Kazutoshi Furikado · Hiroyuki Fujioka
Masahiro Kurosaka · Shinichi Yoshiya
Takeshi Makino · Kenji Fujita

Comparison of mechanical and histological properties between the immature and mature tendon attachment

Accepted: 1 May 2002 / Published online: 22 June 2002
© Springer-Verlag 2002

Abstract We tested the tensile strength of the Achilles tendon attachment to the calcaneus in skeletal mature and immature rabbits. The mean ultimate failure load was 179.6 ± 28.6 N in the mature animals, and 138.5 ± 36.1 N in the immature animals ($P < 0.05$). Although direct insertion was clearly observed histologically in the mature animals, direct insertion was not obvious in the immature animals. In the mature animals, failure occurred partly at the attachment zone (50%) and partly at the tendon zone (50%). In the immature animals, failure occurred at the attachment zone in 85% and at the tendon zone in 15%.

Résumé Nous avons testé la résistance à la tension de l'insertion du tendon Achille au Calcanéum chez les lapins matures et immatures au plan squelettique. La charge de rupture moyenne était 179.6 ± 28.6 N chez les animaux matures, et 138.5 ± 36.1 N chez les immatures ($P < 0.05$). Bien que l'insertion directe ait été clairement observée histologiquement chez les animaux matures, l'insertion directe n'était pas évidente chez les animaux immatures. Chez les animaux matures, la rupture s'est produite en partie à la zone d'attachement (50%) et en partie dans la zone tendineuse (50%). Chez les animaux immatures la rupture s'est produite à la zone d'attachement dans 85% et en zone tendineuse dans 15%.

Introduction

The tendon and ligament attachment to bone is a complex structure encompassing the association of two areas consisting of phenotypically different cells and extracellular matrices [14]. There are two types of morphologi-

cal distinct insertions, direct and indirect, which can be distinguished microscopically. Direct insertion is by way of attachment to the epiphysis at a right angle and is composed of four zones: tendon or ligament, fibrocartilage, calcified fibrocartilage, and bone. Examples of direct insertion are the Achilles tendon, supraspinatus tendon, the femoral side of the medial collateral ligament (MCL) of the knee, and the anterior cruciate ligament (ACL) [1, 3, 6, 14]. Indirect insertion attaches to the periosteum of the diaphysis by Sharpy fibers.

In a biomechanical study of bone-tendon-bone complex of MCL, in skeletally immature rabbits with open epiphysis, all specimens are avulsed at the tibial attachment, which is an example of indirect insertion, while tearing of the tendon mid-substance or femoral attachment, which is an example of direct insertion, is not observed [12]. Therefore, it is considered that the biomechanical property of direct insertion is superior to that of indirect insertion.

Changes of cell morphology at the tendon attachment site during development have been reported [4, 10]. Although a four-zone structure in direct insertion has not been established in neonatal animals, it has been established in skeletally mature ones. Type II collagen is localized in the cartilaginous rudiment of the calcaneus in the neonatal rat and the distribution of this protein during development spreads progressively from the calcaneus and the attachment site into the Achilles tendon [10]. A different type of collagen, type X, which is shorter than type II collagen, is not detectable immunologically at the Achilles tendon attachment site in immature rats; however, the cells that express type X collagen are arranged in rows along the tendon fibers in skeletally mature rats [4].

We hypothesize that biomechanical properties of immature tendon direct insertion might be different from that of mature ones due to the difference of cell morphology. To understand biomechanical properties of direct insertion during development, we performed tensile testing of the Achilles tendon attachment to the calcaneus taken from mature and immature rabbits and analyzed failure mode.

K. Furikado · H. Fujioka (✉) · M. Kurosaka · S. Yoshiya
T. Makino · K. Fujita
Department of Orthopaedic Surgery,
Kobe University Graduate School of Medicine,
7-5-1, Kusunoki-cho, Chuo-ku, Kobe, 650-0017, Japan
e-mail: hfujioaka@med.kobe-u.ac.jp
Tel.: +81-78-3825985, Fax: +81-78-3516944

Fig. 1 Tensile tests were performed with the tendon pulled parallel to the long axis of the calcaneus nonphysiologically at a strain rate of 20 mm/min until failure

Materials and methods

To analyze the change of biomechanical properties of direct insertion during skeletal maturation, we have tested the Achilles tendon attachment to the calcaneus taken from rabbits (Kitayama Labes Co., Ltd., Ina, Japan). For the skeletally mature group, 12 female Japanese white rabbits with an average weight of 3.2 kg (range 3.0–3.3 kg) at 6 months of age (closed epiphysis), and for the skeletally immature group, 12 rabbits with an average weight of 2.0 kg (range 1.7–2.2 kg) at 2 months of age (open epiphysis) were used. After injection of a fatal dose of pentobarbital sodium, the entire calcaneus and the Achilles tendon, including the calf muscles, were taken from both hind legs of each animal.

For mechanical testing, 20 specimens in each group were double-wrapped in gauze soaked in a normal saline solution and were stored in an airtight plastic bag at -20°C . For the noninjured control in histological examination, 4 specimens in each group were used.

Tensile testing

To test the mechanical properties, each specimen was thawed overnight at 4°C and analyzed using a conventional universal mechanical testing machine (AGS-5kNG, Shimazu, Kyoto, Japan) at room temperature. The Achilles tendon and calcaneus complex was isolated in preparation for testing and mounted to the testing machine. The calcaneus was fixed in the resin and the proximal stump of the Achilles tendon was fixed in the custom-made tissue fixation devices (Fig. 1). Tensile testing was performed with the Achilles tendon pulled parallel to the long axis of the calcaneus,

Fig. 2 Mean ultimate failure load of the skeletally mature group was 179.6 ± 28.6 N, and that of the immature group 138.5 ± 36.1 N. These differences were statistically significant ($P < 0.05$)

which is not the physiological direction, at a strain rate of 20 mm/min until failure.

Histological examination of the failure site

To analyze failure patterns after tensile testing, the calcaneal side of the ruptured specimen was examined histologically. As a control, the noninjured Achilles tendon attachment to the calcaneus was taken from both mature and immature animals. Both the non-injured control and the ruptured specimens were fixed in 10% neutral buffered formalin for 7 days, decalcified in 0.25-molar ethylenediaminetetraacetic acid in phosphate buffered saline at pH 7.5, dehydrated in graded alcohols, and embedded in paraffin wax. Sections 7 μm thick were cut sagittally, stained with hematoxylin and eosin, and examined microscopically.

Statistical analysis

To analyze statistically ultimate failure force at rupture of the mature and immature tendon-bone complex, unpaired *t*-tests were used to assess any differences in the ultimate force of the two groups. A value of $P < 0.05$ was considered significant. To compare difference of failure mode either in the mature tendon-bone complex or in the immature tendon-bone complex, χ^2 tests were used. A value of $P < 0.05$ was considered significant.

Results

Tensile testing

The mean ultimate failure load of the skeletally mature group was 179.6 ± 28.6 N and of the immature group 138.5 ± 36.1 N (Fig. 2). The ultimate failure load of the skeletally mature group was higher than that of the immature group, and these differences were statistically significant ($P < 0.05$). However, if the factor of body weight was taken into consideration, the difference between the two groups was not observed.

Histological examination of the failure site

In the noninjured Achilles tendon attachment of skeletally mature animals, the direct insertion composed of ten-

Fig. 3 In the Achilles tendon attachment of skeletally mature animals, direct insertion and a tidemark between fibrocartilage and calcified fibrocartilage was clearly observed (A). In the tendon attachment of immature animals, direct insertion was not completed (B). To analyze the failure mode, specimens were divided into three zones: the Achilles tendon (*t* tendon), the tendon attachment site to bone (*a* attachment), and the calcaneus (*b* bone)

Fig. 4 Representative failure pattern, failure at the tendon attachment zone of immature specimens is shown

Table 1 Mode of failure in tensile testing. To compare difference of failure mode either in the mature tendon-bone complex or immature tendon-bone complex, χ^2 test was used. A value of $P < 0.05$ was considered significant; ns = not significant

Group	Tendon	Attachment	<i>P</i> -value
Mature (<i>n</i> : 20)	10 (50.0%)	10 (50.0%)	ns
Immature (<i>n</i> : 20)	3 (15.0%)	17 (85.0%)	<0.05

don, fibrocartilage, calcified fibrocartilage, bone, and a tidemark between fibrocartilage and calcified fibrocartilage was clearly observed (Fig. 3). In the immature animals, direct insertion composed of fibrocartilage and a tidemark was not clearly observed. To analyze the failure mode, specimens were divided into three zones: the Achilles tendon (tendon), the fibrocartilagenous tendon attachment site to bone (attachment), and the calcaneus (bone).

Different modes of failure were observed between the two groups. In the skeletally mature group, failure at the attachment zone was observed in ten specimens (50%) and at the tendon zone in ten (50%) (Table 1). By contrast, in the skeletally immature group, failure at the attachment zone occurred in seventeen specimens (85%) and at the tendon in three (15%). Among the immature animals, failure at the tendon attachment occurred frequently compared to failure at the tendon mid-substance. These differences were statistically significant ($P < 0.05$). Failure at the bone, such as fracture at the calcaneus, was not observed in any specimen in either the mature or the immature groups. Representative failure patterns of immature specimens are shown in Fig. 4.

Discussion

In this study, the mechanical properties and failure patterns of the Achilles tendon attachment to the calcaneus taken from skeletally immature rabbits were analyzed and compared to those of mature rabbits. Although direct insertion composed of tendon, fibrocartilage, calcified fibrocartilage, and bone, was clearly observed at the tendon attachment of mature animals, these structures were not clearly visible in immature specimens. The tensile strength of the tendon-bone complex in the immature group was lower than that of the mature group. The most failure tended to occur at the tendon attachment zone in immature specimens compared with mature specimens.

The cell morphology of immature direct insertion is different from that of mature direct insertion, and maturation of direct insertion progresses during skeletal development after birth [4, 10]. In this study, we confirmed the histological changes of the tendon attachment between skeletally mature and immature specimens.

It is known that tensile properties of the tendon and the tendon-bone complex change during development. The tensile strength of the immature tendon has been shown to be significantly lower than that of the mature

tendon in both humans and rabbits [2, 7]. Biomechanical studies of the tendon-bone complex, femur-MCL-tibia and femur-ACL-tibia, show that higher tensile property is associated with gaining of body weight during development, resulting in the superior tensile property of the mature tendon-bone complex compared with that of the immature tendon-bone complex [9, 11, 12]. In this study, we tested the Achilles tendon-calcaneus complex taken from skeletally immature and mature rabbits. The tensile strength of the mature tendon-bone complex was higher than that of the immature tendon-bone complex.

As for the failure mode in tensile testing, it is shown that rupture of femur-ACL or MCL-tibia complex predominantly occurs at tendon mid-substance in young adults and that fracture or tendon avulsion from bone frequently occurs in the subjects with osteoporotic condition due to ageing or immobilization [8, 13]. We considered that no failure at bone was observed in the present study because the specimens were taken from immature and young mature animals.

In the tensile testing of young adult femur-ACL-tibia complex, the previous studies show that 60% of failure occur as a result of rupture at tendon mid-substance, 20–30% as a result of tibial avulsion, and 3–10% as a result of femoral avulsion [8]. In a biomechanical study of femur-MCL-tibia complex, all specimens are avulsed at the tibial attachment [12]. The ACL attachment to the femur and tibia, and the MCL attachment to the femur, are examples of direct insertion. In contrast, tibial attachment of the MCL possesses the structure of indirect insertion. Therefore, it is considered that biomechanical property of direct insertion is superior to that of indirect insertion.

In this study, we presented that immature direct insertion is histologically different from mature direct insertion, and the mechanical properties of immature direct insertion is inferior to that of mature direct insertion. Failure occurred at both the tendon and the attachment zone in the tendon-bone complexes taken from skeletally mature rabbits, while failure predominantly occurred at the fibrocartilagenous zone at the tendon attachment in the immature rabbits.

Therefore, injury at the fibrocartilagenous area of the tendon and ligament attachment site of direct insertion may occur frequently in immature patients compared with mature patients, while fracture and dislocation of the joint occur in children. We previously have reported that an enchondral ossification with appearance of type X collagen is observed at early phase of the healing pro-

cess after injury at the tendon attachment [5]. Injuries at the tendon attachment may have some influence on the repair process after fracture and dislocation of the joint in children.

References

1. Benjamin M, Evans EJ, Copp L (1986) The histology of tendon attachments to bone in man. *J Anat* 149:89–100
2. Blanton PL, Biggs NL (1970) Ultimate tensile strength of fetal and adult human tendons. *J Biomechanics* 3:181–189
3. Cooper RR, Misol S (1970) Tendon and ligament insertion. A light and electron microscopic study. *J Bone Joint Surg [Am]* 52:1–20
4. Fujioka H, Wang G-J, Mizuno K, Balian G, Hurwitz SR (1997) Changes in the expression of type X collagen in the fibrocartilage of rat Achilles tendon attachment during development. *J Orthop Res* 15:675–681
5. Fujioka H, Thakur R, Wang G-J, Mizuno K, Balian G, Hurwitz SR (1998) Comparison of surgically attached and non-attached repair of the rat Achilles tendon-bone interface. Cellular organization and type X collagen expression. *Conn Tissue Res* 37:205–218
6. Liu SH, Panossian V, al-Shaikh R, Tomin E, Shepherd E, Finerman GA, Lane JM (1997) Morphology and matrix composition during early tendon to bone healing. *Clin Orthop* 339:253–260
7. Nakagawa Y, Hayashi K, Yamamoto N, Nagashima K (1996) Age-related changes in biomechanical properties of the Achilles tendon in rabbits. *Eur J Appl Physiol* 73:7–10
8. Noyes FR, DeLucas JL, Torvik PJ (1974) Biomechanics of anterior cruciate ligament failure: An analysis of strain-rate sensitivity and mechanisms of failure in primates. *J Bone Joint Surg [Am]* 56:236–253
9. Noyes FR, Grood ES (1976) The strength of the anterior cruciate ligament in humans and rhesus monkeys. *J Bone Joint Surg [Am]* 58:1074–1082
10. Rufai A, Benjamin M, Ralphs JR (1992) Development and ageing of phenotypically distinct fibrocartilages associated with the rat Achilles tendon. *Anat Embryol.* 186:611–618
11. Tipton CM, Matthes RD, Martin RK (1978) Influence of age and sex on the strength of bone-ligament junctions in knee joints of rats. *J Bone Joint Surg [Am]* 60:230–234
12. Woo SLY, Orlando CA, Gomez MA, Frank CB, Akeson WH (1986) Tensile properties of the medial collateral ligament as a function of age. *J Orthop Res* 4:133–141
13. Woo SLY, Gomez MA, Sites TJ, Newton PO, Orlando CA, Akeson WH (1987) The biomechanical and morphological changes in the medial collateral ligament of the rabbit after immobilization and remobilization. *J Bone Joint Surg [Am]* 69:1200–1211
14. Woo SLY, Maynard J, Bulter D, Lyon R, Torzilli P, Akeson W, Cooper R, Oakes B (1988) Ligament, tendon, and joint capsule insertions to bone. In: Woo SLY, Buckwalter JA (eds) *Injury and repair of the musculoskeletal soft tissues* American Academy of Orthopaedic Surgeons, Park Ridge, Illinois, pp 133–166