

Effects of Prolonged Stretching and Thermotherapy on Muscle Contracture of Immobilized Rat Soleus Muscle

YASUTAKA KONDO, MS, PT¹⁾, JIRO NAKANO, PhD, PT²⁾, JUNYA SAKAMOTO, PhD, PT³⁾,
HIDEKI KATAOKA, MS, PT⁴⁾, SHINGO YOKOYAMA, MS, PT⁴⁾, YUICHIRO HONDA, MS, PT⁴⁾,
TOMOKI ORIGUCHI, PhD, MD⁴⁾, TOSHIRO YOSHIMURA, PhD, MD⁴⁾, MINORU OKITA PhD, PT⁴⁾

¹⁾ Department of Rehabilitation, Japanese Red Cross Nagasaki Genbaku Hospital

²⁾ Department of Physical Therapy Science, Unit of Physical and Occupational Therapy Sciences,
Nagasaki University Graduate School of Biomedical Sciences

³⁾ Department of Rehabilitation, Nagasaki University Hospital

⁴⁾ Department of Locomotive Rehabilitation Science, Unit of Rehabilitation Sciences, Nagasaki
University Graduate School of Biomedical Sciences: 1-7-1 Sakamoto, Nagasaki, 852-8520 Japan.
TEL: +81 95-819-7965, FAX: +81 95-819-7965, E-mail: mokita@nagasaki-u.ac.jp

Abstract. [Purpose] We examined the effects of prolonged stretching and thermotherapy on muscle contracture of immobilized rat soleus muscles by determining ankle joint range of motion (ROM), collagen fibril arrangement in the endomysium and insoluble collagen content. [Methods] In the experimental group, bilateral ankle joints of each rat were fixed in full plantar flexion with plaster casts for four weeks. Then, the experimental group animals were randomly partitioned into five groups: 1) immobilization alone; and 2) spontaneous recovery, 3) prolonged stretching, 4) thermotherapy, and 5) prolonged stretching immediately following thermotherapy for 2 weeks after cast removal. Prolonged stretching of the soleus muscle (30 min/day) was conducted by maintaining the dorsiflexion position of the ankle joint. Thermotherapy consisted of immersion of bilateral hindlimbs (20 min/day) in hot water (42 °C). Under anesthesia, both treatments were performed six days per week during the two-week remobilization. [Results] Immobilization led to decreased ROM, collagen fibril movement in the endomysium and increased insoluble collagen. Prolonged stretching and the combination of stretching and thermotherapy accelerated amelioration of the ROM limitation and diminished collagen fibril movement. [Conclusion] Our findings suggest that while thermotherapy does not exert a specific effect on muscle contracture, prolonged stretching can promote recovery.

Key words: Stretching, Thermotherapy, Muscle contracture

(This article was submitted Feb. 2, 2012, and was accepted Feb. 12, 2012)

INTRODUCTION

Clinically, cases involving bed rest for prolonged periods or immobilization of a joint are characterized by decreased muscle elasticity and reduced range of motion (ROM). This condition, known as muscle contracture, is quite possibly the most frequently encountered complication among rehabilitation professionals. Muscle contracture is thought to result from changes in intramuscular connective tissue¹⁻⁴⁾. Intramuscular connective tissue displays a three-tier organization: the epimysium or fascia surrounds whole muscles, the perimysium binds muscle fibers into bundles, and the endomysium outlines individual muscle cells. Collagen is the main fibrous protein in intramuscular connective tissue, and collagen elasticity depends not only on intra- and intermolecular cross-links but also on the arrangement of fibrils^{5, 6)}. Purslow and Trotter⁷⁾ reported that the orientation of collagen fibril distribution in the endomysium exhibits a progressive shift in the circumferential direction at short sarcomere length and in the longitudinal direction

at long sarcomere length. Therefore, collagen fibrils in the endomysium run in various directions during muscle relaxation, and move longitudinally to the axis of the muscle fiber in muscle stretching. This re-orientation of the fibrils may reflect the role of the endomysium in terms of providing mechanical support to the fiber's surface and its action as an elastic device for contraction-relaxation cycles⁵⁾. Moreover, fibril arrangement in the endomysium is believed to be related to muscle elasticity. Our previous studies demonstrated changes in collagen with respect to the mechanism underlying muscle contracture based on morphological and biochemical analyses^{8, 9)}. A morphological assessment by Okita et al.⁸⁾ disclosed that collagen fibril arrangement in the endomysium 1 and 2 weeks after immobilization in the rat soleus was longitudinal to the axis of the muscle fibers, whereas at 4, 8 and 12 weeks after immobilization, the fibril arrangement was circumferential. Such changes in collagen fibril arrangement are indicative of decreased collagen fibril movement in the endomysium of immobilized muscle. The biochemical approach of Hibino et al.⁹⁾ revealed that

insoluble collagen, that which was not dissolved by salt, acid or pepsin, increased significantly in the rat soleus after 3 weeks immobilization relative to untreated muscle. These results are consistent with an increase in collagen characterized by enhanced intermolecular cross-links in immobilized muscle. Therefore, the reduction in collagen fibril movement in the endomysium and the increase in insoluble collagen content in skeletal muscle are thought to be related to the mechanism governing muscle contracture.

Commonly, prolonged stretching and/or thermotherapy are applied in rehabilitation programs following muscle contracture to increase the extensibility of skeletal muscle. Several studies consisting of randomized controlled trials involving prolonged stretching and thermotherapy with respect to the limitation of ROM have been conducted; however, the results obtained so far are controversial¹⁰⁻²⁰. Data from animal experiments suggest that the application of intermittent stretching for 30 min per day provides adequate stimulus for the prevention of ROM limitation²¹. Furthermore, Williams²² demonstrated that intermittent stretching for 15 min per day during a 10-day immobilization period prevented increase in connective tissue. However, little has been reported on the effect of prolonged stretching in terms of changes in intramuscular connective tissue related to the mechanism governing muscle contracture.

In general, thermotherapy for muscle contracture is often administered prior to stretching exercise because increased extensibility of connective tissue was attributed to warming in an earlier investigation²³. In particular, Lehman et al.²³ described a greater increase in residual length in rat tail tendon under increasing loading in a 45 °C hot water bath, whereas progressive lengthening was not observed in a 25 °C water bath. In addition, the tension of rat tail tendon dropped to very low values within minutes in a 45 °C bath whereas very little tension decrease was detected at 25 °C. However, thermotherapy which warms soft tissue to 45 °C is difficult in the clinical setting as it may induce tissue injury and/or the degeneration of protein. Therefore, we concluded that evidence supportive of prolonged stretching and thermotherapy for muscle contracture is incomplete.

The objective of this study was to clarify the effects of prolonged stretching and thermotherapy on muscle contracture of immobilized rat soleus muscles.

SUBJECTS AND METHODS

Thirty 8-week-old male SPF Wistar rats (weight, 287.3 ± 7.0 g) obtained from Kyudo (Tosu, Saga, Japan) were randomly divided into the experimental (n = 25) and control (n = 5) groups. Control rats were untreated. Animals of the experimental group were anesthetized with pentobarbital sodium (40 mg/kg), and their bilateral ankle joints were fixed in full plantar flexion with plaster casts for four weeks with the soleus muscles immobilized in a shortened position. The plaster cast, which was positioned from above the knee joint to the distal foot, was changed weekly to reduce loosening due to muscle atrophy. Rats in the experimental group were randomly divided into five groups: 1) immobilization alone (group I, n = 5); 2) spontaneous recovery (no treatment) for

2 weeks after cast removal (group NT, n = 5); 3) prolonged stretching exercise for 2 weeks after cast removal (group S, n = 4); 4) thermotherapy for 2 weeks after cast removal (group H, n = 6); and 5) prolonged stretching immediately following thermotherapy for 2 weeks after cast removal (group SH, n = 5).

Animals were housed in cages inside a room with a 12 h-12 h light-dark cycle. The temperature and relative humidity of the room were maintained at 25 °C and 50%, respectively. Food and water were available *ad libitum*. Animal care and experimental procedures were performed in accordance with the Guidelines for Animal Experimentation of Nagasaki University with approval of the Institutional Animal Care and Use Committee.

The prolonged stretching exercise followed to that described by Williams²¹. Both soleus muscles of group S and SH rats were stretched by dorsiflexion of the ankle joint with a tensile force of 0.9 N using a spring scale, which was installed vertical to the plantar, and this position was fixed with non-elastic tape. This exercise was conducted for 30 min each day, 6 days per week over two weeks following the immobilization period. Thermotherapy was performed via the following methods. Bilateral hindlimbs of group H and SH rats were immersed in hot water (42 °C) for 20 min. Our pilot study confirmed that the muscle temperature of the triceps surae gradually increased during immersion in hot water, reaching 40.0 °C after 20 min. Therefore, we concluded that adequate heating was applied to the soleus by this method. The frequency of thermotherapy was based on that of prolonged stretching (6 days per week over 2 weeks). In group SH, the prolonged stretching exercise was performed immediately after thermotherapy. All treatments were administered under pentobarbital sodium anesthesia (40 mg/kg). Furthermore, rats of group NT were anesthetized with the identical frequency.

After the 4-week immobilization and the subsequent 2-week remobilization periods, animals of all groups were anesthetized with pentobarbital sodium (40 mg/kg). Following body weight measurements, the range of motion (ROM) of dorsiflexion of the ankle joint was measured with a goniometer as described in a previous report⁸.

Measurement of ROM was defined as the angle (0 to 180 degrees) between a straight line connecting the fifth metatarsal and the malleolus lateralis of the fibula to a line connecting the malleolus lateralis of the fibula and the center of the knee joint when the ankle was passively dorsiflexed under a tensile force of 0.3 N using a spring scale with the knee joint in the fully flexed position. For each group, a comparison between the 4-week immobilization and the subsequent 2-week remobilization values was conducted with the paired Student's t-test. Differences among groups were assessed employing one-way analysis of variance (ANOVA) followed by Scheffe's F post hoc test. Differences were considered significant at p < 0.05.

At the end of each experimental period, the left soleus muscle was excised, stretched with a 4 g sinker as described in our previous study⁸, and fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). The cell maceration method of Ohtani et al.²⁴ was utilized. After initial fixation

Table 1. Changes in the range of motion of ankle dorsiflexion and concentrations of hydroxyproline in insoluble collagen

Groups	Range of Motion (degrees)		Hydroxyproline concentrations of insoluble collagen ($\mu\text{g}/\text{mg}$)
	immediately after 4 weeks of immobilization	after 2 weeks of remobilization	after 2 weeks of remobilization
Control group	160		0.4 ± 0.1 ¶
Group I	74.5 ± 4.4 *		1.4 ± 0.4
Group NT	78.0 ± 4.8 *	118.5 ± 4.8 †	0.6 ± 0.3 ¶
Group S	78.1 ± 4.6 *	129.4 ± 9.4 †, ‡, §	0.4 ± 0.3 ¶
Group H	78.8 ± 6.1 *	120.0 ± 9.8 †	0.6 ± 0.2 ¶
Group SH	79.0 ± 6.1 *	134.5 ± 9.8 †, ‡, §	0.5 ± 0.2 ¶

Values are mean \pm SD. *: vs. control group ($p < 0.05$). †: vs. immediately after immobilization ($p < 0.05$). ‡: vs. group NT after 2-week remobilization ($p < 0.05$). §: vs. group H after 2-week remobilization ($p < 0.05$). ¶: vs. Group I ($p < 0.05$)

in 3% glutaraldehyde, left soleus muscles were cut into $3 \times 3 \times 3$ mm blocks and immersed in 10% aqueous sodium hydroxide for 14 days at room temperature. Specimens were then rinsed in distilled water for 3 days. These samples were placed in a 1% aqueous tannic acid solution for 2 h and rinsed in distilled water for 1 h, followed by post-fixing in a 1% aqueous solution of osmium tetroxide for 2 h. Subsequently, specimens were dehydrated in a series of graded concentrations of ethanol and dried by the t-butyl alcohol freeze-drying method²⁵). The dried specimens were mounted on metal stubs and coated with gold, after which the collagen fibril arrangement in the endomysium was examined and micrographs produced with a scanning electron microscope (SEM; JSM-6700F/IV, JEOL, Tokyo, Japan) at a final magnification of $\times 20,000$. SEM micrographs of the longitudinal surfaces of the cut strips were generated. Care was taken to ensure that micrographs used to analyze collagen fibril orientation in the endomysium were obtained from specimens oriented completely perpendicular to the line of view. The upper acute angles (0 to 90 degrees) formed by the two lines of the collagen fibril segment and the direction parallel to the muscle fiber axis on the photomicrographs were determined as described by Purslow and Trotter⁷). The photomicrograph area was $27 \mu\text{m}^2$ ($4.5 \times 6 \mu\text{m}$). All angles formed by the two lines on the photomicrograph were measured, and 20 photomicrographs of 20 random muscle fibers per soleus muscle were evaluated. More than 7000 angles were obtained for each group.

The extraction of insoluble collagen in muscle tissue was conducted employing the method of Hibino et al.⁹). The right soleus muscles of all rats were extracted at the end of each experimental period and minced with a razor. Subsequently, specimens were placed in 1.0 M NaCl in 0.05 M Tris-HCl buffer that was 10 times the wet weight and homogenized. Homogenates were stirred for 24 h at 4 °C and centrifuged at $15,000 \times g$ for 60 min at 4 °C. Next, sediments were introduced to 0.5 M acetic acid that was 5 times the wet weight, stirred for 24 h at 4 °C, and centrifuged at $15,000 \times g$ for 60 min at 4 °C. Then, sediments were added to 0.5 M acetic acid containing pepsin (1 mg/ml) that was 5 times the wet weight and stirred for 24 h at room temperature. They were then centrifuged at $15,000 \times g$ for 60 min at 4 °C. Finally, sediments were collected as insoluble collagen.

Determination of collagen content: In general, hydroxyproline is thought to be exclusive to collagen; thus, the concentration of collagen was determined via measurement of hydroxyproline content. Collagen content was estimated via the modified technique of Reddy et al.²⁶). Samples, which were lyophilized for 24 h, were hydrolyzed in 6 N HCl for 15 h at 110 °C. Next, the samples were hydrolyzed in alkali for 20 min at 90 °C. The hydrolyzed specimens were then mixed with buffered chloramine-T reagent, and left to stand to allow oxidation at room temperature. The chromophore was developed by adding Ehrlich's aldehyde reagent, after which the absorbance of each sample was measured at 540 nm with a spectrophotometer. Absorbance values were plotted against the concentration of standard hydroxyproline; the presence of hydroxyproline in sample extracts was determined from the standard curve. The hydroxyproline concentration of samples was calculated as the content per wet weight ($\mu\text{g}/\text{mg}$ wet weight).

ANOVA was utilized for statistical evaluation. When ANOVA established a significant F value ($p < 0.05$), pair-wise comparisons were conducted with Scheffé's F method.

RESULTS

Table 1 presents the data of ROM of dorsiflexion of each group. The ROM of dorsiflexion in the control group was 160 degrees. Means \pm SD of ROM of dorsiflexion immediately after the 4-week immobilization period were 74.5 ± 4.4 degrees in group I, 78.0 ± 4.8 degrees in group NT, 78.1 ± 4.6 degrees in group S, 78.8 ± 6.1 degrees in group H and 79.0 ± 6.1 degrees in group SH. After four weeks of immobilization, ROM of dorsiflexion of all the experimental groups was significantly smaller than that of the control group; additionally, there were no significant difference among the experimental groups.

Means \pm SD of ROM of dorsiflexion at the end of the 2-week remobilization period were 118.5 ± 4.8 degrees in group NT, 129.4 ± 9.4 degrees in group S, 120.0 ± 9.8 degrees in group H and 134.5 ± 9.8 degrees in group SH. After two weeks of remobilization, ROM of dorsiflexion had increased significantly compared to that observed at the end of immobilization in each group. Furthermore, ROM of dorsiflexion in groups S and SH was significantly

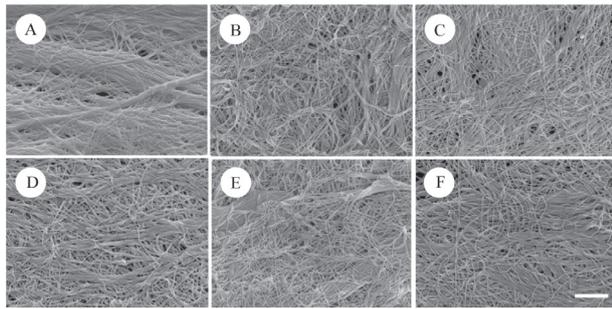


Fig. 1. Scanning electron micrographs of endomysium in all groups.

(A) Control. (B) Group I. (C) Group NT. (D) Group S. (E) Group H. (F) Group SH. Bar = 1 μ m. The muscle fiber axis runs horizontally. Many longitudinal collagen fibril components are present in the control group. Among the experimental groups, this arrangement is apparent only in groups S and SH. Group I exhibits increased circumferential collagen fibril components. Groups NT and H also display increased circumferential collagen fibril components.

greater than that in groups NT and H; however, there was no significant difference between groups S and SH.

A representative scanning electron micrograph of collagen fibril arrangement in the endomysium is exhibited in Figure 1. The muscle fiber axis runs horizontally. Many longitudinal collagen fibril components are present in the control group (Fig. 1A). Among the experimental groups, this arrangement was apparent only in groups S and SH

(Fig. 1D and 1F). In contrast, circumferential collagen fibril components in the I, NT and H groups increased (Fig. 1B, 1C and 1E). The percent distributions of the form angle of the direction parallel to the muscle fiber axis and directional angle of the collagen fibrils are shown in Figure 2. In the control, S and SH groups, a peak was observed for angles between 20 to 50 degrees (Fig. 2A, 2D and 2F). On the other hand, peaks of 40 to 60 and 90 degrees were evident in group I (Fig. 2B). In group NT, a peak was observed for the angle between 30 to 40 degrees; however, peaks of 40 to 60 and 90 degrees remain in evidence (Fig. 2C). In group H, a peak occurred for angles between 20 to 40 degrees and a peak at 90 degrees was also present (Fig. 2E).

Table 1 presents the data of hydroxyproline concentrations of insoluble collagen in the soleus muscle for each group. Means \pm SD values of hydroxyproline concentration were 0.4 ± 0.1 μ g/mg wet weight in the control group, 1.4 ± 0.4 μ g/mg wet weight in group I, 0.6 ± 0.3 μ g/mg wet weight in group NT, 0.4 ± 0.3 μ g/mg wet weight in group S, 0.6 ± 0.2 μ g/mg wet weight in group H and 0.5 ± 0.2 μ g/mg wet weight in group SH. The hydroxyproline concentration of group I had increased significantly compared to that of the control group. Furthermore, the hydroxyproline concentration of the four intervention groups decreased significantly relative to that of group I; however, no significant differences were detected among the four intervention groups.

DISCUSSION

The current findings demonstrated a reduction in ROM of dorsiflexion in group I to half of the control value, as reported by Okita et al.⁸⁾. A recent review proposed that

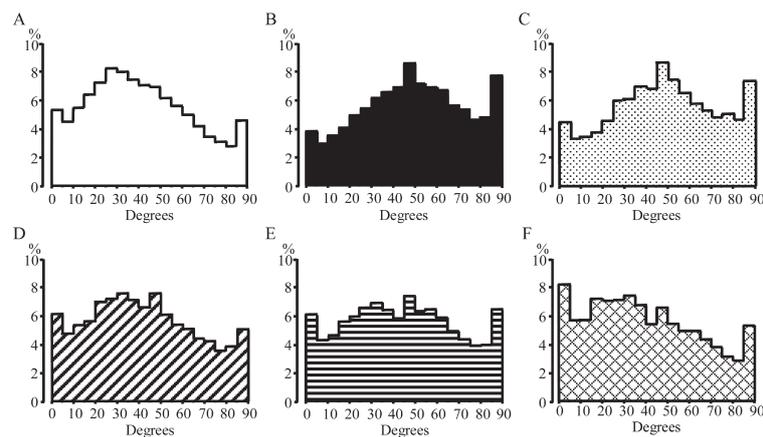


Fig. 2. Percent distributions of the angle of direction formed parallel to the muscle fiber axis and the directional angles of collagen fibrils in all groups. (A) Control. (B) Group I. (C) Group NT. (D) Group S. (E) Group H. (F) Group SH. A peak at angles between 20 to 50 degrees is apparent in the control group. Groups S and SH also exhibit a peak at the same angles. Peaks at 40 to 60 and 90 degrees are evident in group I. In group NT, a peak is observed at angles between 30 to 40 degrees in contrast to group I; however, peaks at 40 to 60 and 90 degrees remain in evidence. A peak is observed at angles between 20 to 40 degrees in group H, in contrast to group I. Group H displays an angle peak at 90 degrees.

myogenic limitation predominates during the first 90 days of immobility, whereas the limitation is mainly arthrogenic beyond 90 days of immobility²⁷). According to this description, the limitation in ROM after four weeks of immobilization in this investigation would be the result of myogenic change.

Purslow and Trotter reported that collagen fibrils' orientation in the endomysium display a progressive shift in the circumferential direction at short sarcomere lengths and in the longitudinal direction at longer sarcomere lengths⁷). Namely, collagen fibrils in the endomysium are oriented in various directions during muscle relaxation and move longitudinally toward the axis of the muscle fiber during muscle stretching^{5, 7}). In the current study, muscle samples were stretched with a 4 g sinker, then fixed for comparison of the collagen fibril arrangement in the endomysium among all the groups during stretching under the same force. Many collagen fibrils in the endomysium of the control group were longitudinal. However, the majority orientation of collagen fibrils in group I had shifted to the circumferential direction, a result which is consistent with that of our previous investigation⁸). This change is suggestive of decreased collagen fibril movement and reduced extensibility in the endomysium.

An earlier report noted that intermolecular cross-link formation in collagen fibers reduces the flexibility of the tissue²⁸). Generally, enhancement of intermolecular cross-links affects collagen solubility and induces an increase in insoluble collagen^{9, 29}). In the present study, the insoluble collagen content of group I increased significantly in comparison with that of the control, a result which is consistent with the findings of Hibino et al.⁹). This result is indicative of an increase in intermolecular cross-links with a stronger molecular structure at four weeks of immobilization. Therefore, given that the limitation in ROM and various changes in collagen occurred as described in previous reports^{8, 9}), we conclude that group I affords an appropriate experimental model for muscle contracture.

ROM of dorsiflexion in the four treatment groups immediately after four weeks of immobilization showed no significant differences relative to group I. Based on this result, we expected various changes in intramuscular collagen related to the mechanism of muscle contracture in the four intervention groups after four weeks of immobilization. Accordingly, we examined the effect of prolonged stretching and thermotherapy on muscle contracture.

Our findings demonstrate that ROM of dorsiflexion in group S after 2 weeks remobilization was significantly larger than that in group NT. This result suggests that prolonged stretching accelerates amelioration of limitation of ROM. On the other hand, Okita et al.³⁰) also examined whether prolonged stretching of rat ankle joints in full plantar flexion promoted recovery from limitation of ROM after 4 weeks of immobilization. Prolonged stretching was applied for 30 min per day for two weeks (6 days/week) as in the current study. In the study of Okita et al.³⁰), however, no significant difference was observed between the stretching group and the spontaneous recovery group in terms of ROM of dorsiflexion, and this result was distinct from that of our

present study. Consequently, we speculate that establishing the conditions for the angle of dorsiflexion under prolonged stretching plays an influential role. Specifically, Okita et al.³⁰) applied prolonged stretching daily at the maximal angle of dorsiflexion, but the conditions for this angle are vague. In contrast, we clearly defined the conditions under which prolonged stretching was conducted: the ankle joint was dorsiflexed by a tensile force of 0.9 N.

In group S, many collagen fibrils in the endomysium exhibited longitudinal orientation toward the axis of the muscle fiber, and a peak of 20 to 50 degrees in the percent distributions of the form angle of the direction parallel to the muscle fiber axis was observed. These results are similar to those of the control group. In group NT, however, many collagen fibrils displayed circumferential orientation; in addition, the percent distributions of the form angle of the direction parallel to the muscle fiber axis were similar to those of group I. Therefore, these data suggest that prolonged stretching may improve diminished collagen fibril movement in the endomysium due to immobilization.

In terms of insoluble collagen content, groups S and NT displayed significantly reduced levels in comparison with group I; however, no significant difference was detected between groups NT and S. Although this result indicates that the number of collagen fibers with strong intermolecular cross-links in the NT and S groups had decreased during the remobilization process, prolonged stretching did not demonstrate specific effects with respect to the enhancement of intermolecular cross-links in intramuscular collagen.

First, in reference to the result in group H (thermotherapy alone), no significant difference was found between groups NT and H regarding ROM of dorsiflexion after the 2 weeks remobilization. Moreover, in group H, many collagen fibrils in the endomysium displayed circumferential orientation toward the axis of the muscle fiber, and a peak of 90 degrees in the percent distributions of the form angle of the direction parallel to the muscle fiber axis was evident. Furthermore, these results were similar to those of group NT. In addition, insoluble collagen content in group H declined significantly in comparison with group I; in contrast, group H exhibited no meaningful difference relative to group NT. Following the report of Lehman et al.²³), it was believed that thermotherapy was effective at increasing extensibility of soft tissue, and thermotherapy has become widely utilized in current clinical rehabilitation programs for muscle contracture. However, few studies have examined whether thermotherapy improves recovery from muscle contracture. From the results of the recovery of ROM after remobilization and the changes in intramuscular collagen related to the mechanism governing muscle contracture, we conclude that thermotherapy alone is not effective in the promotion of recovery from muscle contracture.

On the other hand, Okita et al.³¹) examined the effects on rat triceps surae of continuous mode ultrasound treatment (frequency, 1 MHz; intensity, 1.0 W/cm²) for 15 min per day over a 4-week immobilization period during which ankle joints were fixed in full plantar flexion. The ultrasound intervention inhibited the deterioration of ROM of dorsiflexion of the ankle joint and collagen fibril movement

in the endomysium. Okita et al.³¹⁾ argued that the effect of ultrasound in terms of progressive inhibition of muscle contracture was attributable exclusively to a thermal effect. In the present study, however, the effects of thermotherapy on the recovery process from muscle contracture were unclear. It is possible that the speed of heating of the soleus muscle accounts for the difference between our results and those of Okita et al.³¹⁾, since the time required for the triceps surae to reach a temperature of 40.0 °C was approximately 20 min under our approach, whereas it was only 6 min for the continuous ultrasound method³¹⁾. Thus, assessment of the thermotherapy methodology will be necessary in future experiments.

Second, ROM of dorsiflexion of group SH (combination therapy with prolonged stretching and thermotherapy) was significantly larger than that of groups NT and H after the 2-week remobilization; however, no significant difference was detected between the S and SH groups. In addition, many collagen fibrils in the endomysium of group SH exhibited longitudinal orientation, and a peak of 20 to 50 degrees in the percent distributions of the form angle of the direction parallel to the muscle fiber axis was observed in the SH group. The results of group SH were similar to those of group S. Group SH displayed markedly reduced insoluble collagen content in comparison with that of group I; however, no significant difference was found among the four treatment groups. These findings suggest that recovery from muscle contracture was more striking in animals of group SH than in animals of the NT and H groups. To be sure, neither the SH nor the S group demonstrated a significant difference in recovery, based on the muscle contracture parameters examined in the present study. Therefore, we assume that the combination of prolonged stretching and thermotherapy affords little multiplier effect. Moreover, the recovery promotion factor of muscle contracture in group SH may be appropriate in relation to the influence of prolonged stretching.

In the clinical setting, combination therapy involving prolonged stretching and thermotherapy is often included in rehabilitation programs associated with muscle contracture. Several studies consisting of randomized controlled trials for this combination therapy have been conducted; however, the majority of the subjects in those studies were healthy adults and the evidence supporting the effectiveness of combination therapy for muscle contracture is unclear^{15–20)}. Moreover, the specific effect of combination therapy on intramuscular collagen related to the mechanisms underlying muscle contracture was not proven in the present study. Therefore, we hypothesize that thermotherapy for skeletal muscle may be effective for the promotion of muscle relaxation and the enhancement of blood flow, but not for the stimulation of intramuscular collagen.

Our results suggest that prolonged stretching and combination therapy involving prolonged stretching and thermotherapy promotes recovery from muscle contracture. Little specific effect of thermotherapy on muscle contracture was evident, and the promotion factor for recovery from muscle contracture found in the combination therapy may arise from the influence of the prolonged stretching. We

also speculate that the impact of thermotherapy on muscle contracture is influenced by the speed of heating of skeletal muscle. Further studies will be necessary in order to examine other thermotherapy methodologies.

ACKNOWLEDGEMENTS

The authors thank Mr. T. Suematsu of the Microscopic Center, Nagasaki University School of Medicine, for his assistance with scanning electron microscopy. This research was supported in part by a grant-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (grant no. 21300200).

REFERENCES

- 1) Stolov WC, Fry LR, Riddell WM, et al.: Adhesive forces between muscle fibers and connective tissue in normal and denervated rat skeletal muscle. *Arch Phys Med Rehabil*, 1973, 54: 208–213. [[Medline](#)]
- 2) Melichna J, Gutmann E: Stimulation and immobilization effects on contractile and histochemical properties of denervated muscle. *Pflügers Arch*, 1974, 352: 165–178. [[Medline](#)] [[CrossRef](#)]
- 3) Williams PE, Goldspink G: Connective tissue changes in immobilised muscle. *J Anat*, 1984, 138: 343–350. [[Medline](#)]
- 4) Józsa L, Kannus P, Thöring J, et al.: The effect of tenotomy and immobilisation on intramuscular connective tissue. *J Bone Joint Surg Br*, 1990, 72: 293–297. [[Medline](#)]
- 5) Borg TK, Caulfield JB: Morphology of connective tissue in skeletal muscle. *Tissue Cell*, 1980, 12: 197–207. [[Medline](#)] [[CrossRef](#)]
- 6) Rowe RW: Morphology of perimysial and endomysial connective tissue in skeletal muscle. *Tissue Cell*, 1981, 13: 681–690. [[Medline](#)] [[CrossRef](#)]
- 7) Purslow PP, Trotter JA: The morphology and mechanical properties of endomysium in series-fibred muscles: variations with muscle length. *J Muscle Res Cell Motil*, 1994, 15: 299–308. [[Medline](#)] [[CrossRef](#)]
- 8) Okita M, Yoshimura T, Nakano J, et al.: Effects of reduced joint mobility on sarcomere length, collagen fibril arrangement in the endomysium, and hyaluronan in rat soleus muscle. *J Muscle Res Cell Motil*, 2004, 25: 159–166. [[Medline](#)] [[CrossRef](#)]
- 9) Hibino I, Okita M, Inoue T, et al.: Effect of immobilization on insoluble collagen concentration and type I and type III collagen isoforms of rat soleus muscle. *J Jpn Phys Ther Assoc*, 2008, 11: 1–6. [[CrossRef](#)]
- 10) Harvey LA, Byak AJ, Ostrovskaia M, et al.: Randomised trial of the effects of four weeks of daily stretch on extensibility of hamstring muscles in people with spinal cord injuries. *Aust J Physiother*, 2003, 49: 176–181. [[Medline](#)]
- 11) Winters MV, Blake CG, Trost JS, et al.: Passive versus active stretching of hip flexor muscles in subjects with limited hip extension: a randomized clinical trial. *Phys Ther*, 2004, 84: 800–807. [[Medline](#)]
- 12) Moseley AM, Herbert RD, Nightingale EJ, et al.: Passive stretching does not enhance outcomes in patients with plantarflexion contracture after cast immobilization for ankle fracture: a randomized controlled trial. *Arch Phys Med Rehabil*, 2005, 86: 1118–1126. [[Medline](#)] [[CrossRef](#)]
- 13) Gajdosik RL, Vander Linden DW, McNair PJ, et al.: Effects of an eight-week stretching program on the passive-elastic properties and function of the calf muscles of older women. *Clin Biomech (Bristol, Avon)*, 2005, 20: 973–983. [[Medline](#)] [[CrossRef](#)]
- 14) Horsley SA, Herbert RD, Ada L: Four weeks of daily stretch has little or no effect on wrist contracture after stroke: a randomised controlled trial. *Aust J Physiother*, 2007, 53: 239–245. [[Medline](#)] [[CrossRef](#)]
- 15) Lentell G, Hetherington T, Eagan J, et al.: The use of thermal agents to influence the effectiveness of a low-load prolonged stretch. *J Orthop Sports Phys Ther*, 1992, 16: 200–207. [[Medline](#)]
- 16) Brodowicz GR, Welsh R, Wallis J: Comparison of stretching with ice, stretching with heat, or stretching alone on hamstring flexibility. *J Athl Train*, 1996, 31: 324–327. [[Medline](#)]
- 17) Draper DO, Miner L, Knight KL, et al.: The carry-over effects of diathermy and stretching in developing hamstring flexibility. *J Athl Train*, 2002, 37: 37–42. [[Medline](#)]
- 18) Peres SE, Draper DO, Knight KL, et al.: Pulsed shortwave diathermy and prolonged long-duration stretching increase dorsiflexion range of motion

- more than identical stretching without diathermy. *J Athl Train*, 2002, 37: 43–50. [[Medline](#)]
- 19) Draper DO, Castro JL, Feland B, et al.: Shortwave diathermy and prolonged stretching increase hamstring flexibility more than prolonged stretching alone. *J Orthop Sports Phys Ther*, 2004, 34: 13–20. [[Medline](#)]
 - 20) Lee GP, Ng GY: Effects of stretching and heat treatment on hamstring extensibility in children with severe mental retardation and hypertonia. *Clin Rehabil*, 2008, 22: 771–779. [[Medline](#)] [[CrossRef](#)]
 - 21) Williams PE: Use of intermittent stretch in the prevention of serial sarcomere loss in immobilised muscle. *Ann Rheum Dis*, 1990, 49: 316–317. [[Medline](#)] [[CrossRef](#)]
 - 22) Williams PE: Effect of intermittent stretch on immobilised muscle. *Ann Rheum Dis*, 1988, 47: 1014–1016. [[Medline](#)] [[CrossRef](#)]
 - 23) Lehmann JF, Masock AJ, Warren CG, et al.: Effect of therapeutic temperatures on tendon extensibility. *Arch Phys Med Rehabil*, 1970, 51: 481–487. [[Medline](#)]
 - 24) Ohtani O, Ushiki T, Taguchi T, et al.: Collagen fibrillar networks as skeletal frameworks: a demonstration by cell-maceration/scanning electron microscope method. *Arch Histol Cytol*, 1988, 51: 249–261. [[Medline](#)] [[CrossRef](#)]
 - 25) Inoue T, Osatake H: A new drying method of biological specimens for scanning electron microscopy: the t-butyl alcohol freeze-drying method. *Arch Histol Cytol*, 1988, 51: 53–59. [[Medline](#)] [[CrossRef](#)]
 - 26) Reddy GK, Enwemeka CS: A simplified method for the analysis of hydroxyproline in biological tissues. *Clin Biochem*, 1996, 29: 225–229. [[Medline](#)] [[CrossRef](#)]
 - 27) Harburn KL, Potter PJ: Spasticity and contracture. *Phys Med Rehabil State Art Rev*, 1993, 7: 113–132.
 - 28) Woo SL, Matthews JV, Akeson WH, et al.: Connective tissue response to immobility. Correlative study of biomechanical and biochemical measurements of normal and immobilized rabbit knees. *Arthritis Rheum*, 1975, 18: 257–264. [[Medline](#)] [[CrossRef](#)]
 - 29) Sugama S, Tachino K, Haida N: Effect of immobilization on solubility of soleus and gastrocnemius muscle collagen —Biochemical studies on collagen from soleus and gastrocnemius muscles of rat—. *J Jpn Phys Ther Assoc*, 1999, 2: 25–29. [[CrossRef](#)]
 - 30) Okita M, Yoshimura T, Nakano J, et al.: Effects of short duration stretching on disuse muscle atrophy in immobilized rat soleus muscles. *J Jpn Phys Ther Assoc*, 2001, 4: 1–5. [[CrossRef](#)]
 - 31) Okita M, Nakano J, Kataoka H, et al.: Effects of therapeutic ultrasound on joint mobility and collagen fibril arrangement in the endomysium of immobilized rat soleus muscle. *Ultrasound Med Biol*, 2009, 35: 237–244. [[Medline](#)] [[CrossRef](#)]