The Effect of Donor Age and Low-Dose Gamma Irradiation on the Initial Biomechanical Properties of Human Tibialis Tendon Allografts

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Background: Most tissue banks recover and irradiate tibialis tendon allografts from donors aged up to 65 years. It is unknown whether donor age and low-dose gamma irradiation affect the initial biomechanical properties of tibialis allografts.

Hypothesis: Donor age up to 65 years and low-dose gamma irradiation do not significantly affect the initial biomechanical properties of tibialis allografts.

Study Design: Controlled laboratory study.

Methods: One hundred twenty-six tibialis tendon allografts (63 pairs, 37 human donors) were divided into 3 age groups: young (<45 years), middle (46–55 years), and old (56–65 years). Within each age group, half of the paired tendons underwent tensile testing as single-strand grafts and the other half as double-strand grafts. One tendon from each donor pair was randomly assigned to undergo terminal sterilization with an absorbed dose of 1.46 to 1.80 Mrad (14.6-18.0 kGy) gamma irradiation, whereas the other tendon received no irradiation. All tendon grafts were preconditioned with a cyclic load and tested to failure in tension.

Results: Irradiated single-strand tendons in the old age group had a longer displacement at failure compared to the middle but not the young age group. Nonirradiated double-strand tendons in the old age group had a lower failure stress. Single-strand irradiated old tendons had a lower stiffness, and all irradiated young tendons and old double-strand tendons had a higher failure stress compared to nonirradiated tendons.

Conclusion: Donor age up to 65 years does not significantly affect the initial failure load, stiffness, or displacement at failure of tibialis allografts. An age-related decrease in failure stress was observed among nonirradiated tendons but not in tendons subjected to irradiation.

Clinical Relevance: The results provide biomechanical evidence for use of tibialis allografts from donors up to 65 years of age. Low-dose gamma irradiation does not negatively influence the initial biomechanical properties of tibialis allografts. Further studies examining age and irradiation effects after submaximal cyclic loading conditions are recommended.

Keywords: allograft; anterior cruciate ligament (ACL); gamma irradiation; tibialis tendons

The decision to use autograft or allograft tissue for knee ligament reconstructions is controversial, and there is an ongoing debate about this issue among orthopaedic surgeons who perform reconstructive knee ligament surgery.[15] Autograft tissue has the advantage of being readily available and carries no risk of disease transmission. Animal studies have also demonstrated that autograft tissue undergoes more rapid biological remodeling than do allograft tissues.[16] However, the number and size of autografts that can be harvested from an individual patient are limited, and harvest of

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autograft tissue can result in donor site morbidity. Advantages of allograft tissue include the absence of donor site morbidity, the availability of an unlimited number of tendon grafts for a given patient, the availability of different types of graft tissue, smaller incision size, less surgical dissection, and decreased operating time. However, there are concerns about the use of allograft tissue. These concerns include the potential for allograft tissue to transmit disease, the possible deleterious effects of secondary sterilization and processing methods on the initial biomechanical properties of allograft tissue, delayed biological remodeling of allograft tissue, and a belief by some surgeons that use of allograft tissue results in a higher clinical failure rate (F. R. Noyes, unpublished data, 2005). Use of allograft tissue also adds a significant cost to the procedure, and allograft tissue may not always be available.[31]

In spite of the above issues, the use of allograft tissue for reconstructive knee ligament surgery appears to be increasing. According to Garrett (unpublished data, 2005), 20% to 30% of all primary ACL reconstructions in the United States are currently performed using allograft tissue. Because of the increasing demand for allograft tissue, tissue banks are interested in increasing the eligible pool of donors and providing additional types of graft tissue. Presently, the upper age limit for donation of soft-tissue allografts for most tissue banks is 50 years, with approximately 17% of tissue banks accepting donors from 50 to 65 years.[31]

Few studies have evaluated the effect of donor age on the initial biomechanical properties of tendon grafts commonly used for knee ligament reconstruction. Several investigators have reported an inverse relationship between age and the strength of the intact human femur-ACL-tibia complex.[21,34] Because of the well-known inverse relationship between bone mineral density and increasing age, the above finding is not unexpected.[24] Although the tensile strength of bone–patellar tendon–bone allografts recovered from donors aged 17 to 55 years decreased with age by 20%, Blevins et al[4] found this trend did not reach statistical significance. This age-related decrease in initial graft strength has not been reported for hamstring allografts.[13]

Bone–patellar tendon–bone and Achilles tendon allografts are the most common allograft tissues used for reconstructive knee ligament surgery.[15] To increase the available supply of allograft tissue, anterior and posterior tibial tendons (tibialis tendons) have recently been studied to determine their suitability for use in reconstructive knee ligament surgery.[14,23] Biomechanical testing of double-strand (DS) tibialis tendon grafts demonstrated structural, material, and viscoelastic properties that were equivalent to or better than those of doubled gracilis and semitendinosus tendon grafts.[13,14] Double-stranded tibialis tendon grafts recovered from donors with a mean age of 78 years have also been shown to have an ultimate tensile strength and stiffness equal to or exceeding that of commonly used ACL graft sources.[23] On the basis of these findings, tibialis tendons have been recommended as replacement grafts for the ACL, PCL, medial collateral ligament, and the posterolateral corner.[14,15,18,22]

With the potential to obtain 1 anterior and 1 posterior tibial tendon from each donor leg, 4 additional soft-tissue grafts can be recovered per donor. The use of tibialis tendon allografts from donors in older age groups may help tissue banks meet the increasing demand for allograft tissue. However, there have been no controlled biomechanical studies comparing the initial tensile properties of soft-tissue allografts recovered from donors in older age groups with those recovered from younger age groups.

Although soft-tissue allografts are recovered and processed aseptically, aseptic processing does not entirely remove all risk of bacterial or viral contamination.[30,31] Many tissue banks treat musculoskeletal allograft tissues with additional measures such as gamma irradiation in an attempt to reduce the risk of bacterial contamination and to provide an additional layer of safety.[30] Gamma irradiation has excellent tissue penetration, ensuring that its effect will occur throughout the structure of the tissue. The primary mechanism by which gamma irradiation provides a bactericidal effect is by the direct alteration of nucleic acids leading to dysfunction and destruction of the genome.[30] Gamma irradiation of tissue at room temperature also results in the production of free radicals from liquid water in the tissue, which have a direct antimicrobial effect.[30] In a survey of 36 tissue banks, Vangsness et al[31] reported that a range of 1 to 3.5 Mrad of gamma irradiation was used. It has been reported by some investigators that a dose of 2 Mrad provides complete elimination of bacterial contamination from musculoskeletal allografts.[8,29] However, much higher radiation doses are required to inactivate viruses such as HIV. In bone-tendon-bone allografts, Fideler et al[9] reported a dose of 4.0 Mrad was needed to inactivate the HIV virus, whereas Grieb et al[12] demonstrated a dose of 5.0 Mrad was needed to inactivate HIV along with hepatitis C, hepatitis A, and 7 other microorganisms.

However, gamma irradiation has been shown to have dose-related deleterious effects on the initial biomechanical properties of soft-tissue allografts. Biomechanical studies have shown that radiation doses greater than 2 Mrad significantly decrease the initial failure loads in bone–patellar tendon–bone allografts and bone-ACL-bone allografts.[25,26] Gibbons et al[10] showed that doses of 2 Mrad did not affect the structural properties of bone–patellar tendon–bone grafts, whereas higher doses of 3 Mrad reduced the maximum stress, maximum strain, and strain energy density to maximum stress. However, Curran et al[7] found that bone–patellar tendon–bone allografts irradiated with 2 Mrad elongated 27% more after 1000 cycles and 20% weaker than were nonirradiated grafts.

There is a trade-off between providing tissue sterility and maintaining the biomechanical integrity of the tissue by using lower doses of radiation. At the present time, there is lack of agreement on the lowest acceptable dose for treating soft-tissue allografts. A study by Baker et al[1] recently validated a dose of 0.92 Mrad for terminal sterilization, and the authors thought that this dose would be effective in eliminating bacterial contamination. Although no biomechanical testing was performed, they speculated that this dose was unlikely to have a significant effect on the initial biomechanical properties of the tissue. At the present time, there have been no published studies examining the effects of low-dose (<2 Mrad) irradiation on the initial biomechanical properties of soft-tissue allografts.

The goal of the present biomechanical study was to determine the effect of donor age and low-dose irradiation

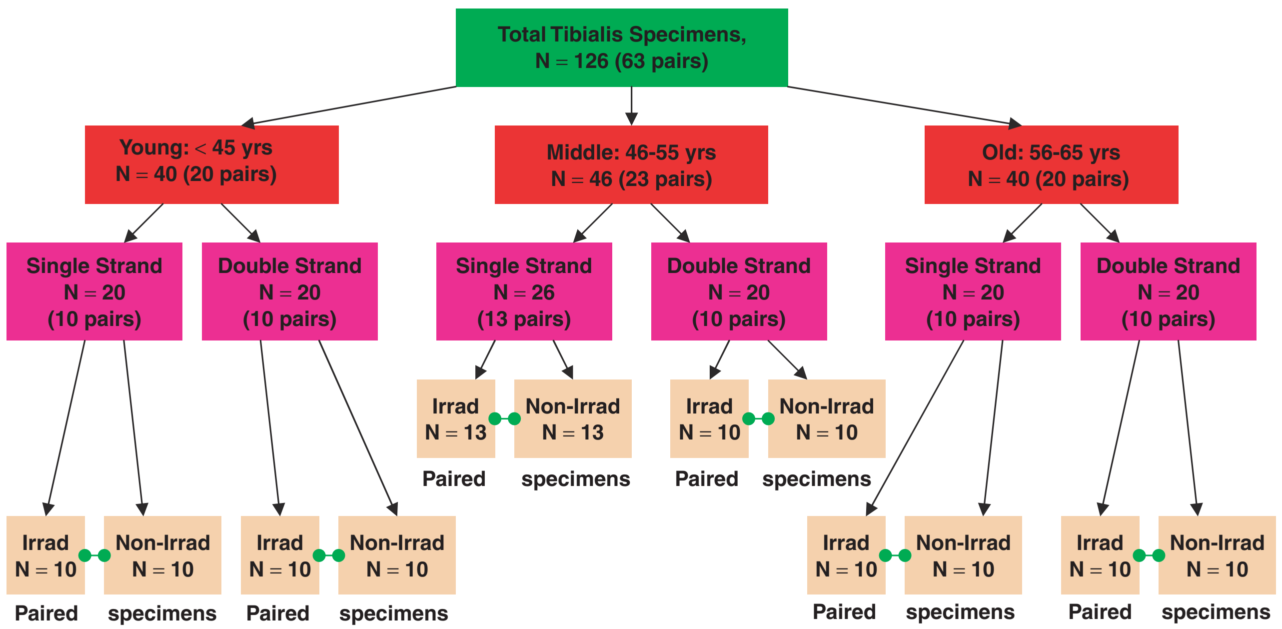

Figure 1. Study design. Irrad, irradiated.

on the initial biomechanical properties of single-strand (SS) and DS human tibialis tendons. We hypothesize that donor age up to 65 years and low-dose (<2 Mrad) gamma irradiation will not significantly affect the initial biomechanical properties of SS and DS human tibialis tendon allografts. This information may validate tissue banks' current upper age limit of 65 years for tibialis donors and may help provide tissue banks with some guidelines for proper irradiation levels.

METHODS

Experimental Design

One hundred twenty-six anterior and posterior fresh-frozen tibialis tendons (63 pairs) were recovered from 37 human donors. Donors ranged in age from 20 to 65 years. All donors met organ bank criteria for tissue donation. The paired specimens were divided into 3 groups based on age: young (20-45 years, n = 40), middle (46-55 years, n = 46), and old (56-65 years, n = 40). The tendons were treated using an Allowash (LifeNet Health, Virginia Beach, Va) sterilization process that uses ultrasonics and centrifugation in combination with several reagents.[19] Within each age group, half of the paired tendons were randomly assigned to be tested as SS or DS tendon grafts. Within each of the age groups, half of the paired SS and DS tendons underwent gamma irradiation at dry ice temperatures with an absorbed graft dosage of 1.46 to 1.80 Mrad (14.6-18.0 kGy). It is claimed that this dose of irradiation provides terminal sterilization with a sterilization assurance level of 10^{-6} in bone–patellar tendon–bone allografts.[19] The opposite member of the pair did not receive radiation (Figure 1).

Specimen Preparation

The tendon grafts were thawed to room temperature immediately before preparation and mechanical testing. All tendon grafts were visually inspected and verified to be intact. Within each age group, the paired tendons were randomly assigned to be tested as either an SS or DS tendon graft. Each tendon graft was cut an equal distance from the proximal and distal end. The SS tendons were cut to a length of 12 cm and the DS tendons to a length of 24 cm. The free ends of the DS tendon grafts were tubularized over a 6-cm length with 5 throws of a "baseball-type" whipstitch using a No. 2 nonabsorbable suture. The cross-sectional areas of the SS and DS tendon grafts were measured using a custom-built area micrometer.[13,17,20] The thickness of the tendon was measured using a dial indicator that monitored plunger height. This measurement was taken 2 minutes after a 0.12-MPa compressive load was applied.[13,17,20] The cross-sectional area was calculated by multiplying the graft thickness and the slot width of the area micrometer.[20] Cross-sectional area of SS tendons was measured by taking the mean of measurements made 15 mm proximal and 15 mm distal to the center of the graft. Cross-sectional area of DS tendons was measured by taking the mean of measurements 30 mm and 60 mm both distal and proximal to the center of each tendon graft. These locations marked the site at which the tendons were clamped and tensioned to failure and aligned the tendon in the grips such that a grip-to-grip distance of 30 mm was maintained. We chose a distance of 30 mm to simulate the intra-articular length of the normal ACL.[11,13,17] During preparation of the tendons, measurement of the cross-sectional area, and biomechanical testing, the specimens were kept moist with normal saline maintained at room temperature.

Mechanical Testing

The tendon graft constructs were tested to failure in tension with a servo-hydraulic materials testing system (model 8521, Instron Corp, Canton, Mass). To minimize soft-tissue slippage or failure at the grip-tendon interface,

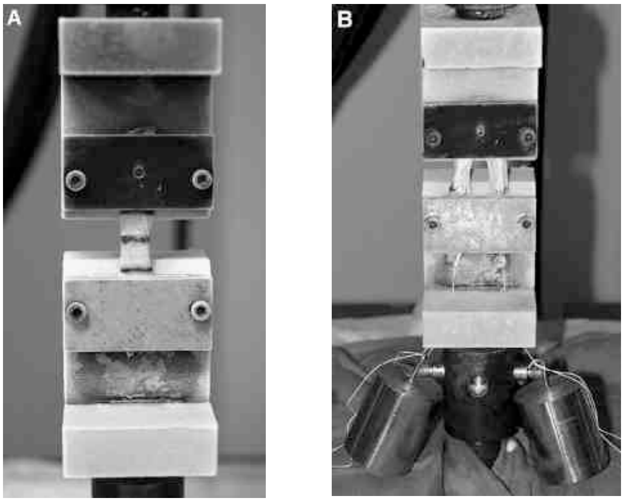

Figure 2. Testing configuration for single-strand (A) and double-strand (B) tibialis tendon grafts.

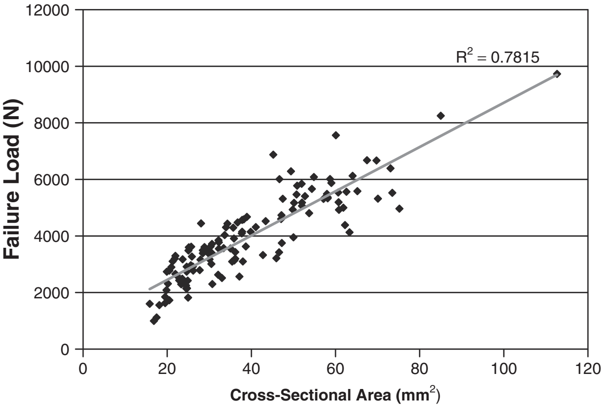

Figure 3. The linear dependence of failure load with tendon cross-sectional area.

specially designed soft-tissue grips were used.[13,17] The SS tendons were tested by placing the free ends in the grips at the marks previously placed 15 mm from the center of the tendon. The DS tendons were tested by placing the center of the tendon over the post in the upper clamp. The DS tendon was allowed to freely slide over the post. The sutures placed in the free ends of the DS tendon were tied together on each end of the tendon graft creating a suture loop. To equalize the tension in the 2 strands of the DS grafts, a 0.5-kg mass was hung from the suture loop on each end of the graft (Figure 2). The upper and lower chambers of the grips were filled with dry ice and the serrated lower and upper clamps tightened. The tendon grafts were preconditioned with cyclical loading of 50 N to 250 N for 100 cycles at 1 Hz; thereafter, a load of 50 N was maintained. After the preconditioning, additional dry ice was placed in the grip chambers, and the clamps were then maximally tightened. The tendon grafts were tested to failure at a strain rate of 100% elongation per second, which simulates the strain applied to the ACL during injury.[3,13,17,20,33] The site and mode of graft failure for all tests were recorded based on visual inspection of the failed specimen. Failure load, displacement at failure, and stiffness were measured from the load-elongation curve using data acquisition and analysis software (Series IX, Instron Corp). Failure load was defined as the applied force at which the load-displacement curve deviated substantially from a straight line. Displacement at failure was defined as the corresponding displacement of the upper clamp when the failure load was reached. Stiffness was defined as the slope of the linear region of the load-displacement curve. Failure stress was calculated by dividing the corresponding failure load by the initial (unloaded) graft cross-sectional area.

Statistical Analysis

A power analysis was performed before testing. This analysis determined that a sample size of 20 tendons in each age group would provide an 80% power to detect a 15% difference in the effects of donor age and irradiation status. A 1-way analysis of variance with least squared difference was used to detect differences in mechanical properties between the young, middle, and old age groups. A paired *t* test was used to determine the effects of irradiation with a given age group on the graft failure properties. Statistical significance was set at $P < .05$.

RESULTS

Biomechanical Properties

The mean ± SD failure load, stiffness, displacement at failure, failure stress, and cross-sectional area are shown in Table 1. The failure loads of all tendons tested were plotted against tendon cross-sectional area, and a strong linear dependence ($r^2 = 0.78$) was found (Figure 3).

Effect of Age

A summary of the significant findings is shown in Table 2. Age had no significant effect on failure load (Figure 4), stiffness, or displacement at failure for nonirradiated SS and DS tendons. A significantly ($P < .05$) lower failure stress was demonstrated in nonirradiated DS tendons in the old age group compared with nonirradiated DS grafts in the young and middle age groups. However, this finding was not found in irradiated DS tendons from the old age group. Age had no significant effect on failure load (Figure 4), stiffness, or failure stress for irradiated SS and DS tendons. However, a significantly longer displacement at failure ($P < .05$) was found in irradiated SS grafts in the old age group compared with irradiated SS grafts in the middle age group but not the young group. Although no significant differences were observed, there was a trend for graft cross-sectional area to increase with increasing age regardless of strand configuration or irradiation treatment (Table 1).

Effect of Gamma Irradiation

A summary of significant findings is shown in Table 2. Among the SS tendons in all 3 age groups, gamma irradiation had no significant effect on failure load or displacement

TABLE 1
Mean ± SD of Initial Tensile Properties

Property	Young		Middle		Old	
	Nonirradiated	Irradiated	Nonirradiated	Irradiated	Nonirradiated	Irradiated
Failure load, N						
Single strand	2843 ± 694	3062 ± 699	2823 ± 573	2729 ± 995	2988 ± 787	3004 ± 603
Double strand	5074 ± 1032	5124 ± 1206	5255 ± 706	5262 ± 845	4971 ± 1980	5334 ± 1353
Stiffness, N/mm						
Single strand	587 ± 105	569 ± 107	639 ± 174	630 ± 213	698 ± 138	525.0 ± 57.7[a]
Double strand	930 ± 186	886 ± 194	947 ± 139	943 ± 189	931 ± 223	966 ± 202
Displacement at failure, mm						
Single strand	6.10 ± 1.59	6.09 ± 1.32	5.62 ± 1.39	5.21 ± 1.68	5.87 ± 1.72	6.83 ± 1.03[b]
Double strand	6.40 ± 0.69	6.91 ± 1.21	6.57 ± 0.74	7.00 ± 1.53	6.37 ± 1.48	7.04 ± 1.62
Failure stress, N/mm²						
Single strand	105 ± 17.8	127 ± 27.9[c]	102 ± 20.9	104 ± 21.4	99.8 ± 23.6	111 ± 16.1
Double strand	98.1 ± 18.4	113 ± 17.5[d]	99.2 ± 17.7	103 ± 11.7	81.5 ± 12.1[e]	103 ± 18.0[f]
Cross-sectional area, mm²						
Single strand	27.2 ± 5.45	24.4 ± 3.64	28.5 ± 7.87	25.6 ± 6.53	30.0 ± 6.38	27.4 ± 6.13
Double strand	52.4 ± 9.58	45.4 ± 9.05	54.1 ± 9.40	51.8 ± 10.1	61.0 ± 22.2	54.4 ± 19.2

[a]Effects of irradiation: significantly lower ($P < .01$) than nonirradiated in same age group.
[b]Effects of age: significantly greater ($P < .05$) than irradiated middle age group.
[c]Effects of irradiation: significantly greater ($P < .05$) than nonirradiated in same age group.
[d]Effects of irradiation: significantly greater ($P < .05$) than nonirradiated in same age group.
[e]Effects of age: significantly lower ($P < .05$) than nonirradiated young and middle age groups.
[f]Effects of irradiation: significantly greater ($P < .005$) than nonirradiated in same age group.

at failure. A significantly higher failure stress ($P < .05$) was observed among irradiated SS grafts in the young age group compared with the nonirradiated tendons in the same age group, although a significantly lower stiffness ($P < .01$) was found in irradiated SS tendons in the old age group compared with nonirradiated SS tendons in the same age group. Among the DS tendons, irradiation had no significant effect on failure load (Figure 4), stiffness, or displacement at failure. A higher failure stress was observed among irradiated DS grafts in the young and old age groups ($P < .05$ and $P < .005$, respectively) compared with the nonirradiated DS tendons in the same age group.

Failure Modes

Midsubstance failure was defined as any rupture occurring within the graft substance between the 2 tendon grips. Grip failure was defined as a graft rupture occurring entirely at the grip-tendon junction. Graft slippage was defined as a tendon completely slipping from 1 of the tendon grips exposing the serrated portion of the tendon. Of the 63 nonirradiated tibialis tendon grafts tested, there were 42 (67%) midsubstance tears, 5 (8%) combination tears consisting of grip failure and graft slippage, 11 (17%) grip failure tears, and 5 (8%) cases of graft slippage. Of the 63 irradiated tibialis tendon grafts tested, there were 46 (73%) midsubstance tears, 8 (13%) combination tears consisting of grip failure and graft slippage, 7 (11%) grip failure tears, and 2 (3%) cases of graft slippage.

DISCUSSION

The goal of the present biomechanical study was to study the effects of donor age and low-dose gamma irradiation on the initial biomechanical properties of SS and DS tibialis tendon allografts. We found that neither age nor irradiation had a significant effect on the failure load of SS or DS tendon grafts. Focusing further on the more clinically relevant DS graft group, age and irradiation had no significant effect on failure load as well as stiffness and displacement at failure.

Previous investigators have documented the biomechanical properties of the tibialis tendon[14,23] (Table 3). Haut Donahue et al[14] used a similar testing protocol; however, they used a liquid nitrogen freeze clamp tendon gripping system that allowed the grips to remain frozen for a longer time period compared with grips that use dry ice. This feature allowed them to perform viscoelastic testing as well as tensile testing. In this study, tendons were recovered from donors with a mean age of 26 years. The failure load of DS anterior and posterior tibialis tendons, respectively, was reported to be 4122 and 3594 N, stiffness was 460 and 379 N/mm, displacement at failure was 12 and 12.5 mm, failure stress was 89.8 and 89.1 MPa, and tendon cross-sectional area was 48 and 42 mm². For comparison, we report mean values for nonirradiated DS tendons from the 3 age groups in the present study. We report a mean failure load of 5100 N (32% higher than Haut Donahue et al), stiffness of 936 N/mm (123% higher), displacement at failure of 6.45 mm (47% lower), failure stress of 93 MPa (4% higher), and cross-sectional area of 55.8 mm² (24% higher). Pearsall et al[23] tested nonirradiated DS anterior and posterior tibialis

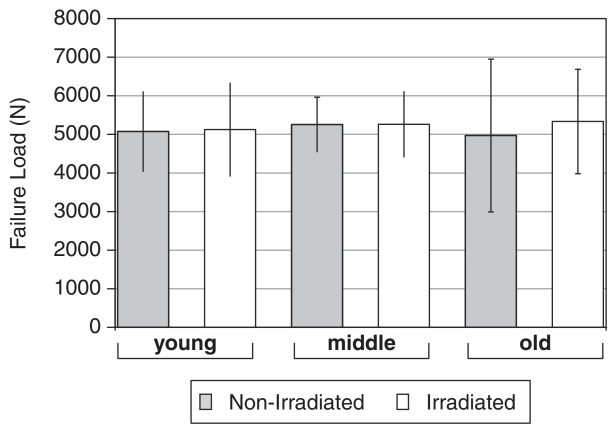

Figure 4. Failure load of double-strand tibialis tendon grafts. No significant differences between age or radiation status.

tendons recovered from human donors with a mean age of 78 years using gripping clamps frozen with dry ice similar to ones used in this study. The failure load for anterior and posterior tibialis tendons, respectively, was reported to be 3412 and 3391 N, stiffness of 344 and 302 N/mm, and tendon cross-sectional area of 38 and 48 mm^2. Failure stress of these tendons ranged from 85 to 108 MPa. Comparing the findings of Pearsall et al[23] with those of the present study, we report mean failure loads that are 50% higher, mean stiffness that is 190% higher, mean failure stress that is 4% higher, and a mean cross-sectional area that is 30% higher.

Differences between the biomechanical properties of nonirradiated DS tibialis tendons in the above studies and our data may reflect differences in the cross-sectional area of the tendons, differences in the tendon length tested, and differences in the applied strain rate used during tensile testing (Table 3). The mean cross-sectional area of the nonirradiated DS tendons in our study was 56 mm^2, which was greater than that of the tendons in the study of Haut Donahue et al[14] (48 mm^2 for anterior tibialis and 42 mm^2 for posterior tibialis tendons) and Pearsall et al[23] (38 mm^2 for anterior and 48 mm^2 for posterior tibialis tendons). As demonstrated in Figure 3, increasing the cross-sectional area of the tendons increases failure load. The mean tendon length tested in the hydraulic materials testing system (ie, distance between upper and lower clamp) also varied, with Haut Donahue et al testing tendons with a length of 75 mm and Pearsall et al testing tendons with a mean length of 185 mm (65-340 mm) and 165 mm (35-370 mm) for anterior and posterior tibialis tendons, respectively; whereas in the present study, the tested portion of the tendons was set at 30 mm in length. Differences may have also occurred because of the different strain rates applied to the tendons during tensile testing. In the present study, a high strain rate was chosen to simulate ACL injury.[3,20,21]

Previous authors have documented the biomechanical strength of the human ACL and various tissues commonly used in reconstructive knee ligament surgery.[6,13,20,21,28,33,34] Noyes and Grood[21] found the failure load of the femur-ACL-tibia complex in young and old human specimens to be 1730 and 734 N, respectively, with stiffness values reported at 182 and 129 N/mm, respectively. Woo et al[34] found the failure load of the femur-ACL-tibia complex in the young adult to approach 2160 N with a stiffness of 242 N/mm. Cooper et al[6] reported the failure load of 10-mm-wide bone–patellar tendon–bone grafts from young donors to approach 2977 N with a mean stiffness of 440 N/mm. Hamner et al[13] reported a mean failure load of 4590 N and stiffness of 861 N/mm for doubled gracilis and semitendinosus grafts recovered from donors with a mean age of 60 years. The failure load of 10-mm-wide central sections of the quadriceps tendon–bone complex and the bone–patellar tendon–bone complex, which were preconditioned with a load of 50 to 800 N at 0.5 Hz for 200 cycles, has been reported to be 2353 and 2376 N, respectively, by Staubli et al,[28] with mean stiffness values of 570 and 904 N/mm, respectively.

TABLE 2
Summary of Statistically Significant ($P < .05$) Findings[a]

	Graft Configuration			
	Single Strand		Double Strand	
	Effect of Age	Effect of Irradiation	Effect of Age	Effect of Irradiation
Failure load, N	NS	NS	NS	NS
Stiffness, N/mm	NS	Decreased in irradiated old age group (compared to nonirradiated)	NS	NS
Displacement at failure, mm	Increased in irradiated old age group (compared to middle age group)	NS	NS	NS
Failure stress, N/mm^2	NS	Increased in irradiated young age group (compared to nonirradiated)	Decreased in nonirradiated old age group (compared to young and middle age groups)	Increased in irradiated young and old age groups (compared to nonirradiated)

[a]NS, no significant findings.

TABLE 3
Comparison of Methods and Results With Other Tibialis Research Data

	Pearsall et al[23]		Haut Donahue et al[14]		
	Anterior	Posterior	Anterior	Posterior	Present: Both
Failure load, N	3412	3391	4122	3594	5100
Displacement at failure, mm			12	12.5	6.45
Stiffness, N/mm	344	302	460	379	936
Failure stress, MPa	85-108	85-108	89.8	89.1	93
Cross-sectional area, mm^2	38	48	48.2	41.9	55.8
Donor age, y	78		26		51
Tendon length tested, mm	185	165	75		30
Strain rate, mm/s	1		1.5		30

The mean strength and stiffness of nonirradiated DS tibialis tendon grafts reported in our study (5100 N and 936 N/mm, respectively) exceed that of the native ACL, 10-mm central-third bone–patellar tendon–bone grafts, 10-mm quadriceps tendon grafts, and doubled gracilis and semitendinosus grafts (Figure 5). Also of note is the strength and stiffness of the irradiated DS tibialis tendons from the old age group (5334 N and 966 N/mm, respectively). This group, generally thought to be a worst-case scenario, has higher strength and stiffness than do nonirradiated DS tendons recovered from young and middle-aged donors (Figure 4). Furthermore, the mechanical properties of the irradiated, old DS tibialis tendons in our study are higher than those of tendon grafts commonly used for ACL and PCL reconstructions.[6,13-15,20,23,28,33,34]

Smith et al[27] investigated the relationship between lyophilization and irradiation on the strength of porcine toe extensor tendons by examining the mechanical properties of tendons that were irradiated with 2.5 Mrad after either tendon freeze-drying or freezing. They found that the ultimate tensile stress of the freeze-dried irradiated group was reduced by approximately 90% compared with the frozen irradiated group, indicating that freeze-drying should be avoided when irradiating tendons to maintain mechanical properties. The present study investigated a low gamma irradiation dose of 1.46 to 1.80 Mrad after freezing, and it was shown that the mechanical properties of the tibialis tendon were not significantly compromised. This low irradiation dose has been previously validated to provide effective bactericidal coverage.[1] However, a previous study demonstrated that grafts subjected to 2 Mrad irradiation had a significantly increased graft elongation after 1000 cycles when compared with nonirradiated controls.[7] Therefore, further investigation is warranted to study the effect of irradiation on the displacement at failure of tibialis tendon allografts under submaximal cyclic loading conditions. Our soft-tissue gripping system required freezing the ends of the tendons with specially designed soft-tissue clamps. As the freezing temperature of the soft-tissue clamps was maintained by manually packing dry ice inside the clamps before cyclically loading the specimens, the number of cycles we could perform was limited by the melting time of the dry ice. Empirical testing revealed that our gripping system was limited to only 100 cycles of cyclic loading before conducting load to failure testing.

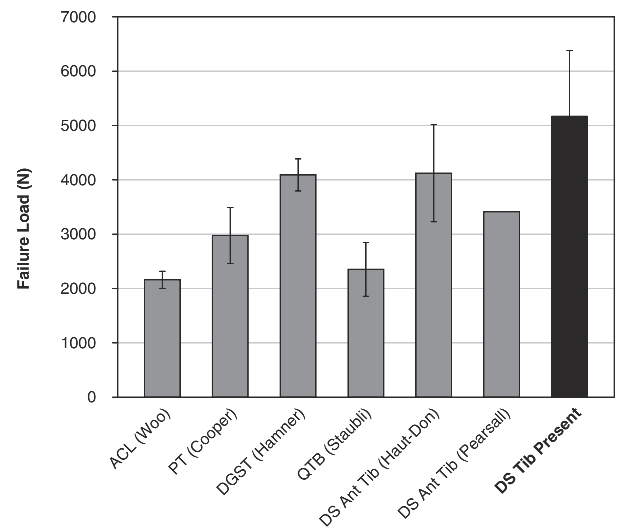

Figure 5. Failure loads of different grafts compared with the double-strand tibialis tendon. DGST, doubled gracilis and semitendinosus; DS Ant Tib, double-strand anterior tibial tendon; DS Tib, double-strand tibialis tendon; PT, patellar tendon; QTB, quadriceps tendon–bone complex.

Tibialis tendons are most commonly used by surgeons as DS grafts for ACL and PCL reconstructions. However, SS grafts represent a common point of reference by which the biomechanical properties of other grafts used for ACL reconstruction are compared. We chose to study the mechanical properties of both SS and DS tibialis tendon grafts in an attempt to address the needs of both clinicians and basic science researchers. The initial biomechanical properties of the tendons were affected by the configuration type (SS or DS). The mean increase in failure load of the DS versus SS tibialis tendons for nonirradiated and irradiated tendons was 77% and 80%, respectively. Theoretically, the failure load of DS grafts should be twice that of SS grafts. The lower values for DS grafts may have resulted because of differences in the site of testing. The SS tendons were tested at the midsection, whereas the DS tendons were tested approximately 4.5 cm from either side of the centerline as the testing configuration required the center of the tendon to be looped around a post and clamped in the soft-tissue grips. This test design may also provide an explanation for the inequality of statistically significant findings among the SS and DS configurations.

The strengths of the present study include the use of a single observer performing all of the biomechanical testing and cross-sectional area measurements (LLG), a large sample size, the use of paired tendons from the same donor in the nonirradiated and irradiated groups, and the randomization among tendons tested as SS or DS configurations. However, experimental error might have been introduced by the dry ice gripping system. Although the present study used a similar gripping technique used by other investigators to perform tensile tests of soft-tissue grafts, we did not always achieve midsubstance failures. The dry ice gripping system previously described by Hamner et al[13] resulted in 90% of tensile tests producing midsubstance failures in hamstring tendons, whereas the present study had only 67% and 73% midsubstance failures in tendons from the nonirradiated and irradiated groups, respectively. In some cases, tendon slippage was unavoidable as the dry ice clamping system was not designed to withstand failure loads more than 7000 N, a value occasionally achieved by some of our DS tendons. Therefore, the lower incidence of midsubstance failures achieved in the present study might reflect limitations of the gripping system due to the higher failure loads of tibialis tendon grafts compared with the SS, DS, and 4-strand hamstring tendon grafts. When the tendon undergoes a grip failure, a stress concentration from the grip occurs at the tendon-grip interface, which causes the tendon to fail at a lower load than would have occurred if the tendon had undergone a midsubstance failure. Therefore, the values we report may underestimate the failure load. The variability in failure load when the tendon fails at midsubstance versus a grip failure is currently unknown. However, because we tested such a large number of tendons (N = 126) and the modes of failure were fairly consistent across the different groups, we speculate that this effect would be comparable among the different age and irradiation groups.

Another limitation of the present study was the use of a single load to failure test to measure the failure load of the tendon grafts. The application of submaximal cyclic loads (or fatigue conditions) more closely simulates the loading pattern to which in vivo ACL grafts are subjected. Although ACL grafts might fail during a single event in which the load exceeds the ultimate failure load, the more likely mode of graft failure occurs when the graft is subjected to submaximal cyclic loading conditions during activities such as walking, jogging, running, and stair climbing.

The dry ice clamps used in the present study caused the midportion of the hamstring tendon construct to be cooled to 13°C. Hamner et al[13] reported no significant difference in linear stiffness and failure stress for patellar tendons tested at room temperature and those tested at 13°C. It was therefore assumed that the cooling of tendon grafts to 13°C did not significantly affect the additional mechanical property of displacement at failure.

This study investigated the mechanical effects of age and low-dose irradiation, but we did not address the possible underlying biological mechanisms by which these effects may contribute to the alteration of mechanical properties. Viidik[32] associated aging of connective tissues with increasing collagen cross-links. Viidik suggested that as age increases, the critical number of cross-links is eventually reached, and the connective tissue then becomes more brittle and weak.[32] Further investigation aimed at determining exactly how the composition of the tibialis tendon is altered with irradiation and increasing age might provide additional useful information for tissue banks.

The favorable mechanical properties of the tibialis tendon found in the present study are supported clinically with a retrospective study that examined the outcomes of 18 recipients of cryopreserved DS anterior tibialis allografts for ACL reconstruction 2 years after surgery.[22] Normal or near-normal grades for manual knee ligament tests were found in 94% of the subjects. Most subjects (83%) rated their current function at greater than 91% of preinjury levels, and all subjects continued to participate at their perceived preinjury activity levels.[22]

Although previous studies showed an inverse relationship between age and strength of the bone–ACL ligament–bone complex, it is important to note that the inferior biomechanical properties demonstrated in older specimens are suspected to be related to bone quality, which is known to decrease with age.[21,24,34] Previous investigators have reported biomechanical properties for SS gracilis and semitendinosus grafts recovered from elderly donors (mean age, 80 years; range, 70–102 years) that were similar to those from young donors.[13] These results suggest that age may have less of an effect on the initial biomechanical properties of soft-tissue grafts. The findings of our study provide additional support for this hypothesis.

An age-related decrease in failure stress was found among DS nonirradiated specimens in the old age group; however, an age-related decrease in failure load in the old age group was not found (Figure 4). This finding may be explained by the observed trend of the increase in graft cross-sectional area with age (Table 1). An age-related increase in displacement at failure was determined among SS old, irradiated tendons; however, this finding was not found among DS irradiated specimens. As the tibialis tendon is used in a DS configuration clinically, this increase in displacement at failure is perhaps not clinically relevant. The irradiation-induced decrease in stiffness among the SS grafts is perhaps also of little clinical concern as the stiffness we report for this group is higher than that of other commonly used ACL reconstruction grafts.[6,13,20,28] Furthermore, because graft fixation is the weak link until biological incorporation of the graft material occurs, it is the type of graft fixation used that determines the stiffness of the femur–ACL graft–tibia complex. Although irradiation resulted in a decrease in the stiffness of SS tendons in the old age group, the stiffness for this group is still 2 to 60 times higher than the stiffness reported for commonly used knee ligament fixation devices.[2,5]

In summary, our study demonstrates that donor age up to 65 years has no significant effect on the initial biomechanical properties of nonirradiated and irradiated SS and DS tendons. Terminal sterilization with 1.46 to 1.80 Mrad gamma irradiation significantly increased the failure stress in DS young and old specimens and SS young specimens;

however, stiffness among old, irradiated SS specimens was significantly decreased. Finally, our data suggest that low-dose gamma irradiation in the range of 1.46 to 1.80 Mrad does not significantly affect the initial biomechanical properties of SS and DS tibialis tendon grafts. Further investigation is required to study whether age and irradiation affect the displacement to failure after submaximal loading.

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