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Original Article

Study on change of rat soleus extensibility caused by reduction in joint movement with unweighting of the hind limbs

Yuta Sato, MS, RPT[1)*], Takeya Ono, PhD, RPT[1, 2)], Hideki Ishikura, PhD, RPT[3)], Kazuki Aihara, PhD, RPT[4)], Tomohiro Matsumoto, MS, RPT[5)], Namiko Umei, PhD, RPT[1, 2)], Wakako Tsumiyama, PhD, RPT[2)], Sadaaki Oki, PhD, MD[1, 2)]

[1)] *Graduate School of Comprehensive Scientific Research, Prefectural University of Hiroshima: 1-1 Gakuen-cho, Mihara City, Hiroshima 723-0053, Japan*
[2)] *Department of Physical Therapy, Faculty of Health and Welfare, Prefectural University of Hiroshima, Japan*
[3)] *Department of Rehabilitation/Physical Therapist, Faculty of Health Sciences, Hiroshima Cosmopolitan University, Japan*
[4)] *Department of Rehabilitation Science, Osaka Health Science University, Japan*
[5)] *Department of Rehabilitation Science, Kobe University Graduate School of Health Sciences, Japan*

Abstract. [Purpose] The purpose of this study was to investigate the change in soleus extensibility from a joint contracture caused by a reduction in joint movement with unweighted lower limbs and to interpret the results to aid in the treatment of human joint contractures. [Subjects and Methods] The subjects of this study were twenty-one 10-weeks-old male Wistar rats. Twenty-one rats were divided into one control (C) and two experimental groups. The first experimental group included fixed right ankle joints in full plantar flexion (F). The second experimental group's hind limbs were suspended and the right ankle joints were fixed in full plantar flexion (FS). The period of this study was one week. On the first and last day of this study, all of the rats' ankle dorsiflexion angles were measured. On the last day of this study, all of the rats' soleus extensibilities were measured. [Results] On the last day of this study, the ankle dorsiflexion angles and the soleus extensibility in the FS group were significantly more decreased than those of the F group. [Conclusion] It was shown that the skeletal muscle extensibility from joint contracture caused by reduction of joint movement with unweighted lower limbs was more markedly decreased than that from joint contracture caused by reduction of joint movement.
Key words: Joint contracture, Joint fixation, Hind limb suspension

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INTRODUCTION

Joint contracture is one of the symptoms of the disuse syndrome[1, 2)] caused by inactivity from a reduction in joint movement[3)]. The definition of joint contracture is a limitation in joint ROM derived from an organic change in periarticular soft tissues, such as skeletal muscle, skin, ligament, tendon, and joint capsule[4)]. Since patients with joint contractures have many difficulties in daily life[5, 6)] and an increase in the possibility of falling[7)], it is important to treat joint contractures

On the other hand, unweighting of the lower limbs, such as a inactivity resulting from bed rest, is one of the causes of the disuse syndrome[1, 2)]. Unweighting the lower limbs from the microgravity environment of the universe may cause joint contractures in the human body[8)]. Furthermore, joint movement decreases with hind limb suspension[9–16)] during an animal

*Corresponding author. Yuta Sato (E-mail: pdntk7562@yahoo.co.jp)

experiment model that reproduces the unweighting of the lower limbs[9]. From this, it is considered that unweighting the lower limbs might cause joint contracture in humans as well. Therefore, in a previous study[15, 16], we performed a combination of hind limb suspension and joint fixation[3, 4, 13–26], an animal experiment model that reproduces the reduction of joint movement. As a result, it was shown that the occurrence of joint contracture when performing joint fixation and hind limb suspension was more pronounced than when compared with only joint fixation.

The main factor for joint contracture caused by reduction of joint movement for one week is the reduction of the extensibility of skeletal muscle[3, 17]. Therefore, when a joint contracture was caused by reduction of joint movement with unweighting lower limbs for one week, it was estimated that reduction of the extensibility of the soleus muscle was severer than joint contractures caused by the reduction of joint movement only. However, how the change in the extensibility of skeletal muscle from joint contracture was caused by reduction in joint movement with unweighting lower limbs was not clarified.

Treatment for joint contractures depends on the type of tissue that is shortened. For example, a method for treatment of skeletal muscle is electrical stimulation[19]. On the other hand, the method for treating joint contractures caused by the skin is stretching[26]. Therefore, for effective treatment, it is very important to identify the involved tissue in the joint contracture caused by reduction of joint movement with unweighted lower limbs.

The purpose of this study was to try to determine a treatment for joint contractures by clarifying the cause of change in the soleus extensibility from a joint contracture when there is a reduction of joint movement with unweighted lower limbs.

SUBJECTS AND METHODS

Twenty-one 10-weeks-old male Wistar rats with a body weight of 335.3 ± 9.9 g (average ± SD) were used. The animals were housed in cages and given free access to standard rat food and water. A constant room temperature of 23°C in the animal care room was maintained by air conditioning, and the day-night cycles were artificially regulated by setting lights-on for 12 h and lights-off for 12 h every day. This experiment was performed according to the Regulations on Animal Experiments of the Prefectural University of Hiroshima and was approved by the University's Animal Experiments Committee (Approval number: 13MA009).

The period of this study was one week. Twenty-one rats were divided into one control (C, n=7) and two experimental groups. The first experimental group included fixed right ankle joints in full plantar flexion (F, n=7). The second experimental group's hind limbs were suspended, and the right ankle joints were fixed in full plantar flexion (FS, n=7).

The right hind limbs of all the rats in the F and FS groups were fixed in full plantar flexion by using non-elastic tape. The rats' right toes of the F and FS groups were exposed for confirmation of the presence or absence of edema. Protection for the joint fixation was performed by using wire mesh. The rewinding of the non-elastic tape was performed as appropriate when the tape loosened or edema was observed.

For the hind limb suspension, a 1.0 mm diameter Kirschner wire was inserted into the rat's tail to form a triangle and then combined with a swivel hook. Hind limb suspension was performed by hanging the swivel hook to the horizontal bar at the top of cage. The rats' hind limbs were thus set in an unweighted state. The swivel hook allowed 360° rotation for the rats. These rats could freely access standard food and water by using their fore limbs. All treatments for hind limb suspension were performed for the FS group. The pain in the rat's tail during hind limb suspension may have affected the joint contracture[15]. Therefore, insertion of a Kirschner wire to the rats' tails were also performed on the C and F groups.

On the first and last days of this study, all of the rats' angles for dorsiflexion of the right ankle joint were measured. On the last day of this study, the measurements of ankle joint ROM were performed after the joint fixation and hind limb suspension had been removed. Marking for measurement of ROM of ankle joint was performed on the head of fibula and the lateral malleolus of the rat. For the measurement of the ROM of the ankle joint, the rat was positioned on its side with the hip and knee joint fixed in flexion by using fixation equipment[27]. A force of 0.3 N was applied perpendicularly to the sole of the foot for passive dorsiflexion by using a tension meter (LTS-1KA; Kyowa Electronic Instruments Co., Ltd., Japan). This dorsiflexion force of 0.3 N is a force that is enough to move a normal rat's ankle joint to the full dorsiflexion position[18]. While the force was applied, a digital camera recorded the test from directly above the hind limb. Recorded videos were downloaded to a computer to extract still images of dorsiflexion at the moment of application of the 0.3 N force. The ankle dorsiflexion angles were measured from the still images using computer software (ImageJ version 1.48v; USA). The ankle dorsiflexion angles were defined as the angle obtained from a line connecting the head of fibula and the lateral malleolus and a line parallel to the bottom of the heel to eliminate forefoot movement from the measurement. The ankle dorsiflexion angles were measured 3 times, and the mean value was used in the analysis.

On the last day of this study, to measure the degree of soleus extensibility, the length-tension curve of the soleus was determined by using an Autograph (AG-50kNG, Shimadzu Co., Japan). The ankle was positioned in full plantar flexion, and the tibia and calcaneus were fixed with a 0.7 mm diameter Kirschner wire. After the rat was sacrificed, the skin on the right hind limb and the gastrocnemius were removed surgically. The femur was cut above the origin of the gastrocnemius and was mounted on the Autograph with the upper clamp. The tarsal bone was mounted with the lower clamp. The fibula, tibia, and all lower leg muscles, except for the soleus, were cut. Tensile test of soleus was performed at an extension rate of 10 mm / min. Since the soleus was extended approximately 10 mm from the ankle in full plantar flexion to the ankle in full dorsiflexion[19, 22], the value of the measurement was the tension torques of the soleus when it was in the 10 mm lengthened

Table 1. The ROM of ankle joint dorsiflexion (in degrees)

Groups	The first day of the study	The last day of the study
C	130.0 ± 2.5	129.2 ± 5.3
F	129.9 ± 2.5	101.5 ± 10.0*,a
FS	131.0 ± 4.1	74.6 ± 8.2*,a,b

Values are Mean ± SD.
*Significant decrease compared with the first and last day of the study (p<0.05).
[a]Significant decrease compared with the C group (p<0.05).
[b]Significant decrease compared with the F group (p<0.05).

Table 2. The changes in soleus muscle extensibility (in Newton)

Groups	The measured tension torque (N)
C	0.23 ± 0.13
F	0.58 ± 0.32[a]
FS	2.13 ± 0.44[a,b]

Values are Mean ± SD.
[a]Significant increase compared with the C group (p<0.05).
[b]Significant increase compared with the F group (p<0.05).

position. The tensile test was performed within 20 minutes after being sacrificed. Joint fixation, hind limb suspension, the measurement of ROM of ankle joint, and sacrifice in this study were performed under anesthesia.

A two-way analysis of variance was used for the analysis of the ankle dorsiflexion angle. The independent variables were the day of the ROM measurement (the first day of the study × the last day of the study) and the groups (C group × F group × FS group). The dependent variable was the ankle dorsiflexion angle. If a significant interaction was found, the simple main effect was calculated for each independent variable. The Tukey's test was used for the post hoc comparison. Also, the non-parametric Kruskal-Wallis test was used for the analysis of the soleus extensibility. The Steel-Dwass test was used for the post-hoc comparison. All statistical analyses were performed by using the SPSS ver. 20 for Windows (SPSS Inc., Chicago, IL, USA) and Ekuseru Toukei 2012 ver. 1.01 (Social Survey Research Information Co., Ltd., Tokyo, Japan). A significant difference was concluded to exist at a probability value of less than 5% in all of the statistical tests.

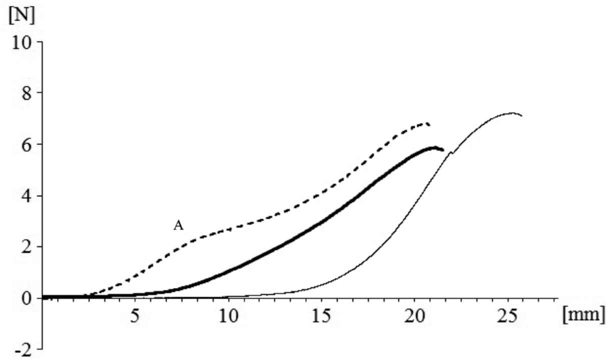

Fig. 1. The length-tension curve of soleus muscle (central value).
The thin line is the C group.
The thick line is the F group.
The dotted line is the FS group.
A: The tension torque of the FS group showed a marked increase at this point.

RESULTS

The mean and standard deviation of the ankle dorsiflexion angle of each group on the first and last days of this study are shown in Table 1. Statistically, it was shown that there was an interaction between the independent variables. Therefore, the simple main effect was calculated for each independent variable. First, the independent variable for the day of the ROM measurement of the first day of this study showed no significant difference between all groups. But on the last day of this study, the ankle dorsiflexion angles in the F and FS groups were significantly lower than in the C group. The decreased angles of dorsiflexion in the FS group were significantly severer than that in the F group. Second, the independent variable the groups showed a significant difference between the first and last days of this study in the F group and FS group, but not in the C group.

The mean and standard deviation of the tension torques at the 10 mm lengthened point of the soleus muscle of each group are shown in Table 2. The soleus extensibility of the F and FS groups was significantly smaller than those of the C group. The soleus extensibility of the FS group was significantly smaller than that in the F group. The central value of the length-tension curve of soleus in each group are shown in Fig. 1. The length-tension curves in the F and FS groups showed more of a left shift than that in the C group. The length-tension curve in the FS group showed more of a left shift than that of the F group. The tension torque in the FS group showed a sharp increase at an earlier stage as compared with the other groups, and gradually increased thereafter.

DISCUSSION

When ankles were immobilized in full plantar flexion for one week, the ankle dorsiflexion angles were severely decreased by unweighting the lower limbs[15, 16]. In the joint contractures caused by joint fixation for one week, the skeletal muscle composed approximately 80.5% of the proportion[17]. Therefore, the main factor of the joint contractures caused by joint fixation for one week was the reduction in skeletal muscle extensibility, and it is supposed that it was the same even in cases with unweighing the lower limbs. In this study, we examined the change in soleus extensibility for joint fixation with hind limb suspension for one week.

First, we considered the ankle dorsiflexion angle. The ankle dorsiflexion angles in the F and FS groups on the last day of this study were significantly less than those on the first day. On the last day of this study, the ankle dorsiflexion angles in the FS group were significantly less than that in the F group. The results of the ankle dorsiflexion angle in this study were the same as in previous studies[15, 16]. Therefore, also in this study, it was clarified that unweighting the lower limbs caused severe joint contractures induced by joint fixation.

Next, we considered the soleus extensibility in the 10 mm lengthened position. Generally, skeletal muscle extensibility is assessed by obtaining the length-tension curve when the muscle is stretched passively by using an Autograph[3, 16, 19, 22–24]. The tension torque of the soleus muscle of normal rats in the length-tension curve gradually increased, but did not increase until the 10 mm lengthened position. The length-tension curve of the soleus muscle when reducing the extensibility by joint fixation shifts more to the left than that of a normal soleus, and the tension torque increases earlier[22, 24]. In this study, the length-tension curves in the F and FS groups shifted further left than that in the C group, and the tension torque in the F and FS groups increased earlier than that in the C group (Fig. 1). Also, the results showed that in the 10 mm lengthened position the soleus extensibility of the F and FS groups was significantly more decreased than that in the C group. Without muscle contraction, the skeletal muscle extensibility decreased through the fascia[4]. The main component in the fascia is collagen[4]. The soleus showed an increased collagen[4] content and decreased extensibility[3] after joint fixation for one week. Therefore, it was considered that the soleus extensibility in the F and FS groups were decreased by the increased collagen in the fascia. So why was the soleus extensibility in the FS group decreased more than that of the F group? Performing hind limb suspension for one week caused an increase in collagen[10]. Therefore, it was considered that the soleus extensibility in the FS group was decreased by a markedly increased collagen in the fascia.

Next, we considered the length-tension curve of soleus in the FS group. The length-tension curve of the soleus muscle of the FS group shifted more to the left than that of a normal soleus, and the tension torque increased markedly earlier as the period of joint fixation was extended[22]. In this study, the length-tension curve of soleus in the FS group shifted more to the left than that in the F group, and the tension torque markedly increased until point A, as seen on the graph (Fig. 1). Therefore, it is assumed that the change which occurred in the soleus muscle of the FS group may be a result of the joint fixation being more longer than one week. Collagen increase differs for endomysium and perimysium with the extension period of joint fixation[4]. The collagen of the perimysium is increased by joint fixation, but is not increased further if the period of joint fixation is extended[4]. On the other hand, the collagen of the endomysium is increased by joint fixation and increases further if the period of joint fixation is extended[4]. Therefore, it was considered that the decreased skeletal muscle extensibility by joint fixation was particularly affected by the endomysium. In other words, it is assumed that the extensibility of skeletal muscle is reflected particularly in the extensibility of the endomysium. In this study, it was estimated that the tension torque would increase more markedly earlier and the length-tension curve would increase more markedly in the FS group due to a more marked decrease in the endomysium extensibility as compared with those in the other groups. Therefore, the soleus extensibility in the FS group decreased more than that in the F group.

The new finding in this study is that the joint contracture progressed in a short period since the skeletal muscle extensibility for joint contractures markedly decreased, which was caused by a reduction in joint movement with unweighting of the lower limbs. Therefore, it is suggested that the treatment for joint contractures caused by a reduction in joint movement with unweighted lower limbs should be performed for skeletal muscles. The limitation in ROM was restored nearly completely when the period of reduction in joint movement was within 30 days, incompletely for 40 days, and no recovery for 60 days or more[20]. Therefore, it is considered that the prognosis of joint contracture depends on whether the period of reduction of joint movement is shortened as much as possible. Moreover, if there was an exacerbating factor that affected the occurrence of joint contracture, it is suggested that it is necessary to determine the cause. In this study, we clarified that unweighting the lower limbs affects the skeletal muscle in particular. The result of this study is very meaningful for considering treatment for patients with low weight-bearing frequency.

The limitation of this study was the lack of consideration for effects other than the soleus muscle on joint contractures. Previous studies on joint contractures caused by a reduction in joint movement investigated the effects of joint capsules[21] and ligaments[25] as well as skeletal muscle. Since the influence of the joint components other than skeletal muscle on a joint contracture caused by reduction of joint movement with unweighting of the lower limbs was not investigated in this study, it is necessary to investigate the effects on extensibility of other joint components (e.g. joint capsule, ligament, etc.) in future studies.

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