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The Effect of Donor Age on Structural and Mechanical Properties of Allograft Tendons

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Background: Allograft tendons are commonly used in surgical ligament reconstruction. While it is commonly accepted that donor age will affect mechanical properties of graft tissue, the apparent age threshold is unknown.

Hypothesis: Donor age will significantly influence the structural and mechanical properties of tibialis posterior allograft tendons.

Study Design: Controlled laboratory study.

Methods: A total of 550 allograft posterior tibialis tendons were examined. Linear stiffness, ultimate tensile force, ultimate displacement, tensile modulus, ultimate tensile strength, and ultimate tensile strain were calculated for specimens from donors in each of 6 age groups: 15-29, 30-39, 40-49, 50-59, 60-69, and 70-79 years. Both first- and second-order polynomial regressions were performed to determine the correlation between structural and mechanical properties and age. Welch analyses of variance with Games-Howell post hoc tests were performed to facilitate comparisons among age groups.

Results: All parameters displayed a weak correlation with age, with the highest R^2 term being 0.063 for ultimate tensile strength. Linear stiffness, ultimate tensile force, and tensile modulus displayed almost no correlation with age. Ultimate tensile strength increased slightly with age up to 40-49 years and then decreased with further increases in age. Slight decreases in ultimate displacement and ultimate tensile strain were observed with increasing age. Numerous statistically significant differences were observed between age groups for each outcome parameter; however, the magnitudes of the differences between age groups are relatively small (<15%) for all outcome parameters.

Conclusion: Age explained at most 6% of the variation in structural and mechanical properties of tibialis posterior allograft tendons.

Clinical Relevance: Posterior tibialis tendons from all age groups displayed structural properties superior to the native anterior cruciate ligament, with higher stiffness and ultimate force, and less displacement to failure. Although statistically significant differences in structural and mechanical properties were observed between age groups, the magnitudes of the differences are small and most likely not clinically relevant. The age of the donor will not likely affect the suitability of a graft for use in surgical reconstruction.

Keywords: allograft; biomechanical properties; age; tibialis posterior

The ideal graft used in ligament reconstruction procedures should have structural and biomechanical properties similar to those of the native ligament.[27] The mechanical properties of tendons make them well suited: they are relatively stiff (ie, they have a high elastic modulus), can sustain high tensile stress (force per cross-sectional area), can stretch elastically up to approximately 5% strain (length increment relative to initial length), and are very resilient.[24]

Autografts have been shown to substantially decrease retear rates among the younger population; however, the reduced pain and harvest site morbidity, along with the reliable clinical results of allograft, may favor its consideration as a graft choice in older or lower demand patients.[27] The advantages of using allografts include the lack of donor-site morbidity, a reduction in surgery time and hospitalization, and a better cosmetic result.[10] Allografts may also represent a good graft choice in complex cases, such as multiligamentous injury, or during revision surgery in which the autograft of choice has been used in the primary surgery.[27] Bone–patellar tendon–bone grafts, hamstring,[8] anterior tibialis tendon,[6,22] posterior tibialis,[11] Achilles tendon,[18] and fascia lata[19] represent some commonly used graft choices.

It is commonly accepted that donor age will affect mechanical properties of graft tissue, but the apparent

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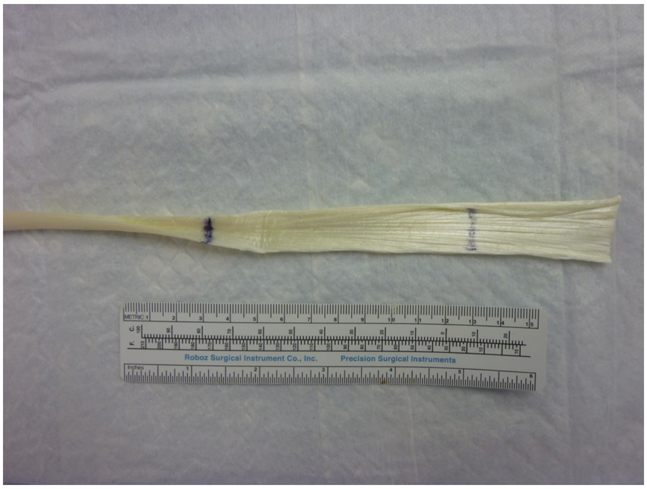

Figure 1. A 100-mm section of posterior tibialis tendon used for biomechanical evaluation.

age threshold is unknown.[16] Thus, many orthopaedic surgeons prefer to obtain tissue from younger donors when using allografts for procedures such as anterior cruciate ligament (ACL) reconstruction. Results of studies relating age and mechanical properties of allograft tendons are varied.[4] This may be due to small sample sizes, multiple methods of tendon sterilization and preparation, or testing parameters.

Tissue banks historically have used 1 of 2 methods of sterilization: ethylene oxide or gamma radiation.[26] Ethylene oxide, which was shown by Jackson et al[14] to produce high graft failure rates and persistent synovitis, is no longer used. Unlike other tissue sterilization processes for which the mechanical properties of the tissues have been studied, the BioCleanse tissue sterilization process (RTI Surgical) does not use heat or irradiation; it is a low-temperature chemical and mechanical sterilization process that achieves a sterility assurance level (SAL) of 10^{-6} and is validated to inactivate or remove human immunodeficiency virus (HIV), hepatitis, fungi, and spores. There is currently a lack of data concerning the material properties of allograft tendons processed with the BioCleanse system.

The purpose of this investigation was to perform a comprehensive biomechanical comparison of the initial properties of posterior tibialis allograft tendons to determine an appropriate donor age threshold for reconstructive procedures.

MATERIALS AND METHODS

A total of 550 tibialis posterior tendons processed with BioCleanse (donated by RTI Surgical) were stored in a –80°C freezer before testing. Immediately before testing, specimens were thawed at room temperature and kept moist in phosphate-buffered saline (PBS). Specimens were cut to approximately 100 mm in length, with the same anatomic section of tendon harvested from each donor tendon (Figure 1). The central "flat" region of the tendon was used for analysis.

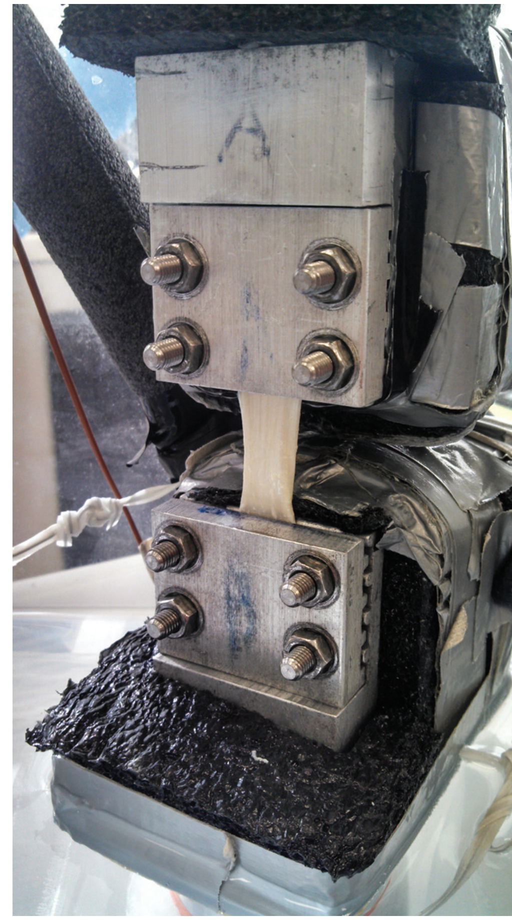

Figure 2. Experimental setup used for mechanical testing. The water bath and cyro grip insulation have been removed for clarity.

Specimen width and thickness were recorded using digital calipers with the specimen under a preload of approximately 10 N. Measurements were taken at 3 locations along the gauge length (central 32 mm) of the tendon. The average cross-sectional area, assuming a rectangular shape, was used in subsequent calculations.

Mechanical Testing

Testing was conducted on an ElectroPuls E10000 test system using a 10-KN Dynacell load cell (Instron Corp). The accuracy of the load cell is better than ±0.5% down to 1/100th capacity. The accuracy of the actuator position encoder on the test system is ±30 μm. Load string compliance was subtracted from the machine actuator position readout to provide a more accurate measurement of displacement.

The specimens were clamped in temperature-controlled, liquid nitrogen–cooled cryo grips (Figure 2) similar in design to those described by Sharkey et al.[25] A torque wrench was used to ensure the same holding force was applied to each specimen. Specimen gauge length was set to approximately 32 mm to replicate mean ACL length.[1]

TABLE 1
Posterior Tibialis Tendon Group Definitions and Geometry

Group	No. of Samples	Donor Age, y		Tendon Cross-Sectional Area, mm^2, Mean ± SD	Tendon Length, mm, Mean ± SD
		Mean ± SD	Range		
1	92	22 ± 4	15-29	22.9 ± 6.5	31.9 ± 0.4
2	92	35 ± 3	30-39	24.9 ± 8.6	32.0 ± 0.5
3	92	46 ± 3	40-49	18.9 ± 4.8	32.0 ± 0.4
4	90	55 ± 3	50-59	20.1 ± 6.2	32.0 ± 0.4
5	92	63 ± 2	60-69	22.5 ± 7.1	32.0 ± 0.5
6	92	73 ± 2	70-79	25.3 ± 7.7	32.0 ± 0.4
All data	550	49 ± 17	15-79	22.4 ± 7.3	32.0 ± 0.4

During testing, the specimens were immersed in PBS maintained at 37°C. To simulate pretension applied in surgery, we first subjected the specimens to a tensile ramp at 10 mm/min to 90 N, followed by a hold at 90 N for 60 seconds.[16] Specimens were then cyclically loaded from 50 to 250 N at 1 Hz for 1000 cycles. Cyclic loading served to further precondition the specimens, as well as simulate loading encountered during early rehabilitation.[7] Finally, specimens were pulled at a rate of 100% strain per second to failure,[7,20] replicating the short duration loading interval typically seen in ACL ruptures in vivo.[3,5]

Ultimate tensile force (N) was determined directly from the load-to-failure portion of the test. Ultimate tensile strength (MPa) was calculated by dividing the ultimate tensile force by the specimen cross-sectional area. Ultimate tensile strain (%) was computed by dividing the change in specimen length at ultimate load (ultimate displacement, mm) by the specimen gauge length. The tensile modulus (GPa) was determined by performing a linear curve fit of the stress versus strain curve between 2% and 6% strain (linear section of the stress vs strain curve) during the load-to-failure portion of the test. Linear stiffness (N/mm) was calculated from the following equation: $k = E*A/L$, where k is the linear stiffness, E is tensile modulus, A is the cross-sectional area, and L is the gauge length. Specimen creep (mm) was defined as the difference in maximum displacement between cycle 1000 and cycle 3 from the cyclic loading sequence.

Statistical Analysis

Posterior tibialis tendons were separated by donor age: 15-29, 30-39, 40-49, 50-59, 60-69, and 70-79 years (groups 1-6, respectively). Calculations to determine group sample size were carried out based on the hypothesis that age affects the tendon structural and mechanical properties. Using t tests with Bonferroni corrections to compare each age group with the "gold-standard" 15- to 29-year age group, a sample size of 92 specimens per group was calculated based on preliminary data (critical effect size = 0.51, based on a 15-MPa difference in ultimate tensile strength between groups).

Both first- and second-order polynomial regressions were performed to determine the correlation between structural and mechanical properties and age. In addition to the t tests with Bonferroni corrections described above, Welch analyses of variance (ANOVAs) with Games-Howell post hoc tests were performed to include comparisons between age groups other than the gold-standard 15- to 29-year age group. For all but one of the outcome parameters, preliminary statistical analysis revealed that the significant findings from t tests matched significant findings for the same comparisons of the Welch ANOVA. As the Welch ANOVA analysis provides comparisons between all age groups, not just comparisons between older age groups and the gold-standard group, findings from the Welch ANOVA are presented in the subsequent sections. Significance was set at $P < .05$.

RESULTS

Average age and geometry for each group are presented in Table 1. Only 90 specimens were included for group 4, as 2 specimens were from repeat donors. Most specimens exhibited failure within the gauge length; however, 6% of the specimens (n = 33) exhibited failure within the grips. These specimens were included in the analysis, as additional t tests revealed no difference in properties between these and the other specimens tested in this study. Structural and mechanical properties for each age group are presented in Tables 2 and 3, respectively. The average magnitude of specimen creep during the cyclic loading portion of testing was negligible; consequently, creep data were excluded from further analysis.

Scatter plots of structural and mechanical properties versus age are presented along with the regression fits in Figures 3 and 4, respectively. For 3 of the properties (ultimate displacement, tensile modulus, and ultimate tensile strain), the addition of a second-order term did not substantially increase the regression fit. For this reason, the first-order polynomial regression results were selected for these variables. All parameters displayed a weak correlation with age, with the highest R^2 term being 0.063 for ultimate tensile strength. Ultimate tensile strength increased slightly with age up to 40-49 years and then decreased with further increases in age. Linear stiffness (R^2 = 0.029), ultimate tensile force (R^2 = 0.012), and tensile modulus (R^2 = 0.004) displayed almost no correlation with age. Slight decreases in ultimate displacement (R^2 = 0.057) and

TABLE 2
Structural Properties of Posterior Tibialis Tendons Organized by Age Group[a]

Age Group, y	Linear Stiffness, N/mm	Ultimate Tensile Force, N	Ultimate Displacement, mm
15-29	951 ± 181	3255 ± 751	5.24 ± 0.80
30-39	979 ± 178	3437 ± 711	5.42 ± 0.70
40-49	904 ± 166	2980 ± 671	4.81 ± 0.65
50-59	910 ± 198	2991 ± 762	4.84 ± 0.64
60-69	1006 ± 247	3283 ± 941	4.87 ± 0.57
70-79	1034 ± 228	3349 ± 825	4.89 ± 0.63
All data	964 ± 206	3217 ± 797	5.01 ± 0.70

[a]Values are reported as mean ± SD.

TABLE 3
Mechanical Properties of Posterior Tibialis Tendons Organized by Age Group[a]

Age Group, y	Tensile Modulus, GPa	Ultimate Tensile Strength, MPa	Ultimate Tensile Strain, %
15-29	1.37 ± 0.25	146 ± 29	16.4 ± 2.5
30-39	1.34 ± 0.30	146 ± 30	16.9 ± 2.2
40-49	1.57 ± 0.24	161 ± 26	15.0 ± 2.1
50-59	1.51 ± 0.25	154 ± 30	15.1 ± 2.0
60-69	1.48 ± 0.24	150 ± 26	15.2 ± 1.7
70-79	1.37 ± 0.25	137 ± 25	15.3 ± 2.0
All data	1.44 ± 0.27	149 ± 29	15.7 ± 2.2

[a]Values are reported as mean ± SD.

ultimate tensile strain (R^2 = 0.059) were observed with increasing age. Excluding the slope coefficient for the first-order polynomial regression of tensile modulus (P = .122), all coefficient terms were statistically significant ($P \leq 0.01$). A second-order polynomial regression revealed minimal correlation between age and cross-sectional area (R^2 = 0.057).

When specimen data were stratified by age group, numerous significant differences were observed between age groups for each outcome parameter; however, the magnitudes of the differences between age groups are relatively small for all outcome parameters. The percentage difference between the age groups displaying the largest and smallest values for each outcome parameter was 12.5% for linear stiffness (groups 3 vs 6, $P < .001$), 13.3% for ultimate tensile force (groups 2 vs 3, $P < .001$), 11.1% for ultimate displacement (groups 2 vs 3, $P < .001$), 14.6% for tensile modulus (groups 2 vs 3, $P < .001$), 14.7% for ultimate tensile strength (groups 3 vs 6, $P < .001$), and 11.1% for ultimate tensile strain (groups 2 vs 3, $P < .001$). All above-mentioned percentage difference calculations were calculated relative to the group with the larger magnitude value. While no single age group displayed superior properties compared with all other age groups, group 3 exhibited greater ultimate tensile strength and modulus and less ultimate tensile strain compared with groups 1 and 2 ($P < .01$ for all comparisons). Group 3 also demonstrated greater ultimate tensile strength and modulus compared with group 6 ($P < .001$). Finally, the cross-sectional area for group 3 was significantly less than that of groups 1, 2, 5, and 6 ($P \leq .001$ for all comparisons), and cross-sectional area for group 4 was significantly less than that of groups 1 ($P < .05$), 2, and 6 ($P < .001$).

DISCUSSION

This study evaluated both the structural and mechanical properties of allograft posterior tibialis tendons. The mechanical properties are a function of the tendon material, while tendon structural properties are a function of both material and geometry. Structural properties are more relevant to the surgeon, as these parameters define how an allograft tendon will behave in vivo.

Analysis revealed that allograft posterior tibial tendon structural and material properties displayed a weak correlation with age. Age explained at most 6% of the variation in tendon properties. In addition, it is important to note that mechanical properties, including linear stiffness, ultimate tensile force, and ultimate tensile strength, were better fit with a second-order polynomial regression, indicating that these parameters both increase and decrease with age. Consequently, one cannot simply conclude that because 1 tendon is from a younger donor than another, it will display superior structural and mechanical properties.

Results of past studies relating age and mechanical properties of allograft tendons are varied. Blevins et al[4] tested bone–patellar tendon–bone allografts procured from donors aged 17 to 54 years at strain rates of 10% and 100% elongation per second. No significant correlation

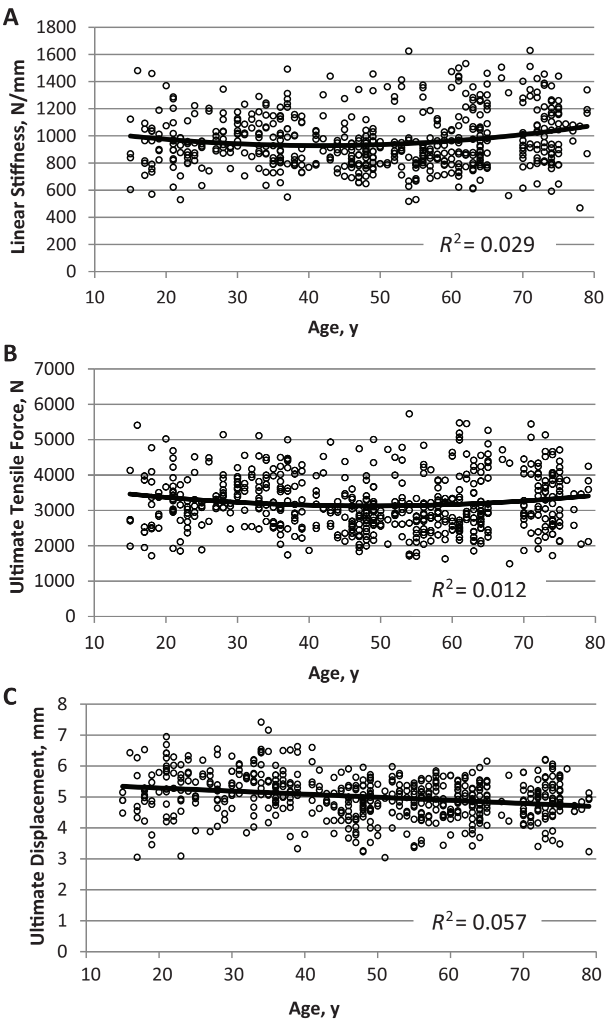

Figure 3. Scatter plots with regression fit curves for posterior tibialis tendon structural properties versus age: (A) linear stiffness, (B) ultimate tensile force, and (C) ultimate displacement.

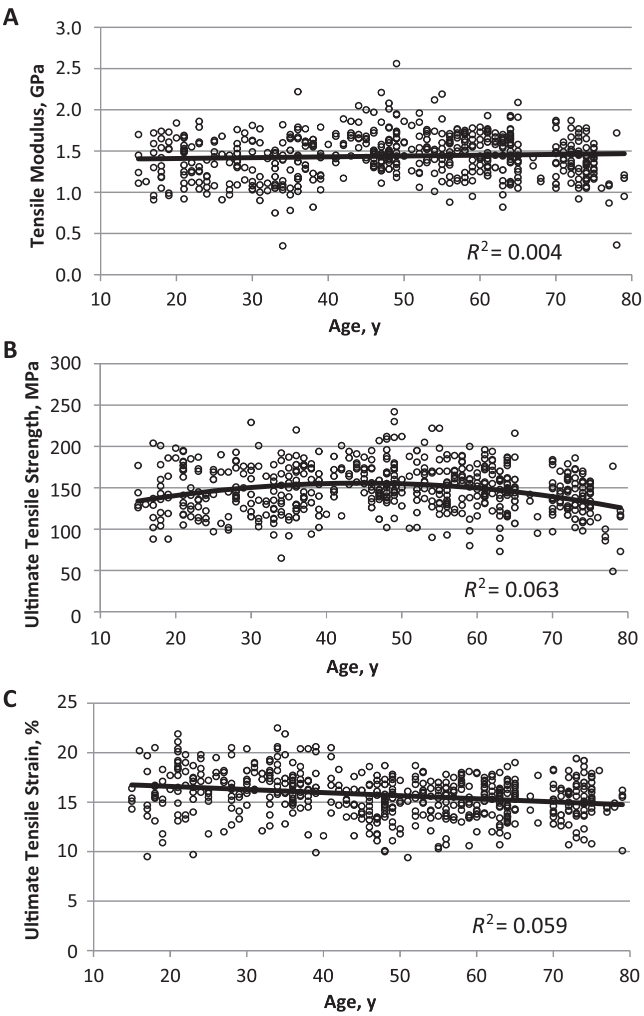

Figure 4. Scatter plots with regression fit curves for posterior tibialis tendon mechanical properties versus age: (A) tensile modulus, (B) ultimate tensile strength, and (C) ultimate tensile strain.

between tensile strength and age was found. While specimens tested at 100% per second exhibited a weak, negative, linear correlation between modulus and age, modulus measurements made at 10% per second showed no significant correlation with age. Flahiff et al[9] found no significant correlation between mechanical properties and age in patellar complexes from donors aged 18 to 55 years. Although slightly decreasing tensile strength was observed with age, the difference was not significant. Johnson et al[15] found significant differences in patellar tendon complex tensile strength between younger (29-50 years) and older (64-93 years) age groups. Balsly et al[2] evaluated the mechanical properties of radiation-treated patellar tendon, anterior tibialis tendon, semitendinosus tendon, and fascia and found no significant correlation between strength and age or between modulus and age. Greaves et al[11] evaluated the mechanical properties of tibialis tendons, grouping the results of both anterior and posterior tendons together. Specimens were separated into 3 age groups (young: 20-45 years, middle: 46-55 years, old: 56-65 years). While no difference in tensile force, stiffness, and ultimate displacement was observed between age groups for the nonirradiated single- and double-strand tendons, the reported failure stress was found to decrease with age for the double-strand tendons tested in this study. In the present study, ultimate tensile strength was best fit with a second-order polynomial regression and was found to decrease in specimens from donors older than 50 years. This matches the trend observed by other authors.[9,11,15]

The reported mechanical and structural properties of the posterior tibialis tendons evaluated in this study are generally higher than those reported by other authors. In the study conducted by Greaves et al,[11] nonirradiated single-strand tendons exhibited slightly lower ultimate tensile force (young: 2843 N, middle: 2823 N, old: 2988 N), stiffness (young: 587 N/mm, middle: 639 N/mm, old: 698 N/mm), and ultimate tensile strength (young: 105 MPa, middle: 102 MPa, old: 100 MPa) than the average values of ultimate tensile force (3217 N), stiffness (964 N/mm), and ultimate tensile strength (149 MPa) determined in the current study.

Pearsall et al[23] and Haut Donahue et al[12] evaluated the mechanical and structural properties of doubled posterior tibialis tendons, while Giannini et al[10] examined single-strand posterior tibialis tendons. With adjustment to allow for comparison among single-stranded tendon specimens, the average failure loads from these studies are 1696 N, 1797 N, and 2517 N, respectively. The modulus and ultimate strength reported in these studies were also lower than those observed in the present study. These differences do not appear to be due to cross-sectional area measurements, as the measurements reported in these previous studies are similar to the values obtained in the current study. Variations in the aforementioned properties may be due to differences in displacement rate to failure,[29] the chemical treatment of the tendons, and the testing environment.[13] The ultimate tensile strain reported in the current study falls in the range of values reported by previous authors.[10,11,12,23]

While data stratification by age showed numerous significant differences between test groups for all properties analyzed, the magnitude of differences between test groups was small (<15%). In addition, no single age group displayed superior structural properties compared with all other age groups. Considering mechanical properties, group 3 (donor age, 40-49 years) exhibited both higher tensile strength and modulus, with less ultimate strain than the other age groups; however, the differences in mechanical properties for group 3 compared with all other groups were not statistically significant for all group comparisons. Higher magnitude strength and modulus values can be attributed to the smaller cross-sectional area of group 3 compared with the other age groups.

For all age groups considered in this study, the posterior tibialis tendon displayed higher stiffness and ultimate force and exhibited less displacement to failure than the native ACL. In testing ACL bone-ligament-bone specimens, Woo et al[28] found the stiffness of the ACL in younger individuals (22-35 years old) to be 242 N/mm, while the stiffness in older individuals (60-97 years old) dropped to 180 N/mm. Similarly, ultimate tensile force was higher in younger (2160 N) compared with older individuals (658 N). Noyes and Grood[21] observed slightly lower stiffness and failure loads in the ACL and found the strain at failure of the ACL in younger individuals (16-26 years old) to be 60.25%. Kennedy et al[17] tested the ACL substance alone and found slightly lower failure loads (626 N) and failure strain (35.8%). The lower failure strain observed by Kennedy et al can be explained by lack of deformation at the tendon insertion, as the ACL substance was tested alone. For comparison, in this study, the age groups displaying the lowest posterior tibialis tendon stiffness (904 $\pm$ 166 N/mm, group 3) and ultimate tensile force (2980 $\pm$ 671 N, group 3) exhibited approximately 3.7 times and 1.4 times the stiffness and failure load, respectively, of the ACL in young humans.[28] Moreover, the posterior tibialis tendon age group displaying the highest ultimate strain (16.9%, group 2) was approximately 47% of that observed in the ACL substance.[17]

Limitations of this study include the use of only posterior tibialis tendons treated by the BioCleanse process. The results obtained in the current study may not reflect trends in untreated allograft posterior tibialis tendons. The specimen gauge length used in this study was selected to mimic the length of the native ACL. Use of longer specimens would minimize the effects of small displacements at the grip interface and reduce displacement errors.

CONCLUSION

Donor age has only a minor, if any, influence on the structural and mechanical properties of allograft posterior tibialis tendons. Although statistically significant differences exist between age groups, the differences are small in magnitude. The age of the donor will not likely affect the mechanical properties of posterior tibialis tendons for use in surgical reconstruction.

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