The Biomechanical, Biochemical, and Morphological Properties of 19 Human Cadaveric Lower Limb Tendons and Ligaments

An Open-Access Data Set

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Background: Methodological heterogeneity hinders data comparisons across isolated studies of tendon and ligament properties, limiting clinical understanding and affecting the development and evaluation of replacement materials.

Purpose: To create an open-access data set on the morphological, biomechanical, and biochemical properties of clinically important tendons and ligaments of the lower limb, using consistent methodologies, to enable direct tendon/ligament comparisons.

Study Design: Descriptive laboratory study.

Methods: Nineteen distinct lower limb tendons and ligaments were retrieved from 8 fresh-frozen human cadavers (5 male, 3 female; aged 49-65 years) including Achilles, tibialis posterior, tibialis anterior, fibularis (peroneus) longus, fibularis (peroneus) brevis, flexor hallucis longus, extensor hallucis longus, plantaris, flexor digitorum longus, quadriceps, patellar, semitendinosus, and gracilis tendons; anterior cruciate, posterior cruciate, medial collateral, and lateral collateral ligaments; and 10 mm–wide grafts from the contralateral quadriceps and patellar tendons. Outcomes included morphology (tissue length, ultrasound-quantified cross-sectional area [CSA_{US}], and major and minor axes), biomechanics (failure load, ultimate tensile strength [UTS], failure strain, and elastic modulus), and biochemistry (sulfated glycosaminoglycan [sGAG] and hydroxyproline contents). Tissue differences were analyzed using mixed-model regression.

Results: There was a range of similarities and differences between tendons and ligaments across outcomes. A key finding relating to potential graft tissue suitability was the comparable failure loads, UTS, CSA_{US}, sGAG, and hydroxyproline present between hamstring tendons (a standard graft source) and 5 tendons not typically used for grafting: fibularis (peroneus) longus and brevis, flexor and extensor hallucis longus, and flexor digitorum longus tendons.

Conclusion: This study of lower limb tendons and ligaments has enabled direct comparison of morphological, biomechanical, and biochemical human tissue properties—key factors in the selection of suitable graft tissues. This analysis has identified 6 potential new donor tissues with properties comparable to currently used grafts.

Clinical Relevance: This extensive data set reduces the need to utilize data from incompatible sources, which may aid surgical decisions (eg, evidence to expand the range of tendons considered suitable for use as grafts) and may provide congruent design inputs for new biomaterials and computational models. The complete data set has been provided to facilitate further investigations, with the capacity to expand the resource to include additional outcomes and tissues.

Keywords: autograft; allograft; reconstruction; anterior cruciate ligament (ACL); tissue mechanics

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Tendon and ligament injuries are a common and significant problem, accounting for approximately half of the 61 million musculoskeletal injuries reported each year in the United States alone.[42] The incidence of tendon, ligament, and joint reconstructions is increasing,[45] driven

largely by increased sports participation, an aging population, and the emergence of new surgical techniques.

There are numerous studies on human tendon and ligament properties relevant to their utility as grafts; however, the research focus is generally narrow and limited to a single modality of assessment, on 1 or a limited number of different tissues. The majority of laboratory-based studies characterize the biomechanical properties of a single clinically relevant tissue, one that is either commonly injured[8,44] or used as a graft,[26] or they compare 2 different graft options.[26] Therefore, the body of literature is heavily weighted toward the most common tissues involved in major surgical reconstructions, and there is a tendency to focus more on the grafting tissues rather than the injured native tissue being replaced or augmented. The focus on graft properties versus native tissue is exemplified by the greater number of biomechanical studies on the patellar, hamstring, and quadriceps tendons[26] relative to the ACL.[27] Furthermore, meaningful comparisons of the characteristics of tendons and ligaments across different studies are confounded by the use of different methods, which can affect the results and compromise the validity of direct comparisons. Cross-study comparisons are particularly challenging for biomechanical properties, as reported measurements are highly sensitive to testing methodologies, such as the technique used to measure cross-sectional area (CSA),[20,30] the presence or absence of preconditioning[38] and the specific preconditioning parameters used,[10] strain rate,[23] and analysis methods (eg, calculating elastic modulus at a defined force[38] or strain,[29] from the "linear" region,[34,36] from the maximum gradient,[7,28] or not reported[5,43]). Unfortunately, it is not uncommon for studies to omit details that can have significant effects on outcomes, and typically, only studies performed by the same research laboratory report comparable methods including similar storage and handling conditions,[9] measurement equipment, testing parameters, and analyses. A single data set with measurements of a wide range of tendons and ligaments from the same tissue donors, using consistent methodologies, would be a valuable resource for surgeons and tissue banks to facilitate direct comparisons between graft options, explore new indications for existing tendon grafts, and expand the range of tendons as allograft candidates. Such a data set would also be an important resource for computer modeling and tissue engineering by defining tissue properties for a range of samples and providing design targets for natural and synthetic engineered grafts to be better tuned or matched to recipient tissues.

The present study aimed to characterize the morphological, biomechanical, and biochemical properties of 19 human tendons and ligaments of the lower limb. It is the first installment in the creation of a larger comprehensive open-access repository (linked at https://dataverse.harvard.edu/dataverse/human_musculoskeletal_tissue_datasets#). The repository will include a wider range of properties and additional musculoskeletal tissues from both the upper and the lower limbs, with a focus on tissues commonly injured, used as grafts for reconstruction, or having the potential to be used as grafts. All tissues were sourced from the same donors and tested using consistent methodologies across outcomes. In this study of baseline ex vivo tissue properties, comparisons between all 19 tissues were conducted, although the results and discussion are primarily discussed in the context of grafts relevant to the reconstruction of the ACL. The statistical analyses for all comparisons are included in the Appendices (available in the online version of this article) so that readers may review other comparisons of interest (eg, similarities and differences relative to the MCL). Appendix 1 provides supplementary information, summarized data values, and summarized statistics that directly support the study. Appendix 2 provides the complete adjusted and unadjusted statistical outputs. Appendix 3 (available online; https://doi.org/10.7910/DVN/XUTODT) provides the complete data set.

METHODS

In this study, all measurements are reported to the precision allowed by each instrument's resolution or are rounded to 2 decimal places when the resolution was >0.01 of the relevant unit. For example, the "free" length of each tissue measured using a ruler (1-mm resolution) is reported rounded to the nearest 1 mm.

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TABLE 1
List of Tissue Names and Abbreviations

Tissue	Abbreviation
Ankle tendons	
Achilles tendon	AT
Tibialis posterior	TP
Tibialis anterior	TA
Fibularis/peroneus longus	FL
Fibularis/peroneus brevis	FB
Flexor hallucis longus	FHL
Extensor hallucis longus	EHL
Plantaris	Plt
Flexor digitorum longus	FDL
Knee tendons	
Semitendinosus tendon	ST
Gracilis tendon	GT
Quadriceps tendon (intact)	QT
Quadriceps tendon (middle 10 mm)	mQT
Patellar tendon (intact)	PT
Patellar tendon (middle 10 mm)	mPT
Knee ligaments	
Anterior cruciate ligament	ACL
Posterior cruciate ligament	PCL
Medial collateral ligament	MCL
Lateral collateral ligament	LCL

Tissue Acquisition

Paired left and right lower limbs (midfemur to toe-tip) were sourced fresh-frozen (stored at –20°C) from 8 human donors (5 male, 3 female; age, 49-65 years) with no known history of musculoskeletal injuries or conditions. Donor information is summarized in Appendix 1 Table A1.1 (available online). All cadaveric tissue was sourced from Science Care Inc with ethics approval granted by the Northern Sydney Local Health District Human Research Ethics Committee.

Specimen Retrieval

Lower limbs were thawed at room temperature for 36 hours. Seventeen anatomically distinct tendons and ligaments were retrieved from both limbs of each donor (see Table 1 for a list of tissue names and abbreviations). All specimens were retrieved by an orthopaedic surgeon (P.H.) in the same sequence to facilitate accurate identification, labeling, and safe removal (full dissection protocol in Appendix 1 Methods, available online). One left or right limb per donor was randomly allocated to the outcomes included in this study. Any specimens that were damaged during retrieval were replaced by the same tendon in the contralateral limb. For the quadriceps and patellar tendons, both left and right tendons were included in this study: one intact sample as per all other tissues (QT and PT) and one 10 mm–wide graft sample dissected from the middle of the respective contralateral tendon (designated mQT and mPT), for a total of 19 tissue groups (Table 1). After removal, all tissues were wrapped in saline-soaked gauze, sealed in double ziplocked plastic bags, and stored at –20°C. All specimens were labeled with donor number, limb side, and tissue name.

Specimen Preparation

Tendons and ligaments were thawed at room temperature for 60 to 75 minutes. Muscle, fat, and loose connective tissue were meticulously removed with a scalpel, and bone blocks were removed from the relevant specimens at the point of insertion to ensure that the measured mechanical properties would not be confounded by test failures occurring at the enthesis, or by failed mechanical potting; the implications of this are included in the discussion. For tendons, the distal aspect of the aponeurosis was marked with picrosirius red dye, and this was used to define the proximal boundary of the "free" tendon. For ligaments, the free length defined the total length of the isolated ligament (ie, after bone block removal). The transverse centerline of each free tendon and ligament was identified and marked with red dye. The gauge length for mechanical testing was marked by 2 lines equidistant from the centerline. The target gauge length was set at 50 mm except for the shorter QT, mQT, PT, mPT, and knee ligaments, which had reduced gauge lengths defined by the shortest sample for each tendon or ligament (see Table A1.3 for tissue-specific target gauge lengths).

Morphology Measurements

The free length of each tendon and ligament was recorded with a ruler. For the tendons with a gauge length of $\geq$50 mm, the specimens were submerged in a saline bath and transverse ultrasound images were captured at 10-mm increments along the gauge region with a linear array transducer (Lumify L12-4; Phillips Healthcare) using the "Superficial" setting (mechanical index, 0.9; frame rate, 24 Hz; gain, 54; depth, 35 mm; power, –0.3 dB). For tendons and ligaments with a target gauge <50 mm, a single image was taken at the center of the gauge region. The same operator (D.M.A.) collected all images.

A semiautomatic image analysis macro was developed using ImageJ (Version 1.53t; National Institutes of Health) to identify the tissue border on each image and perform cross-sectional measurements. The tissue border was outlined using a default minimum threshold of 80 and a mask created for the region of interest (ROI) that met specific particle parameters (area, >0.8 pixel^2; circularity, >0.4). The ROI was overlaid onto the original image to visually confirm the specimen had been identified correctly. If the specimen was not appropriately identified, the operator adjusted the default threshold and circularity values until a suitable region was selected (ranges: threshold, 60-110; circularity, 0.1-0.4). Five specimens not selected correctly using the semiautomated method were manually outlined using the hand tracing tool. Measurements were calibrated using the scale bar included in each ultrasound image. The ROI was analyzed using inbuilt ImageJ measurement tools to measure (1) CSA, (where CSA measured in this manner from utrasound is referred to as CSA_{US}); and (2) the major and minor axis lengths, which were determined from an ellipse fitted using the least squares method with respect to area. For

the purpose of comparing analogous regions between tissues, only measurements from the image taken at the center of the free tendon or ligament are reported in the results of this paper. After ultrasound imaging, CSA was measured again at the narrowest region within the gauge length using a contact-based protocol.[11] Briefly, the tendon was placed under a glass slide attached to a rachet micrometer. The slide was lowered until the torque limit was reached and the height of the specimen was read from the micrometer. The specimen was photographed through the glass slide and the width was measured using ImageJ. CSA was calculated by assuming a rectangular cross section and multiplying the measured height and width and is referred to as CSA_M.

Biomechanical Testing

Specimens were gripped in custom cryogenic clamps, cooled with dry ice, and attached to a servohydraulic testing machine (Instron 8874; Instron Corp); this is a typical clamping method for high-strength soft tissues,[22] which we used for all specimens to standardize the fixation across the data set. Specimens were first secured in the top clamp (using the dye line to align the clamps at the desired gauge length), before being lowered and fastened in the bottom clamp, ensuring the specimen was slack when tightening the grips. Specimens were loaded without preconditioning under uniaxial tension to failure at 5% strain/s. Throughout the test, force was measured at 100 Hz using a 25-kN load cell (Instron Dynacell 2527-201; Instron Corp), and crosshead displacement was measured at 100 Hz (Instron 8874). Mode of failure was determined visually with the aid of the red dye lines and was recorded as midsubstance, edge of clamps, within clamps, or grip slip (data exclusion based on mode of failure described in Appendix 1 Results, available online).

Maximum load at failure was defined as the maximum measured force. The working gauge length was defined as the distance between clamps when a force threshold of 4 N was reached. Displacement data were converted to strain by dividing the displacement by the working gauge length. Force data were converted to stress by dividing the force by the CSA_M. A custom MATLAB program (Version 9.4; The MathWorks Inc) generated stress-strain curves for each specimen to determine ultimate tensile strength (UTS; stress at maximum measured force) and failure strain (strain at maximum measured force). Elastic modulus was determined as the maximum slope over a 2% strain range, as calculated from moving linear regressions fitted across 2% strain on overlapping increments of 0.05% strain (ie, sequential data points). The regression line corresponding to the maximum calculated elastic modulus was plotted on the stress-strain curve to visually confirm it was calculated within what would subjectively be classified as the "linear" region.

Biochemical Analysis

After mechanical testing, approximately 50 to 100 mg of tissue (wet weight) was collected from the midregion of the gauge length and stored at –80°C for compositional analyses using microtiter plate adaptations of published methods.[6,14,40] The samples were dried under vacuum for 3 hours at room temperature, and the dry weight was recorded before papain digestion. The sulfated glycosaminoglycan (sGAG) content was measured as an indication of proteoglycan content using the 1,9-dimethylmethylene blue assay[6,14] and absorbance measured at 650 nm. Aliquots of the digest (100 μL) were tested in duplicate, and chondroitin sulfate from bovine trachea (Sigma-Aldrich) was used as the standard. The total hydroxyproline content was measured as an indication of collagen content using the *p*-dimethylaminobenzaldehyde assay[40] and absorbance measured at 562 nm. Aliquots of the digest (200 μL) were vacuum dried, hydrolyzed, and neutralized before 50 μL of the hydrolysate was tested in duplicate with *trans*-4-hydroxy-L-proline (Sigma-Aldrich) as the standard. The sGAG and hydroxyproline content were expressed as micrograms per milligram of tissue dry weight.

Statistical Analysis

An a priori power analysis (G*Power 3.1; Heinrich Heine Universität)[15] of the linear regression models used to compare all tissue groups determined that a total sample size of 90 was required to detect a 2-tailed moderate effect size ($f^2 = 0.15$)[12] with 80% power and an α of .05; the current study included a total sample size of 152. The same model variables were used for all outcomes. All other statistical analyses were performed using Stata (Version 13.1; StataCorp). All outcomes were analyzed using mixed-effects linear regression including donor age, sex, height, and weight as covariates. A random intercept term was included to account for the nonindependence of tendons retrieved from the same donor. Pairwise comparisons between all tendons and ligaments (19 groups, 171 comparisons) (Table 1) were adjusted using the Šidák correction.

Pearson correlation coefficients were generated to determine associations between outcomes of interest, primarily biomechanical versus biochemical outcomes. A Benjamini-Hochberg correction with a false discovery rate of 0.05 was applied to adjust for multiple comparisons. Statistical significance was defined as a *P* value smaller than the corresponding Benjamini-Hochberg critical value.

A summary of statistical comparisons between all tissues (β coefficient; significance (y/n) after adjustment) is provided in Appendix 1 Table A1.4 (available online). While this study reports adjusted *P* values, complete adjusted and unadjusted statistical outputs (β coefficient; *P* value; 95% CI) are provided in Appendix 2 (available online).

Additional Outcomes Included in the Data Set

Additional morphological, biomechanical, and biochemical outcomes not reported in this study are described in Appendix 1 Methods and are provided in the data set (Appendix 3, available online; https://doi.org/10.7910/DVN/XUTODT).

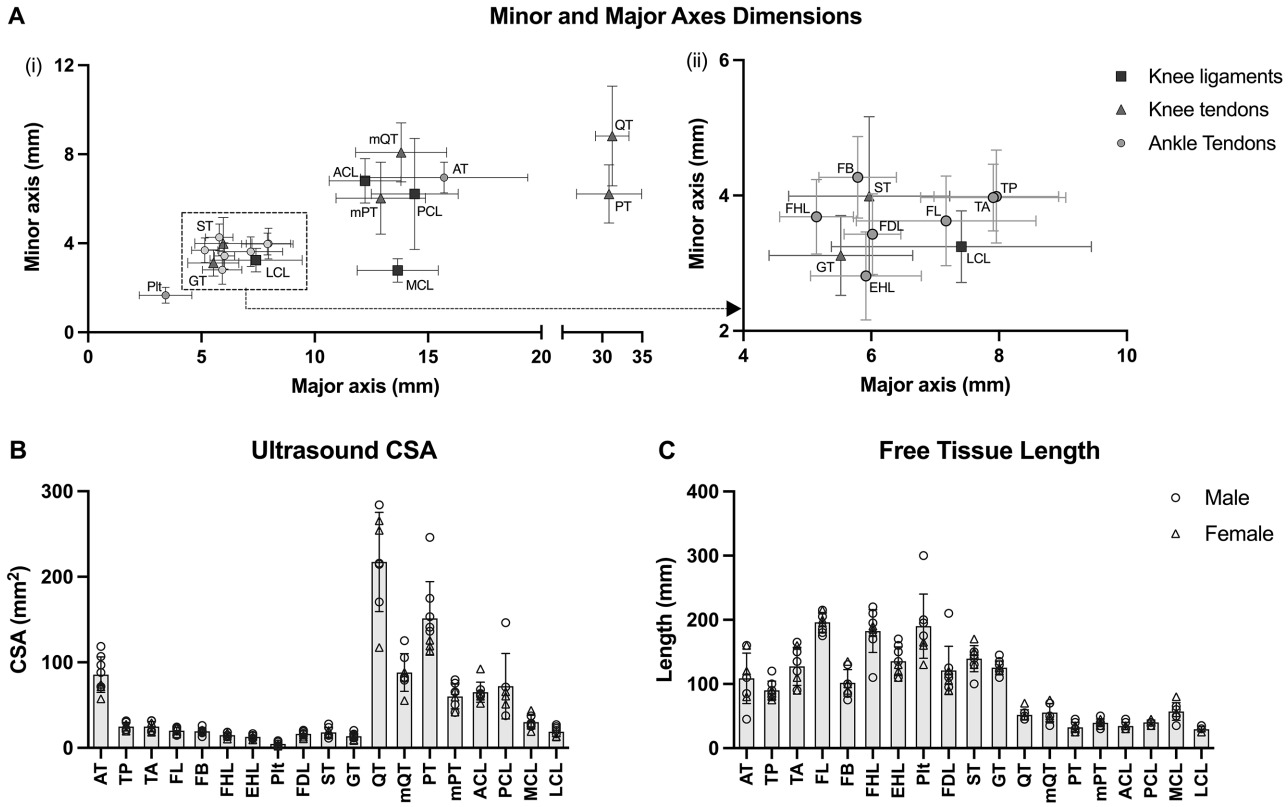

Figure 1. Morphological characterization of human tendons and ligaments of the lower limb presented as mean ± SD. In panels A and B, measurements were from transverse ultrasound images at the center of the free tissue length. (A) Minor and major axis dimensions: (i) all tissues and (ii) scaled axes to distinguish the overlapping cluster of tissues highlighted by the box in i. (B) Cross-sectional area (CSA). (C) Free tissue length. Measurements in panel A are categorized as knee ligaments (solid square), knee tendons (triangle), and ankle tendons (circle). Measurements in panels B and C show individual data points categorized by donor sex (circles for male, triangles for female). Significant differences between tissues are not annotated in the figure given the large number and are instead summarized in Appendix 1 Table A1.4, with complete statistical outputs provided in Appendix 2 (available online).

RESULTS

All 19 tissue specimens were present and retrieved from each of the 8 donors. Data were excluded across a number of tissues and outcomes for various reasons, as detailed in Appendix 1 Results—Sample Exclusions (available online). The final sample sizes per tissue and outcome (n = 3-8) are specified alongside the result summaries (mean ± SD) in Appendix 1 Tables A1.2 and A1.3 (available online). Of note, all tissues that were tested with a reduced gauge length <50 mm (QT, mQT, PT, mPT, ACL, PCL, LCL, and MCL) were excluded from strain-based analyses as the working gauge length could not be confidently determined (see Discussion). The complete data set is available in Appendix 3 (available online).

Morphology

On average, the minor and major dimensions as measured from ultrasound imaging ranged from 1.67 and 3.42 mm (Plt) to 8.82 and 31.26 mm (QT) (Figure 1A; Appendix 1 Table A1.2, available online). Correspondingly, the mean CSA_{US} ranged from 4.58 mm^2 (Plt) to 217.48 mm^2 (QT) (Figure 1B). Many of the tissues had similar midlength cross sections with no significant differences in minor and major axial lengths, or CSA_{US}, observed between the tissues displayed in Figure 1A(ii): TP, TA, FL, FB, FHL, EHL, FDL, ST, and GT (Appendix 1 Table A1.4, available online). No differences were observed in minor/major dimensions and CSA_{US} between the cruciates (ACL and PCL) or between the cruciates and the mQT and mPT grafts, with the single exception of the minor dimension of the PCL, which was significantly smaller than the mQT ($\beta = -1.94$ mm; $P = .0107$). The minor axis dimension was significantly larger for mQT than mPT ($\beta = 2.34$ mm; $P < .0001$), reflecting the dimensional differences observed between the intact QT and PT.

On average, the free tissue length ranged from 29 mm (LCL) to 196 mm (FL). All ankle tendons (AT, TP, TA, FL, FB, FHL, EHL, Plt, and FDL) and the hamstring tendons (ST and GT) were significantly longer than all 4 knee ligaments (ACL, PCL, MCL, and LCL), with the exception of TP versus MCL (Appendix 1 Table A1.4, available online). No significant differences were observed when

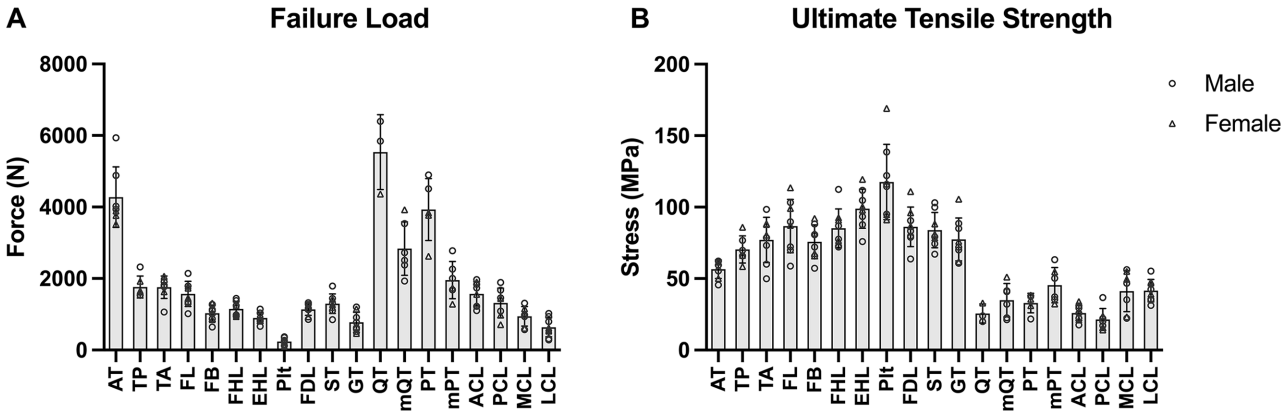

Figure 2. Biomechanical properties of human tendons and ligaments of the lower limb: (A) failure load and (B) ultimate tensile strength, presented as mean ± SD. Measurements show individual data points categorized by donor sex (circles for male, triangles for female). Significant differences between tissues are not annotated in the figure given the large number and are instead summarized in Appendix 1 Table A1.4, with complete statistical outputs provided in full in Appendix 2 (available online).

comparing the remaining knee tendons (QT, mQT, PT, and mPT) with all 4 knee ligaments. On average, the total specimen length for tendons was 51% longer than the free tendon length (Appendix 2 Table A2.1, available online).

Biomechanics

On average, the failure loads ranged from 236.48 N (Plt) to 5533.74 N (QT) (Figure 2A; Appendix 1 Table A1.3, available online). The ACL had a significantly lower failure load compared with AT (β = −2729.95 N; $P < .0001$), QT (β = −3883.41 N; $P < .0001$), PT (β = −2356.91 N; $P < .0001$), and mQT (β = −1213.41 N; $P < .0001$); and a significantly higher failure load compared with EHL (β = 677.66 N; $P = .0322$), Plt (β = 1335.70 N; $P < .0001$), GT (β = 792.68 N; $P = .0022$), and LCL (β = 937.45 N; $P = .0001$) (Appendix 1 Table A1.4, available online).

On average, UTS ranged from 21.39 MPa (PCL) to 117.63 MPa (Plt) (Figure 2B). The ACL had a significantly lower UTS ($P < .0001$ unless otherwise stated) compared with all ankle tendons and the hamstring tendons, including AT (β = −29.49 MPa; $P = .0002$), TP (β = −42.93 MPa), TA (β = −51.15 MPa), FL (β = −60.72 MPa), FB (β = −49.68 MPa), FHL (β = −59.23 MPa), EHL (β = −72.88 MPa), Plt (β = −91.64 MPa), FDL (β = −60.22 MPa), ST (β = −57.94 MPa), and GT (β = −51.48 MPa) (Appendix 1 Table A1.4, available online).

On average, failure strain ranged from 14.15% (Plt) to 23.58% (AT) (Figure 3A). The AT had significantly higher failure strain compared with all other ankle and hamstring tendons (TP, TA, FL, FB, FHL, EHL, Plt, FDL, ST, and GT) (Appendix 1 Table A1.4, available online). The ST also had significantly higher failure strain compared with the FHL (β = 4.23%; $P = .0330$) and Plt (β = 4.54%; $P = .0127$) tendons. All other comparisons were not statistically significant after adjustment.

On average, the elastic modulus ranged from 374.46 MPa (AT) to 1007.05 MPa (Plt) (Figure 3B). Other than TP, the AT had a significantly lower modulus compared with all other ankle and hamstring tendons (TA, FL, FB, FHL, EHL, Plt, FDP, ST, and GT) (Appendix 1 Table A1.4, available online), whereas the Plt had a significantly higher modulus compared with all other ankle and hamstring tendons (AT, TP, TA, FL, FB, FHL, EHL, FDP, ST, and GT).

Biochemistry

On average, the hydroxyproline content ranged from 73.7 μg/mg dry tissue (QT) to 96.8 μg/mg dry tissue (PCL) (Figure 4A; Appendix 1 Table A1.2, available online). Hydroxyproline content was similar across all tissue groups, with the exception of the PCL, which had significantly higher hydroxyproline content when compared with AT (β = 18.5 μg/mg dry tissue; $P = .0069$), TA (β = 19.0 μg/mg dry tissue; $P = .0042$), and QT (β = 22.2 μg/mg dry tissue; $P = .0003$).

On average, mean sGAG content ranged from 1.0 μg/mg dry tissue (Plt) to 7.1 μg/mg dry tissue (PCL) (Figure 4B). Of 29 significant differences observed, 27 were between a tendon and a ligament and most involved comparisons with the ACL or PCL. For example, both the ACL and the PCL had significantly higher sGAG content when compared with all tendons except FB and the mQT. Of the exceptions, the content of Plt was significantly lower than that of all 4 knee ligaments, ACL (β = −5.5 μg/mg dry tissue; $P < .0001$), PCL (β = −6.0 μg/mg dry tissue; $P < .0001$), MCL (β = −3.2 μg/mg dry tissue; $P = .0291$), and LCL (β = −3.2 μg/mg dry tissue; $P = .0068$), as well as FB (β = −3.4 μg/mg dry tissue; $P = .0039$) (Appendix 1 Table A1.4). The PCL also had significantly higher sGAG content compared with the LCL (β = 2.8 μg/mg dry tissue; $P = .0343$).

Associations

As summarized in Table 2, sGAG content was negatively correlated with UTS ($r = −0.60$; $P < .0001$) and elastic

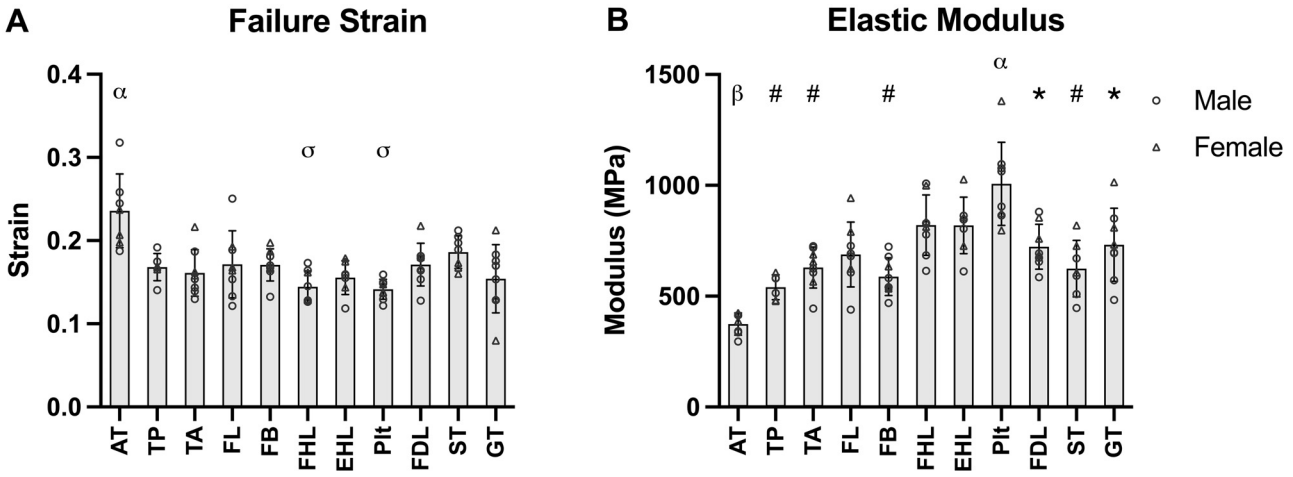

Figure 3. Biomechanical properties of human tendons of the lower limb: (A) failure strain and (B) elastic modulus, presented as mean ± SD. Measurements show individual data points categorized by donor sex (circles for male, triangles for female). Significant differences after the Šidák correction: (A) α = different from all and σ = different from ST; (B) α = different from all, β = different from all except TP, # different from FHL and EHL, and * = different from TP. All statistical outputs are summarized in Appendix 1 Table A1.4 and provided in full in Appendix 2 (available online).

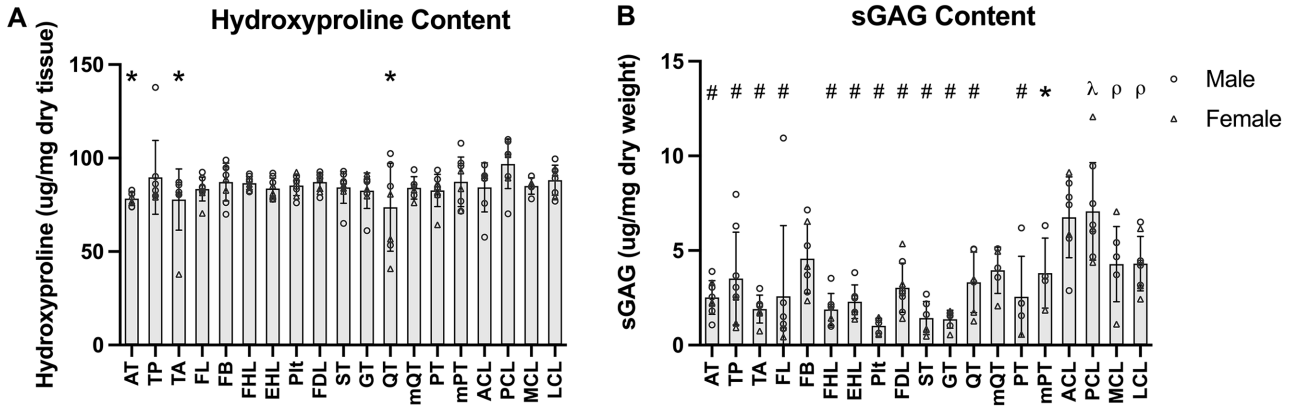

Figure 4. Biochemical properties of human tendons and ligaments of the lower limb: (A) hydroxyproline content and (B) sulfated glycosaminoglycan (sGAG) content, presented as mean ± SD. Measurements show individual data points categorized by donor sex (circles for male, triangles for female). Significant differences after Šidák correction: * = different from PCL, # = different from ACL and PCL, ρ = different from plantaris, and λ = different from LCL. All statistical outputs are summarized in Appendix 1 Table A1.4 and provided in full in Appendix 2 (available online).

modulus ($r = -0.30$; $P = .0381$) and positively correlated with hydroxyproline content ($r = 0.29$; $P = .0061$).

DISCUSSION

This study presents data on a large range of human tendons and ligaments of the lower limb, using consistent methodologies that enable direct comparisons of characteristics between a wide range of tendons and ligaments. Summarized data values are provided in Appendix 1, complete statistical comparisons for all 19 tissues are provided in Appendix 2, and the entire data set is available in Appendix 3 (available at https://doi.org/10.7910/DVN/XUTODT). All have been provided as resources for future investigations. As the project continues, additional outcomes and musculoskeletal tissues of the upper and lower limbs will be added to the data set. Because such a wide range of tissues were evaluated, not all comparisons can be discussed. In this paper, the focus was the ACL and graft options for ACL reconstruction to demonstrate the importance and value of this resource.

The ACL failure load (1572 N) and UTS (26 MPa) in this study lie within the range of previously published values for human ACL (Figure 5),[8,23,34,41,44] although given the high variability in reported values, this is not surprising. Mechanical testing results are highly sensitive to testing

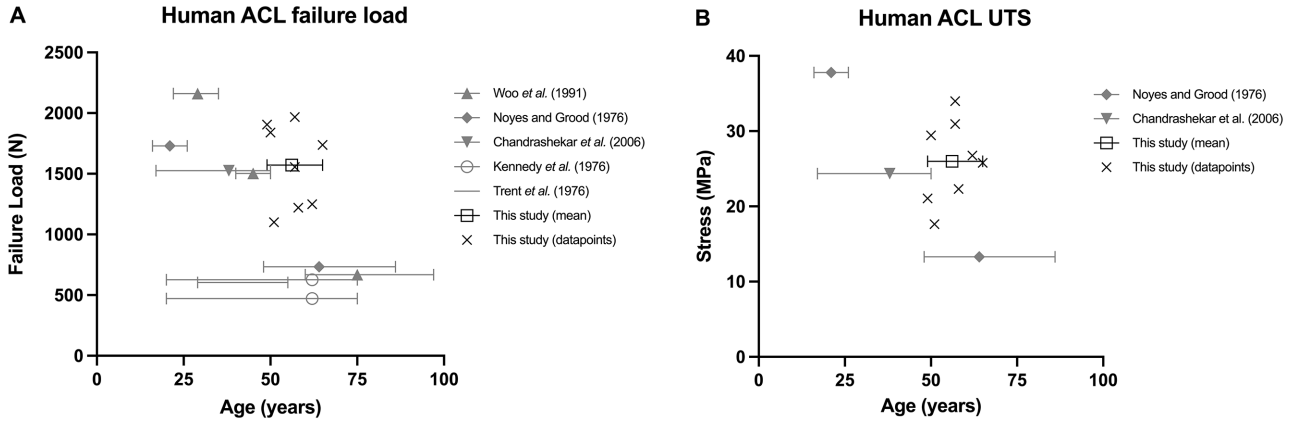

Figure 5. Comparison with literature values on human anterior cruciate ligament (ACL) (A) failure load and (B) ultimate tensile strength (UTS),[8,23,34,41,44] reported as the mean by donor age, where age is annotated by age range (whiskers), median age (circle), and mean age (triangle, diamond, square) where reported. Open symbols represent studies using isolated ACL specimens, and solid symbols represent femur-ACL-tibia complex specimens. Two studies reported on multiple age ranges,[34,44] and 1 study reported on 2 strain rates (2.0 and 8.3 mm/s) for the same age group.[23]

TABLE 2
Pearson Correlation Coefficients (r) and Adjusted P Values for Associations Between Outcomes After the Benjamini-Hochberg Correction[a]

	Hydroxyproline Content		sGAG Content	
Outcome	r	Adjusted P	r	Adjusted P
CSA_{US}	–0.03	.8032	0.20	.0721
Failure load	–0.10	.3980	0.03	.8032
UTS	–0.10	.3980	**–0.60**	**<.0001**
Failure strain	–0.23	.0878	0.03	.8041
Elastic modulus	0.05	.8032	**–0.30**	**.0381**
sGAG content	**0.29**	**.0061**		

[a]Statistical significance is annotated in bold font. Strength of association: weak ($0.30 \leq |r| < 0.50$); moderate ($0.50 \leq |r| < 0.70$).[24,31] CSA_{US}, ultrasound-quantified cross-sectional area; sGAG, sulfated glycosaminoglycan; UTS, ultimate tensile strength.

conditions, and individual studies of the ACL have demonstrated that outcomes are notably affected by strain rate,[23] sex,[8] and particularly age.[34,44] Of these studies, only that by Kennedy et al[23] tested isolated human ACLs, as in the present study, reporting strain rate–dependent failure loads of 472 N and 626 N at 2.0 mm/s and 8.3 mm/s, respectively. While demonstrating an increase in failure load with increasing strain rates, both values are >50% lower than the ACL failure load of the present study (1572 N), which was loaded at a slower rate (5% strain/s) equivalent to 0.5 mm/s. The reasons for this discrepancy may be due to a number of factors, such as different testing fixtures (noncryogenic[23] vs cryogenic) or donor characteristics (eg, age, which was not reported by Kennedy et al). The present results are closer to studies using bone blocks to test the ACL, that is, the femur-ACL-tibia complex (FATC).[8,34,41,44] However, in one study using 100% strain/s, our ACL failure loads were closest to the FATC values reported for younger donors aged 16 to 26 years (1730 kN) rather than the more similarly aged older donors at 48 to 86 years (734 N).[34] The alignment with younger donors, irrespective of strain rate, is also seen when compared with the results of Woo et al[44] when the FATC was tested in a tibial orientation (22-35 years: 1602 N; 40-50 years: 1160 N; 60-97 years: 495 N). In contrast, when the FATC was tested in an anatomic orientation, the results aligned with the comparably aged group (22-35 years: 2160 N; 40-50 years: 1503 N; 60-97 years: 658 N).[44] These data highlight how difficult it is to perform and interpret critical comparisons across ACL studies given the variabilities in donor age, specimen orientation, measurement techniques, and loading rate.[8] This issue is not limited to the ACL and captures the challenges of comparing properties across studies for tendons and ligaments in general, emphasizing the need and rationale for this project, and highlighting the benefits of within-study comparisons of donor-matched tissues tested using the same equipment and methodologies.

The design of this study allows direct intertendon and interligament biomechanical comparisons to be made with confidence and provides additional morphological and compositional comparative data. Although clinical practice and research studies typically focus on the mPT, mQT, and hamstring tendons (ST and GT) as grafts for ACL reconstruction, our results demonstrate that there are several other tendons that possess similar baseline ex vivo properties. Of the common graft tissues evaluated, the mPT and mQT at the time of retrieval may be the closest biomechanical, morphological, and biochemical matches to the ACL itself, yet hamstring tendons are the most common graft source[1,2] and at baseline are comparable to a number of smaller but longer tendons that could be utilized in a similar looped, multibundle construct. While recommendations based on these findings require further

testing, the results support expanding the range of tendons used as allografts for ACL reconstruction to include FL, FB, FHL, EHL, and FDL as they have comparable or superior strength (failure load and UTS) and comparable morphological and biochemical properties to tendons in current clinical use. These findings agree with our previous work on ankle tendons retrieved from donors across a wider range of ages.[3] Expanding the range of tendons used as allografts not only addresses limitations in supply but also would increase the range of dimensions available to the surgeon, providing more flexibility for graft construction when working within the constraints of variable patient anatomies.

Tendon CSA and length will independently dictate how many loops are required to meet a specific graft diameter and how many loops are possible, respectively. Together with mechanical data, this resource provides the means to predict possible graft configurations for the new candidate tendons. For example, the longer fibularis longus tendon could be looped to form a 3-stranded graft with a greater CSA and higher single-strand material strength than a double-stranded tibialis tendon allograft, while still meeting a target graft length of 70 mm.[13] Of note, the free tendon lengths reported reflect the tendon-only length, and while similar to lengths in other studies,[21] they are generally shorter than lengths in studies that use a length-maximizing tendon stripper.[17] Additionally, the results could be used to predict optimal combinations of different tendons to construct grafts of specific dimensions and higher strengths where the single tendon is insufficient in size. It is important to consider that increasing the number of graft strands may not change the biomechanical properties linearly and that the failure load of a multistrand graft may not reach the combined failure loads of individual strands.[19] However, increasing graft strands is reported to improve graft fixation and creep, both of which are associated with lower rates of reinjury.[19,35] This highlights the need for further research to confirm graft-specific surgical procedures including fixation, in situ biomechanical testing, and in vivo validation before the new candidate tendons are ready for clinical adoption.

The hydroxyproline content and collagen content by proxy were fairly consistent across all tissues, while the sGAG content was more tissue specific. Although it may seem intuitive to consider matching the composition of tendon grafts to the composition of the ACL, this is not typically prioritized in the selection of grafts. In this study, hamstring tendons were found to have some of the lowest sGAG levels compared with the ACL, and yet they are one of the most common tendons used in ACL reconstructions.[1,2] Interestingly, when considering all tendon and ligament tissues, a moderate negative association was observed between sGAG content and UTS, and for the subset of 10 tissues with results for elastic modulus, a weak negative association was observed between sGAG and elastic modulus. A similar inverse relationship between sGAG levels and biomechanical properties has been shown in pathological equine tendons induced by focal surgical injury.[11] Notably, the reductions in UTS and modulus were partially reversible through the use of chondroitinase to lower sGAG levels, suggesting that there could be some benefits to the selection of grafts with lower starting sGAG. However, hamstring tendon grafts have been reported to have a higher failure rate than other grafts (eg, PT)[32] that have more closely aligned sGAG content. More evidence is needed to determine whether graft composition directly predicts graft success.

While we have highlighted the benefits and use of this study's data set, the consistent testing of such a wide range of different tissues using the same methods is not without some limitations and considerations. To conduct the study, a number of freeze-thaw cycles were required, 2 to facilitate consistent morphological and biomechanical testing after dissection/retrieval, and a third for biochemical analysis. Under the storage and testing conditions used, this was not expected to modify the tendon or ligament properties characterized, as 10 freeze-thaw cycles had no effect on biomechanical outcomes or CSA in a previous study.[4] Area micrometry is useful for measuring irregularly shaped soft tissues, as it deforms the tissue into a flat, regular shape using constant pressure; however, it may excessively deform smaller tissues and underreport CSA relative to noncontact methods.[37] The assumption of a rectangular cross section may overestimate CSA for larger tendons (eg, AT, QT, and PT) that resist deformation and retain a more rounded shape. However, the rectangular cross section was robust across the range of tissues tested, and the relative error between rectangular- and elliptical-based calculations was considered negligible. To standardize the mechanical fixation across the entire data set, and to prevent failure at the enthesis or within the bone, bone blocks were removed from the relevant specimens. This decision was supported both by the literature[33,34] and by pilot studies that confirmed a primary mode of failure at the enthesis. Removing the entheses forces the tissue to fail midsubstance, which may not reflect the clinical failure location. This difference needs to be taken into consideration, but it allows a standardized fixation across the whole data set. Cryogenic clamps assist the mechanical testing of tissues under high loads while minimizing slippage; however, there is the risk of tissue freezing resulting in cryotrauma or artificial rigidity. Mitigation strategies included checking each tissue for freezing within the gauge region before failure loading, and only analyzing samples that failed midsubstance or midsubstance near the edge. As reported in the Appendix 1 Results – Sample Exclusions (available online), the intact QT had the lowest sample size included in the analysis (n = 3) given the high number of within-clamp failures (n = 5) occurring at the proximal convergence of the subtendons.[18] Thus, the interpretation of QT biomechanical outcomes needs to consider the small sample size. The target gauge length of the mQT was subsequently shortened to increase the grip of the free tendon and reduce the number of test failures to 1. For specimens with gauge lengths <50 mm, the closer proximity of the upper and lower clamps exacerbated the cooling effect and some tissues experienced freezing that extended into the gauge region. This made determining the working gauge length ambiguous and strain calculations uncertain. Thus, for this subset of tissues, strain-based outcomes were dropped, and failure load and UTS were reported only

when the failure occurred in a nonfrozen region. A limitation inherent to most in vitro uniaxial tests and analyses is the assumption of uniform tensile loading of the tissue, which does not necessarily mimic in vivo conditions or reflect the more complex, multidirectional loading patterns within the tissue structure, especially in multibundle tissues. For example, strain was calculated from the change in crosshead displacement, assuming a homogeneous strain field between the grips, which may under- or overreport local strains detectable using markers. Biochemically, a sizable number of sGAG samples was excluded for factors associated with incomplete digestion and/or residual fatty tissue components. This particularly affected QT and PT, although sample numbers are available across both the intact (QT and PT) and the graft (mQT and mPT) tissues. While this study was powered to detect a moderate effect size between tissues, the effects of donor age and sex were not specifically investigated, as there were only 8 donors (3 female, 5 male) of a narrow age range (49-65 years). From our own, larger study of ankle tendons from 35 donors, there were limited effects of donor characteristics, and these were notably tendon specific, affecting only AT, TP, and TA.[3] This suggests that while some tissue sample populations may be directly comparable between studies, others require careful consideration and/or adjustment for specific donor effects. Finally, this study only characterizes ex vivo tendon and ligament properties. Baseline properties of native and graft tissues will change during the healing process after injury and surgery.[25,39] In the context of grafts relevant to ACL reconstruction, remodeling time frames have been shown to vary for different tissues,[16] and it may be important to better understand which baseline graft characteristics are needed to optimize the healing and ligamentization process.

CONCLUSION

The present study of 19 human lower limb tendons and ligaments has enabled direct comparisons of morphological, biomechanical, and biochemical tissue properties, key factors in the selection of suitable graft tissues. This analysis has identified 6 potential new donor tissues with properties comparable with currently utilized grafts for ACL reconstruction. Further research is needed to confirm graft-specific procedures and validation with in vivo studies.

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