cross sections of 10 μ m thickness were prepared using a cryostat (Shiraimatu, Osaka, Japan) and mounted on glass slides. Some sections were stained with hematoxylin and eosin. Others were stained using a histochemical method for myofibrillar adenosine triphosphatase (ATPase), with preincubation at pH 4.1. Type I, IIA, and IIB fibers were morphologically identified according to the criteria by Brook and Kaiser²¹⁾. Cross-sectional areas of each muscle fiber type were measured using an image analyzer (ImageJ, NIH, USA). The composition ratio of each muscle fiber type was also calculated.

Statistical analysis

Means and standard deviations (SDs) were calculated from individual values. Statistical differences were determined using a one-way analysis of variance (ANOVA). When an ANOVA was significant, group differences were determined using Scheffe's post hoc test. $p < 0.05$ was considered statistically significant.

RESULTS

Muscle weights

The mean \pm SD of the wet and relative muscle weights of the soleus and plantaris muscles is presented in Fig. 1. These values of the soleus muscle were 41.4 (mg) and 0.24 (mg/g) in the HU group and 43.2 (mg) and 0.24 (mg/g) in the HUM group; those of the plantaris muscle were 156.2 (mg) and 0.89 (mg/g) in the HU group and 150.8 (mg) and 0.84 (mg/g) in the HUM group. These values were significantly smaller in the HU and HUM groups than in the CON group, but there were no statistically significant differences in these values between the HU and HUM groups. The means of the wet and relative weights of the soleus muscle were 55% and 47% lesser in the HU group and 53% and 47% lesser in the HUM group than those in the CON group, while the means of the wet and relative weights of the plantaris muscle were 30% and 17% lesser in the HU group and 32% and 26% lesser in the HUM group than those in the CON group. It appears that the reduction rate of the soleus muscle in the HU and HUM groups was more than that of the plantaris muscle in these groups.

ATPase histochemistry

Muscle fibers in the soleus and plantaris muscles were divided into 3 types by ATPase histochemistry: type I, type IIA, and type IIB.

1) Cross-sectional area of muscle fibers

The cross-sectional area of each fiber type of the soleus and plantaris muscles in the CON, HU, and HUM groups is presented in Fig. 3. With regard to the soleus muscle, the cross-sectional areas of types I, IIA, and IIB

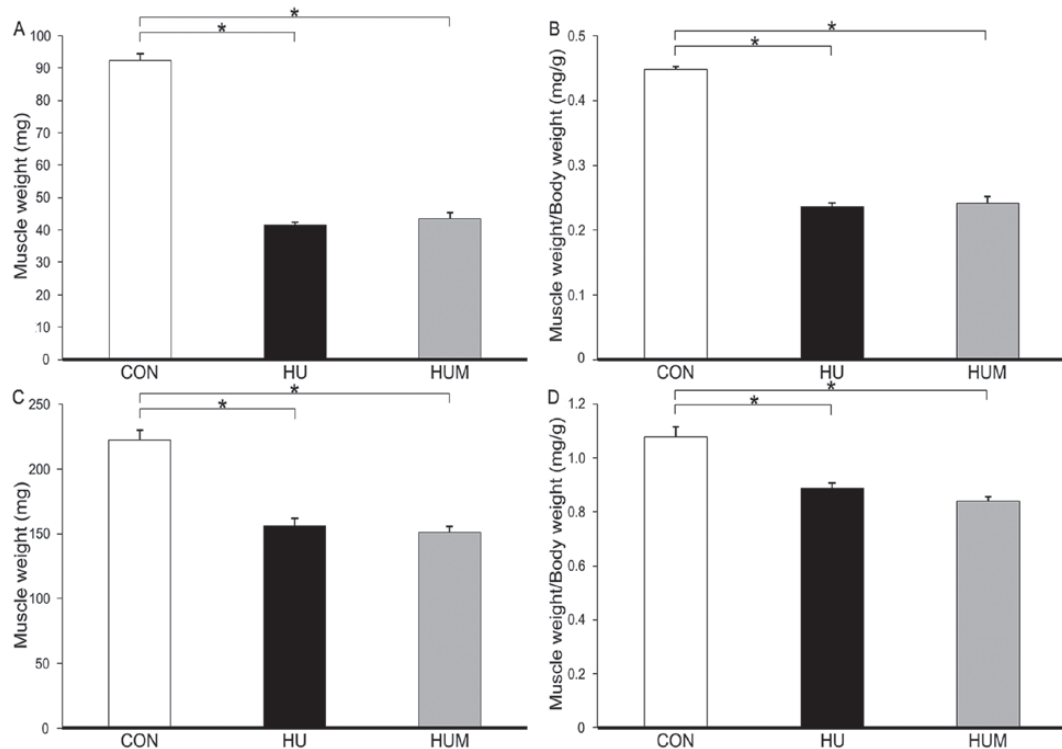


Figure 1. Muscle weight (mg; A, C) and relative muscle weight (mg/g; B, D) of the soleus (A, B) and plantaris (C, D) muscles. The wet and relative weights of the soleus muscle were 55% and 47% less in the HU group and 53% and 47% less in the HUM group than those in the CON group. Whereas, the wet and relative weights of the plantaris muscle were 30% and 17% less in the HU group and 32% and 26% less in the HUM group than those in the CON group. *: significantly different from the CON group at $p < 0.05$

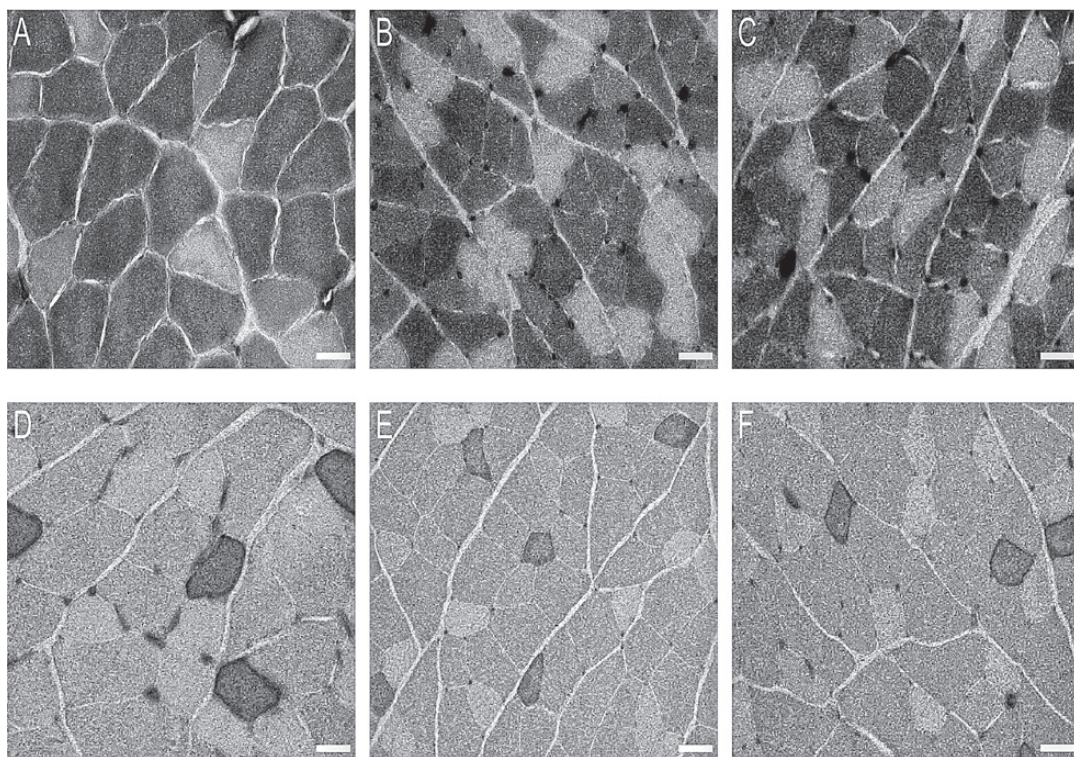


Figure 2. Photographs of cross sections of histochemical myosin ATPase staining in the soleus (A–C) and plantaris (D–F) muscles. A, D: CON group; B, E: HU group; C, F: HUM group. Bar = 30 μm

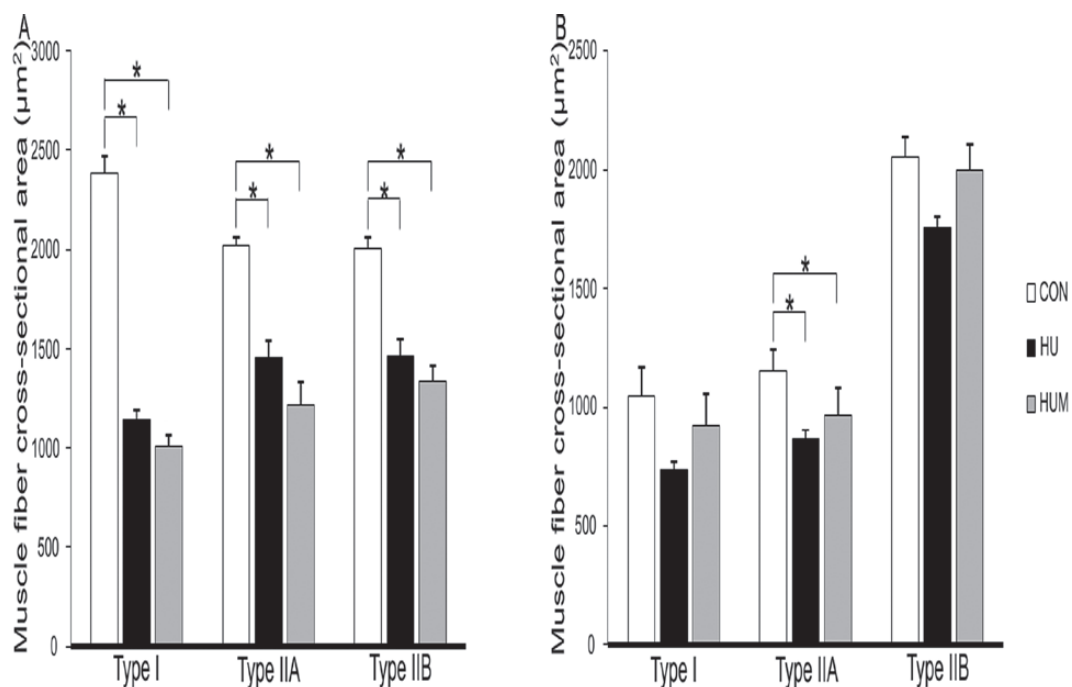


Figure 3. The mean values of the cross-sectional areas (μm^2) of each fiber type of the soleus (A) and plantaris (B) muscles. With regard to the soleus muscle, the cross-sectional areas of types I, IIA, and IIB were 52%, 28%, and 27% smaller in the HU group and 58%, 40%, and 33% smaller in the HUM group than those in the CON group. With regard to the plantaris muscle, these values of the type IIA fiber were 25% and 16% smaller in the HU and HUM groups, respectively, than those in the CON group. *: significantly different from the CON group at $p < 0.05$.

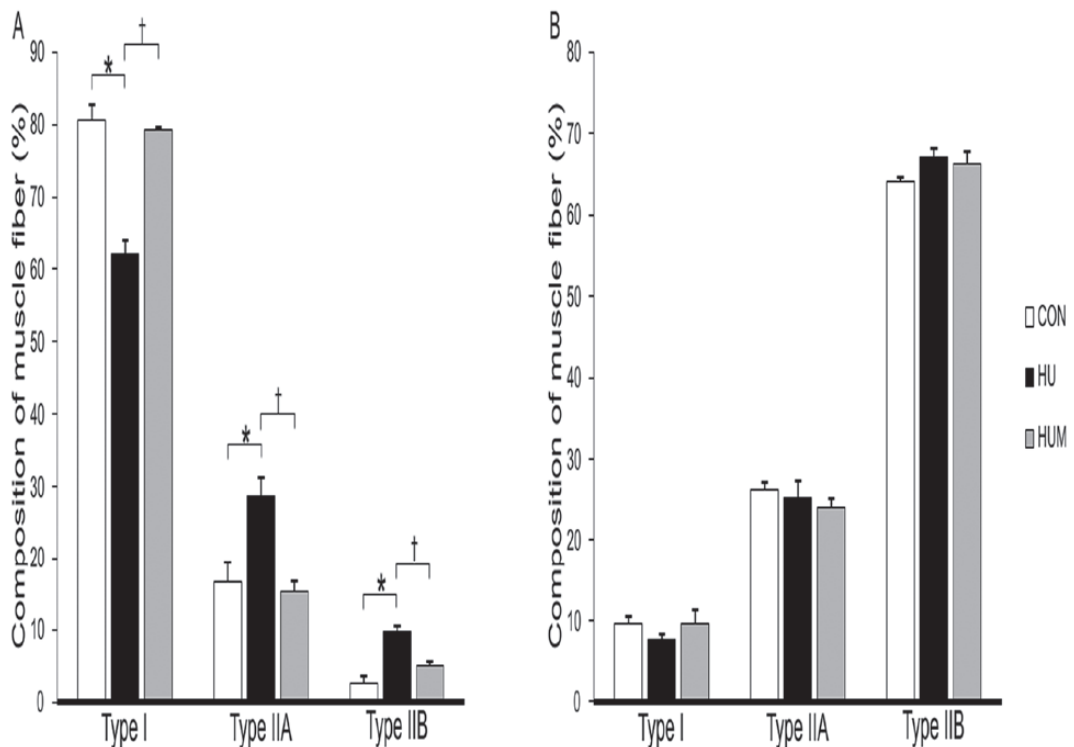


Figure 4. Composition ratios (%) of each muscle fiber type in the soleus (A) and plantaris (B) muscles. * and †: significantly different from CON or HU group, respectively, at $p < 0.05$

were 52%, 28%, and 27% smaller in the HU group and 58%, 40%, and 33% smaller in the HUM group than those in the CON group (Fig. 3A). These values of the soleus muscle in the HU and HUM groups were not significantly different. With regard to the plantaris muscle, the cross-sectional areas of type I and IIB were not significantly different among the three groups (Fig. 3B), but those of type IIA appeared to be significantly smaller in the HU and HUM groups than in the CON group. These values of the type IIA fiber were 25% and 16% smaller in the HU and HUM groups than those in the CON group.

2) Composition of fiber types

The composition of fiber types of the soleus and plantaris muscles in the CON, HU, and HUM groups is presented in Fig. 4. The soleus muscle in the CON group consisted of 80.7% type I, 16.6% type IIA, and 2.7% type IIB fibers (Fig. 4A). In the HU group, the percentage of type I was significantly lower (18.7%) and the percentages of types IIA and IIB were significantly higher (12.1 and 7%, respectively) than those in the CON group (Fig. 4A). In addition, the percentage of type I fiber of the soleus muscle in the HUM group was 17.3% higher and the percentages of types IIA and IIB fibers were significantly lower (13.4% and 4.7%, respectively) than those in the HU group (Fig. 4A), indicating that muscle fiber type transformation was prevented by the magnetic stimulation. The plantaris muscle in the CON group was composed of 9.7% type I, 26.3% type IIA, and 64.0% type IIB fibers (Fig. 4B). There were no significant differences in the percentages of type I, IIA, and IIB fibers among the CON, HU, and HUM groups.

DISCUSSION

Hindlimb unloading through the tail suspension method has been widely used as a measure to induce muscle

atrophy^{1-6, 8-12)}. In this study, muscle atrophy was induced by the hindlimb unloading method, and the influence of magnetic stimulation on such atrophy was examined in the rat soleus and plantaris muscles.

Although the wet and relative muscle weights evidently decreased in both the soleus and plantaris muscles after 14 days of hindlimb unloading, muscle atrophy was more apparent in the former than in the latter. It was reported that type I muscle fiber atrophied more severely than other fiber types due to hindlimb unloading⁸⁻¹⁰⁾. Furthermore, cross-sectional areas of the type I fiber were more severely reduced than those of other muscle types. It is well known that the soleus muscle is mainly composed of type I muscle fibers, while the plantaris muscle contains only a few type I fibers⁸⁻¹⁰⁾. This may be the main reason why atrophy of the soleus muscle is more severe than that of the plantaris muscle.

Boonyarom et al.¹²⁾ reported a partial compensation of muscle atrophy using electrical stimulation (20 Hz, 15 min) based on morphological examination. According to histochemical findings, Shimada et al.¹⁶⁾ reported a partial recovery of muscle atrophy by applying magnetic stimulation (20 Hz, 60 min). However, there were no significant differences in wet muscle weight, relative muscle weight, or the cross-sectional areas of any muscle type of the soleus and plantaris muscles between the HU and HUM groups in this study. Our findings suggest that the magnetic stimulation used in this study was unable to reduce the muscle atrophy induced by hindlimb unloading. It has been reported that muscle atrophy is often accompanied by changes in the composition ratio of the muscle fiber types^{3, 5-7)}. Therefore, this study has succeeded in producing identical histochemical characteristics with disuse muscle atrophy similar to previously reported studies^{1-6, 8-12)}. Our histochemical observation revealed that the composition ratio of type I decreased, while that of types IIA and IIB increased in the soleus muscle due to hindlimb unloading (HU group). These changes were significantly reduced by the magnetic stimulation applied during hindlimb unloading (HUM group). These results suggest that magnetic stimulation may be effective in reducing muscle fiber type transformation in disused soleus muscles.

As mentioned above, although magnetic stimulation could not reduce muscle fiber atrophy, it may be effective in reducing muscle fiber type transformation in disused soleus muscles. It has been reported that maintenance of muscle mass is controlled by a balance between protein synthesis and the protein degradation pathway²³⁻²⁵⁾, and the shift toward protein degradation results in muscle atrophy^{25, 26)}. On the other hand, it has been reported that the ATPase activity of muscle fibers is largely determined by the MHC isoforms expressed in each fiber²⁷⁾, and MHC gene expression is regulated at the transcriptional and/or pretranslational level²⁸⁾. These findings suggested that there may not be common regulators for the mechanisms involved in muscle atrophy and the muscle fiber type transformation, and that magnetic stimulation may only be able to affect the regulation mechanism of MHC gene expression, which determines muscle fiber type at transcriptional and/or pre-translational level.

The results of magnetic stimulation in this study, which was performed under selected experimental conditions, are valuable for elucidating the repair or prevention of skeletal muscle atrophies. This painless stimulation may find extensive application in pediatric patients, electric-sensitive patients, and patients with skin lesions. The characters of deep stimulation are beneficial for trigger effect of muscle contraction in an antigravity muscle bulk or immobilized extremity in a plaster cast. Magnetic stimulation may provide an effective treatment mode for retarding disuse muscle atrophy.

Acknowledgments

We would like to thank Dr. Mitsuru Miyamoto, Miss Momoko Nagai, Miss Yuna Miyagi, Mr. Ryo Takagi, and other members of our laboratory staff for their assistance with the experiments.

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