The effect of eccentric exercise on injured patellar tendon healing in rats: a gene expression study

The effect of eccentric exercise on injured patellar tendon healing in rats: a gene expression study

Department of Orthopedic Surgery, Graduate School of Medical Science, Kanazawa University
Masafumi Yagishita

Abstract

Recently, clinical studies have suggested that eccentric exercise can be beneficial for patellar tendinopathy. It is known that loading induces collagen synthesis in tendon, but the mechanisms responsible for mediating this effect are still unclear. We hypothesized that loading-induced expression of collagen depends on a specific contraction type. Eccentric exercise induces a more beneficial healing response than concentric exercise. Two longitudinal incisions were made in rat patellar tendons and the rats were then divided into three groups: eccentric exercise group (group E; n=35); concentric exercise group (group C; n=35); non-exercise group (group N; n=35). Each rat was taught to run downhill (eccentric) or uphill (concentric) for 14 days. Patellar tendons were collected at 1, 4, 7, 14, 21, 28 and 42 days following injury. Type I and III collagen, transforming growth factor-β-1 (TGF-β-1), and matrix metalloproteinase-13 (MMP-13) were measured by reverse transcription polymerase chain reaction. The gene expression level of type I collagen of group E was significantly greater than that of group N on day 4. The gene expression level of type III collagen of group E was significantly greater than that of group N on days 7 and 14. The gene expression level of TGF-β-1 of group E was significantly greater than that of group C and group N on days 4 and 7. The gene expression level of MMP-13 of group C and group N was significantly greater than that of group E on day 21. Eccentric exercise can more effectively induce TGF-β-1 expression compared to concentric exercise and subsequently cause high expression of type I and III collagen. In addition, our results showed that eccentric exercise also reduces MMP-13 gene expression. In conclusion, eccentric exercise can aid tendon repair.

Key words eccentric exercise, patellar tendinopathy, collagen synthesis

Introduction

Patellar tendinopathy occurs in several sports and the condition can severely limit or even end an athletic career. This condition particularly affects elite athletes in jumping sports[1]. The etiology and pathogenesis of chronic tendon pain is not fully understood, although histopathological and biochemical evidence indicates that it is not an inflammatory condition[2]. A previous report about patellar tendonitis concluded that it was caused by dissociation of fibers rather than partial fiber rupture[3]. The initial treatment of patellar tendinopathy typically includes rest, ice, electrotherapy, taping, anti-inflammatory medication, and/or corticosteroid injection. However, these treatment regimens have not been demonstrated to be effective and thus they have no evidence-based support[4].

Recently, clinical studies have suggested that eccentric exercise can be beneficial for several tendinopathies including patellar tendinopathy[5], Achilles tendinopathy[6], and lateral humeral epicondylalgia[7], but the mechanism remains unclear. An eccentrically loaded isometric contraction was defined as a contraction during which the musculotendinous unit was active in an attempt to

平成23年5月18日受付，平成23年6月17日受理

Abbreviations: COL1A1, collagen type I a 1; COL3A1, collagen type III a 1; TGF-β-1, transforming growth factor-β-1; MMP-13, matrix metalloproteinase-13; GAPDH, glyceraldehyde-3-phosphate dehydrogenase

maintain a specific joint position as it resisted the lengthening imposed by an external load. Conversely, a concentrically loaded isometric contraction was defined as an isometric contraction during which the contractile apparatus attempted to shorten while joint motion was restricted. Previously, we showed that eccentric exercise helps to stabilize angiogenesis during the early phase of tendon injury. Conversely, concentric exercise, which destabilizes angiogenesis, leads to a delayed healing response. Initiation of eccentric exercise immediately after tendon injury may improve healing by reducing vascularity[8].

The fibrillar collagens, types I and III, are the major components of tendon. Loading induces collagen synthesis in tendon, but the mechanisms responsible for mediating this effect are still unclear[9]. Several observations point to transforming growth factor-β-1 (TGF-β-1) as an essential mediator of mechanically-induced collagen synthesis in a variety of cell types including patella tendon fibroblasts and this growth factor may act as a link between loading and collagen synthesis in tendons[10][11]. Mechanical loading of tendons has also been suggested to induce certain changes in collagen structure, possibly involving increased collagen cross-linking and degradation[12][13]. Therefore, exercise may change the expression of enzymes involved in collagen processing such as matrix metalloproteinase-13 (MMP-13), which regulates degradation of collagen molecules[14].

The aim of the present study was to investigate the specific effects of concentric exercise and eccentric exercise on expression of type I and III collagen mRNA in tendon in rats. Furthermore, to identify possible mediators of loading-induced collagen regulation in these tissues, we studied the changes in expression of TGF-β-1 and MMP-13. We hypothesized that loading-induced expression of collagen, TGF-β-1 and MMP-13 in tendon would depend on contraction type.

Materials and Methods

***Tendon injury model*:** 105 female Wistar rats (180-188g, Charles River Laboratories, Yokohama, Japan) aged 7 weeks old were used in this study. The rats were anesthetized by an intramuscular injection of ketamine (25 mg/kg). We used a fiber dissociation model to mimic patellar tendon injury. To create a fiber dissociation model of patellar tendon injury, we made a small incision in the skin to expose the patella tendon and created two longitudinal splits in each tendon for the patellar tendon injury model[8]. Rats were assigned randomly to three groups: an eccentric exercise group (group E; n=35), a concentric exercise group (group C; n=35), and a non-exercise group (group N; n=35). Rats were given a full day to recover from surgery before beginning the exercise training program. The protocol was approved by the Institution Animal Care and Use Committee of Kanazawa University.

***Exercise training*:** While running down an incline, extensor muscles primarily perform eccentric contractions in which the muscles lengthen while they are actively developing tension. The major function of these extensor muscles during downhill running is to decelerate the animal's center of mass to maintain a constant average running velocity. Therefore, the eccentric exercise group ran down a 15 °incline. On the other hand, the extensor muscles perform concentric contractions during uphill running[15]. The concentric exercise group ran on a treadmill up a 15 °incline. Each rat exercised once daily on a motor-driven rodent treadmill (Muromachi Kikai,. Tokyo, Japan) for 1 hr at 15 m/min in room air for a maximum of 14 days. After exercise, each rat was returned to the same environment as those in the non-exercise group. Rats in the non-exercise group were allowed to walk freely in their cages without restriction for the entire duration of the experiment.

***Tissue collection and preparation of mRNA*:** To study the time course of RNA expression in response to exercise, patellar tendons of each rat were collected at 1, 4, 7, 14, 21, 28 and 42 days following injury (five rats for each time point). To prepare mRNA, about 30 mm^3 rat tendon was acquired and dissolved in 1ml trizol (Invitrogen, Carlsbad, USA). To extract RNA, 200μl chloroform was added. RNA was then precipitated from the aqueous phase

Table 1. PCR conditions of each gene

Gene name	Course of temperature and time	Cycle
COL1A1	94 ℃ (15s)- 57 ℃ (30s)- 72 ℃ (30s)	25
COL3A1	94 ℃ (15s)- 57 ℃ (30s)- 72 ℃ (30s)	35
TGF-β-1	94 ℃ (15s)- 55 ℃ (30s)- 72 ℃ (30s)	35
MMP-13	94 ℃ (15s)- 60 ℃ (30s)- 72 ℃ (30s)	35
GAPDH	94 ℃ (15s)- 57 ℃ (30s)- 72 ℃ (30s)	25

COL1A1; collagen type I a 1: COL3A1; collagen type III a 1: TGF-β-1; transforming growth factor-β-1: MMP-13; matrix metalloproteinase-13: GAPDH; glyceraldehyde-3-phosphate dehydrogenase.

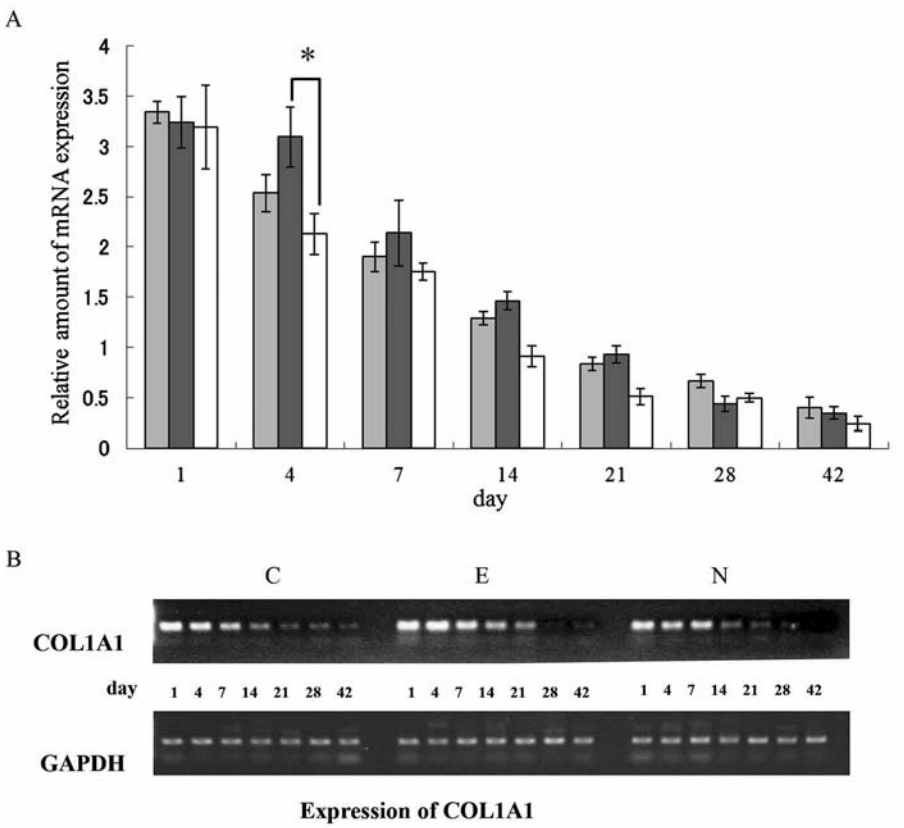

Fig. 1. The bands were visualized and analyzed quantitatively by densitometry (Image Gauge Version 4.0, Fuji Film, Tokyo, Japan). Values were then normalized to GAPDH RNA expression. Expression of type I collagen mRNA peaked at day 1 and decreased gradually. The gene expression level of the eccentric exercise group was significantly greater than that of the non-exercise group on day 4 (A and B). * P<0.05. C; concentric exercise group ▒. E; eccentric exercise group ■. N; non-exercise group □.

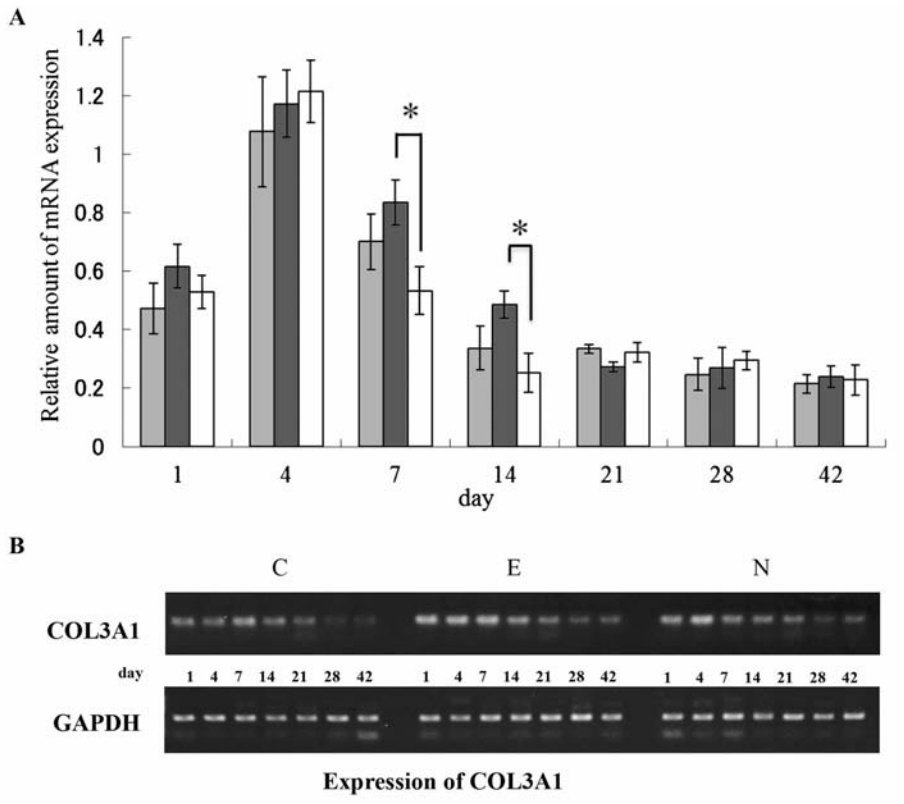

Fig. 2. Expression of type III collagen mRNA peaked at day 4 and decreased gradually. The gene expression level of the eccentric exercise group was significantly greater than that of the non-exercise group on days 7 and 14 (A and B. * P<0.05. C; concentric exercise group ▒. E; eccentric exercise group ■. N; non-exercise group □.

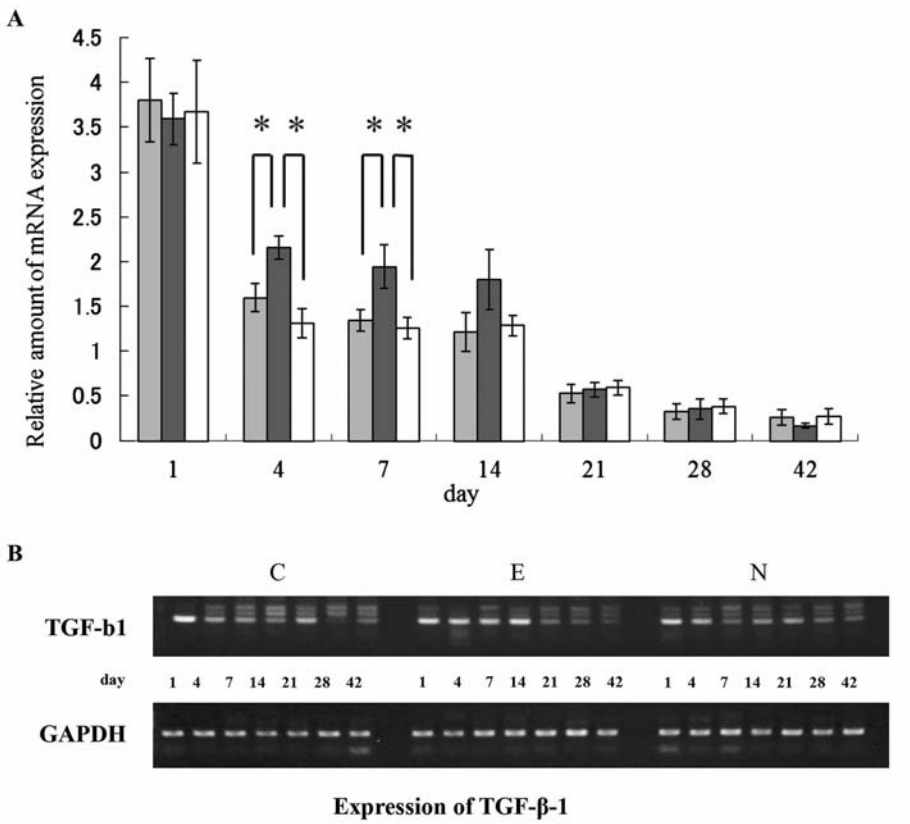

Fig. 3. Expression of transforming growth factor-β-1 mRNA peaked at day 1 and decreased gradually. The gene expression level of the eccentric exercise group was significantly greater than that of the concentric exercise and non-exercise groups on days 4 and 7 (A and B). * P<0.05. C; concentric exercise group ▒. E; eccentric exercise group ■. N; non-exercise group □.

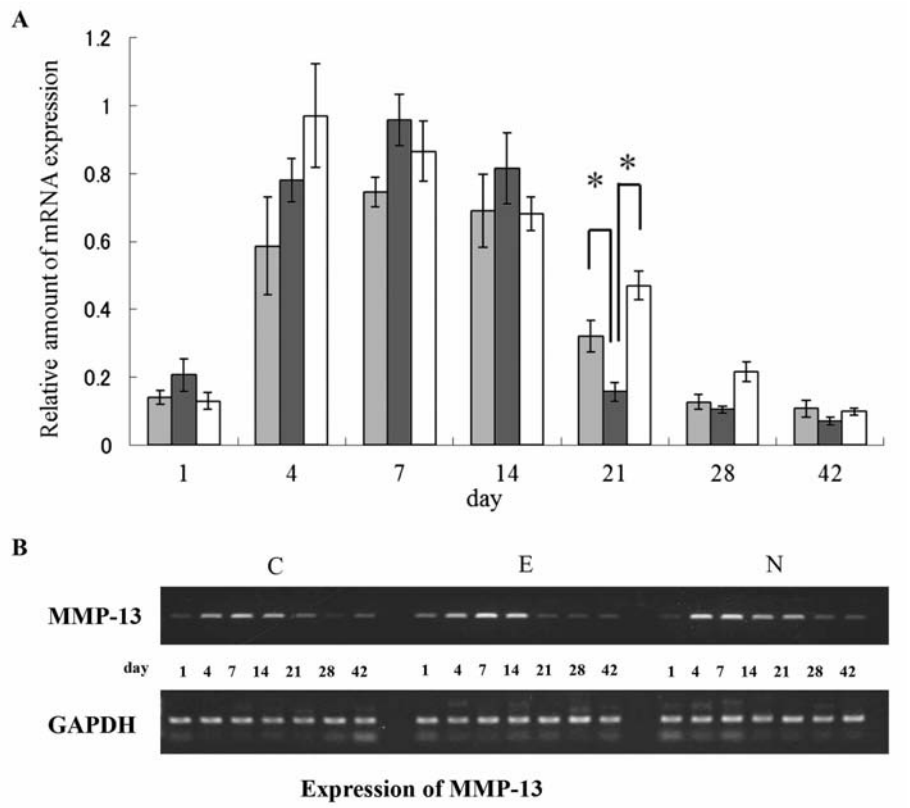

Fig. 4. Expression of matrix metalloproteinase-13 mRNA peaked from day 4 to day 14 and decreased gradually. The gene expression level of the concentric exercise group and non-exercise group was significantly greater than that of the eccentric exercise group on day 21 (A and B). * P<0.05. C; concentric exercise group ▒. E; eccentric exercise group ■. N; non-exercise group □.

by treating with the same amount of isopropyl alcohol and the pellet was washed with ethanol.

***Primer design*:** The genes examined in this study were collagen type I α1 (COL1A1), collagen type III α1 (COL3A1), transforming growth factor-β-1 (TGF-β-1), matrix metalloproteinase-13 (MMP-13), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Primers for all genes were designed using gene sequences published on the NCBI website. The sequence of each primer was as follows: COL1A1: 5'-TGA GCC AGC AGA TTG AGA AC-3' (sense), 5'-AAC CTT CGC TTC CAT ACT CG-3' (antisense); COL3A1: 5'-TGG CAT CAA AGG ACA TCG-3' (sense), 5'CAC CTC CAA CTC CAG CAA TG-3' (antisense); TGF-β-1: 5'-ATA CAG GGC TTT CGC TTC AG-3' (sense), 5'-GTG TGT CCA GGC TCC AAA TG-3' (antisense); MMP-13: 5'-GAT GTC AGG CAT TAA AGG AAG GGG ATA ACC-3'(sense), 5'-GCA CCA AGT GTC AGT CAC TAA GGA AAG CAG-3'(antisense); GAPDH: 5'-CTC CGC TCA CAT TCT GTT TG-3' (sense), 5'-ACC ATC GCC CAT CTT CAT C-3' (antisense).

***Semi-quantitative RT-PCR*:** Total RNA (1 μg) was converted to cDNA with Superscript III reverse transcriptase (Invitrogen) and oligo(dT)12-18 primers (GE Healthcare Bio-Sciences). RT-PCR was performed for COL1A1, COL3A1, TGF-β-1 and MMP-13. The thermal cycling program consisted of an initial denaturation step of 94 ℃ for 2 min, followed by the conditions listed in Table 1 and a 30s extension step at 72 ℃. It was confirmed that measurement by using the listed condition is performed within exponential phase. Aliquots of each RT-PCR product were electrophoresed on a 1.5% agarose gel containing ethidium bromide. The bands were visualized and analyzed quantitatively by densitometry (Image Gauge Version 4.0, Fuji Film, Tokyo, Japan). Values were then normalized to GAPDH RNA expression.

***Statistical analysis*:** Analysis of variance (two-way ANOVA) with Fisher's post-hoc analysis was used to determine if significant differences in mRNA levels existed among the experimental periods (at 1, 4, 7, 14, 21, 28, and 42 days after injury). Significance was taken as $P<0.05$.

Results

***Expression of mRNAs*:** Expression of COL1A1 peaked at day 1 after injury and decreased gradually in all groups. The gene expression level of the eccentric exercise group was significantly greater than that of the non-exercise group on day 4, but there was no significant difference between the concentric exercise group and the non-exercise group (Fig. 1). Expression of COL3A1 peaked at day 4 after injury and decreased gradually in all groups. The gene expression level of the eccentric exercise group was significantly greater than that of the non-exercise group on days 7 and 14, but there was no significant difference between the concentric exercise group and the non-exercise group (Fig. 2). Expression of TGF-β-1 peaked at day 1 after injury and decreased gradually in all groups. The gene expression level of the eccentric exercise group was significantly greater than that of the concentric exercise and non-exercise groups on days 4 and 7 (Fig. 3). Expression of MMP-13 peaked from day 4 to day 14 after injury and decreased gradually in all groups. The gene expression level of the concentric exercise group and the non-exercise group was significantly greater than that of the eccentric exercise group on day 21 (Fig. 4).

Discussion

Eccentric exercise is a treatment option for patellar tendinopathy and was first presented by Curwin and Stanish in 1985 with encouraging results[16]. Recently, eccentric exercise has been found to be effective in pilot studies of patients with patellar tendinopathy, but the mechanism remains unclear.

In this study, we developed a tendon injury model in rats. The patellar tendon of rats was incised with two longitudinal splits using a scalpel following the method used by Nakamura in a tendonitis model[8]. There are several animal models of tendonitis. Dahlgren et al.[17] developed collagenase-induced tendonitis lesions in the tensile region of the flexor digitorum superficialis tendon of adult horses using filter-sterilized bacterial collagenase type I. The collagenase-induced model is a well-established model. The acute swelling, matrix destruction, and increase in the cross-sectional area of the tendon are similar to those seen in naturally occurring tendon injuries. Kobayashi used external fixation and pneumatic cylinders in the patellas and tibias of domestic rabbits[3]. They developed a tendonitis model in which the patellar tendon underwent quantitative and cyclic tensile loading. These models are thought to closely reproduce real tendonitis. As mentioned previously, we used rats with

longitudinally incised patellar tendons as a tendonitis model because small animals are well suited for treadmill exercise. In addition, external fixation devices are difficult to place on rats. The longitudinal incision procedure has major advantages such as its ability to easily disrupt collagen fibers, which causes tendonitis, and its high reproducibility. The disadvantage is that, unlike collagenase type I, it does not reproduce naturally occurring tendon injuries.

Tendon healing occurs in three overlapping phases[18]. The first stage is the inflammatory phase in which monocyte and macrophage infiltration occurs within 24 hours after injury. The second stage is the remodeling phase in which synthesis of type III collagen begins 2-4 days after injury and continues for a few weeks. The third stage is the modeling phase, which begins 6 weeks after injury, where consolidation and maturation occur. A high proportion of type I collagen is synthesized during this phase.

In human and animals, mechanical loading by exercise has been shown to induce collagen synthesis in both tendon and muscle tissues. Heinemeier et al.[9] found a noticeably increased expression of type I and III collagen in tendon in response to short-term strength training in rats. However, very few *in vivo* results had been published with regard to tendon expression of collagen mRNA in response to loading. Moreover, there have not been any animal experiments examining collagen synthesis in the remodeling phase 6 weeks after exercise loading. Therefore, we focused on how various exercises affect collagen synthesis in the remodeling phase.

Type III collagen plays a central role in the remodeling phase and it begins to be expressed on the first day of injury. In our study, its expression peaked at 4 days after injury and gradually decreased thereafter. However, Oshiro et al.[19] reported that type III collagen expression peaked on the 14th day after injury and gradually decreased thereafter. The time to reach its peak expression differed because the injury model of Oshiro et al. involved transection of the flexor tendon of the third toe in rats, a single stitch suture using 9-0 Ethilon, and no exercise loading. In our study, exercise loading is thought to have promoted early expression of type III collagen. We also found that synthesis of type I collagen peaked on the first day after injury and gradually decreased thereafter. Previously, it had been thought that a high proportion of type I collagen is synthesized in the modeling phase at 6 weeks or more after injury. Archambault et al.[20] used 11 weeks of high-frequency loading and found increased type III collagen expression in rabbit Achilles tendon. However, they found no change in type I collagen expression. Our results showed almost no expression of type I collagen at 4 weeks or more after injury. Heinemeier et al.[9] showed an early increase in type I collagen expression with exercise loading of rat Achilles tendon. Their results are consistent with ours and indicate that type I collagen serves as a repair component in early remodeling, similar to type III collagen.

The mechanism is still unknown as to what induces collagen synthesis when tendon is loaded via exercise. Chiquet et al.[21] reported have indicated that exercise-induced collagen synthesis relies on increased expression of collagen-inducing stress/strain-responsive growth factors including transforming growth factor-β-1(TGF-β-1) and connective tissue growth factor. In human and animal cells and tissues, TGF-β-1 expression has been found *in vitro* and *in vivo* in response to mechanical loading[10)22)]. In humans, it has been reported that type I and/or type III collagen induced by exercise loading is directly dependent on TGF-β-1. Our results showed that TGF-β-1 expression peaked on the first day and decreased thereafter, corresponding to the results of type I collagen. We found that the group with eccentric exercise had significantly higher TGF-β-1 expression until 7 days after injury compared to the groups with concentric exercise and non-exercise. Legerlotz et al.[23] observed no difference in TGF-β 1 expression by the type of exercise, but this observation was made 12 weeks after injury. Heinemeier et al.[9] found that an eccentric exercise group had significantly higher TGF-β-1 expression in muscle tissue after four days of exercise. However, they did not find any difference in the tendon among the eccentric, concentric, and isometric exercise groups, but their study used normal tendons. Thus, our results showed significant differences among such groups because we used injured tendons. In our study, the eccentric exercise group had significantly higher TGF-β-1 expression. This higher expression level might promote type I collagen expression on the 4th day and type III collagen expression on the 7th and 14th day. These results also suggest that collagen synthesis is dependent on TGF-β-1.

Matrix metalloproteinases (MMPs) are enzymes essential in remodeling of tendons. Their excessive and long-term expression enabled hydrolysis of the extracellular matrix, which is thought to cause tendonitis[24]. Matrix-metalloproteinase-13/collagenase 3 (MMP-13) cleaves the triple-helix of type I, II, and III collagen fibers[25]. Stress on tendon induces MMP-13 expression. Arnoczky et al. reported that tendon injury can be prevented by MMP inhibitor administration[26]. MMP-13 is reported to be expressed at a high level 7-14 days after stress and to decrease gradually thereafter[19]. Our results showed that MMP-13 expression was high at 4-14 days after injury. The eccentric exercise group had significantly lower MMP-13 expression at 21 days after injury compared to the other groups. This suggests that eccentric exercise can be beneficial in promoting remodeling by suppressing MMP-13 expression at an early stage.

We used a small-animal treadmill for the exercise regimen. Downhill running was used as the eccentric exercise and uphill running as the concentric exercise. We established the treadmill inclination, duration of exercise, and speed based on our previous experimental methods[8)27)]. Eccentric and concentric exercises have clearly different effects on muscles, but such differences are not apparent in tendons. Some reports have shown that eccentric exercise promoted clinical healing of tendonitis, but no report has clarified the reason. Load on tendons is said to be twice as much in eccentric exercise compared to concentric exercise[9)28)]. It is conceivable that the difference in tension between these exercises affects healing, but much clarification is need.

Because the incision model we used with two longitudinal splits might induce acute inflammation, it is controversial whether this model reflects the true pathology of chronic tendonitis. Further studies are therefore necessary to elucidate the reason why eccentric exercise is beneficial for the treatment of tendonitis.

Conclusions

Our results suggest that eccentric exercise can more effectively induce TGF-β-1 expression compared to concentric exercise and subsequently cause high expression of type I and III collagen. Our results also showed that eccentric exercise reduces MMP-13 expression. In conclusion, eccentric exercise might aid good tendon repair.

Acknowledgments

My heartfelt appreciation goes to Professor Hiroyuki Tsuchiya and Professor Katsuro Tomita whose comments and suggestions were of inestimable value for my study. I especially would like to express my deepest appreciation to Dr. Kitaoka and Dr. Kabata whose comments made enormous contribution to my work.

I thank Y. Kasai for her assistance. I also appreciate the technical advice and expertise of Y. Takao.

I did not receive and will not receive any benefits or funding from any commercial party related directly or indirectly to the subject of this article.

References

1) Lian OB, Engebretsen L, Bahr R. Prevalence of jumper's knee among elite athletes from different sports: a cross-sectional study. Am J Sports Med 33: 561-567, 2005

2) Alfredson H. The chronic painful Achilles and patellar tendon: research on basic biology and treatment. Scand J Med Sci Sports 15: 252-259, 2005

3) Kobayashi T. Effect of cyclic tensile loading on ligament and ligament-bone junction. J. Juzen Med Soc 106: 236-248, 1997 (in Japanese)

4) Peers KH, Lysens RJ. Patellae tendinopathy in athletes: current diagnostic and therapeutic recommendations. Sports Med 35: 71-87, 2005

5) Bahr R, Fossan B, Løken S, Engebretsen L. Surgical treatment compared with eccentric training for patellar tendinopathy (Jumper's Knee). A randomized, controlled trial. J Bone Joint Surg Am 88: 1689-1698, 2006

6) Mafi N, Lorentzon R, Alfredson H. Superior short-term results with eccentric calf muscle training compared to concentric training in a randomized prospective multicenter study on patients with chronic Achilles tendinosis. Knee Surg Sports Traumatol Arthrosc 9: 42-47, 2001

7) Svernlöv B, Adolfsson L. Non-operative treatment regime including eccentric training for lateral humeral epicondylalgia. Scand J Med Sci Sports 11: 328-334, 2001

8) Nakamura K, Kitaoka K, Tomita K. Effect of eccentric exercise on the healing process of injured patellar tendon in rats. J Orthop Sci 13: 371-378, 2008

9) Heinemeier KM, Olesen JL, Haddad F, Langberg H, Kjaer M, Baldwin KM, Schjerling P. Expression of collagen and related growth factors in rat tendon and skeletal muscle in response to specific contraction types. J Physiol 582: 1303-1316, 2007

10) Nakatani T, Marui T, Hitora T, Doita M, Nishida K, Kurosaka M. Mechanical stretching force promotes collagen synthesis by cultured cells from human ligamentum flavum via transforming growth factor-beta1. J Orthop Res 20: 1380-1386, 2002

11) Yang G, Crawford RC, Wang JH. Proliferation and collagen production of human patellar tendon fibroblasts in response to cyclic uniaxial stretching in serum-free conditions. J Biomech 37: 1543-1550, 2004

12) Kubo K, Kanehisa H, Fukunaga T. Effects of resistance and stretching training programmes on the viscoelastic properties of human tendon structures in vivo. J Physiol 538: 219-226, 2002

13) Langberg H, Rosendal L, Kjaer M. Training-induced changes in peritendinous type I collagen turnover determined by microdialysis in humans. J Physiol 534: 297-302, 2001
14) Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res 92: 827-839, 2003
15) Armstrong RB, Ogilvie RW, Schwane JA. Eccentric exercise-induced injury to rat skeletal muscle. J Appl Physiol 54: 80-93, 1983
16) Stanish WD, Curwin S, Rubinovich M. Tendinitis: the analysis and treatment for running. Clin Sports Med 4: 593-609, 1985
17) Dahlgren LA, Mohammed HO, Nixon AJ. Temporal expression of growth factors and matrix molecules in healing tendon lesions. J Orthop Res 23: 84-92, 2005
18) Sharma P, Maffulli N. Biology of tendon injury: healing, modeling and remodeling. J Musculoskelet Neuronal Interact 6: 181-190, 2006
19) Oshiro W, Lou J, Xing X, Tu Y, Manske PR. Flexor tendon healing in the rat: a histologic and gene expression study. J Hand Surg Am 28: 814-823, 2003
20) Archambault JM, Hart DA, Herzog W. Response of rabbit Achilles tendon to chronic repetitive loading. Connect Tissue Res 42: 13-23, 2001
21) Chiquet M, Renedo AS, Huber F, Flück M. How do fibroblasts translate mechanical signals into changes in extracellular matrix production? Matrix Biol 22: 73-80, 2003
22) Lindahl GE, Chambers RC, Papakrivopoulou J, Dawson SJ, Jacobsen MC, Bishop JE, Laurent GJ. Activation of fibroblast procollagen alpha 1(I) transcription by mechanical strain is transforming growth factor-beta-dependent and involves increased binding of CCAAT-binding factor (CBF/NF-Y) at the proximal promoter. J Biol Chem 277: 6153-6161, 2002
23) Legerlotz K, Schjerling P, Langberg H, Brüggemann GP, Niehoff A. The effect of running, strength, and vibration strength training on the mechanical, morphological, and biochemical properties of the Achilles tendon in rats. J Appl Physiol 102: 564-572, 2007
24) Lavagnino M, Arnoczky SP, Frank K, Tian T. Collagen fibril diameter distribution does not reflect changes in the mechanical properties of in vitro stress-deprived tendons. J Biomech 38: 69-75, 2005
25) Armstrong DG, Jude EB. The role of matrix metalloproteinases in wound healing. J Am Podiatr Med Assoc 92: 12-18, 2002
26) Arnoczky SP, Lavagnino M, Egerbacher M, Caballero O, Gardner K. Matrix metalloproteinase inhibitors prevent a decrease in the mechanical properties of stress-deprived tendons: an in vitro experimental study. Am J Sports Med 35: 763-769, 2007
27) Nakamura R. Effect of eccentric and concentric exercise on tendons. J Juzen Med Soc 112: 19-27, 2003 (in Japanese)
28) Jones DA, Rutherford OM. Human muscle strength training: the effects of three different regimens and the nature of the resultant changes. J Physiol 391: 1-11, 1987