

Histochemical Study on the Atrophy of the Quadriceps Femoris Muscle Caused by Knee Joint Injuries of Rats

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ABSTRACT

Atrophy developing in the quadriceps femoris muscle following knee injury is one of the serious problems not only in the field of orthopedics but also of rehabilitation. However the pathogenesis of this atrophy has not yet been elucidated. The author therefore produced a complex ligament injury model using the knee joints of rats in order to study the pathogenesis of this atrophy. After severing the anterior cruciate ligament, the medial collateral ligament and tibial insertion of the medial meniscus of rats, these animals were sacrificed at 4, 8 and 12 weeks. After removing the vastus lateralis muscle, vastus medialis muscle, and rectus femoris muscle, specimens of these muscles were stained for ATPase. The transection area of the muscle fibers was measured and the fiber type composition was determined. At 4 weeks the vastus medialis muscle and at 12 weeks the vastus lateralis muscle showed marked atrophy. The rectus femoris muscle exhibited the least atrophy throughout the entire observation period. In examining the atrophy of the quadriceps femoris muscle by muscle fiber type, the degree of atrophy was found to differ among the venters and even the same venter showed a different reaction depending on the elapsed time after sustaining the injury. Neither changes in the fiber type composition nor neurogenic findings could be observed.

Key words: *Muscle atrophy, Knee injury, Histochemistry, ATPase*

In patients who sustain injury to the knee joint ligaments, marked atrophy develops in the quadriceps femoris muscle and this atrophy prevents the patients from resuming sports or returning to work early and furthermore induces new osteoarthritis as a secondary complication. In order to alleviate this atrophy of the quadriceps femoris muscle, various kinds of physical exercises have been advocated, but their effect has been found to be unsatisfactory, and the pathogenesis of this atrophy remains to be unclear. The author therefore designed a complex ligament injury model using the rat knee joints in order to study the atrophy of the quadriceps femoris muscle mainly by means of histochemical method.

MATERIALS AND METHODS

Thirty 5-week-old male Wistar rats were used in this study. Under general anesthesia by intraperitoneal injection of Somnopentil at a dose of 25 mg per kg, a skin incision about 1.0 cm in length was made medially on the right knee joint. The anterior cruciate ligament (ACL), the medial collateral ligament and tibial insertion of the medial meniscus were sharply cut under the microscope and thereafter the wound was closed. Thus an unstable knee joint was prepared (Fig. 1). No particular fixation was used for the lower extremity treated in this manner, but the animals were

housed separately one per cage. At 4, 8 and 12 weeks after the operation, the quadriceps femoris muscle, tibialis anterior muscle and extensor digitorum longus muscle on both sides were removed

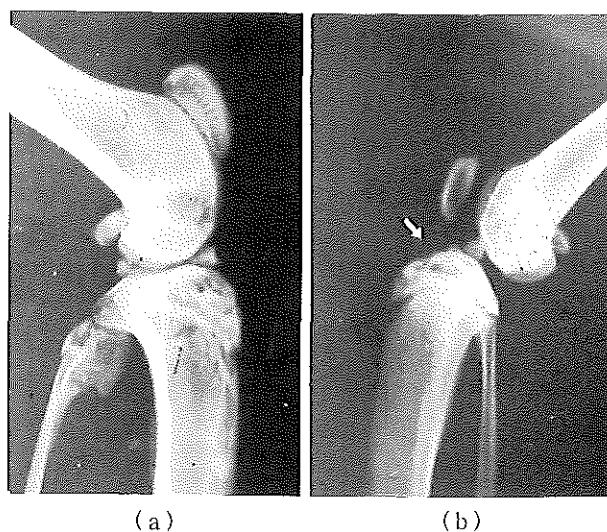


Fig. 1. Roentgenogram of the knee joint 8 weeks after operation. (a) Non-treated knee joint and (b) treated knee joint. In the treated knee joint, anterior drawer sign was observed and there was marked malalignment between the femur and tibia as indicated by the arrow.

from the origin to the point of attachment, and their respective wet weight was measured in units of 1 mg on a chemical balance (Sartorius 2474). At a point about 1 cm centrally from the upper end of the patella, three venters of the quadriceps femoris muscle were separated and the specimens were taken from each venter at the same height on both the non-treated and treated sides. After placing the muscle slices thus obtained in Hexene cooled with dry ice and acetone quickly freezing, Eight micron-thick transections were prepared with a cryostat. Following preincubation at pH of 10.3, a histochemical staining of myofibrillar ATP-ase⁴ was performed at pH of 9.6, and micrographs were taken at 40x magnification. These photos were enlarged and the transection areas of 200 muscle fibers of type I and type II were measured with an image analyzer (SPORTIAS GP-2000). The average section area for both types of muscle fibers was compared between the treated and non-treated sides, and the rate of the muscle fiber atrophy was obtained by dividing the value of the treated side by that of the non-treated side. The number of type I muscle fibers contained in 400 muscle fibers was determined for each venter and the proportion of the total was taken as the fiber type composition.

RESULTS

1. Macroscopic findings of the quadriceps femoris muscle

The external appearance of the quadriceps femoris muscle on the treated side 4 weeks after the operation showed virtually no change from that of the non-treated side, but after 8 weeks slight atrophy began to appear and after 12 weeks the

degree of atrophy became quite marked. However no changes were noted in the color tone of the muscles throughout the entire period.

2. Changes in wet weight

The wet weight of the quadriceps femoris muscle on the treated side when compared with that of the non-treated side was $102 \pm 7\%$ after 4 weeks, $93 \pm 5\%$ after 8 weeks, and $87 \pm 6\%$ after 12 weeks (mean \pm S.E.). The wet weight of the tibialis anterior muscle and extensor digitorum longus muscle which are not involved in the movement of the knee joint was $93.7 \pm 7\%$ and $100 \pm 10\%$ after 4 weeks, $96 \pm 9\%$, and $95 \pm 8\%$ after 8 weeks, and $88 \pm 7\%$ and $90 \pm 6\%$ after 12 weeks, respectively, being less than that for the quadriceps femoris muscle (Fig. 2). Atrophy was noted in the muscle of the lower leg not directly involved in the movement of the knee joint, but the quadriceps femoris muscle was found to show the greatest decrease.

3. Histochemical findings of the quadriceps femoris muscle

The venters of the normal quadriceps femoris muscle consist of two types of muscle fiber: type I and type II muscle fibers which are both arranged in a chessboard pattern. Neither neurogenic findings such as type grouping, group atrophy, and angular muscle fibers nor myogenic findings such as scattered muscle fiber atrophy were observed (Figs. 3, 4 and 5).

4. Fiber type composition in the quadriceps femoris muscle.

In the present study, the fiber type composition was expressed as component rate of type I muscle fibers among a group of 400 muscle fibers of the venters on both the treated and non-treated sides. In first examining the fiber type composition of the vastus medialis muscle, the non-treated side, 4, 8 and 12 weeks after the operation showed values of $36 \pm 6\%$, $41 \pm 10\%$ and $29 \pm 10\%$, respectively, while the corresponding values for the treated-side were $35 \pm 7\%$, $45 \pm 9\%$ and $32 \pm 8\%$, respectively, demonstrating no significant differences between both sides (Fig. 6-a). Looking the fiber type composition for the vastus lateralis muscle 4, 8 and 12 weeks after the operation, the non-treated side had values of $8 \pm 11\%$, $11 \pm 10\%$ and $18 \pm 8\%$, respectively, compared with $13 \pm 10\%$, $15 \pm 10\%$ and $17 \pm 7\%$, respectively, for the treated side, thus showing no significant difference between both sides (Fig. 6-b). Lastly in examining the fiber type composition for the rectus femoris muscle at 4, 8 and 12 weeks after the operation, the non-treated side had values of $15 \pm 7\%$, $15 \pm 8\%$, $11 \pm 8\%$, respectively, and the treated side $12 \pm 8\%$, $15 \pm 6\%$ and $9 \pm 7\%$, respectively, showing no uniform tendency (Fig. 6-c) (mean \pm S.E.).

5. Time-course change in the rate of muscle fiber atrophy

Type I and Type II muscle fibers of the vastus

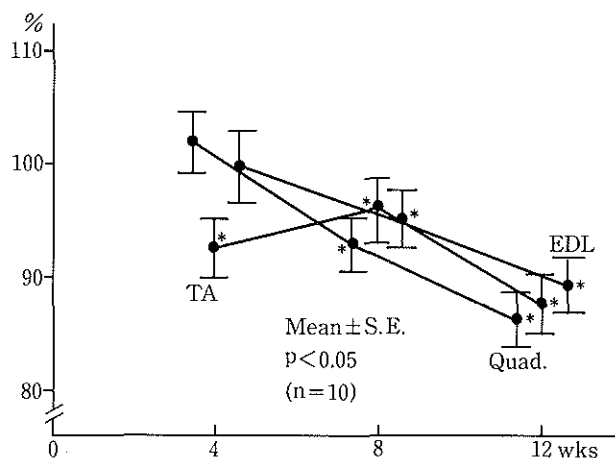


Fig. 2. Changes in wet muscle weight.

The decrease in wet weight of the tibialis anterior and extensor digitorum longus muscle was smaller than the decrease in wet weight of the quadriceps femoris muscle except for the observation period of 4 weeks after the operation. A significant difference was observed between the treated and the non-treated side at each period.

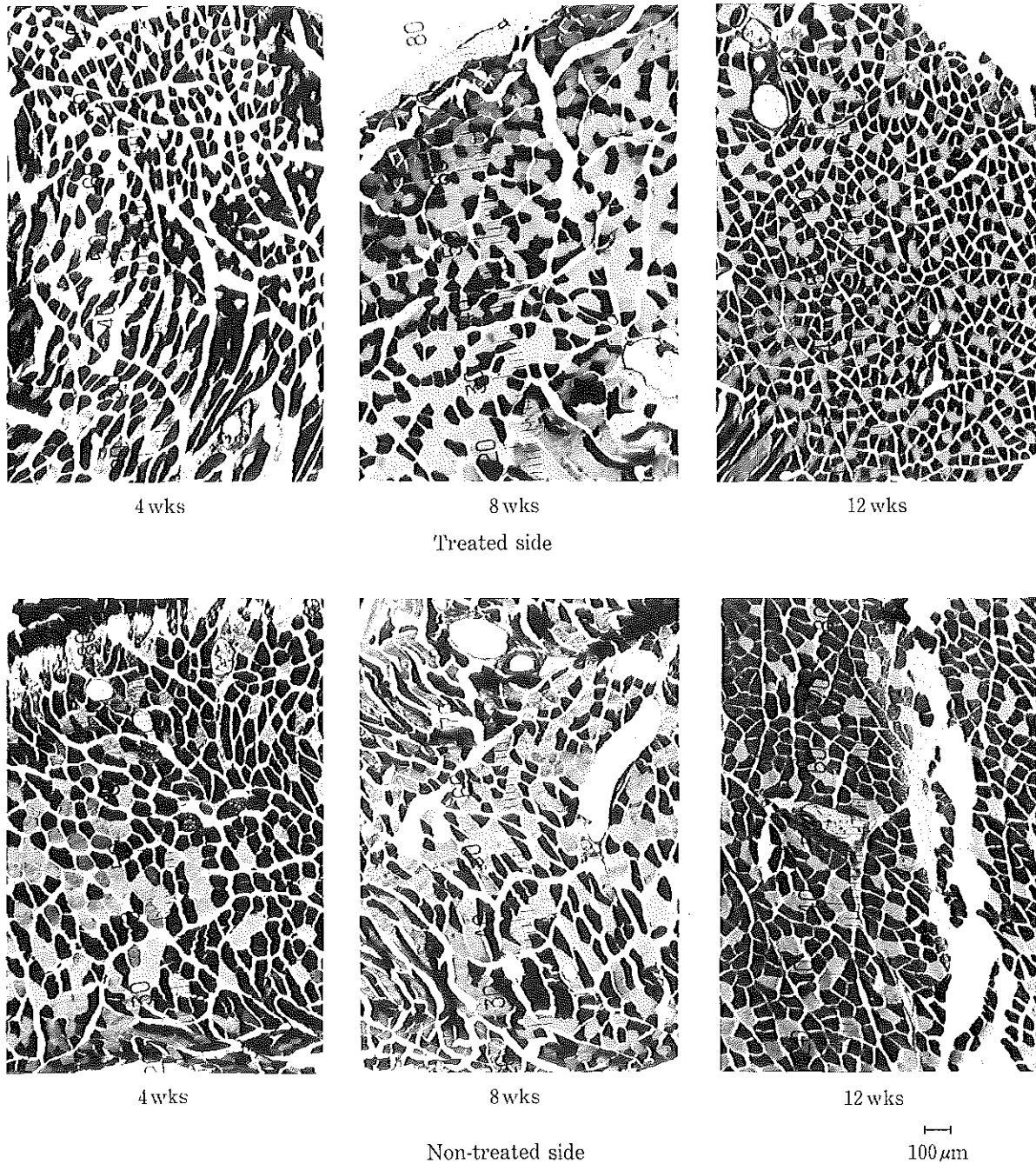


Fig. 3. Histochemical findings of the vastus medialis muscle ($\times 40$). The lightly stained portions of the figure are type I muscle fibers, while the darkly stained portions are type II muscle fibers. Both type I and type II muscle fibers are arranged in a chessboard pattern. No neurogenic findings such as type grouping, group atrophy, and angular muscle fibers nor myogenic findings such as scattered muscle fiber atrophy could be observed.

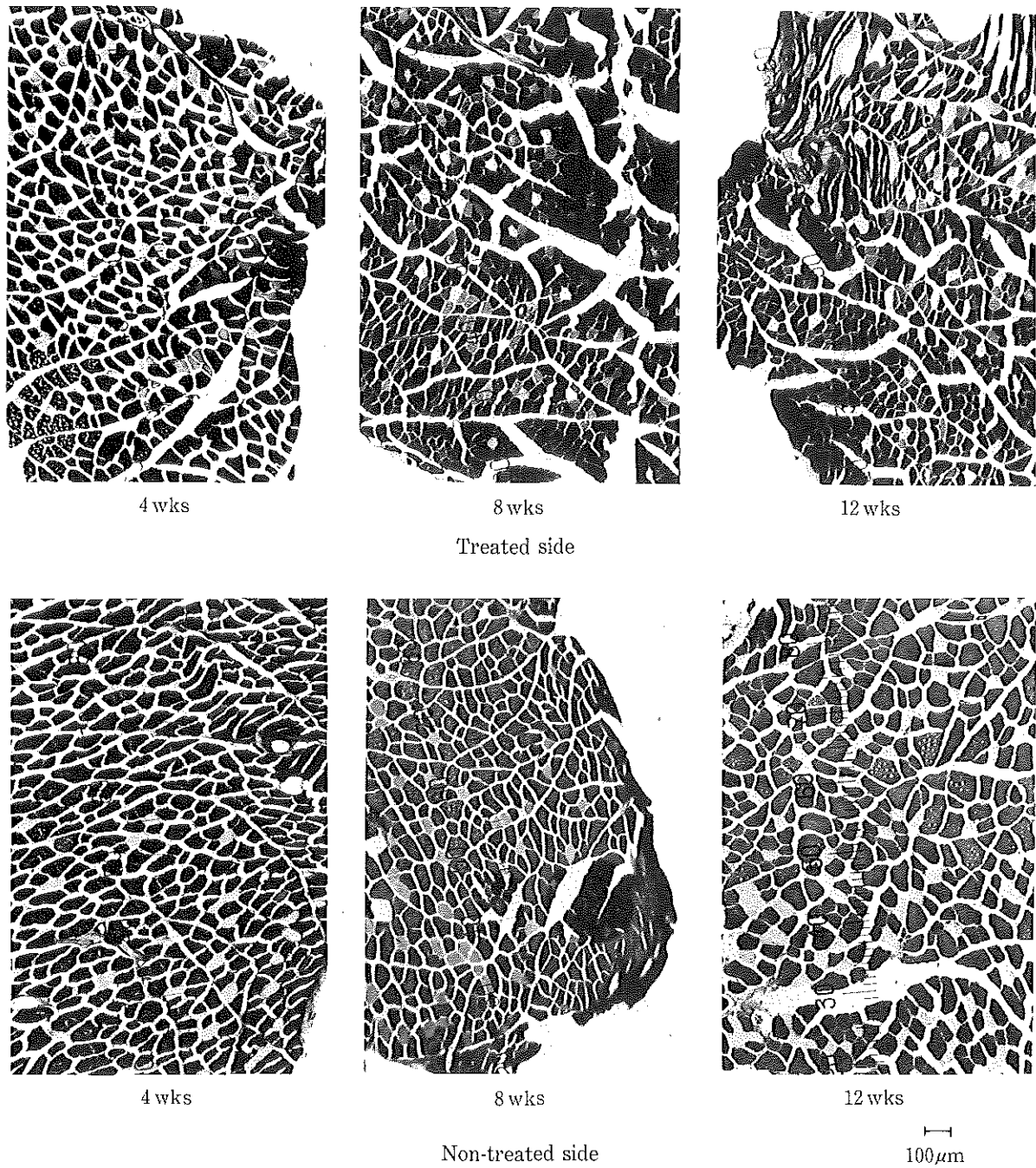


Fig. 4. Histochemical findings of the vastus lateralis muscle ($\times 40$)
The neurogenic and myogenic findings were negative as in the case of the vastus medialis muscle.

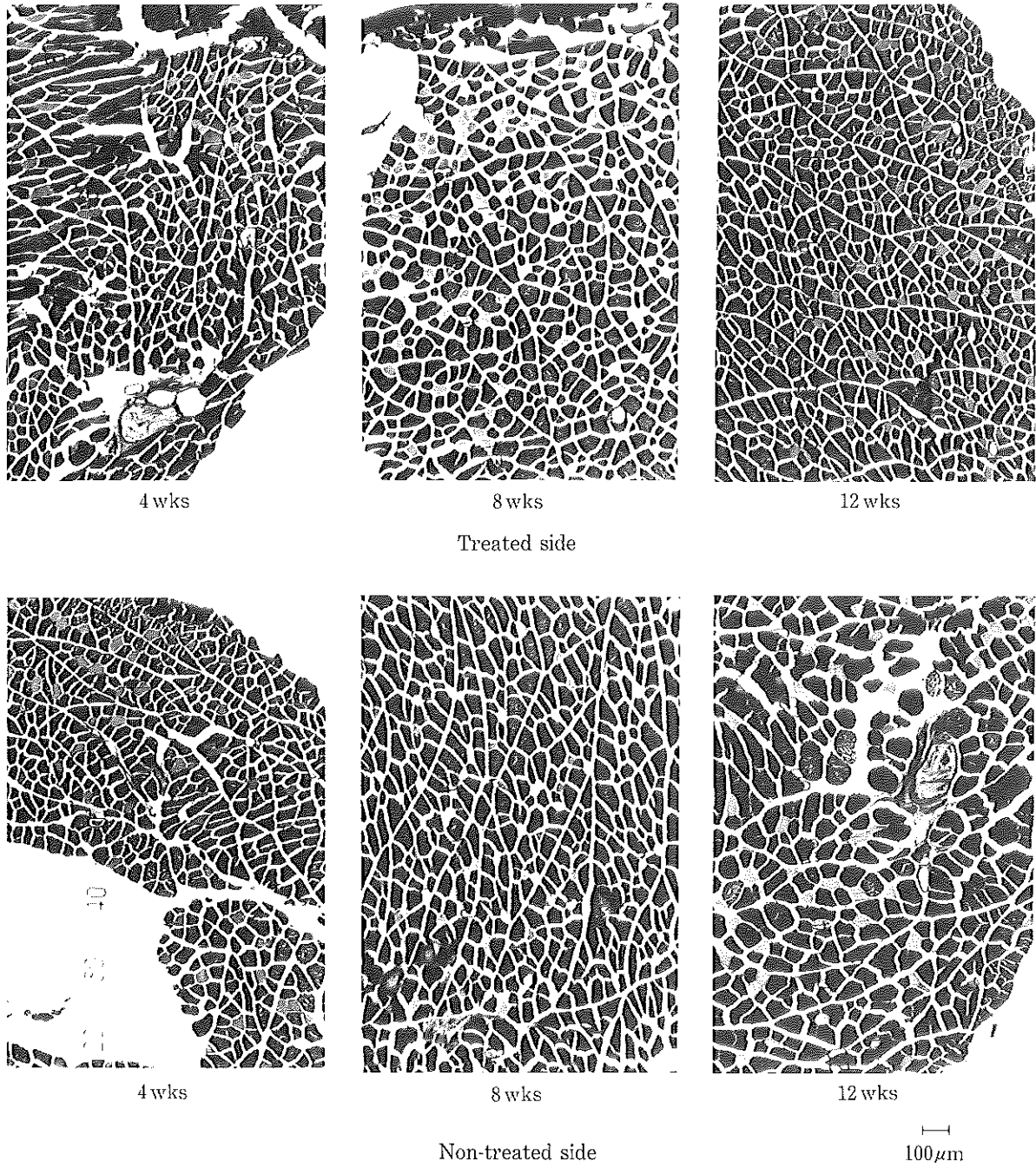


Fig. 5. Histochemical findings of the rectus femoris muscle ($\times 40$)
The neurogenic and myogenic findings were negative as in the case of the vastus medialis muscle.

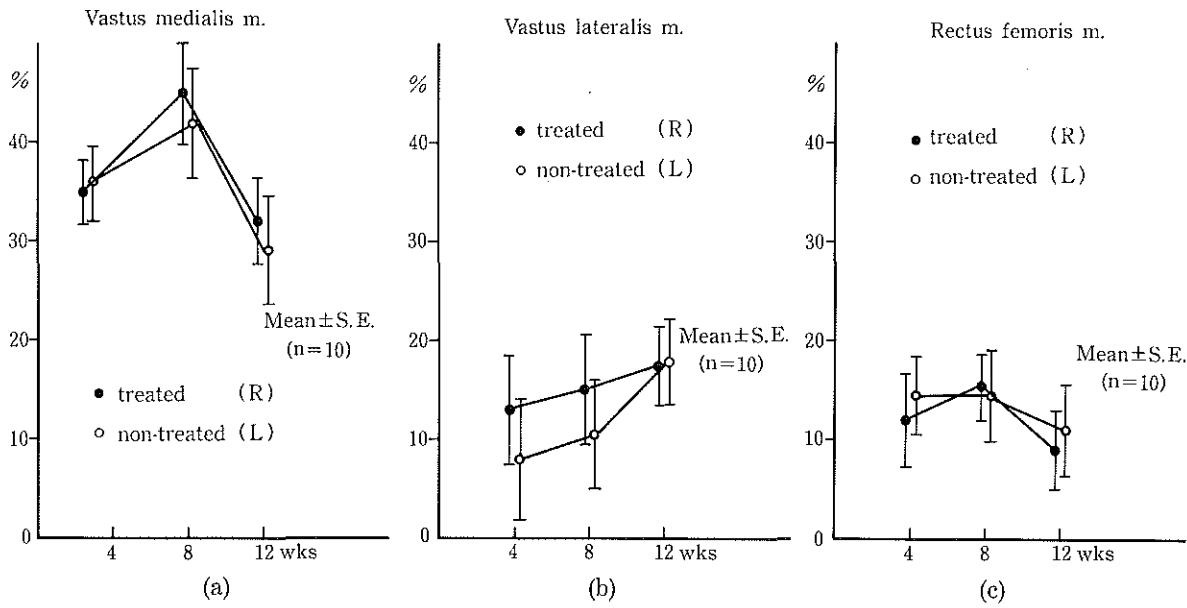


Fig. 6. Fiber type composition.

Fiber type composition showed no significant difference between the treated and non-treated sides for the vastus medialis, vastus lateralis muscle and rectus femoris muscle.

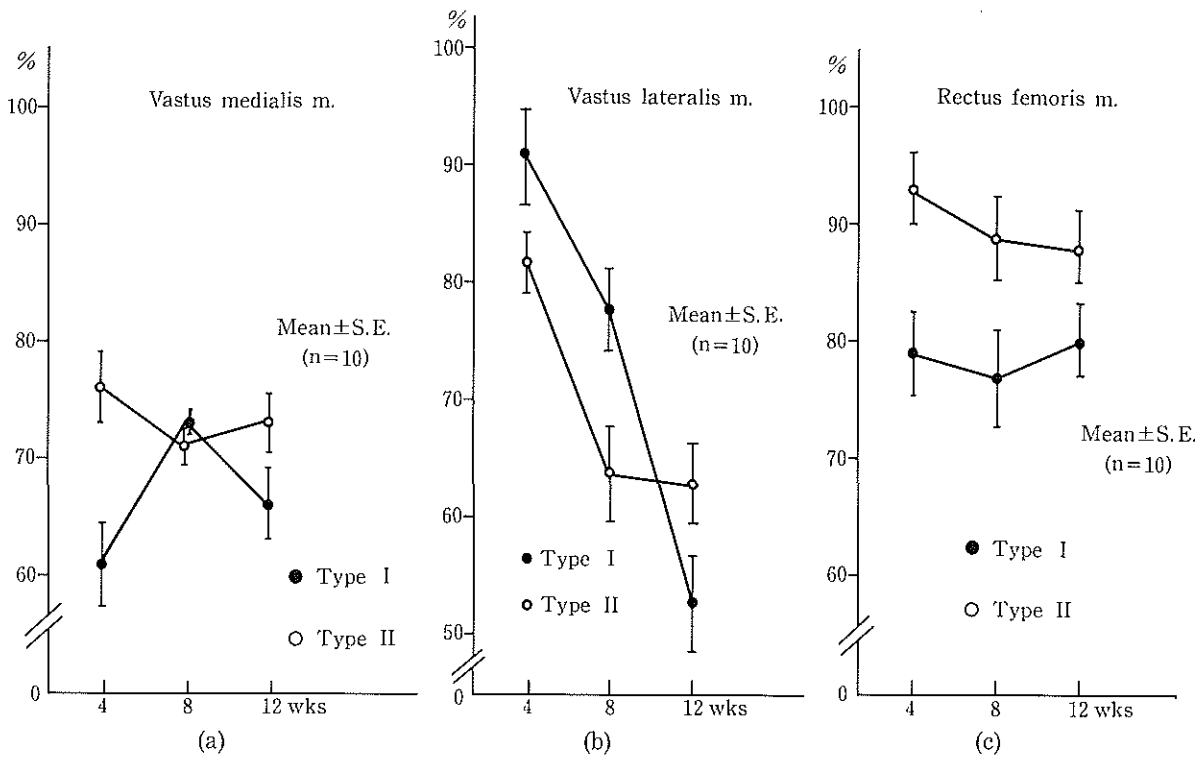


Fig. 7. Time course changes in the muscle fiber type atrophy rate.

The vertical axis in Fig. 7 represents the muscle fiber atrophy rate. Both type I and type II muscle fiber of the vastus medialis muscle were markedly atrophied even after 4 weeks with atrophy predominating in type I muscle fibers. After 12 weeks, atrophy of type I muscle fibers was greater than that of type II muscle fibers (Fig. 7-a). In the vastus lateralis muscle, type I and type II muscle fibers showed a rapid decrease in size and finally became the most atrophic among all the venter (Fig. 7-b). Throughout the entire observation period, muscle atrophy of type I muscle fibers was more pronounced than that of type II muscle fibers. Moreover, both type I and type II muscle fibers showed the least atrophy (Fig. 7-c).

medialis muscle showed marked atrophy of $61 \pm 7\%$ and $76 \pm 6\%$, respectively, after 4 weeks compared with the non-treated side, with type I mus-

cle fiber atrophy predominating. Although type II muscle fibers showed further atrophy with $71 \pm 3\%$ after 8 weeks, it is interesting to note that the

size of type I muscle fibers had reached $73 \pm 2\%$ and increased over the value after 4 weeks. However, after 12 weeks the size of type I muscle fibers had again decreased to $66 \pm 6\%$ to show further atrophy, which was more marked compared with the value of $73 \pm 5\%$ for type II muscle fiber (Fig. 7-a). In examining the size of type I and type II muscle fibers of the vastus lateralis muscle, the values after the initial observation period of 4 weeks were $91 \pm 8\%$ and $82 \pm 5\%$, respectively, but the atrophy rate was slight compared with that of the other venters. However, the size of type I and type II muscle fibers after 8 weeks had decreased to $78 \pm 7\%$ and $64 \pm 8\%$ and after 12 weeks to $53 \pm 8\%$ and $63 \pm 7\%$, respectively, showing a rapid atrophy after both 8 and 12 weeks, and finally the atrophy rate of the vastus lateralis muscle reached the highest value of all three venters. After 4 weeks and 8 weeks, the atrophy rate of type II muscle fibers was greater than that of type I muscle fibers, but after 12 weeks the atrophy rate of type I muscle fibers finally became greater than type II muscle fibers (Fig. 7-b). The size of type I and type II muscle fibers of the rectus femoris muscle 4 weeks after the operation was $79 \pm 7\%$ and $93 \pm 6\%$ and changed to $77 \pm 8\%$ and $89 \pm 7\%$ after 8 weeks and to $80 \pm 6\%$ and $88 \pm 6\%$ after 12 weeks, respectively, exhibiting an extremely slight degree of atrophy rate compared with the vastus medialis muscle and vastus lateralis muscle. Throughout the entire course, muscle atrophy of type I muscle fibers was more pronounced than that of type II muscle fiber (Fig. 7-c) (mean \pm S.E.)

DISCUSSION

Atrophy of the quadriceps femoris muscle is a finding more important than any other clinical symptom in knee joint injuries. Several clinical studies have thus been conducted with the aim of pursuing this problem by histochemical methods using human biopsied muscles. However, the conditions of the patients vastly differ in each study and only one venter among the four venters of the quadriceps femoris muscle has been examined in each study. Therefore, in this study the degree of muscle fiber atrophy was compared between each venter under almost the same condition. If there is a difference in atrophy between the venters of muscle fibers, the rehabilitation program for training the quadriceps femoris muscle becomes different. These are thus subjects of not only purely academic interest but also of importance in practice. The present experimental model is considered to provide important clues in ascertaining the pathogenesis of atrophy of the quadriceps femoris muscle.

The first point to consider in attempting to elucidate the action mechanism of marked atrophy of the quadriceps femoris muscle is that the action of

the quadriceps femoris muscle differs from that of other muscles. Since such muscles as the hamstring or gastrocnemius muscles are diarthric muscles that involve not only movement of the knee joint but also of ankle joints, movement involving joints other than the knee joint may not be disturbed even if the knee joint is injured or becomes fixed. However, in examining the quadriceps femoris muscle, only the rectus femoris muscle among the four venters is a diarthric muscle, while the other venters that constitute the great bulk of the quadriceps femoris muscle are monoarthric muscle. Another reason why atrophy of the quadriceps femoris muscle in ACL injury is more pronounced is that the quadriceps femoris muscle serves as ACL antagonist and constitutes an antigravity muscle⁹. It is thus assumed that quadriceps femoris muscle sustains marked atrophy. The author therefore examined the atrophy and strength of human quadriceps femoris muscle and hamstrings muscles after operation for knee ligament injuries¹⁰.

It is well known that the vastus medialis muscle is markedly atrophied among the four venters in the quadriceps femoris muscle, but the difference in atrophy between each venter has not yet been quantitatively investigated. In comparing the degree of atrophy between each venter of the quadriceps femoris muscle, it was found that the vastus medialis muscle in rats was most atrophied 4 weeks after knee joint injury and after 12 weeks the vastus lateralis muscle became fairly extensively atrophic. The rectus femoris muscle was found to show the least atrophy throughout the entire observation period as Wolf et al. pointed in patients who were placed in a cast¹⁸. Atrophy of the rectus femoris muscle is the least because it is the only diarthric muscle among the four venters of the quadriceps femoris muscle and can participate in the movement of the hip joint even though the knee joint is injured, thus resulting in only a slight degree of atrophy.

Muscle atrophy is described in a single term, but mechanism of contraction and metabolic characteristics are various depending on the type of muscle fiber. In reviewing the reports on the nature of atrophy of the quadriceps femoris muscle accompanying knee joint injury, two theories have been presented, one stating that the degree of muscle atrophy differs according to the type of muscle fibers involved and the other claiming that there is no difference in atrophy between type I and type II muscle fibers. In the former theory, some claim that type I muscle fibers become more atrophic than type II muscle fibers, while others claim that the atrophy of type II muscle fiber is greater than that of type I muscle fiber. There is an endless controversy between the two theories. These theories were derived from different conditions of the quadriceps femoris muscle and thus a definite con-

clusion can not be reached. The theory that atrophy of type I muscle fibers is predominant has been strongly supported by several workers^{5,6,10}. Eriksson (1981)⁹ explained this phenomenon as a result of irreversible degeneration in neuromuscular units and emphasized that it might be different from simple functional atrophy. More recently Nakamura et al (1986)¹³ proposed another mechanism that atrophy of type I muscle fibers develops if the damage is complicated by ACL injury or ACL and medial meniscus injury and that atrophy of type II muscle fibers commonly develops in all types of knee joint injury. They offered the following explanation for these phenomena. Both the movement pattern of the joint and the tension of the muscles which contracted in order to maintain the joint stability change after ACL injury, and these resulted in atrophy of type I muscle fibers. However, as ACL injuries are usually not associated with such severe pain that it prevents movement of the joint, maintenance of activity may lessen the development of type II fiber atrophy after ACL injuries. There are several reports that type I muscle fiber chiefly becomes atrophic, but these studies are inadequate in resolving the present proposition. This is because in these studies the site and time of muscle biopsy were not taken in account at all. In discussing the difference of atrophy between type I and type II muscle fibers, these factors are the most important as shown in this study. As far as the present experiment is concerned, the theory that atrophy of type I muscle fiber is greater than that of type II muscle fiber is valid only for atrophy of the rectus femoris muscle, but the cause of this predominant atrophy of type I muscle fiber is still unknown.

Baughner et al (1984)¹¹ in carrying out biopsy of the vastus medialis muscle during surgery on 14 patients with ACL injury observed more pronounced atrophy of type II muscle fibers, whereas type I muscle fibers exhibited hypertrophy. Mutoh et al (1982)¹² conducted out muscle biopsy from the vastus lateralis muscle in cases complicated by injury of the medial collateral ligament, ACL, and lateral meniscus and found pronounced atrophy of type II muscle fibers. In case of ACL injury movement requiring maximum contraction of the muscles is difficult, but since such movements as standing and walking which mainly rely on type I muscle fibers are not greatly impeded, it has been assumed that atrophy is likely to be greater in type II muscle fibers. In this study it was only in the vastus lateralis muscle at 4 weeks and 8 weeks after operation that atrophy of type II muscle fiber was greater than type I muscle fibers.

Young (1982)¹⁹ and Gerber (1985)⁸ emphasized that the nature of atrophy by muscle fiber type must be assessed with caution because even if either muscle fiber type is greater than the other, the size of the muscle fibers differs between the

sites where the muscle is located. They concluded that there was no difference of atrophy between type I and type II muscle fibers. In addition, the possibility of pain, inactivation and nerve damage¹⁶ in the muscle should be considered as a factor influencing the atrophy of the quadriceps femoris muscle. The degree of pain is said to play a very important role in muscle atrophy from the fact that the degree of atrophy in type I muscle fiber is well correlated with duration of pain. If the main cause of atrophy of the quadriceps femoris muscle is pain, relief of the pain could be the optimal therapy to protect muscle fiber from atrophy⁷. Inactivation has been found to cause mainly type I muscle fiber atrophy from the biopsied muscles in patients where cast was applied¹⁷. Since the size of type I muscle fibers is related to the degree of muscle tension in the cast⁹, placement of the muscle in a tense position could be the optimal treatment, if the main cause of atrophy is immobilization. As for nerve damage, it might be assumed that the nerves in the quadriceps femoris muscle sustain some mechanical damages because of the unstable knee, but, in this study the neurogenic changes such as group atrophy, type grouping, and angular muscle fibers could not be observed.

Since type I and type II muscle fibers are controlled by their own anterior horn cells in the spinal cord²⁰, the fiber type composition is determined at the time of birth. In rats Okada et al (1981)¹⁴, based on their research on growth differentiation reported that all muscle fibers complete their differentiation about 30 days later, when type I and type II muscle fibers reach a certain rate. The reason why 5-week-old rats were selected as experimental animals for this study is that at this point in time the fiber type composition becomes definite. Even the pre-determined muscle fiber types can change their type under special conditions such as cross-innervated muscle³, electrical stimulation to nerves and muscles at a frequency different from that of the original muscle contraction pattern¹¹, and immobilization. Let us now consider the possibility of changes in fiber type composition in the present experiment with marked joint instability. The axis of joint movement and the rotating moment during movement should have changed and moreover the various venters of the quadriceps femoris muscle also altered their original function after the operation. However, immobilization should not be seriously considered, because rats were not placed in the cast after the operation. The results showed no changes in fiber type composition. In order to detect a reversal in this control relationship, a much longer observation period might be required, because the type of muscle fibers is determined by the nerves controlling them. This study is the first report to compare the difference in atrophy of muscle fiber type between each

venter of the quadriceps femoris muscle. It is concluded that the atrophy rate of muscle fiber types differs not only by the venter but also by the time when muscle samples are obtained.

The results of this experiment indicated that the best rehabilitation program to prevent the muscle atrophy of the quadriceps femoris muscle would be to combine endurance exercise and muscle strengthening exercise with maximum resistance, particularly for monoarthric venters of the quadriceps femoris muscle. The exercise should be commenced as early as possible after injury.

CONCLUSION

1. An unstable knee joint model was produced and the atrophy of muscle fibers developing in the quadriceps femoris muscle was observed by histochemical methods 4, 8, and 12 weeks after operation.
2. The decrease in wet muscle weight was most pronounced in the quadriceps femoris muscle.
3. The most marked atrophy was noted after 4 weeks in the vastus medialis muscle and after 12 weeks in both the vastus medialis muscle and vastus lateralis muscle. The rectus femoris muscle showed the least atrophy throughout the entire observation period.
4. The degree of atrophy of the quadriceps femoris muscle by muscle fiber type differed according to the individual venter and also differed for the same venter depending on the time elapsed after sustaining the injury but in the rectus femoris muscle atrophy of type I muscle fibers was greater than of type II muscle fibers throughout the entire observation period.
5. No significant changes were observed in the fiber type composition in the quadriceps femoris muscle.
6. No neurogenic changes could be observed.

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