Correlation between change in muscle excursion and collagen content after tendon rupture and delayed repair

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Correlation between change in muscle excursion and collagen content after tendon rupture and delayed repair

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<TABLE OF CONTENTS>

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

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Understanding the excursion change after tendon rupture is important in selection of optimal treatment time and options. The objectives of the present study were, first, to learn whether the change in collagen content, thought to determine passive stiffness of muscle, is related to change in muscle excursion after tendon rupture with delayed repair. The second objective was to determine the contribution of the collagen subtypes to passive stiffness after tenotomy. Tenotomy on the extensor digitorum muscle of the second toe of New Zealand White rabbits was performed with or without subsequent repair. Muscle excursion, total collagen, and collagen subtype were assessed at various times after tenotomy. Total collagen and type I collagen significantly increased with time after tenotomy ($p < 0.05$), excursion and type III collagen decreased significantly ($p < 0.05$), and there was no significant change in type IV collagen ($p = 0.106$). Muscle excursion ratio was significantly negatively correlated ($p < 0.05$) with total collagen and type I collagen after tenotomy. Perimysium and endomysium thickening and a greater number of connections between thickened endomysia were noted 6 weeks after tenotomy compared to the control. The total collagen 8

weeks after tendon repair increased significantly and muscle excursion decreased significantly with delay in repair ($p < 0.05$), so that the excursion was significantly negatively correlated with total collagen ($p < 0.05$). The increase in collagen that was observed after tenotomy was not reversed by repair. Improvement in excursion was noted when repair was delayed by 1 or 2 weeks, but when it was delayed by 4 or 6 weeks, excursion decreased. Type I collagen has been associated with decreased excursion after tendon rupture. It may be that the decreased excursion observed after tendon rupture could be avoided if type I collagen accumulation could be prevented. Also, since muscle excursion was not improved when repair was performed more than 4 weeks after rupture, tendon transfer might be the best treatment option for tendon ruptures that have been neglected for more than 4 weeks.

Key words: collagen, collagen subtypes, muscle excursion, tendon rupture, delayed tendon repair

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I. Introduction

The tendon is a dense cord of fibrous tissue that joins the muscle to a bone.[1] Muscular tensile force is transferred to the bone via the tendon to produce joint motion. The tendon is vulnerable to injury, including rupture, because of its distal and superficial location, limited blood supply, and high tensile stress.[2,3] Tendon ruptures are classified as closed or open. Closed tendon injuries include traumatic tendon avulsion, spontaneous mid-substance rupture, attrition rupture, infiltrative tenosynovial rupture, and iatrogenic injury. These tendon injuries are often overlooked and, therefore, untreated and may eventually reduce active joint motion.[4] Current methods of tendon repair that involve post-operative rehabilitation and prevention of tendon adhesion have attracted a great deal of attention recently. It is necessary, however, to view the tendon as part of the myotendinous unit. Understanding the effect of tendon

rupture on this unit, including the muscle, is important in predicting the clinical outcome.

When a tendon ruptures, the muscle retracts. Prolonged muscle retraction leads to muscle shortening and decreased excursion, which makes normal end-to-end repair of the ruptured tendon impossible.[5-7] For treatment of neglected tendon rupture, repair must be performed with excessive joint flexion and may require additional procedures, such as tendon grafting or tendon transfer.[5] In experiments with rabbit soleus muscles, decreases in muscle excursion were mainly observed during the first four weeks after tenotomy.[8] In this study, the time of tendon repair after tenotomy and the muscle excursion at the time of repair independently influenced the recovery of muscle excursion.[8] However, the major mechanism of excursion change and its recovery after tenotomy with delayed tendon repair is not known.

Prolonged muscle retraction after tendon rupture causes intracellular changes, including Z band streaming, shortened sarcomere lengths, central core degenerative changes, changes in the myosin heavy chains, titin isoform changes, and decreases in sarcomere number, as well as extracellular changes. The latter include an increase in collagen and reduced numbers of capillaries.[9-15] In pathologic muscle, increased collagen content is believed to be an important factor in the excursion reduction.[16] In a study using rat muscles, collagen networks increased after tenotomy in rat muscles,[17] and increases in the collagen content of muscles after tenotomy or tendon rupture appear to be related to poor muscle recovery when tendon repair is delayed.

Collagen is an important component of the extracellular matrix (ECM) of the connective tissue layers that surround muscle cells. These are the endomysium, perimysium, and epimysium.[18-20] The endomysium is a reticular layer that encases individual muscle fibers and is composed of type IV

collagen.[21] The perimysium encases muscle fiber bundles and is composed of type I and III collagen. It stretches when the muscle fiber bundle expands and bends into a corrugated form when it contracts.[21] The epimysium, which forms the outer protective sheath of the muscle, is composed of type I and III collagen.[22-24] Type I collagen has a triple helix structure composed of two α1(I) chains and one α2(I) chain with thick fibers. Type III collagen has a triple helix structure composed of the same α1(III) chains with thin fibers.[25] Although fibers tend to form spontaneously depending on collagen type, the thickness of collagen fibers varies depending on the types of collagen and the diameter increases proportionately with type I to type III ratio.[26] The ratio of collagen types differs in the endomysium, perimysium, and epimysium, as well as between muscles and species.[27] The difference in molecular structure and tensile stress between collagen subtypes is thought to influence passive stiffness and excursion. However, no study has been conducted on the changes in ratios of collagen subtypes over time after tenotomy.

Understanding the cause of the excursion change after tendon rupture with delayed repair is important in determining optimal treatment time and making correct treatment choices, specifically, choosing between direct tendon repair, grafting, or tendon transfer. Identifying the area in which the change in the ratio of collagen subtype is initiated may reveal the appropriate therapeutic target of change of muscle excursion. Also, it is important to understand the cells and mechanisms involved. The objectives of the present study were, first, to learn whether the change in collagen content, thought to determine passive stiffness of muscle, is related to change in muscle excursion after tendon rupture with delayed repair. The second objective was to determine the contribution of the collagen subtypes to passive stiffness after tenotomy.

II. Materials and methods

1. Materials

All experimental methods were approved by the Institutional Animal Care and Use Committee. New Zealand White rabbits were used for this study (mean body mass: 3.1 ± 0.5 kg; n = 48 subjects, 96 hind limbs).

2. Experimental design

The extensor digitorum muscle of the second toe (EDII) was chosen as the experimental muscle because it has a long and large tendon that can be easily manipulated surgically. This muscle is easy to define because all muscle fibers of the muscle of the second toe originate from the medial surface of the tibia. The EDII tendon is also an intrasynovial tendon, as are the human finger flexors that are commonly injured.[28]

Animals were randomly assigned to treatment groups of six at the beginning of the study. For the first experiment, tenotomy of the EDII was performed on the right legs and the intact left legs served as the control. Passive muscle excursion and the content of hydroxyproline and type I, III, and IV collagen were measured at 1, 2, 4, or 6 weeks after tenotomy. The legs were also histologically examined. For the second experiment, tenotomy of the EDII was performed on the right leg and the intact left legs served as the control. The tendon was repaired at various times after tenotomy. Passive muscle excursion and hydroxyproline content were measured in both legs 8 weeks after repair.

3. Surgical procedures and measurements

A. Tenotomy

Anesthesia was induced with an intramuscular injection of 15 mg/kg of

Zoletil 50 (Zoletil 50 mg/mL, [tiletamine 125 mg, zolazepam 125 mg]; Virbac, Carros, France) and 5 mg/kg of Rompun (xylazine hydrochloride 23.32 mg/mL; Bayer Korea, Seoul, South Korea), and was maintained with enflurane, which provided approximately 30 min of adequate sedation. The right hind limbs of the animals were shaved and they were positioned supine on the operating table. The knee was in approximately 90° of flexion and the hip was in flexion and external rotation. Under aseptic conditions, a longitudinal skin incision was made over the medial side of the distal tibia and the tendon of the EDII was exposed. The tendon was transected at the level of the metatarsal. The wound was closed and animals were returned to their cages.

B. Tendon repair

At various times after tenotomy was performed as described above, animals were anesthetized, their incisions were re-opened, and the proximal tendon stump of the muscle was completely released from the adhesions with the surrounding tissues. Because end-to-end repair was impossible due to the decreased muscle excursion, the tendon was sutured to the ankle extensor retinaculum at the point of the 50% excursion. After wound closure, animals were allowed to move freely in their cages.

C. Measurement of excursion

The proximal tendon stump of the muscle was completely released from the adhesions with the surrounding tissues before the measurement of muscle excursion. Marking suture was placed in a proximal tendon end in the myotendinous junction, which was selected as a measurement point to avoid tendon length change by stretching of the tendon. The EDII tendon was pulled with a hemostat by an operating surgeon. Traction force was maximally applied

to the EDII tendon to the extent that further plastic deformation did not occur by the stretching of the tendon. A location of marking suture in retracted position and maximal manual traction position were marked in the medial tibia bone. Muscle excursion was obtained by measuring the distance between two bone markers using a digital caliper.

D. Measurement of hydroxyproline in EDII

Animals were sacrificed with an overdose of pentobarbital (150mg/kg i.p.). The EDII were harvested from the bilateral lower limbs after measuring the muscle excursion. Hydroxyproline, a major component of collagen, was quantified as a measure of the collagen content using the Hydroxyproline Colorimetric Assay Kit (BioVision, San Francisco, CA, USA). The standard curve was initially prepared. The hydroxyproline standard was diluted to 0.1 mg/ml by adding 10 μl of the 1 mg/ml standard to 90 μl of distilled water (dH2O). Increasing volumes (0, 2, 4, 6, 8, and 10 μl) were added to a series of wells, and the overall volumes were adjusted to 50 μl/well with dH2O, to generate 0, 0.2, 0.4, 0.6, 0.8, and 1.0 μg/well of the hydroxyproline standard. Muscle samples were homogenized in dH2O, using 100 μl H2O for every 10 mg of tissue. To a 100 μl sample of homogenate, 100 μl of concentrated HCl (~12 N) was added in a pressure-tight, Teflon-capped vial, and hydrolyzed at 120°C for 3 hours. A total of 10 μl of each hydrolyzed sample was transferred to a 96-well plate and evaporated to dryness under a vacuum. A total of 100 μl of the chloramine T reagent was added to each sample and standard, and incubated at room temperature for 5 minutes. Then, 100 μl of the p–dimethylaminobenzaldehyde reagent was added to each well and incubated for 90 min at 60℃. Finally, absorbance at 560 nm was measured in a VersaMax ELISA Microplate Reader (Molecular Devices, Sunnyvale, CA, USA).

E. Measurement of type I, III, and IV collagen content in EDII

Type I, III, and IV collagen were measured using specific enzyme-linked immunosorbent assay (ELISA) kits (www.antibodies-online.com; catalog numbers: ABIN628250, ABIN628251, ABIN628256, respectively). Briefly, the muscle tissues were rinsed in ice-cold phosphate buffered saline (PBS) (0.02 mol/L, pH 7.2) to remove excess blood thoroughly, and weighed before homogenization. The tissues were minced to small pieces and homogenized in ice-cold PBS using a Precellys 24 tissue homogenizer (Bertin Corp, Rockville, MD, USA). The resulting suspension was subjected to 2 freeze-thaw cycles to further break the cell membranes. After that, the homogenates were centrifuged for 15 minutes at 5000 rpm and the supernatants were used for type I, III, and IV collagen assay, according to the manufacturer's instructions.

F. Histological examination

The muscle tissues were processed for histological analysis, embedded in paraffin, and sectioned with a microtome to obtain 5 μm thick cross-sections. The sections were mounted on slides and stained with Masson trichrome to assess the presence of collagen infiltration and fibrosis. Sections were scanned with a virtual microscope (BX51, Olympus, Tokyo, Japan) attached to the charged couple device (CCD) camera VC50, captured by using a 40X objective (equivalent to 400X base magnification), and digitized as virtual slides.

G. Statistical analysis

The SPSS version 20 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. The muscle excursion, the content of hydroxyproline, and the type I, III, and IV collagen content relative to the control were used to counter the individual differences. The values for these three parameters at each

time point after tenotomy were compared with the linear mixed model. The muscle excursion and hydroxyproline content relative to the control value 8 weeks after tendon repair were compared with the linear mixed model, according to the repair timing. Post-hoc analysis was also performed. The muscle excursion ratios to the control value after tenotomy and after delayed tendon repair were compared according to the period after the tenotomy, using the Mann-Whitney test. The hydroxyproline ratios to the control value after tenotomy and after delayed tendon repair was also compared according to the period after the tenotomy, using the Mann-Whitney test. Correlations between the muscle excursion ratio to the control value and hydroxyproline, type I, III and IV collagen content ratio to the control value at each time point after tenotomy and between the muscle excursion ratio to the control value, and hydroxyproline content ratio to the control value 8 weeks after tendon repair were also analyzed. For all analyses, the level of significance was $p < 0.05$.

III. Results

1. Passive muscle excursion after tenotomy

The mean passive muscle excursions after tenotomy are shown in Table 1 and Figure 1. There was a significant difference in the mean muscle excursion relative to the control among groups ($p < 0.05$). Post-hoc analysis showed a significant decrease in the muscle excursion relative to the control between 1 and 6 weeks after tenotomy ($p < 0.05$).

2. Hydroxyproline content of muscle after tenotomy

Hydroxyproline content of the muscle was measured to quantify the total collagen. There was a significant difference in the mean hydroxyproline content

relative to the control after tenotomy among groups ($p < 0.05$). Post-hoc analysis showed a significant increase in the hydroxyproline content relative to the control between 1 and 4 weeks after tenotomy ($p < 0.05$) and between 1 and 6 weeks after tenotomy ($p < 0.05$; Table 1 and Figure 1).

Table 1. Parameters of EDII muscles of rabbits at various times after tenotomy

Time after tenotomy	**1 week**	**2 weeks**	**4 weeks**	**6 weeks**	***p* value**
Passive muscle excursion (mm)	3.7 ± 0.5	3.7 ± 0.3	3.1 ± 0.8	2.4 ± 0.4	
Passive muscle excursion ratio (treated/control)	0.94 ± 0.06	0.92 ± 0.13	0.76 ± 0.25	0.60 ± 0.12	<0.05†
Hydroxyproline content (ng/mL)	591.86 ± 82.52	678.95 ± 94.93	865.21 ±171.95	740.09 ±240.86	
Hydroxyproline content ratio (treated/control)	0.98 ± 0.31	1.16 ± 0.27	1.59 ± 0.43	1.62± 0.10	<0.05*,†
Type I collagen content (ng/mL)	32.85 ± 5.94	49.88 ± 1.59	64.88 ± 2.53	65.39 ± 9.81	
Type I collagen content ratio (treated/control)	1.06 ± 0.22	1.17 ± 0.08	1.55 ± 0.14	1.61 ± 0.43	<0.05*,†
Type III collagen content (ng/mL)	9.03 ± 1.91	7.34 ± 1.96	6.58 ± 1.48	5.62 ± 1.68	
Type III collagen content ratio (treated/control)	0.92 ± 0.20	0.78 ± 0.20	0.68 ± 0.29	0.57 ± 0.33	<0.05†
Type IV collagen content (ng/mL)	1.89 ± 1.62	2.27 ± 1.01	2.68 ± 0.55	2.70 ± 1.79	
Type IV collagen content ratio (treated/control)	0.87 ± 0.48	1.43 ± 0.59	1.24 ± 0.57	1.57 ±1.05	0.106

Data are presented as means ± SE; * significant post-hoc difference between 1 and 4 weeks after tenotomy ($p < 0.05$); †significant post-hoc difference between 1 and 6 weeks after tenotomy ($p < 0.05$).

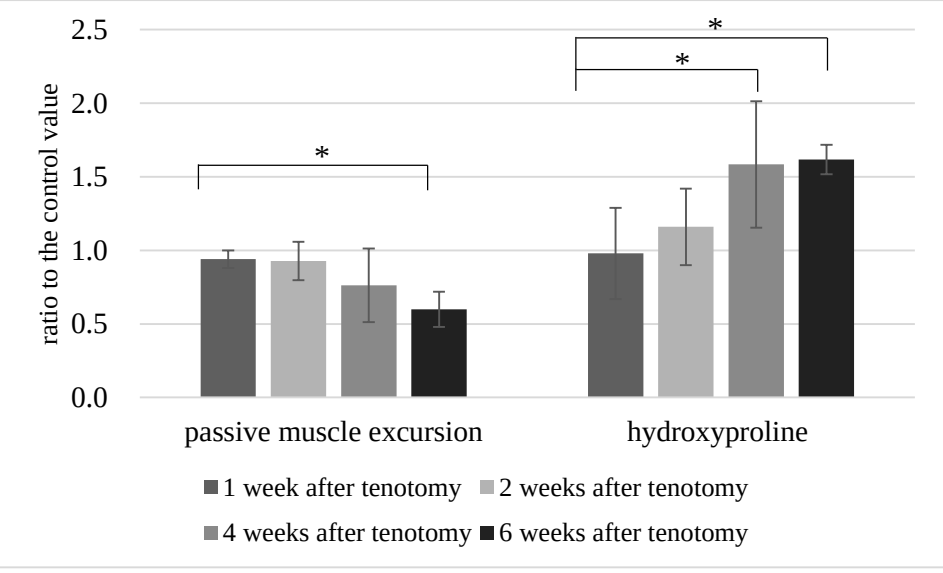


Figure 1. Passive muscle excursion and hydroxyproline content of the EDII muscle of rabbits at various times after tenotomy. Data represent means ± SE and are relative to the control values. *significant post-hoc difference (p < 0.05).

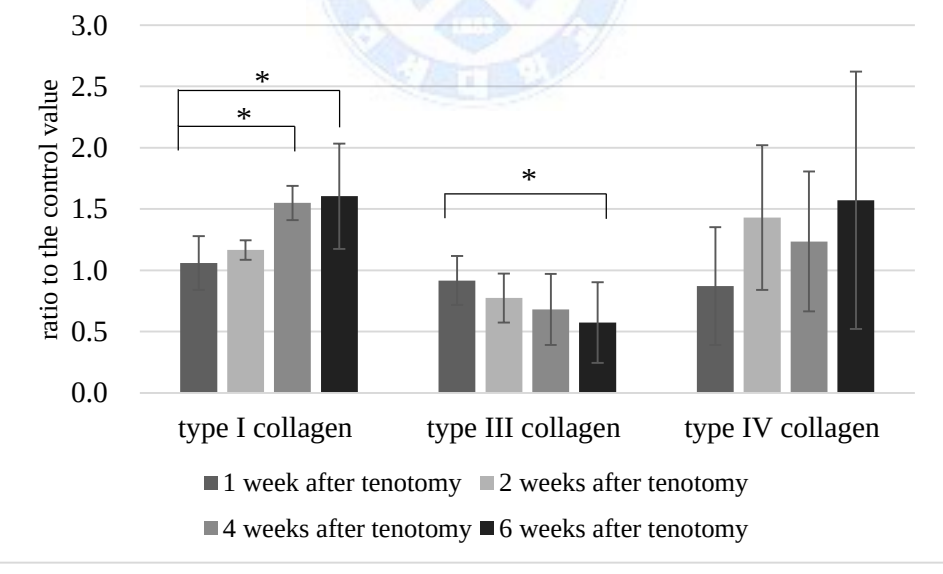


Figure 2. Collagen content of the EDII muscle of rabbits at various times after tenotomy. Data represent means ± SE and are relative to the control values. *significant post-hoc difference (p < 0.05).

3. Collagen content of muscle after tenotomy

Collagen contents of the muscles after tenotomy are shown in Table 1 and Figure 2.

There was a significant difference in the mean type I collagen content relative to the control after tenotomy among groups ($p < 0.05$). Post-hoc analysis showed a significant increase in the type I collagen content relative to the control between 1 and 4 weeks after tenotomy ($p < 0.05$) and between 1 and 6 weeks after tenotomy ($p < 0.05$).

There was significant difference in the mean type III collagen content relative to the control after tenotomy among groups ($p < 0.05$). Post-hoc analysis showed a significant decrease in the type III collagen content relative to the control between 1 and 6 weeks after tenotomy ($p < 0.05$).

There was no significant difference in the mean type IV collagen content ratio after tenotomy among groups ($p = 0.106$).

4. Histological findings after tenotomy

Masson's Trichrome staining of cross-sections of EDII muscle showed no differences 1 week after tenotomy. Two weeks after tenotomy, a slight thickening of the perimysium was seen and fibroblast infiltrations in the endomysium around the atrophic muscle fiber were noted. Four weeks after tenotomy, the perimysium and endomysium were thickened and the endomysium had more fibroblasts than at earlier times. The thickened endomysia were connected to each other and to the perimysia. Six weeks after tenotomy, there were more atrophic muscle fibers than earlier. The perimysium was slightly thickened than at 4 weeks, but the endomysium became more thickened and there were increased connections between them; additionally, fatty infiltration was noted (Fig. 3).

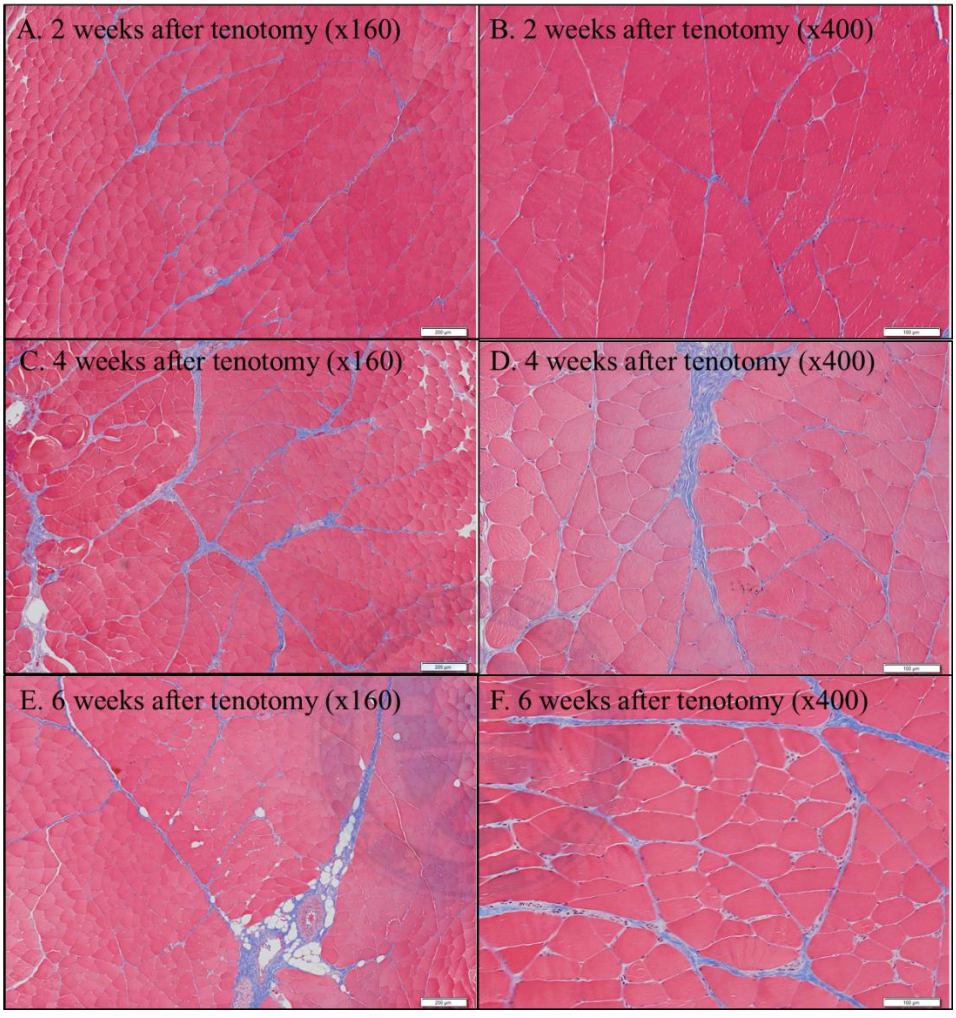


Figure 3. Photomicrographs of cross-sections of rabbit EDII after tenotomy, stained with Masson's trichrome. (A, B) Two weeks after tenotomy, slight thickening of the perimysium with fibroblast infiltrations in the endomysium is apparent. (C, D) By the 4 weeks after tenotomy, thickening of the perimysium is more obvious with increased fibroblast presence. (E) Note fatty infiltration at 6 weeks. (F) Also at 6 weeks, endomysium thickening and increased connections between the thickened endomysia are apparent (Scale bars: A, C and E, 200 μm and B, D and F, 100 μm).

5. Passive muscle excursion after tendon repair

The mean passive muscle excursions after tendon repair at the times measured are shown in Table 2 and Figure 4. There was a significant difference in the muscle excursion relative to the control after tendon repair among groups ($p < 0.05$). Post-hoc analysis showed a significant decrease in the muscle excursion relative to the control between 1 and 4 weeks after tenotomy ($p < 0.05$) and between 1 and 6 weeks after tenotomy ($p < 0.05$). Significant differences in excursion values relative to the control were observed between tenotomy and delayed tendon repair group at all-time points ($p < 0.05$; Fig 5).

Table 2. Passive muscle excursion and hydroxyproline content of the EDII muscle of rabbit after tenotomy with delayed tendon repair

Time of repair after tenotomy	**1 week**	**2 weeks**	**4 weeks**	**6 weeks**	***p* value**
Passive muscle excursion (mm)	3.7 ± 0.5	3.8 ± 0.5	3.0 ± 0.5	2.4 ± 0.3	
Passive muscle excursion ratio (treated/control)	0.97 ± 0.09	0.94 ± 0.16	0.73 ± 0.12	0.58 ± 0.10	<0.05*,†
Hydroxyproline content (ng/mL)	644.27 ± 64.11	684.24 ± 187.93	791.42 ± 152.78	792.10 ± 311.20	
Hydroxyproline content ratio (treated/control)	1.18 ± 0.22	1.20 ± 0.40	1.71 ± 0.96	1.93 ± 1.10	<0.05†

Hydroxyproline was used as a measure of total collagen. *Significant post-hoc difference between 1 and 4 weeks after tenotomy ($p < 0.05$), † significant post-hoc difference between 1 and 6 weeks after tenotomy ($p < 0.05$).

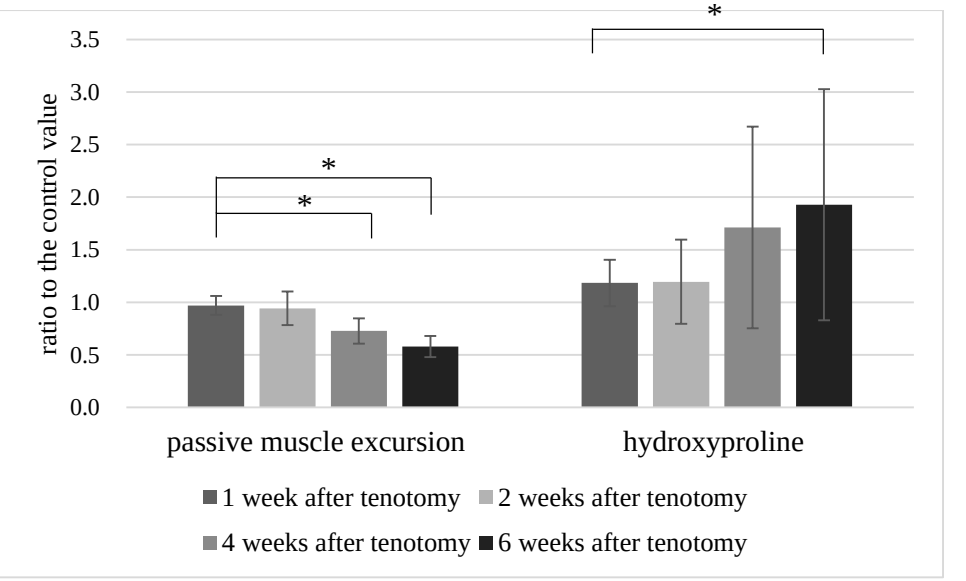


Figure 4. Passive muscle excursion and hydroxyproline content as a measure of total collagen after delayed tendon repair in the EDII muscle of rabbit. Data are expressed as means ± SE and relative to the control values. *significant post-hoc difference ($p < 0.05$).

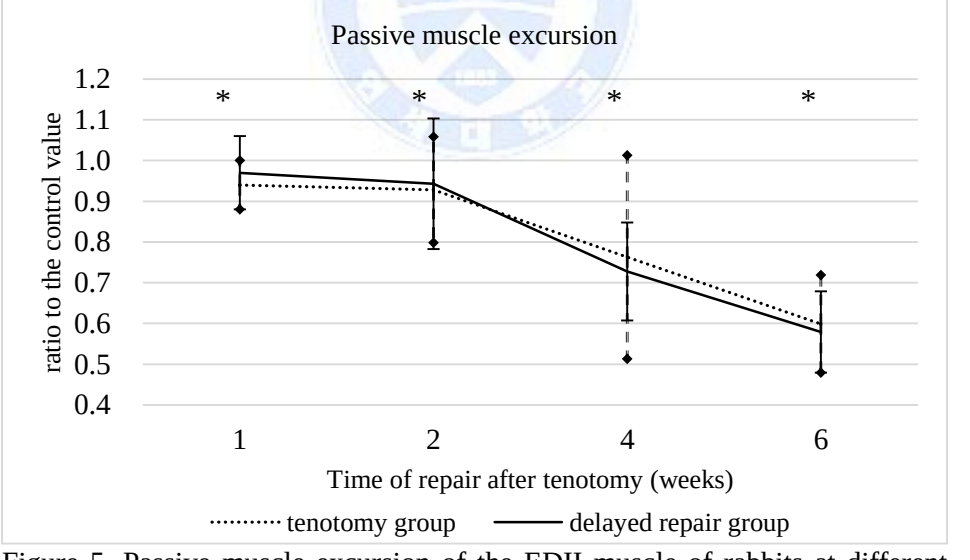


Figure 5. Passive muscle excursion of the EDII muscle of rabbits at different times after tenotomy. The tendon was repaired at the indicated time in the delayed repair group. Data are expressed as means ± SE and relative to the control values. *significant difference between groups ($p < 0.05$).

6. Hydroxyproline content as a measure of total collagen in muscle after tendon repair

The mean hydroxyproline contents in the muscle after tendon repair are presented in Table 2 and Figure 4. There was a significant difference in the mean hydroxyproline content relative to the control after tendon repair among groups ($p < 0.05$). Post-hoc analysis showed a significant increase in the hydroxyproline content ratio between 1 and 6 weeks after tenotomy ($p < 0.05$). Significant differences in hydroxyproline content relative to the control were observed between tenotomy and delayed tendon repair group according to period after tenotomy ($p < 0.05$; Fig. 6).

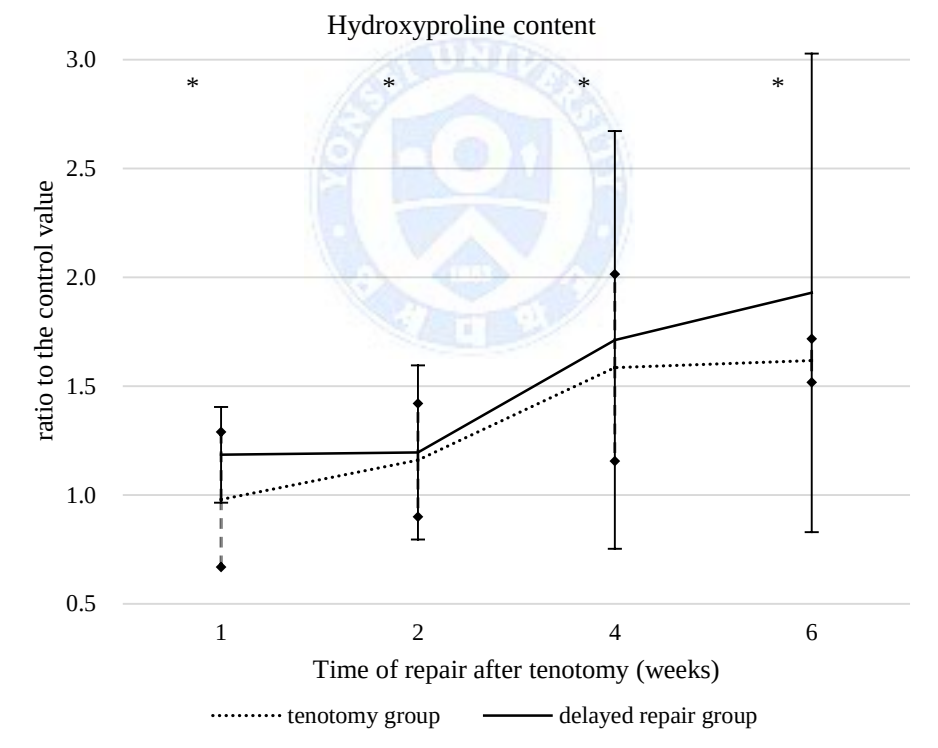
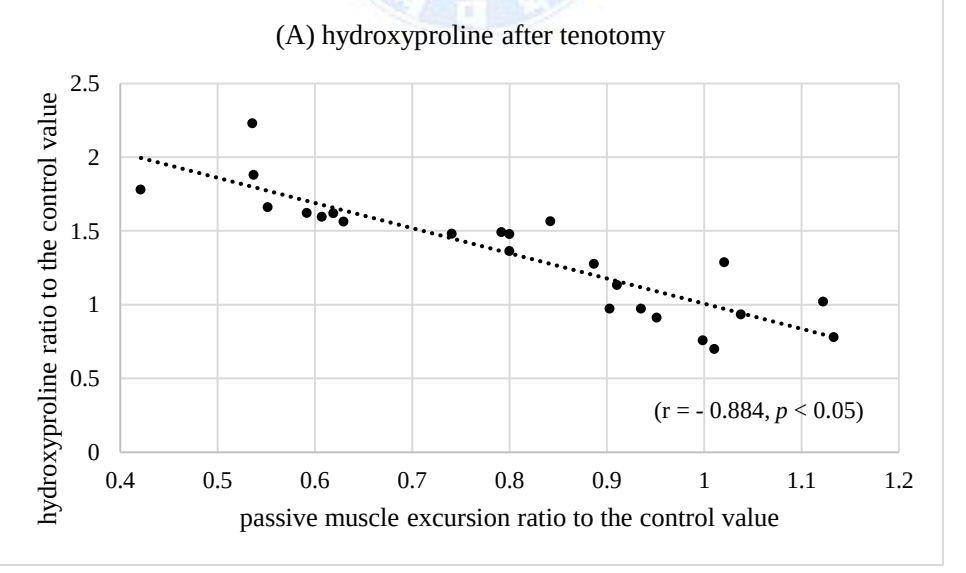
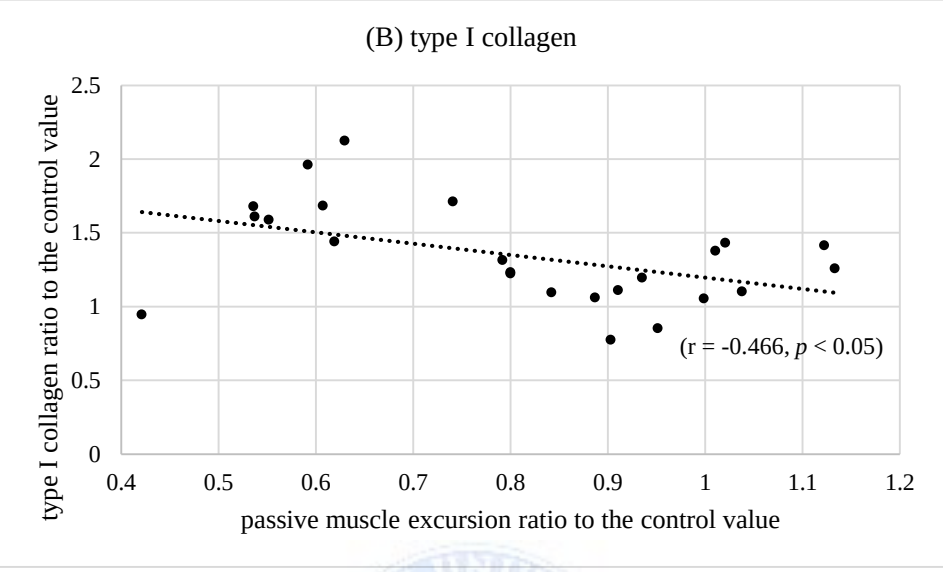


Figure 6. Hydroxyproline content in the EDII muscle as a measure of total collagen at different times in rabbits subjected to tenotomy or tenotomy with delayed repair at different times after tenotomy. Data are expressed as means ± SE and relative to the control values. *significant difference between groups ($p < 0.05$).

7. Correlation analysis

When considering the values relative to the control, we observed a significant correlation between the muscle excursion values and the hydroxyproline content after tenotomy ($r = -0.884$, $p < 0.05$). There was also a significant correlation between muscle excursion values and the amount of type I collagen after tenotomy ($r = -0.466$, $p < 0.05$). We conclude that the decreased muscle excursion was associated with increases in total type I collagen content following tenotomy. There was no significant correlation between the muscle excursion values and the type III collagen content ($r = 0.283$, $p = 0.18$) or with type IV collagen content after tenotomy ($r = -0.19$, $p = 0.373$; Fig. 7). However, we noted a significant correlation between the muscle excursion values after tendon repair and the hydroxyproline content after delayed tendon repair ($r = -0.721$, $p < 0.05$). It may be that the decrease in muscle excursion correlated with the increase in muscle total collagen content over repair time following tenotomy (Fig. 8).





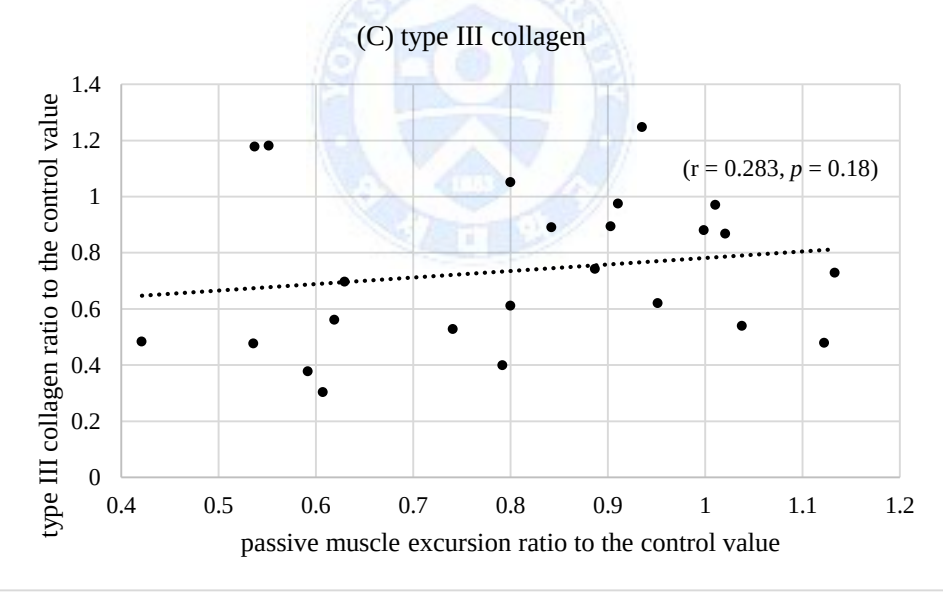


Figure 7. Correlations between the excursion ratios relative to the control values for several parameters in EDII muscles of rabbits after tenotomy.

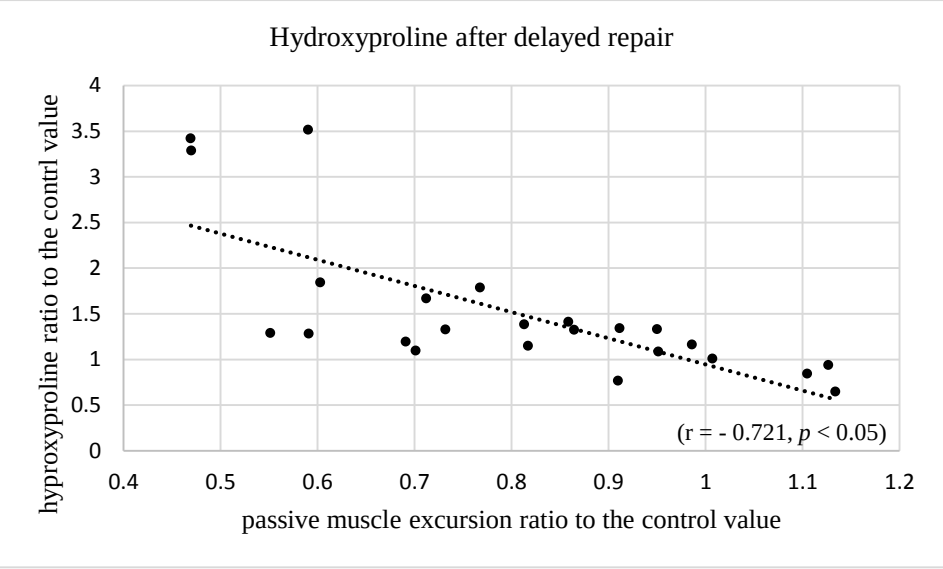


Figure 8. Correlation between muscle excursion values and the hydroxyproline content, both relative to the control in EDII muscles of rabbits after tenotomy with delayed tendon repair.

IV. Discussion

To find out the relationships between the change in muscle excursion and collagen contents after tendon rupture and delayed repair, tenotomy on EDII muscles of rabbits was performed on the right legs with or without subsequent repair. The intact left legs served as the control. The muscle excursion, the content of hydroxyproline, and the type I, III, and IV collagen content relative to the control were used to counter the individual differences. Total collagen and type I collagen significantly increased with time after tenotomy ($p < 0.05$), excursion and type III collagen decreased significantly ($p < 0.05$), and there was no significant change in type IV collagen ($p = 0.106$). Muscle excursion ratio was significantly negatively correlated ($p < 0.05$) with total collagen and type I collagen after tenotomy. Perimysium and endomysium thickening and a greater number of connections between thickened endomysia were noted 6 weeks after

tenotomy compared to the control. The total collagen 8 weeks after tendon repair increased significantly and muscle excursion decreased significantly with delay in repair ($p < 0.05$), so that the excursion was significantly negatively correlated with total collagen ($p < 0.05$). The increase in collagen that was observed after tenotomy was not reversed by repair. Improvement in excursion was noted when repair was delayed by 1 or 2 weeks, but when it was delayed by 4 or 6 weeks, excursion decreased. Type I collagen has been associated with decreased excursion after tendon rupture.

Muscle excursion can be divided into two phases. Active excursion is a change in length caused by muscle contraction, which is 40% of the length of the muscle. Passive excursion is the change in length when maximum passive tension is applied on a tendon under retraction.[29] Active excursion is related to the number of sarcomeres (which is equal to the length of the muscle fibers), and can change so that the length of individual sarcomeres does not change, maintaining their optimal length in the presence of sustained length change in muscles at rest.[30,31] Passive excursion is thought to be related to the number of sarcomeres as well as the passive stiffness of the muscle. Passive muscle stiffness is, in turn, determined by the isoform of titin that is present and the amount of collagen.[32,33] The amount of the titin isoform determines the muscle fiber stiffness within a sarcomere, and the collagen content determines muscle stiffness on the whole-muscle basis.[34] There is disagreement about the role sarcomere number plays in passive excursion under pathological conditions, which was suggested to be due to the importance of the ECM.[35] In the present study, the hydroxyproline content was negatively correlated with excursion over time after tenotomy with delayed tendon repair. It may be that the decrease in passive excursion is determined by the increase in the total collagen after tenotomy.

In a number of studies, most hydroxyproline has been detected in fibrillar collagens, such as types I and III, and to a much lesser extent in non-fibrillar collagens, such as type IV. Nonetheless, hydroxyproline content is considered to represent total collagen.[36,37] In the present study, the ratio of type I to type III collagen 1 week after tenotomy was 3.6:1 and type I was negatively correlated with passive excursion while type III was positively correlated with passive excursion. Type I and III collagen are important components of the perimysium and epimysium and their relative amounts differ between species, and between muscle groups within the same species.[38,39] Therefore, different results might be observed according to ratio of type I to type III collagen content if excursion is analyzed according to hydroxyproline content. Type I collagen, a fibrillar collagen, is composed of a bundle of thick fibrils with an average diameter of 75 nm. It is thought to resist tension and so decreases excursion via increased passive stiffness. In contrast, type III collagen is composed of loosely packed bunches of thin fibers with a diameter of 45 nm and provides structural stability to expandable organs, such as the uterus.[40] The difference in tensile stress between type I and type III collagen may determine passive stiffness and excursion. If there is a high ratio of type I to type III collagen in a muscle, increased passive stiffness and decreased excursion would be expected. Therefore, measures that suppress increases in type I collagen content might prevent a decrease in excursion after tendon rupture.

The amount hydroxyproline and type I collagen relative to the control increased by the fourth week after tenotomy and changed little after that. This result is consistent with the histological examination: thickening of perimysium and the slight thickening of endomysium were noted 4 weeks after tenotomy, but did not continue to increase rapidly after that. The increase in collagen during the fourth week after tenotomy is in agreement with studies that found

that internal changes in the muscle cells were completed 4 weeks after the alterations to muscle use.[31,41] However, a decrease in excursion was observed during the six weeks after tenotomy. The decrease in excursion between weeks 4 and 6 cannot be explained by an increase in collagen content, because the increase of total collagen content was not significant between weeks 4 and 6. It may have been partially caused by structural changes in the collagen. Structural changes in collagen involve pyridinium cross-linking and advanced glycation end products.[42] Cross-linking stiffens collagen fibers.[43] Such structural changes are reportedly irreversible and accumulate over time, contributing to the aging process.[42,44,45] Additional experiments should determine whether a decrease in excursion 4 weeks after tenotomy is related to collagen cross-linking.

Type IV collagen is a non-fibrillar collagen and important component of the endomysium.[18] It forms a reticular meshwork of individual muscle fibers and may function in resistance to multi-directional, rather than longitudinal, tension.[23] In addition, the endomysium connects individual muscle fibers through a basement membrane and is important in lateral force transmission.[46] The collagen of the endomysium and perimysium increases after tenotomy.[15] In the present study, thickening of the endomysium was accompanied by increased fibroblast infiltration 4 weeks after tenotomy and was even more apparent 6 weeks after tenotomy. The type IV collagen content relative to the control tended to increase over time after tenotomy. Although this was not significant, a larger study might be useful to determine if this trend is real. One study reported that, after high-force eccentric contraction, thickness of the endomysium increased as the type IV collagen content of the skeletal muscle increased.[47] Furthermore, because type IV collagen other than that in the ECM is distributed widely in the basal lamina of microvessels and vessel density decreases after tenotomy, changes in microvessels may be an important factor in analysis of

type IV collagen content.[48] Another report also stated that synthesis of type I, III, and IV collagen in muscles decreased after long periods of immobilization.[49] These results are confounding factors in the interpretation of our results. However, an increase of type IV collagen content may be related to the thickening of the endomysium as the amount of type IV collagen tended to increase relative to the control over time. Immunohistochemical analysis might clarify this issue.

After delayed repair, the hydroxyproline content relative to the control increased after repair (Fig. 6). Therefore, the accumulation of collagen in response to tenotomy was not reversed within the 8 weeks of this study. In particular, a gradual increase in hydroxyproline content was observed at 4 and 6 weeks in the delayed tendon repair group. This is considered to be caused by fibrosis resulting from micro-circulation dysfunction of fixed muscle in an overstretched state, which is induced via a decrease in excursion over time. Improvement in excursion ratio was noted 1 and 2 weeks after the repair, whereas a decline in excursion compared to the control was noted 4 and 6 weeks after repair (Fig. 5). Therefore, improvement in excursion may be possible if repair is performed up to 2 weeks after tenotomy. After 4 weeks, the increased collagen content may limit the degree of tendon repair, causing pathologic positive feedback of fibrosis and preventing improvement in excursion. Therefore, end-to-end repair or tendon grafting more than 4 weeks following the tendon rupture is unlikely to result in recovery from excursion. Tendon transfer might be the best available option for the treatment of tendon rupture that has been neglected for more than 4 weeks.

There are several limitations to the present study. First, the applied maximal passive tension may be subjective when measuring passive excursion. Muscle excursion has been measured during full flexion and extension of joints

using ultrasonography or magnetic resonance imaging, but in cases of tendon rupture, excursion measurement by joint motion is impossible.[50,51] In this study excursion was measured by manual traction to the extent that further plastic deformation did not occur. Tendon traction using a specific weight was thought to be a more objective method than manual traction, but because excursion could vary according to the weight applied to a tendon, it is difficult to determine the proper weight and interpret the results. Maximal manual traction force might be subjective, but this measurement method was thought to be more applicable and reproducible in a clinical situation.[8] Second, our study was focused on the relationship between passive muscle excursion and collagen content following tenotomy and delayed tendon repair. Sarcomere number is also considered to be an important factor in muscle excursion and a change in sarcomere number after tenotomy with delayed repair could be important in muscle excursion. Understanding the effect of the amount of collagen on muscle excursion will require assessing the effect of a decreased sarcomere number in passive excursion.

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V. Conclusion

In this study, muscle excursion decreased, the amount of total collagen and type I collagen increased, and type III collagen decreased over time after tenotomy, while little change in type IV collagen content was observed. Total collagen and type I collagen content were negatively correlated with excursion after tenotomy. Type I collagen is considered an important factor in decreased excursion after a tendon rupture. The total collagen did not decrease once the tendon was repaired and increases in excursion were only observed when the repair was performed within 2 weeks after tenotomy. Therefore, in order to

prevent a decrease in excursion after tendon rupture, measures to suppress increased type I collagen content should be developed. In addition, tendon transfer should be considered as a treatment option when tendon rupture has been neglected for more than 4 weeks.

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ABSTRACT(IN KOREAN)

건 파열 및 지연봉합 후 발생한 근 가동역 및 콜라겐 총량 변화의 연관성

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고일현

건 파열 후 및 지연된 건 봉합 후 발생하는 근 가동역 변화의 원인을 이해하는 것은 진구성 건 파열의 치료시기 및 방법을 결정하는데 중요하다. 근육의 수동성 경직도를 결정한다고 알려진 콜라겐의 총량 및 아형의 변화가 건 파열 후 및 지연된 건 봉합 후 근 가동역의 변화와 연관이 있다는 가설 하에, 24 마리 토끼의 우측 제 2 족지 신전건을 절제하고 네 군으로 나누어 1, 2, 4, 6 주 후에 양측 제 2 족지 신전근의 콜라겐 총량, 1, 3, 4 형 콜라겐의 양 및 수동적 근 가동역을 측정하였고 조직학적 검사를 시행하였다. 또한 다른 24 마리 토끼의 우측 제 2 족지 신전건을 절제하고 네 군으로 나누어 1, 2, 4, 6 주 경과 후 지연된 건 봉합술을 실시하고, 양측 제 2 족지 신전근에 대해 봉합 8 주째 콜라겐 총량 및 수동적 근 가동역을 측정하였다. 개체간 차이를 보정하기 위해 좌측의 측정치를 대조군으로 하여, 우측의 측정값을 좌측의 측정값으로 나눈 비율을 통계적으로 분석하였다. 건 절제 후 시간이 지남에 따라 콜라겐 총량 및 1 형 콜라겐의 비율은 증가하였으나($p < 0.05$), 3 형 콜라겐의 비율은 감소하였고($p < 0.05$), 4 형 콜라겐은 의미있는 변화를 보이지 않았다($p = 0.106$). 건 절제술 후 근 가동역의 비율은 콜라겐 총량($r = -0.884$, $p < 0.05$) 및 1 형

콜라겐(r = - 0.466, p < 0.05)의 비율에 대해 통계학적으로 의미있는 음의 상관 관계를 보였다. 건 절제 후 6 주째 근 주막 및 근 내막의 비후 및 비후된 근 내막간의 연결이 증가된 소견 보였다. 건 파열 후 건 봉합이 지연될 수록 봉합 후의 콜라겐 총량의 비율은 증가하였고(p < 0.05), 근 가동역의 비율은 감소하였다(p < 0.05). 지연된 건 봉합을 시행한 군에서도 건 절제술을 시행한 군과 같이, 근 가동역의 비율은 콜라겐 총량의 비율에 대해 의미있는 음의 상관관계를 보였다(r = - 0.721, p < 0.05). 건 파열 상태가 지속된 기간을 기준으로 건 절제군과 지연된 건 봉합군을 비교하였을 때 지연된 건 봉합군에서 모든 기간에서 의미있는 콜라겐 총량의 증가가 있었다(p < 0.05). 건 절제 1, 2 주 경과후 봉합시에는 건 절제군에 비해 근 가동역의 의미 있는(p < 0.05) 호전이 관찰되었으나, 4, 6 주 경과후 봉합시에는 건 절제군에 비해 오히려 근 가동력이 의미 있게(p < 0.05) 감소하였다. 결론적으로 1 형 콜라겐의 증가가 건 파열 후 근 가동역의 감소와 연관이 있으며, 근 가동역의 감소를 막기 위해서는 1 형 콜라겐의 증가를 억제하는 방법이 개발되어야 하겠다. 또한 건 파열 후 치료가 4 주 이상 지연된 경우 근 가동역의 의미있는 호전이 보이지 않으므로, 4 주 이상 지연된 건 파열의 치료시에는 건 이전술의 사용을 고려하는 것이 유용할 것으로 사료된다.

핵심되는 말: 콜라겐, 콜라겐 아형, 근 가동역, 건 파열, 지연된 건 봉합

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