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The Characterization of Mechanical Properties of a Rabbit Femur-Anterior Cruciate Ligament-Tibia Complex During Cyclic Loading*

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The purpose of this study was to investigate the effect of cyclic loading, which produced the condition of ACLs during sports activities, on tensile properties of femur-ACL-tibia complexes (FATCs). Paired FATCs of 40 New Zealand white rabbits were tested on a materials testing machine. One specimen of each pair was designated as a control and loaded until failure. The contralateral specimen was loaded cyclically (1.4 Hz, 1 hr.) with 20%, 30%, 40%, or 50% of ultimate tensile strength (UTS) of the control and then loaded until failure. The UTS and mode of failure were recorded after each test. Five specimens ruptured during cyclic loading in the 50% group. In the 40% group, the mean value of UTS of cycled specimens was significantly lower than that of controls. There was no statistically significant difference in UTS values between control and cycled specimens in the 20% and 30% groups. Cycled specimens had a significantly higher incidence of substance failure than controls. Our results demonstrated that FATCs have the strength to withstand cyclic loading within normal sports activity levels. However, FATCs can be damaged by cyclic loading under strenuous sports activity levels. We speculate that cyclic loading makes the ACL substance weaker than the insertion site.

Key Words: Sports Injuries, Cyclic Loading, Tensile Properties, Ultimate Tensile Strength (UTS), Femur-ACL-Tibia Complexes (FATCs)

1. Introduction

ACL injuries are very common sports injuries[(8),(9),(14),(19),(22)]. Many studies have investigated the properties and biomechanics of the human and animal femur-ACL-tibia complexes (FATCs)[(1),(2),(4),(10),(13),(18),(20),(23),(24),(27),(28),(31),(35),(36)]. Results from those studies have been used in ACL injury prevention and reconstruction surgery protocols. Many researchers have investigated the influence of immobilization, exercise, age, loading direction and rate, and other factors on the tensile properties of human and animal FATCs[(21),(23)-(28),(32),(35),(36)]. However, few studies have focused on the tensile properties of FATCs under cyclic loading, which produced the condition of ACLs during sports activities.

Properties of bone under cyclic loading have been investigated and a relationship between cyclic loading on bone and stress fractures is well known[(6),(7)]. Weisman et al.[(34)] in reporting the effect of cyclic loading on MCLs, said that ligaments lengthened by cyclic loading were weaker ligaments and that the viscoelastic properties of MCLs contributed to the lengthening. They speculated that lengthened ligaments might be easy to be injured clinically.

Haut et al.[(12)] reported the effect of 20 cyclic loadings on patella-patella tendon-tibia complexes. They concluded that cyclic loading did not alter failure characteristics of the specimens. Twenty, however, seems too few cycles to simulate sports activities and to investigate the effect of cyclic loading.

We hypothesized that ACLs were easily to be injured during sports activities due to changes in tensile properties. The purposes of this study were to identify and quantify the effect of cyclic loading on the

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tensile properties of rabbit FATCs.

2. Materials and Methods

2.1 Specimen preparation

Forty New Zealand white rabbits (weighing 3.63 ±0.16 kg, mean±S.D., at sacrifice) were euthanized by an overdose of sodium pentobarbital. The hind legs were immediately removed at the hip joint. The legs were wrapped in saline soaked gauze, double wrapped in plastic bags and stored at −15°C. Several hours before testing, the specimens were removed from the freezer and thawed. All muscle, skin, and connective tissue were dissected from the legs, leaving the joint capsule, infrapatellar fat pad, bilateral menisci, PCL, and ACL intact.

The femur and tibia were osteotomized 20 mm proximal and 40 mm distal from the knee joint, respectively. As much of the bone marrow as possible was removed from the tibia shaft. The tibia was mounted with bone cement (polymethylemethacrylate) in a cylinder, which was 35 mm in length and 15 mm in diameter. Approximately ten minutes before testing, the joint capsule, infrapatellar fat pad, bilateral menisci, and PCL were removed from the knee, leaving the ACL intact. Specimens were kept moist with saline during preparation and testing.

2.2 Mounting

A custom testing fixture was designed and attached to an electro-hydraulic materials testing machine (MTS Systems Corp., Minneapolis, Minn.). To fix a specimen to a fingers device, which minimized the influence of bending of the femur shaft, and to measure the cross sectional area (CSA) of the ACL, bilateral femoral condyles were osteotomized with a small band saw 2 mm from the tip of the condyles with the knee flexed maximally to protect the ACL from the saw. After the CSA of the midportion of the ACL was measured by area micrometer technique, the specimen was fixed to the test fixture with the knee flexed at 35°. The area micrometer technique is to measure CSA of midportion of ligament by applying 5 g of weight on the ligament surface. When this weight is applied, the ligament is supposed to be flat which is approximately rectangular shape. From this rectangular shape, the width and length of ligament were measured and calculated for CSA. Because, load could be applied along the axis of the ACL (anatomical axis)[35],[36] with this fixture when the knee was flexed at this angle (Fig. 1).

2.3 Experimental procedure

The 40 pairs of hind legs were divided into four groups, each consisting of 10 pairs.

One specimen of each pair was chosen randomly as a control. It was fixed to the materials testing machine, cyclically stretched between 0 and 0.5 mm deformation ten times at a rate of 10 mm/min. for preconditioning. The specimen was loaded until failure at a rate of 200 mm/min. as described by Woo et al.[35]. Load was applied along the anatomical axis, because it seemed more physiological to apply tensile loads to specimens along the anatomical axis than along the tibial axis as Woo et al. did in their studies[35],[36].

Load and displacement were monitored with a precalibrated load cell and linear variable displacement transducer (LVDT), respectively. Resolution of the load cell was approximately 0.556 N through the analogue to digital converter. Resolution of the LVDT was approximately 0.025 mm. Output voltage from the load cell bridge was conditioned via a strain amplifier unit. Output voltage from the LVDT was amplified. The data were collected by a DASH-16 (Metrabyte Corporation, MA) and sent to the data acquisition system using LabTech Notebook data acquisition software (Laboratory Technologies Corporation, MA). Data were stored and collected at 100 Hz sampling rate.

The UTS was obtained from the load-displacement curve while stiffness was calculated from a linear region in the curve. The mode of failure was noted and classified as follows: 1) avulsion of bone at the insertion site, 2) insertion site failure without bony fragment, and 3) ligament substance failure.

The contralateral specimen was preconditioned in the same manner, loaded cyclically (1.4 Hz, triangle wave pattern) for 1 hr. at 20%, 30%, 40%, or 50% of the UTS of the control specimen, then loaded immediately until failure at a rate of 200 mm/min. The same calculations were made as described above and the results were compared with the control results. Length change (elongation) compared to the initial length (after preconditioned) was recorded after cyclic loading at the beginning (load=0) of the tensile test.

The frequency of 1.4 Hz was selected based on

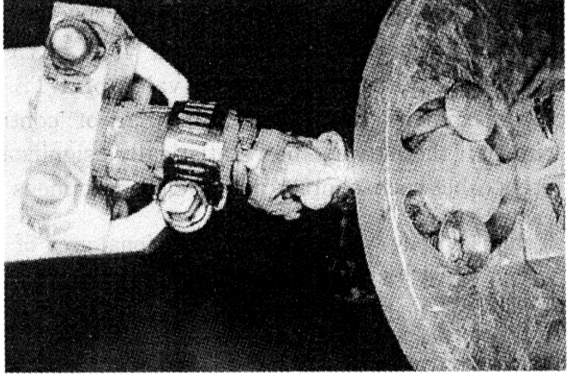

Fig. 1 A rabbit femur-ACL-tibia complex mounted to the materials testing machine with a custom-designed device

Mann et als.[17] estimation that cyclic loading on knee ligaments was 1.4 Hz during running (average speed 4.77 m/sec.). The specimens were cycled for 1 hr. because sports players usually do not continue moving more than 1 hr. at high levels of intensity in most kinds of sports.

During cyclic loading, the ligament was kept at room temperature and hydrated with normal saline spray every five minutes.

2. 4 Statistical analysis

The effects of cyclic loading on tensile properties of FATCs were analyzed using the paired t-test in each group. The difference between groups were analyzed using analysis of variance (ANOVA). The difference in failure mode between control and cycled specimens was analyzed using McNemar's *chi*-square test. Significance was set at $p<0.05$.

2. 5 Histological finding

To observe fiber waviness, histological slides were prepared for light microscopy from intact and cycled ACLs. After fixation in 10% buffered formalin and infiltration with paraffin, the ACLs were cut longitudinally and stained with hematoxylin and eosin by standard techniques.

3. Results

No significant difference was found in body weight or CSA of the midportion of the ACL among the four groups. Nor was any significant difference of CSA found between the control and cycled specimens in either group (Table 1).

Load-displacement curves for one paired specimen in the 40% group were drawn in Fig. 2. The cycled specimen showed 0.64 mm of elongation after cyclic loading.

Five specimens ruptured during cyclic loading in the 50% group. Thus differences between the control and cycled specimens could not be analyzed statistically in the 50% group.

The values of UTS in the 20% group were 465.5 ±109.1 N (mean±S.D.) and 443.2±65.4 N for control and cycled specimens, respectively. In the 30% group, UTS values were 473.8±96.8 N and 461.4±83.5 N, respectively. Although the mean value of UTS of cycled specimens was lower than that of control specimens, there was no statistically significant difference between control and cycled specimens in the 20% and 30% groups. In the 40% group, UTS values were 481.9±96.2 N and 421.9±125.6 N, respectively. The mean value of UTS of cycled specimens was significantly ($p<0.05$) lower than that of control specimens (Fig. 3).

Table 1 Body weight and cross sectional area of each group (mean±S.D.)

group	body weight(kg)	cross sectional area(mm^2) control	cross sectional area(mm^2) Cycled
20%	3.67 ±0.14	4.65 ±0.80	4.41 ±0.63
30%	3.60 ±0.20	4.53 ±0.55	4.53 ±0.36
40%	3.62 ±0.18	4.43 ±0.56	4.34 ±0.53
50%	3.62 ±0.13	4.88 ±0.68	4.77 ±0.63

The values of stiffness in the 20% group were 179.5±33.2 N/mm and 194.6±50.3 N/mm for control and cycled specimens, respectively. There was no statistically significant difference between control and cycled specimens. In the 30% group, stiffness values were 164.6±37.3 N/mm and 191.8±30.6 N/mm, respectively. In the 40% group, stiffness values were 179.6±21.6 N/mm and 206.9±28.3 N/mm. In the 30% and 40% groups, the mean value of stiffness of cycled specimens was significantly ($p<0.05$) higher than that of control specimens (Fig. 4).

The values of elongation were 0.21±0.09 mm, 0.62±0.49 mm, 0.65±0.61 mm, and 0.97±0.44 mm in the 20%, 30%, 40%, and 50% groups, respectively, although the beginning (load=0) of the tensile test could not be recorded for some specimens (Table 2).

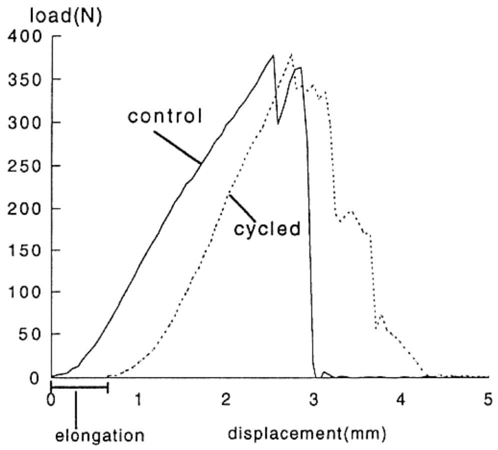

Fig. 2 Load-displacement curves for load to failure tests of control and cycled rabbit femur-ACL-tibia complexes

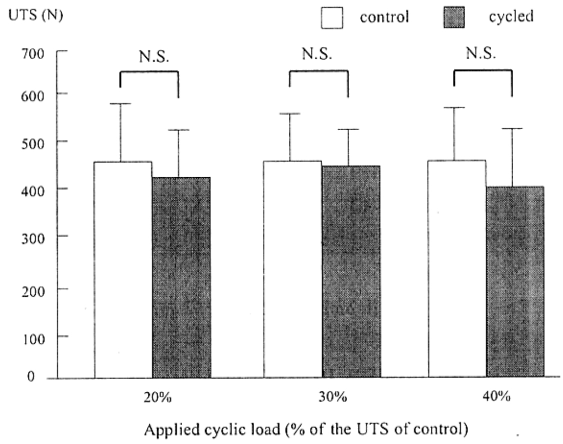

Fig. 3 UTS of control and cycled rabbit femur-ACL-tibia complexes

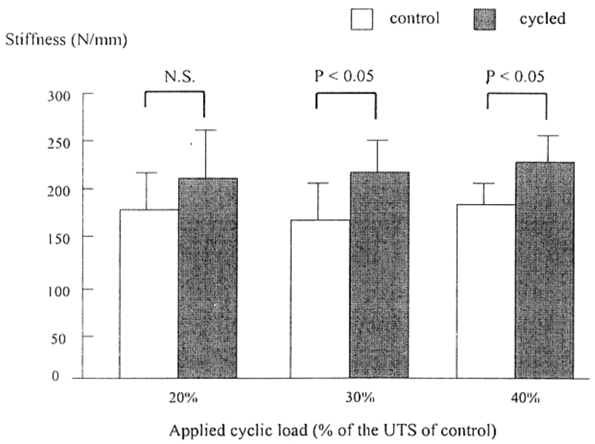

Fig. 4 Stiffness of control and cycled rabbit femur-ACL-tibia complexes

The cycled specimens had a significantly higher ($p<0.005$) incidence of substance failure (22/40, or 55.0%) than the control specimens (11/40, or 22.5%) (Fig. 5). There was no pair in which the control specimen showed substance failure and the cycled specimen showed tibial insertion or avulsion failure.

The fiber waviness of the ACL, which was cyclically loaded with 40% of the UTS of the control specimen, was less than the fiber waviness of the intact ACL (Figs. 6, 7).

Although the CSAs of ACLs were measured, the stresses were not calculated because the failure modes were not uniform.

4. Discussion

Noyes et al.[25] investigated tensile properties of the human ACL. They reported that UTS averaged 734 N in older humans (48 - 86 years old) and 1 730 N in younger humans (16 - 26 years old). Woo et al.[36] reported that the average UTS along the anatomical axis was 2 160 N in younger knees (22 - 35 years old), 1 503 N in middle-aged knees (40 - 50 years old), and 658 N in older knees (60 - 97 years old).

Markolf et al.[18] reporting on loads applied to an ACL, found that approximately 140 N were applied to an ACL in hyperextension with 200 N quadriceps tendon pull. Approximately 260 N were applied in 10 Nm intnernal tibial torque and in hyperextension with no quadriceps tendon pull. Henning[13] calculated loads to the ACL in an in vivo strain gauge study. They reported that isometric quadriceps contraction provided approximately 200 N to the ACL at 0° of flexion with a 20 Lb. weight boot. Running downhill (4.5 degrees decline) at 5 mph provided approximately 380 N.

From these studies, it seems that approximately 200 N, which are approximately 10% of the UTS of the ACL, are applied to the ACL during walking (active knee motion and quadriceps contraction). Approximately 400 N, which are approximately 20% of the UTS of the ACL, are applied during running and sports activities. Noyes et al.[27] said that approximately 25% and 50% of UTS were applied to the ACL during normal and strenuous activity, respectively.

Table 2 The values of elongation in each group

group	effective data number	Ave ±SD(mm)
20%	7	0.21 ±0.09
30%	10	0.62 ±0.49
40%	8	0.65 ±0.61
50%	4*	0.97 ±0.44

*Five specimens were rupted during cyclic loading

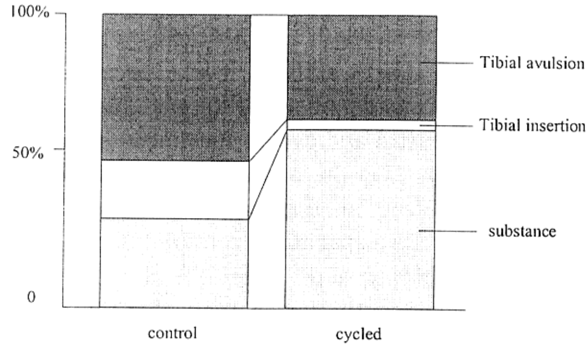

Fig. 5 Histogram of failure modes of control and cycled rabbit femur-ACL-tibia complexes

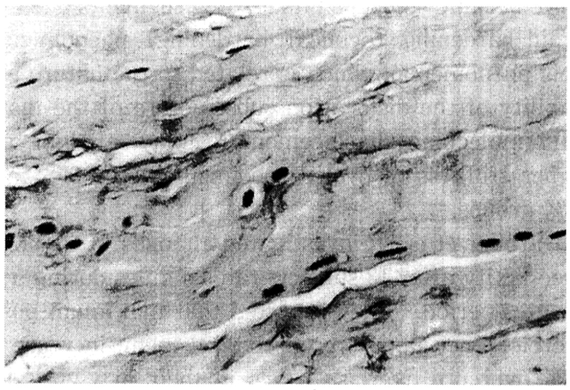

Fig. 6 Histology of intact ACL

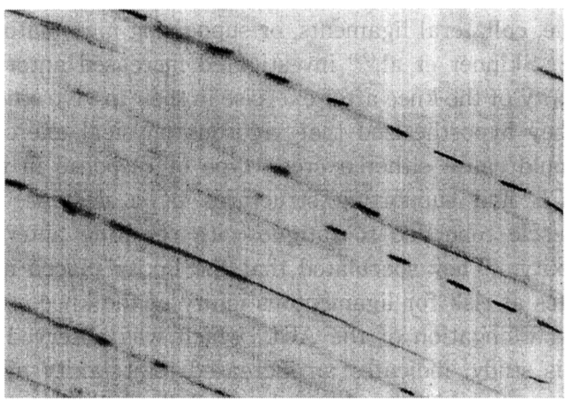

Fig. 7 Histology of cycled ACL

We applied 20%, 30%, 40%, and 50% of the UTS of the control as cyclic loads in this study. Applying 20% and 30% of the UTS simulated the condition of ACLs during normal sports activities and applying 40% and 50% of the UTS simulated the condition of ACLs during strenuous sports activities, although we do not know the in situ load on the ACL exactly during sports activity. Our results demonstrate that FATCs have the strength to withstand cyclic loading within normal sports activity levels, because there was no statistically significant difference for the UTS between the control and cycled specimens in the 20% and 30% groups. On the other hand, FATCs can be damaged by cyclic loading under strenuous sports activity levels. Five specimens ruptured during cyclic loading in the 50% group. The UTS of cycled specimens was significantly lower than that of control specimens in the 40% group.

Viidik[33] said that when loading-unloading cycles were applied to rabbit ACLs, the load-deformation curve became steeper in its loading segments due to a strain-hardening (cyclical hardening) phenomenon. This phenomenon, which is related to the nature and stability of the dislocation substructure of the material (molecular displacement)[3], may explain the higher stiffness of cycled specimens in the 30% and 40% groups.

Stoller et al.[30] investigated changes in torsional knee laxity after 13 subjects ran 3.5 miles during a 30 minutes period. They reported that maximum post-exercise knee laxity represented a mean increase of 14% over pre-exercise levels in their in vivi study. They said that measured increases in knee laxity could be produced from the stretching in the joint capsule, collateral ligaments, or supporting musculature.

Skinner et al.[29] investigated increased anterior laxity of the knee after exercise in their in vivo study. They hyposthesized that vigorous physical exercise would cause either a creep type of response in the ACL and supporting structures or a decrease in muscle tone due to fatigue with resulting anterior laxity. They speculated that the laxity placed athletes at risk for ligamentous injury of the knee.

Elongation of the ACL, which was observed in this study, indicates an increased joint laxity after exercise, which has been observed clinically.

Weisman et al.[34] reported that fiber waviness of the cycled MCL was less than that of the uncycled MCL. They concluded that ligaments lengthened by cyclic loading were weaker ligaments and speculated that lengthened ligaments might be easy to injure. Although elongation was observed in the 20% and 30% groups, which represented normal sports activities, the cycled specimens did not show lower UTS than the controls in theses groups. Therefore, our results indicate that lengthened (elongated) ligaments are not necessarily weaker ligaments.

Clinically, completer ACL tears are generally observed in the substance of the ligament[5],[11],[15],[16]. Woo et al.[35],[36] reported that tibial bony avulsion was the most common failure mode for rabbit and human ACLs when they applied load along the anatomical axis. This finding does not agree with clinical results. Our results may explain this discrepancy. We speculate that cyclic loading makes the substance weaker than insertion site.

This study may not completely simulate the condition of ACLs during sports activities, because biologic materials have the inherent ability to repair themselves and muscle forces can protect the ACL in vivo[34]. On the other hand, when the muscle is fatigued and cannot protect the ACL during sports activities, cyclically loaded ACLs seem particularly susceptible to injury.

The influence of cycling loading should be considered when a graft is selected for an ACL reconstruction.

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