# How Variable Are Achilles Allografts Used for Anterior Cruciate Ligament Reconstruction?

## A Biomechanical Study

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**Background:** Allograft tendon used in anterior cruciate ligament reconstruction (ACLR) requires sterilization before implantation. Low-dose gamma irradiation is a means of sterilization that may minimize tissue damage.

**Purpose:** To quantify the variability in mechanical properties between low-dose irradiated Achilles tendon allografts used for ACLR.

**Study Design:** Descriptive laboratory study.

**Methods:** A total of 15 intact outer-third Achilles tendon allograft specimens were collected from the remains of full Achilles allografts used for intraoperative ACLR at a single hospital. All grafts were obtained from a single tissue bank and underwent proprietary disinfection and low-dose gamma irradiation (1.5-2.5 Mrad). Biomechanical testing was carried out to measure tendon elongation, failure location during tensile testing, maximum stress, maximum strain, and modulus of elasticity. The mean and standard deviation were calculated for each outcome measure, and the variability between specimens was calculated by the coefficient of variation (CV). The effect of donor age on graft material properties was examined by use of linear regression. One-way analysis of variance was performed to compare differences in the mechanical properties across failure locations.

**Results:** During cyclic testing, tendon elongation averaged 1.4% $\pm$ 1.6% with a CV of 118%. During failure testing, the maximum stress averaged 12.2 $\pm$ 4.1 MPa, maximum strain averaged 21.0% $\pm$ 6.3%, and modulus of elasticity averaged 95.5 $\pm$ 30.8 MPa. The CVs for maximum stress, maximum strain, and modulus of elasticity were 34%, 30%, and 32%, respectively. Ten tendons failed in the midsubstance and 5 failed at the tendon-bone enthesis. No differences were noted in mechanical properties between grafts that failed in the midsubstance versus those that failed at the enthesis. Donor age did not correlate with allograft elongation during cyclic load or any of the material property measures during failure testing.

**Conclusion:** The variabilities in the material properties and graft elongation during cyclic loading of Achilles tendon allografts used in ACLR fall within the range of properties reported in the literature for other ACLR allografts. Material properties do not differ by donor age or graft failure location observed during failure testing.

**Clinical Relevance:** Surgeons should be aware that there exists considerable variation in the mechanical properties of Achilles allograft tendons used for ACLR. This variability is difficult to detect by tissue bank screening or the treating surgeon's inspection and may contribute to the heterogeneity in outcomes of allograft ACLR.

**Keywords:** anterior cruciate ligament reconstruction; allograft; variability; low-dose gamma irradiation

Reconstruction of the anterior cruciate ligament (ACL) is the mainstay of treatment for ACL ruptures in active patients who wish to return to cutting and pivoting activities.[40,41] It is estimated that 300,000 to 400,000 ACL reconstructions (ACLRs) are performed each year in the United States, yet considerable controversy exists as to whether autograft or allograft tissue should be used.[16,19,21,23,41] The potential advantages of allografts are well documented and include avoidance of donor site morbidity, predictable graft sizes, the ability to treat multiple ligament injuries, reduced operative times, and less postoperative pain.[13,36,42,44,50] Not surprisingly, the use of human allograft tendon has become more prevalent in

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ACLRs over the past 20 years, with approximately 20% (60,000) of annual reconstructions now using allografts.[10,12,25,30] However, these benefits come with known limitations. Before using an allograft, the surgeon must inform the patient regarding the risk of disease transmission, including viral and bacterial infection.[12,31,44] Allograft tissue also has a biological disadvantage, which causes a delay in host incorporation or "ligamentization" compared with autograft.[2,14,22] Last, recent studies have suggested a 2- to 4-fold increased risk of graft failure if allograft is used in young, highly active patients.[3,23]

Improved donor screening, more sensitive serological testing, and new tissue processing and sterilization techniques have decreased the risk of disease transmission.[12,17,49] Likewise, prior studies have documented the relatively low impact of low-dose irradiation on allograft mechanical properties.[5,37,52] However, little is known regarding the effect of proprietary sterilization techniques on the mechanical properties of allograft tissue.[17] Variability in proprietary sterilization techniques, as well as graft variability related to donor age and sex, suggests that there is likely similar variability in the mechanical properties of allograft tendons being distributed and used in ACLR.[48] Despite the popular use of allografts, there is a paucity of data regarding variability in biomechanical properties of processed allografts that are used intraoperatively.[34,52] A study of Achilles allografts by Penn and colleagues[34] showed significant variability in material properties in nonirradiated Achilles allograft. However, to the best of our knowledge, no prior studies have examined the variation in the material properties of low-dose irradiated Achilles allograft used in the operating room for ACLR.

The purpose of this study was to examine material properties of Achilles tendon allografts that met the screening criteria of the treating surgeon and were used for ACLR in patients. We hypothesized that there would be considerable variability in the maximum stress, maximum strain, modulus of elasticity, and cyclic creep of the Achilles tendon allografts that were subjected to cyclic and failure load testing and that these material properties would not differ by donor age or graft failure location.

## METHODS

### Overview

Fifteen male Achilles tendon allograft specimens, with an average age of 42 years (range, 18-53 years) were collected from the remains of whole Achilles tendon allografts that were used for ACLR at a single institution. All allografts were quality-certified by means of a proprietary sterilization technique and irradiated with low-dose gamma irradiation (1.5-2.5 Mrad) to meet the standards of a single proprietary tissue bank (Community Tissue Services, Dayton, Ohio). After the treating surgeon inspected each graft, the central third of the graft was harvested for ACLR. The intact outer third of the Achilles tendon allograft were retained, and the larger side of each allograft was used for biomechanical testing. Each retained specimen was measured with calipers for length and then at the top, middle, and bottom for width and thickness. Width and thickness were averaged, and these values were used to calculate total graft volume.

### Biomechanical Testing

Specimens were stored at –80°C before testing. On the day of testing, each specimen was thawed at room temperature, and sharp dissection was performed to standardize specimen length to 4.5 cm proximal to the Achilles insertion on the calcaneus. The calcaneal bone block was secured in a custom-designed clamp interfaced with polymethylmethacrylate (PMMA). Proximally, the tendinous portion of the graft was mounted to a custom-designed pneumatic clamp that prevented tissue slippage. The testing setup allowed for direct visualization of the enthesis throughout testing (Figure 1). A consistent potting technique was used for all specimens. This technique included leaving the calcaneal bone of the allograft as long as possible so that the PMMA was in contact with the calcaneal bone and not the Achilles enthesis. The potting technique was modeled from multiple similar studies.[34,51-53] All biomechanical testing was done at room temperature on the same mechanical testing system (MTS). All tissues were kept moist with a normal saline spray (devoid of protease inhibitors) during preparation and testing to avoid desiccation.

Once each specimen was mounted onto the MTS, tendon geometry was measured under a 1-N preload to remove tissue crimp. All measurements were taken by use of a 0.05-mm precision caliper. Cross-sectional area was calculated by taking width and thickness measurements at 3 distinct locations proximal to the tendon enthesis. The 3 measurements of width and thickness were averaged, and the cross-sectional area was calculated assuming an elliptical shape. Although specimen length was standardized before mounting, the length of the tendon specimen was again measured under the 1-N preload.

### Cyclic Loading

Static pretensioning was conducted at 89 N for 10 minutes to simulate the forces applied by the surgeon intraoperatively before and during implantation.[20,38] After preconditioning,

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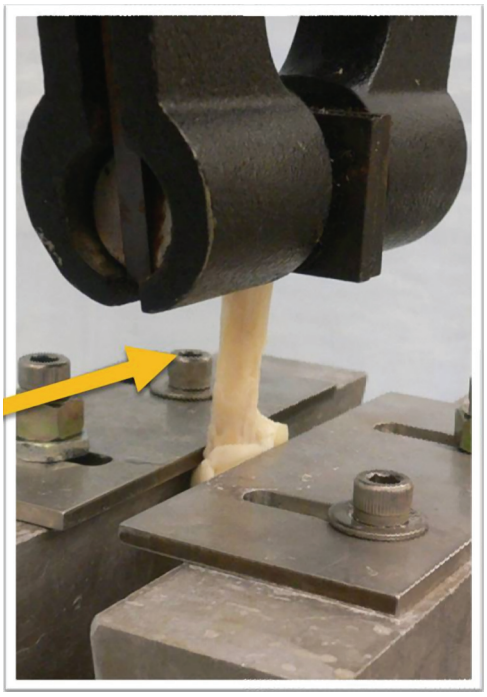

**Figure 1.** Images of the biomechanical testing setup, including the customized clamp to prevent tissue slippage during cyclic loading.

1000 submaximal cycles in the physiologic loading region between 50 N and 250 N were applied as a simulation of in vivo forces that the graft may experience during the early postoperative period.[4]

To account for geometric variability across specimens, the loads were normalized to each graft's cross-sectional area (stress) and the elongation was normalized to the initial length (strain) before testing. Cyclic loading consisted of 1000 cycles performed at 1 Hz. During cyclic testing, graft elongation (creep) was measured by clamp-to-clamp displacement, and maximum elongation was calculated as the difference between initial displacement and the recorded displacement on the 1000th cycle. The tendon was carefully inspected at the tendon-clamp interface for signs of slipping.

### Failure Testing

After cyclic testing, each specimen was returned to a 1-N preload for 1 minute. Failure testing was then performed at a rate of 100% strain per second. The material properties of each graft (maximum stress, maximum strain, and modulus of elasticity) and the mode of failure were recorded during failure testing. Modulus of elasticity was determined by calculating the slope of the linear portion of the stress-strain curve.

### Statistical Analysis

The sample size was selected based on the numbers used in similar studies.[34,51-53] The mean and SD were calculated for each outcome variable (strain, maximum stress, maximum strain, modulus of elasticity, and cyclic creep or elongation). To assess the variability between Achilles allograft specimens, the coefficient of variation (CV) was calculated for each outcome measure.[1] The effect of age on the mechanical outcomes was examined with a linear regression for each measure. A 2-sample $t$ test was performed to compare differences in the mechanical properties between failure locations. An alpha less than .05 was used as the threshold for significance.

## RESULTS

### Graft Characteristics

Graft dimensions and mode of failure are described in Table 1. The mean graft volume was 721.0 ± 182.4 $mm^3$.

### Cyclic Testing

All 15 allografts elongated over the 1000 cycles of cyclic testing (Figure 2). The average normalized elongation was 1.4% ± 1.6% of the original tendon length. The CV for normalized elongation during cyclic testing was 118%. During cyclic testing, each specimen was visually inspected, and the MTS output was interrogated for signs of graft slippage. No apparent tendon slippage was noted during testing. We found no significant correlation between donor age and the magnitude of tendon elongation during cyclic testing ($R^2$ = 0.0211).

### Failure Testing

All 15 specimens sustained catastrophic failure during testing. Ten specimens failed in the tendon midsubstance, while 5 specimens failed at the tendon-bone interface. No difference was found in mechanical properties between those that failed at midsubstance versus those that failed at the enthesis (Table 2). The mean maximum stress during failure testing was 12.2 ± 4.1 MPa. The mean maximum strain was 21.0% ± 6.3%. The average modulus of elasticity was 95.5 ± 30.8 MPa. The CVs for stress, strain, and modulus were 34%, 30%, and 32%, respectively. No significant correlation was found between donor age or failure location and any of the 3 material properties measured (maximum stress, $R^2$ = 0.0883; maximum strain, $R^2$ = 0.3994; modulus of elasticity, $R^2$ = 0.018).

## DISCUSSION

The purpose of this study was to quantify the variability in mechanical properties of low-dose gamma-irradiated Achilles tendon allografts that were used intraoperatively for ACLR. In our study, creep with cyclic loading varied by 118% across the specimens tested. The mechanical behavior of the grafts during failure testing varied by greater

TABLE 1
Summary of the Graft Characteristics Including Graft Dimensions and Mode of Failure

| Specimen No. | Age, y | Width, mm | Thickness, mm | Length, mm | Volume, $mm^3$ | Mode of Failure |
|---|---|---|---|---|---|---|
| 1 | 50 | 6.07 | 3.69 | 25.39 | 567.87 | Midsubstance |
| 2 | 53 | 6.04 | 2.98 | 30.00 | 540.28 | Midsubstance |
| 3 | 51 | 7.64 | 3.88 | 26.00 | 769.73 | Midsubstance |
| 4 | 53 | 4.72 | 3.06 | 25.00 | 360.69 | Midsubstance |
| 5 | 40 | 7.20 | 3.25 | 26.00 | 608.74 | Midsubstance |
| 6 | 43 | 4.52 | 5.35 | 35.00 | 845.22 | Enthesis |
| 7 | 42 | 7.95 | 4.46 | 27.00 | 957.65 | Midsubstance |
| 8 | 45 | 7.41 | 3.11 | 24.00 | 553.92 | Midsubstance |
| 9 | 18 | 6.62 | 6.06 | 24.00 | 963.83 | Midsubstance |
| 10 | 48 | 4.72 | 6.91 | 26.99 | 881.25 | Enthesis |
| 11 | 37 | 7.01 | 4.15 | 23.00 | 668.88 | Midsubstance |
| 12 | 52 | 6.20 | 4.22 | 26.00 | 679.73 | Enthesis |
| 13 | 21 | 8.03 | 4.31 | 26.00 | 900.91 | Enthesis |
| 14 | 44 | 6.18 | 5.21 | 28.00 | 901.63 | Midsubstance |
| 15 | 35 | 7.70 | 2.85 | 28.00 | 614.01 | Enthesis |
| Mean ± SD | 42.1 ± 10.8 | 6.53 ± 1.18 | 4.23 ± 1.20 | 26.69 ± 2.91 | 720.96 ± 182.40 | |

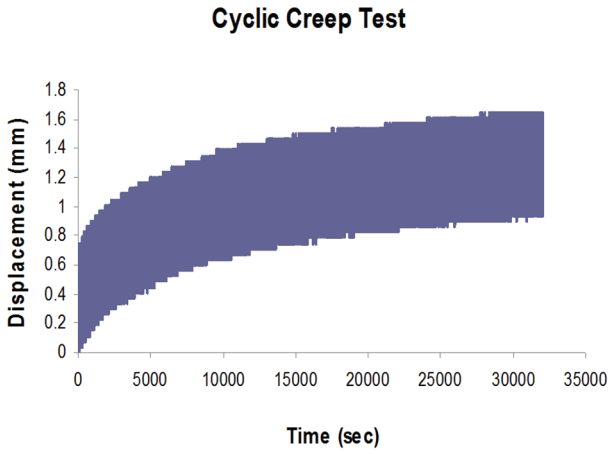


**Figure 2.** Representative graph of allograft elongation during cyclic loading for 1000 cycles at 1 Hz.

TABLE 2
Comparison of Mechanical Properties by Failure Type

| | Enthesis (n = 5)[a] | Midsubstance (n = 10)[a] | *P* Value |
|---|---|---|---|
| Maximum stress, MPa | 12.29 ± 5.26 | 12.09 ± 4.05 | .47 |
| Maximum strain, % | 21.98 ± 9.51 | 19.55 ± 4.98 | .26 |
| Modulus, MPa | 95.01 ± 41.06 | 95.83 ± 28.85 | .48 |
| Cyclic creep, % | 1.77 ± 2.11 | 1.31 ± 1.40 | .31 |

[a]Values expressed as mean ± SD.

than 30% for maximum stress, maximum strain, and modulus of elasticity, results that fall within the range reported in the literature for other types of allografts and associated graft sterilization techniques used in ACLR.[8,9,34]

Graft choice in ACLR continues to be an area of active study and discussion in the literature.[‖] [AQ: 1] The general consensus recommendation from the American Academy of Orthopaedic Surgeons *Management of Anterior Cruciate Ligament Injuries: Evidence-Based Clinical Practice Guideline* published in 2014 states that there is strong evidence that ACL injuries can be treated successfully with autograft or appropriately processed allograft.[41] However, the guidelines caution that the recommendations may not be generalizable to young or highly active patients.[40,41] This caveat regarding young and highly active patients refers to the less predictable performance and higher failure rate of allografts in ACLR in that population. In a prospective longitudinal cohort study, Kaeding et al[23] reported clinically and statistically significant higher risk of failure for allografts versus autografts in young patients. This difference diminished with increasing age.

Non-patient-related factors that affect the variability in results of allograft ACLR include high-dose irradiation, proprietary sterilization techniques, and variable allograft tissue quality. High-dose gamma irradiation has now been removed from the standard sterilization process. Less is known about the proprietary cleansing techniques of tissue banks. Tejwani et al[48] examined proprietary sterilization techniques as potential independent risk factors for revision ACLR and found that irradiation greater than 1.8 Mrad, sterilization with the BioCleanse process, younger age, male sex, and patellar tendon allograft were all associated with higher risk of ACLR failure and the need for revision ACLR.

The one factor that is currently out of the treating surgeon's control is the quality of the allograft tissue. Requesting younger allograft donors may mitigate this risk; however, younger tissue does not always equate to better structural characteristics. In the present study, we were unable to identify a difference in material properties by

[‖]References 15, 16, 18, 19, 21, 23, 26, 41, 45, 46.

graft-specific factors like donor age or location of mechanical failure. This is concordant with the findings of Blevins et al[6] and Swank et al,[47] who found that donor age explained very little, if any, of the variability in bone–patellar tendon–bone and posterior tibialis tendon allografts, respectively. Prior results of allograft biomechanical testing for different graft types and sterilization processes are mixed.[28,34] However, even nonirradiated Achilles tendon allografts demonstrate high variability. Penn et al[34] tested 15 nonirradiated Achilles allografts and found CVs of 31%, 36%, and 39% for strain, stress, and modulus of elasticity, respectively. All of the variability measures in the study by Penn and colleagues[34] were greater than those found in the present study. The present study demonstrates that our current standard of care for proprietary tissue preparation, delivery of allograft to the operating room, and gross intraoperative surgeon inspection of allograft before reconstruction may fail to detect the substantial variability in the tissue that is used to perform ACLR. Recently, variability in the mechanical properties of structural allograft used in spine fusion has been identified, and a recent Instructional Course Lecture called for improved biomechanical testing of grafts.[24] A similar improvement in the processing and delivery of soft tissue allograft should be considered.

The mechanical properties of human tendon allografts used in ACLR, particularly patellar tendon grafts, have been examined previously in the literature. Butler et al[8] reported maximum strain, stress, and modulus of elasticity in vitro for the middle third of bone–patellar tendon–bone samples as 13.5% ± 0.7%, 68.5 ± 6.0 MPa, and 643.1 ± 53.0 MPa, respectively. In the same study, these mechanical properties in native ACLs were reported as 15.0% ± 0.8%, 36.4 ± 2.5 MPa, and 345.0 ± 22.4 MPa, respectively. An in vivo study using nonfailure testing for the tibialis anterior tendon reported peak strain, stress, and modulus of 2.5% ± 0.4%, 25 ± 2.5 MPa, and 1200 ± 150 MPa, respectively.[29] In a direct comparison of common allografts used in ACLR, Penn et al[34] demonstrated no significant difference among the mechanical properties of patellar tendon, tibialis anterior tendon, tibialis posterior tendon, and Achilles tendon allografts. In that study, the strain, stress, and modulus for Achilles tendon allografts were 48.0% ± 15%, 47.40 ± 17.1 MPa, and 266.3 ± 106.83 MPa, respectively. These correlate with CVs of 31%, 36%, and 40%, respectively. The absolute mechanical properties in the present study were lower, perhaps due to tissue processing techniques, as the current study evaluated low-dose irradiated specimens whereas Penn et al[34] evaluated fresh frozen specimens without irradiation. Additionally, the CVs in the current study were higher than those in prior studies, which again points to the incompletely understood deleterious effects of proprietary tissue processing and even low-dose irradiation.

Some of the variability in the mechanical properties reported in the present study may also be explained by regional variability in the Achilles tendon grafts themselves. While we did not specifically compare the collected region of each graft, the specimens we used were varied in that the largest, outer one-third of the graft was retained for testing after the central one-third was harvested for ACLR. Previous work by Yanke et al[51,53] showed that the central portion of bone–patellar tendon–bone grafts is biomechanically stronger than the medial or lateral portions. To our knowledge, this has yet to be evaluated in Achilles allografts. If the findings of patellar grafts hold true in the Achilles tendon, the central portion of the allograft (used for clinical ACLR in the current study) may represent a more durable graft source. Similarly, the medial and lateral portions of the tendon may differ with respect to their innate biomechanical properties. Further work is necessary to evaluate the regional significance of Achilles tendon allografts.

Considerable heterogeneity is found with regard to outcomes after ACLR using allograft tissue. Achilles allografts show a wide variability in survival rates at a minimum of 2 years postoperatively, ranging from 0% to 33%.[¶] It is possible that intergraft differences in biomechanical properties may partially explain this variability. Unfortunately, the clinical relevance of the variability in strain, stress, and modulus of elasticity for Achilles tendon allografts used in ACLR has not been well studied. The CV for each biomechanical property in the current study may very well be within an acceptable range and may not significantly alter outcomes. A study of native ACL biomechanics by Chandrashekar et al[9] found CVs of 25%, 40%, and 40% for strain, stress, and modulus of elasticity, respectively. With the exception of strain, all CVs calculated in the current study as well as those reported by Penn et al[34] were lower than those measured by Chandrashekar et al.[9] Further work is needed to evaluate to what extent this variability may affect failure rates and functional outcomes in vivo. The results presented here represent foundational data necessary to answer these unknowns. Follow-up evaluation of the ACLR patients who had implanted grafts from which we harvested our biomechanical specimens will help to correlate the tissue properties we observed with functional and patient-reported outcomes.

## Limitations

This study has several limitations. Varying biomechanical testing protocols are described in the literature, and thus direct comparison of the current results with prior biomechanical studies is limited. However, in the current study, biomechanical loads were chosen based on practical intraoperative and rehabilitative forces imparted on the allograft tissue. The sterilization method and proprietary graft processing protocol used to treat the grafts examined in this study may differ from the processing techniques used by other accredited tissue banks. It was not our intent to compare various graft sources but rather to quantify the variability among grafts from one commonly used tissue bank. We also did not compare irradiated with nonirradiated specimens, which would have helped to ensure that the data were comparable with other similar studies. Our sample size was small but adequate for our primary goal

[¶]References 7, 11, 27, 32, 33, 35, 39, 43, 54.

to quantify the variability between specimens in 4 biomechanical parameters using CVs. We acknowledge that the portion of the Achilles graft used in this study may not be representative of what is used in actual ACLR, as the material properties of the central third of tendon allografts have been shown to be different from those of the inner third and the outer third.[53] Future studies could involve validation of the outer third of the allograft as a biomechanical surrogate for the central third and could prospectively compare biomechanical parameters (from the outer third) with clinical outcomes (from the corresponding central third). The advantage of our method, however, was that we tested specimens that were used intraoperatively, and in this regard our method met the screening criteria of both the tissue banks and the treating surgeon.

## CONCLUSION

Low-dose gamma-irradiated Achilles tendon allograft sterilized for use in ACLR is highly variable under cyclic loading conditions and in all parameters of failure testing. In the current standard of care, the surgeon may not appreciate the degree of variability in biomechanical properties between acceptable allografts for ACLR. Variability in the mechanical properties of allograft tissue may in part explain the heterogeneity of allograft ACLR results and may contribute to the increased failure rate in the young and active population. Future studies should attempt to delineate the clinical effect of allograft variability in the mechanical properties of allograft tissue. Furthermore, future studies should examine techniques to prospectively identify the mechanical quality of allograft tissue either preoperatively or intraoperatively.

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