Clinical Biomechanics 67 (2019) 90–95

Contents lists available at ScienceDirect

Clinical Biomechanics

journal homepage: www.elsevier.com/locate/clinbiomech

Biomechanical and geometric characterization of peroneus longus allografts with respect to age

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ARTICLE INFO

Keywords:
Peroneus longus tendon
ACL reconstruction
Biomechanics

ABSTRACT

Background: Anterior cruciate ligament injuries are among the most common injuries in high impact sports, and reconstruction is the standard surgical procedure for these ruptures. Reconstructions are often performed using allografts rather than autografts on a case-by-case basis. Controversy exists as to whether or not age of donor tissue plays a factor in the mechanical properties of allografts.
Methods: 38 peroneus longus (PL) tendons were prepared using the two-strand graft technique and then subjected to a cyclic loading test regimen of 1000 cycles to determine material properties. Specimens were grouped based on age to ascertain whether donor age affects the material properties of PL tendons.
Findings: Secant modulus of the first cycle was determined to be 150.43 (SD 40.24) MPa. The average magnitude of the dynamic modulus was determined to be 82.81 (SD 24.65) MPa. Specimens were grouped into three distinct groups for analysis ($x < 40$ yo, 40 yo $\leq x < 60$ yo, 60 yo $< x$).
Interpretation: The need for using intrinsic material properties is highlighted. There is no significant difference in any intrinsic material property with respect to age or the fatigue of the tendon as the cycle count increases. Conversely, the measured stiffness of a tendon decreased as function of age with a large effect size. Based on analysis of graft geometries, it was determined that PL tendons become significantly more slender with increased age which result in the observed decrease in stiffness.

1. Introduction

Ligamentous injury to the athlete usually affects the ankle or the knee, and is a major cause of time away from their sport (Joseph et al., 2013; Mahapatra et al., 2018; Swenson et al., 2013). The Anterior Cruciate Ligament (ACL) is an essential structure of the knee, working alongside other ligaments to stabilize this mechanically complex joint (Dargel et al., 2007). ACL disruptions are one of the most common injuries in high-impact sports, especially those that require sudden changes in momentum (Prodromos et al., 2007; Sanders et al., 2016). ACL reconstruction (ACLR) is the standard surgical procedure for ACL ruptures. Over 130,000 ACLRs are performed annually in the United States (Prodromos, 2008). ACLR is usually done with a substitute for the ACL (either an autograft or an allograft) since repaired ACLs have been shown to fail over time (Mahapatra et al., 2018). Despite decreased functional outcomes compared to autografts, allografts allow numerous advantages including shorter operation time and shorter rehabilitation time (Anderson et al., 2016). One option for transplantation is the peroneus longus (PL) tendon, which when doubled, has been described as suitable for knee reconstructions in terms of its biomechanical properties (Pearsall et al., 2003).

Ligaments and tendons (along with most other tissues) display viscoelastic behavior (Wang, 2006; Wang et al., 2012). When deformed, they display a combination of elastic behavior, where strain is instantaneous and totally recovered, and viscous behavior, where strain is dependent on time, and is not reversible or completely recovered. The inherent viscoelastic properties of a tendon are inherent to their integrity. Due to the viscoelastic nature of tendons, dynamic modulus is a better representation of resistance to deformation than elastic modulus (Beach et al., 2017; Dourte et al., 2013).

The effect of donor age on allograft mechanics is not well established. Studies that have examined the effects of age have reported

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https://doi.org/10.1016/j.clinbiomech.2019.04.017
Received 28 August 2018; Accepted 25 April 2019

inconsistent findings. Some research has shown that aging results in stiffer, more resilient tendons (Vogel, n.d.; Maganaris and Narici, 2005; Shadwick, 1990), while other studies demonstrated that tendons get weaker with age (Carroll et al., 2008; Vafek et al., 2017). Other investigations show that there are negligible changes in mechanical properties with respect to age (Blevins et al., 1994; Greaves et al., 2008; Swank et al., 2015). This lack of consensus extends to the clinical realm where, for example, recent evidence showed donor age to impact post-operative patient outcomes with Achilles tendon allografts (Zaffagnini et al., 2018) but not patellar tendon allografts (Hampton et al., 2012). There is clearly disagreement about the extent of biomechanical changes affect allografts with age, whether there are significant changes at all, and if any changes would have clinical impacts. Therefore, this paper aimed to further elucidate the change in biomechanical properties of PL tendon allografts with respect to donor age so that informed decisions could be made by surgical teams when selecting a graft.

2. Methods

2.1. Specimen preparation

Thirty-eight deidentified PL tendons were donated from Pinnacle Transplant Technologies (Pinnacle Transplant Technologies, Phoenix, AZ, USA). The age range in this study was 22 years old to 74 years old. Tendons were grouped into three age groups: younger than 40 years old, between 40 and 60 years old, and over 60 years old. These age groups were chosen to represent younger-adult, middle-aged, and older-adult groups. Additionally Lansdown et al. used similar age cut-offs, noting negative impacts on biomechanical properties in tendons older than 40 years old and especially older than 65 years old (Lansdown et al., 2017). Thirteen of the tendons were from female donors and twenty-five were from male donors. Donor gender was uniformly distributed among all age groups. All other patient medical information including anthropomorphic characteristics and possible comorbidities was deidentified according to HIPAA regulations, and IRB approval was not required for use of the tissue samples. Allografts were stored at −20 °C as per Pinnacle's recommendations until use. Prior to mechanical testing, allografts were submerged in saline and thawed at room temperature for 2–3 h. Each end of the tendon was then sutured together to create a loop. The non-loop end was stabilized with K-wire and subsequently embedded with bone cement. The loop end was attached to the crosshead of universal testing machine via a carabiner (proof load: 25kN, Fig. 1A).

2.2. Cyclic testing

Prepared specimens were subjected to a cyclic loading protocol using an Instron 5566 (Norwood, MA, USA). A preload of 10 N was applied to the tendons before the test regimen started, and the tendons were allowed to reach equilibrium before the start of the test regimen. Once equilibrated, gage length of the grafts was measured with calipers to the nearest hundredth of a millimeter. Cross sectional area of each strand was then calculated by estimating area as an ellipse (πab), where a and b are the length of major and minor axes determined using calipers tightened until first contact on the tendon surface. Values for a and b were determined by the average of three separate measurements at half the distance of gage length. The two areas were summed to get a total cross-sectional area for the two-strand grafts.

Dynamic modulus, E*, is complex and can be broken down into the storage modulus, E′, and the loss modulus, E″, where the E′ represents energy stored by the material, and E″ represents the energy lost from the system, usually dissipated as heat (Ehrenstein et al., 2004).

$$E^* = E' + iE'' \quad (1)$$

Furthermore, the magnitude of E*, denoted |E*|, is the resultant of the storage and loss moduli. It can also be calculated using the secant

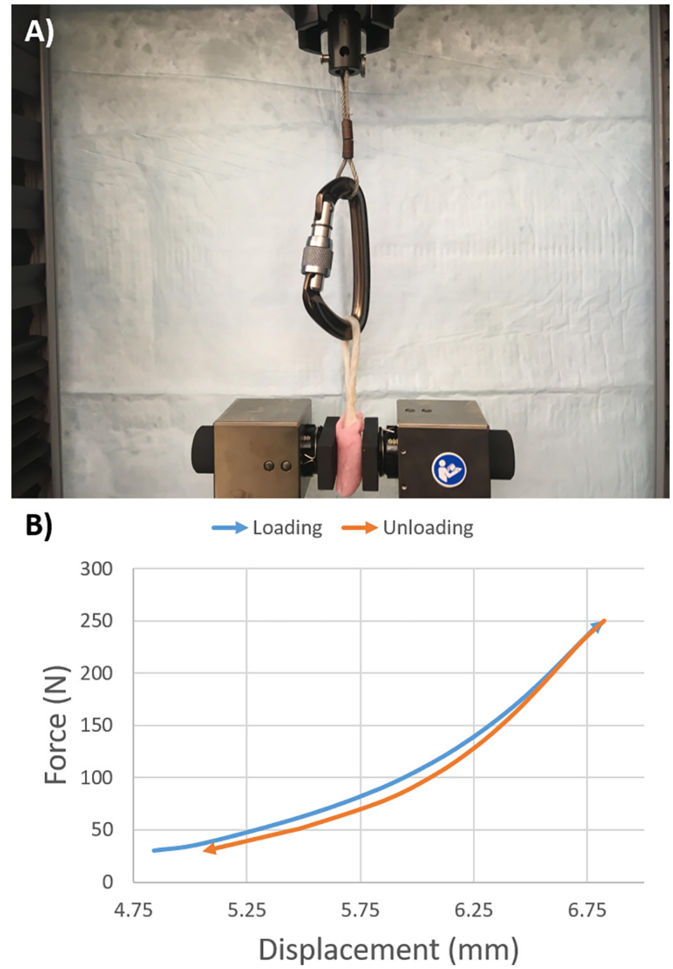

Fig. 1. A) Example experimental setup of the tendon in a universal testing machine. The loop end of the tendon is attached to the crosshead, while the non-loop end is fixed to the lower jaw of the universal testing machine. The non-loop end has also been stabilized with k-wire and embedded in bone cement to prevent slippage of the specimen. B) An example force displacement curve during cyclic testing. The blue colored line represents the loading phase, and the orange colored line represents unloading phase. Area between the blue and the orange lines represents energy loss during cyclic testing. Direction of the arrows on each line represents the direction of time. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

modulus, E_{sec} from the stress and strain amplitudes.

$$a\Big)\, E_{sec} = |E^*| = \frac{\sigma_A}{\varepsilon_A} \quad b\Big)\, |E^*| = \sqrt{E'^2 + E''^2} \quad (2)$$

Cyclic testing consisted of a force controlled (50 N to 250 N) triangular waveform at a frequency of 1 Hz for 1000 cycles. After each test, stress and strain were determined from the force-displacement curve (Fig. 1B). Stress was calculated from the force divided by cross sectional area, and strain was calculated by the change in displacement divided by the original gage length. Secant modulus was calculated based on eq. 2a, given above. In this study, amplitude was determined as half the range between the minimum and maximum loads each cycle, with the upper limit being 250 N. The loss factor, η, was calculated using the relations $k^* = k(1 + i\eta)$ and $\eta = \frac{A_{loop}}{\pi FX}$, where k is the stiffness of the sample, F is applied force, X is displacement, and A_{loop} is the area of the hysteresis loop (Zainudin et al., 2017). The dynamic modulus was calculated based on Eq. 2b. The average value for dynamic modulus in the last 100 cycles is reported as E* in this study. Since viscoelastic materials are known reach steady state under dynamic loading, the last

100 cycles of the loading regimen was chosen to be representative of the dynamic modulus during steady state under dynamic loading. Elastic modulus per specimen was calculated based the stress-strain curve of the loading phase of the first cycle.

2.3. Statistical analysis

Based on a pre-hoc power analysis using one-way analysis of variance with α = 0.05 and β = 0.20, we determined that 14 tendons in each age group would be sufficient to detect statistically significant differences between groups. Due to the dearth of previous literature reporting the relationship between age and the biomechanical properties of PL tendons, we utilized an effect size of 0.51 in the calculations as reported in Swank et al. (Swank et al., 2015). A Kruskal-Wallis test was performed (α = 0.05) with the null hypothesis, "the medians of the groups are equal", being tested against the alternate hypothesis, "at least one group has a different median than at least one other group." Dunn's post hoc tests were then conducted to determine which groups were differing from the other groups. Specimens were grouped into three distinct groups based on donor age ($x < 40$, $40 \le x < 60$, $60 < x$). Regression analysis using first- and second-order polynomials was also performed to assess potential correlation between age and various material properties. Effect size was measured by Hedges' *g*.

3. Results

3.1. Characterization of mechanical properties with age

Selected mechanical properties of tendons were determined through custom MATLAB (MathWorks, Natick, MA, USA) functions (Table I). One tendon was excluded from the analysis because it pulled out the jaw before the cyclic loading regimen was completed. All other tendons completed 1000 loading cycles without rupture (Fig. 2).

As data was determined to be nonparametric, statistical analysis was performed using a Kruskal-Wallis test in conjunction with a Dunn's post hoc test. Between the three groups, there was no statistically significant difference in the calculated E ($P = 0.17$), or |E*| ($P = 0.0676$) (Fig. 3A, B). The lack of statistically significant differences implies that the mechanical properties of a PL tendon do not change with age. Material properties were also compared with gender using a Wilcoxon rank-sum test, which also showed no statistically significant differences in |E*| ($P = 0.288$). Regression analysis was performed on the material properties with respect to age. Both first- and second-order polynomials were fitted against scatter plots of a given material property versus donor age (Fig. 3C, D). Analysis showed a very weak to no correlation with age indicating no appreciable relationship between a given material property and age.

3.2. Stiffness and age

Interestingly, despite a lack of change in mechanical properties, stiffness measurements appeared to significantly decrease with age ($P = 0.030$, $g = 1.07$). A Dunn's post hoc test revealed that tendons from the youngest group (< 40 years of age) were significantly stiffer than the oldest group (Fig. 4A). Furthermore, regression analysis detected a moderate correlation between age and stiffness ($R^2 = 0.11$; $P = 0.044$) (Fig. 4B). The effect size between the youngest and oldest group is considered to be large (Durlak, 2009).

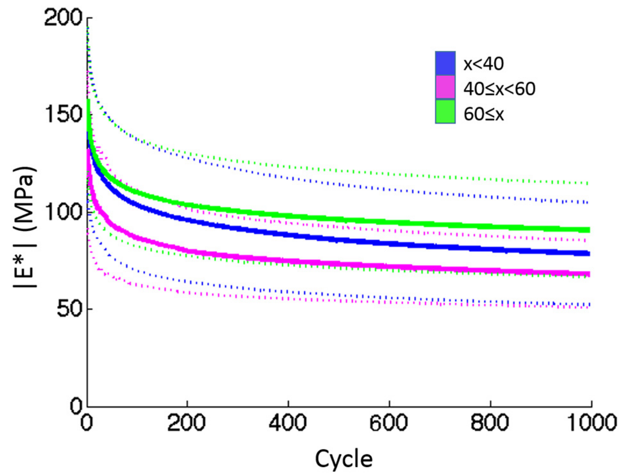

Fig. 2. Dynamic modulus magnitude calculated from secant modulus per cycle. Solid lines represent mean of each group, and the dotted lines represent one standard deviation above or below the mean. There are no statistically significant differences between groups for any cycle number.

4. Discussion

4.1. Mechanical characterization of PL tendons

The results of this investigation suggest that there is no appreciable change between the real component of E* and |E*|. This is because |E*| is the resultant vector of E*, and the imaginary component of E* is sufficiently small for the difference between the real component of E* and |E*| to be obscured by rounding errors. This is most likely due to the strain rate sensitivity of tendons. At lower strain rates, tendons absorb more energy, behaving less like a Hookean spring and more like a viscous dashpot. Conversely, tendons are better able to transmit forces from muscle to bone at higher strain rates, dissipating less energy compared to lower rates (Wang, 2006; Wang et al., 2012). In this study, loss from tendons at any given cycle could be considered negligible, and the elastic behavior of the tendon is the main contributing factor of the dynamic modulus magnitude. Loss was considered negligible due to the useful small angle approximation. Since the difference between |E*| and the recovery modulus are obscured by computer rounding errors, we can infer that $\cos\theta \approx 1$, and therefore |E*| and E′ are approximately equal. However, despite the loss factor being negligible per given cycle, dynamic modulus decreased per cycle. Here we see an example of the tendon stabilizing into a steady state. In a tendon, stress-relaxation

Table I
Mechanical properties of peroneus longus tendons grouped by age.

	Age < 40	Age 40 < 60	Age > 60
E (MPa)	151.8 (SD 42.72)	131.6 (SD 43.21)	157.7 (SD 37.23)
|E*| (MPa)	78.93 (SD 26.41)	68.33 (SD 17.29)	90.95 (SD 24.06)
|E*| Cycle $n = 1000$ (MPa)	78.40 (SD 26.26)	67.93 (SD 17.21)	90.59 (SD 23.97)
Stiffness (N/mm)	73.58 (SD 10.26)	62.86 (SD 11.49)	61.46 (SD 11.49)
n	10	8	19

Means and Standard Deviations of selected mechanical properties for each age range. E represents the elastic modulus of each group. |E*| is the magnitude of the dynamic modulus (average of last 100 cycles). |E*| n = 100 represents the magnitude of the dynamic modulus calculated for loading cycle 1000, the final loading cycle in the testing protocol. Stiffness is also reported for each age range. The last row, n, is the number of specimens per group. Data is presented as mean (SD).

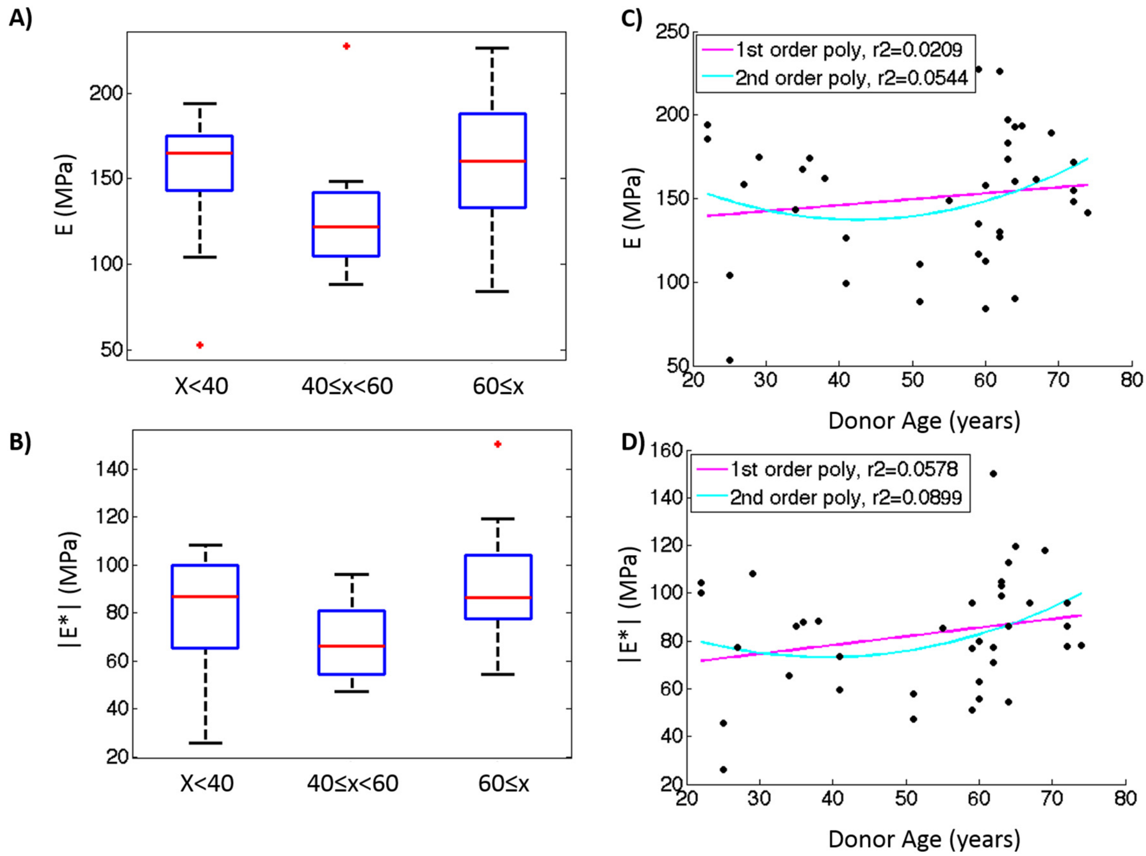

Fig. 3. Boxplots representing distributions of groups used during statistical analysis for initial elastic modulus, E (A) and dynamic modulus magnitude, |E*| (B). There was no statistically significant difference between groups for either E or |E*|. Regression analysis of elastic modulus (C) (P = 0.392 for the first-order poly and P = 0.165 for the second-order poly) and Dynamic modulus magnitude, |E*| (D) (P = 0.152 for the first-order poly and P = 0.071 for the second-order poly) with respect to donor age. No statistically significant correlation was found between these mechanical properties and age.

curves tend to look exponential: a rapid transient decrease in applied stress during a strain-controlled test before stabilizing towards a value lower than the first cycle measurement (Shepherd and Screen, 2013). Despite reaching a steady state, it has been previously shown that fatigue damage is already present (Shepherd and Screen, 2013).

4.2. Effect of age on material properties

Our findings, showing no significant change in material properties with age, agree with various studies performed on other tendons. To the best of our knowledge, this is the first study that compares the material properties of the PL tendon with age. Different tendons may respond differently with aging. Bone-patellar tendon-bone allografts studied by Blevins et al. showed a significant but weak correlation between elastic modulus and age (Blevins et al., 1994). Meanwhile, Greaves et al. showed that tibialis allografts did not have any significant correlation with age (Greaves et al., 2008). Swank et al. also concluded that posterior tibialis allografts did not show any significant changes when correlating mechanical properties with age (Swank et al., 2015). Here, we show by both linear regression and hypothesis testing that PL allografts do not show any significant changes in mechanical properties with age. Therefore, age should not play a factor based only on material properties in the allograft selection process when using PL tendons.

4.3. Separating material properties from geometry

It is important to note that the characterization of PL tendons is focused on intrinsic material properties instead of extrinsic properties as it allows for a more accurate comparison of the tendon's mechanical properties. Intrinsic material properties allow for a description of the material itself as opposed to a combination of the material and external factors. For example, elastic modulus (E) is a better indicator of a material's resistance to deformation than stiffness, k. Although elastic modulus and stiffness are proportional, it is described by Eq. 3c:

$$(a)\,\sigma = E\varepsilon = \frac{F}{A}\;(b)\,k = \frac{F}{\delta}\;(c)\,k = \frac{AE}{L} \tag{3}$$

where k is stiffness, E is Elastic modulus, A is the cross-sectional area of the specimen, σ is the stress in the member, ε is the strain in the member, δ is the displacement of a member, and L is the gage length. Stiffness is dependent on not only the material properties but also the cross-sectional area and gage length. Different stiffness measurements between specimens could be due to either different elastic modulus or differing geometries.

Furthermore, by using intrinsic material properties to describe a tendon, more accurate comparisons can be made between studies and research groups. Although studies that report geometry-dependent readings (i.e., stiffness and force) establish meaningful conclusions based on their measurements, comparing new stiffness measurements to published ones becomes difficult. For example, the average stiffness measurement for this study was 65.04 (SD 12.09) N/mm. Values recorded here were significantly lower than those published by Aguila et al.'s nonirradiated PL tendons (216.1 (SD 59.0) N/mm) (Aguila et al., 2016). However, Aguila et al.'s tendons had an average gage length and diameter of 4 cm and 7.7 mm, respectively. An average tendon in this study was 6.6 mm in diameter and had a gage length of 7.5 cm. Calculating elastic modulus from stiffness using these values, Aguila et al.'s tendons had an elastic modulus of 187.26 MPa which is within one standard deviation of the tests in the study. It would be difficult to determine if there is a statistically significant difference between these

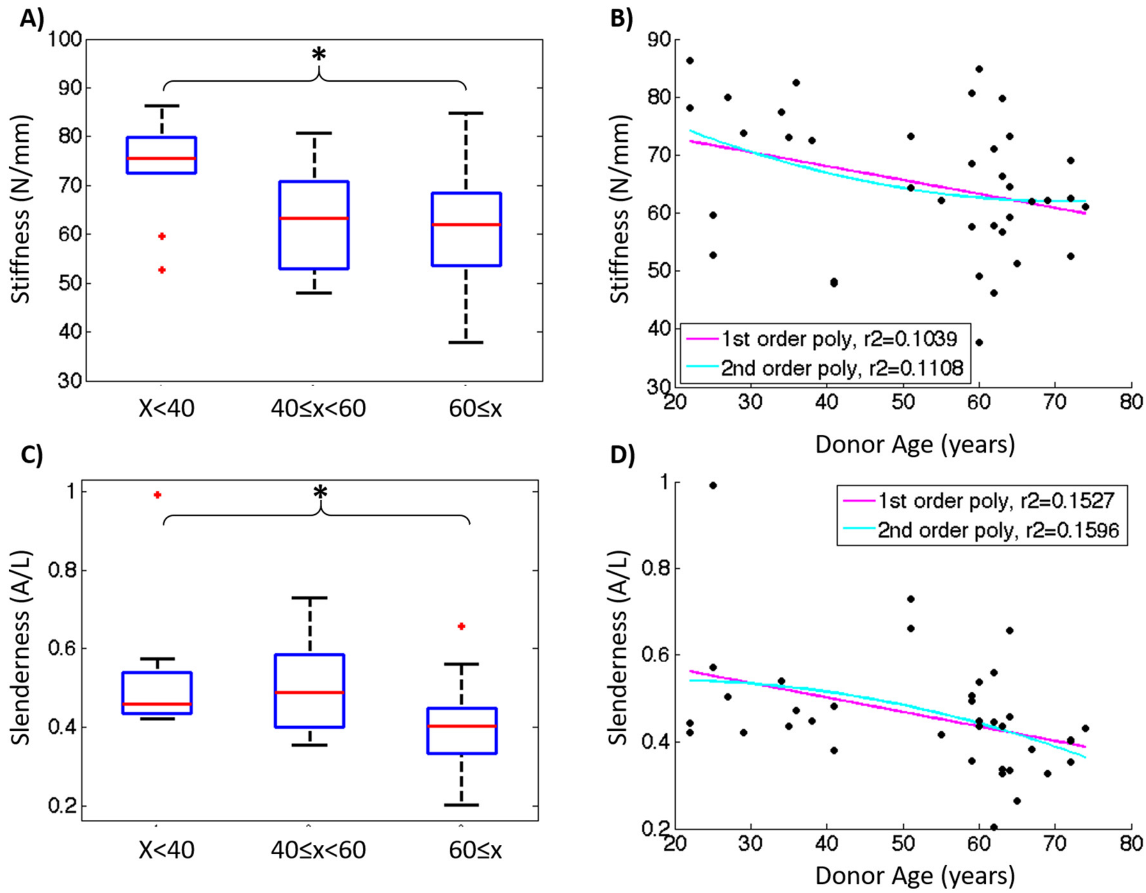

Fig. 4. A) Boxplots representing distributions of groups used during analysis of stiffness. * denotes statistically significant changes between groups. B) Regression analysis of stiffness with respect to age. A first-order polynomial regression showed no statistically significant correlation (P = 0.052); however, a second-order polynomial regression revealed a statistically significant moderate correlation between the age of the allograft donor and stiffness (P = 0.044). C) Boxplots representing distribution of groups used during analysis of slenderness, where * again denotes statistically significant changes between groups. D) Regression analysis of slenderness with respect to donor age. Both a first- and second-order polynomial regression demonstrated statistically significant moderate correlations (P = 0.017 and 0.014), respectively between the age of the allograft donor and slenderness.

material properties and the ones reported by Aguila, but by focusing on properties that are independent of geometries, comparing data between groups becomes much more feasible.

4.4. Stiffness, slenderness, and age

In this study, the material properties that contribute to stiffness do not change with age yet stiffness itself appears to decrease with age (Fig. 4A, B). Interestingly, since stiffness is proportional to elastic modulus, one would expect these two metrics to have similar trends. However, since stiffness is also proportional to the cross-sectional area and the gage length, other factors besides elastic modulus influence stiffness. We can replace A/L in Eq. 3c with some variable, S. Here, S, the ratio of area to length, represents the "slenderness" of the test specimen. With this simple substitution, it becomes clear that the stiffness is proportional to both the elastic modulus and slenderness of the specimen.

There is a statistically significant difference when comparing our slenderness metric with the different age groups with a large demonstrated effect size ($P = 0.0370$, $g = 0.9340$) (Fig. 4C). A Conover-Iman post hoc test showed that the median stiffness of tendons from the oldest group has a statistically significant difference when compared to those from the youngest or the middle-aged groups. Furthermore, there is a moderate correlation between age and our metric for slenderness, S ($R^2 = 0.16$, $P = 0.014$) (Fig. 4D). When using the two-strand technique (Vinagre et al., 2017) simulated in this study, it appears that prepared PL tendons from older donors are more slender than their counterparts from younger donors.

4.5. Clinical significance

Differences in stiffness between the oldest age group (> 60 years old) and the youngest age group (< 40 years old) of donor tendons was attributed to increased slenderness in the older donor tendons. From a clinical standpoint, since peroneus longus allografts from older tendon donors would be expected to be more slender, this could manifest as a potential decrease in the diameter of the graft over time. Conte et al. stated that quadrupled-strand hamstring autografts with a diameter equal to or larger than 8 mm had significantly lower rates of graft failure (Conte et al., 2014). Additionally, Magnussen et al. argued that ACL reconstructions with hamstring autografts of smaller diameter were more likely to require early revision procedures (Magnussen et al., 2012). Snaebjornsson et al. echoed this sentiment, finding an inverse correlation between hamstring tendon autograft diameter and the likelihood of revision surgery (Snaebjörnsson et al., 2017). Moreover, Mariscalco et al. reported that smaller hamstring autograft diameters served as a predictor of worse Knee Injury and Osteoarthritis Outcome Scores (KOOS) in patients 2 years post-operatively (Mariscalco et al., 2013). Although the exact cut-off for diameter associated with poor outcomes is debated in these publications, there is still substantial evidence that smaller graft diameters in ACL reconstruction lead to poorer outcomes across a number of clinical measures. These results are applicable to the present study's findings regarding age-associated geometric changes in peroneus longus tendons. Increased slenderness in tendons from donors over 60 years old could lead to decreases in diameter of the overall graft. This potential reduction in diameter, therefore, may heighten patients' risk of the adverse outcomes mentioned above. These changes in slenderness can be avoided by choosing a peroneus longus tendon from a younger donor if possible. If a peroneus longus from an older donor (over 60) is used, care should be taken by the surgical team when preparing the graft to maximize diameter in order to offset any potential changes in slenderness of the graft.

4.6. Limitations

In this investigation, engineering stress and strain were used instead of true stress and strain for the basis of our modulus calculations. Engineering stress and engineering strain are based on the initial unloaded cross-sectional area and length measurements whereas true stress and true strain are based on the instantaneous cross-sectional area and length. Although engineering stress and strain are overwhelmingly used in material science applications, true stress and true strain would be a more accurate metric of internal stresses and strains of a specimen. A related limitation of this study is that no extensometer was used to measure changes in gage length, and digital imaging correlation was not implemented to determine cross-sectional area during the tensile test. Because the PL tendons were deidentified, there was no data on donor height, weight, or comorbidities that could have affected a specimen's properties. Lastly, although care was taken to prevent specimen slippage (i.e., stabilizing the non-looped end with k-wire and bone cement), the slippage of the tendon between the grips was not monitored. There was one instance of a tendon slipping free from the grip, which was subsequently omitted from the analysis of results.

5. Conclusion

PL tendons were subjected to a cyclic loading regimen to interrogate their material properties. Here, we show that neither age nor gender influenced said material properties; however, older PL tendon grafts become less stiff secondary to geometrical changes (increased slenderness) with significant effect. To our knowledge, this is the first study to assess the effect of age on the mechanical and geometric properties of these particular tendons. Furthermore, we show that an allograft prepared using PL tendons becomes more slender with increasing age, as PL tendons become more slender with age. There is a higher risk of reduced stiffness when older PL allografts are prepared with the two-strand technique. Thus, when preparing an allograft, the surgeon may want to consider the age of the donor as a factor. Lastly, we highlight the importance and advantages of using intrinsic material properties to characterize material properties of tendons.

Conflict of interest disclosure

All authors have no Conflicts of Interest to disclose.

Acknowledgements

We would like to thank Pinnacle Transplant Technologies for generously donating the peroneus longus allografts. We would like to thank Pooja Swami, M.S., and Haixiang Liang, M.D., for helping proofread and edit this manuscript.

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