ORIGINAL ARTICLE



Biomechanics of hamstring tendon, quadriceps tendon, and bone–patellar tendon–bone grafts for anterior cruciate ligament reconstruction: a cadaveric study

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Received: 17 December 2021 / Accepted: 9 March 2022 / Published online: 1 April 2022 © The Author(s), under exclusive licence to Springer-Verlag France SAS, part of Springer Nature 2022

Abstract

Purpose The three most commonly used autografts for anterior cruciate ligament reconstruction (ACL) are: bone–patellar tendon–bone (BTB), hamstring tendons (HT), and quadriceps tendon (QT). A cadaveric study was performed to determine if there were any differences in mechanical and structural properties under biomechanical testing.

Methods Twenty-seven graft specimens were harvested from 9 human cadaveric legs. Mean donor age was 75.2 years (range 53–85 years). Twenty-two specimens (8 HT, 7 QT, and 7 BTB) completed cyclic preconditioning from 50 to 800 N for 200 cycles and a load to failure test at an extension rate of 1 mm/s. Structural and mechanical properties of BTB, HT, and QT grafts were compared using a one-way ANOVA and Tukey's honest significant difference.

Results There was no difference in the ultimate load to failure (N) across all 3 graft types (p=0.951). Quadriceps tendon demonstrated greater cross-sectional area (mm²) when compared to both HT and BTB (p=0.001) and was significantly stiffer (N/mm) than HT but not BTB (p=0.004). Stress (N/mm²) of the HT at ultimate load was greater than QT but not BTB (p=0.036). Elastic modulus (MPa) of HT was greater than both QT and BTB (p=0.016).

Conclusion There was no difference in the ultimate load to failure of BTB, HT, and QT grafts harvested from the same specimens. All 3 grafts had similar loads to failure with a significant increase in stiffness when compared to the native ACL. Furthermore, QT demonstrated more favourable structural properties compared to HT and BTB with greater cross-sectional area to both HT and BTB and greater stiffness compared to HT.

Keywords Anterior cruciate ligament \cdot Reconstruction \cdot Quadriceps tendon \cdot Patellar tendon \cdot Hamstring \cdot Biomechanical properties

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Introduction

Anterior cruciate ligament (ACL) reconstruction is one of the most commonly performed orthopaedic procedures [1, 2]. The choice of autograft for ACL reconstruction is often influenced by surgeon preference and patient characteristics but continues to be extensively studied and subject to debate.

Historically, bone–patellar tendon–bone (BTB) had been considered the gold standard autograft for ACL reconstruction. While its popularity has decreased more recently, it continues to remain a popular graft choice, especially within the USA [3, 4]. Concerns regarding persistent anterior knee pain, patellar fracture, and patellar tendon rupture have led some to prefer other graft options [5–8].

Hamstring tendon (HT) autograft is a commonly used graft choice worldwide with proponents pointing to less

donor site morbidity and avoidance of extensor mechanism disruption with similar outcomes and re-rupture rates when compared to BTB [9–11]. However, variability in graft size, graft truncation during harvest, and increased re-ruptures rates in younger highly active individuals remain problematic [12–14].

More recently, quadriceps tendon (QT) has re-emerged as a viable alternative graft option [15]. Initially introduced by Marshall et al. [16] in 1979, it fell out of favour after Noyes et al. [17] noted graft relative weakness when compared to BTB in a biomechanical study. However, further biomechanical studies a decade later demonstrated improved biomechanical properties of QT when compared to BTB [18, 19]. The advantages of a more dependable graft size with less donor site morbidity to BTB and comparable outcomes have led to a growing enthusiasm for use of QT for primary ACL reconstruction [5, 15, 20].

There have been multiple biomechanical studies examining the various properties of BTB, HT, and QT as graft options, but to our knowledge, there have been no studies with direct mechanical and biomechanical comparisons between BTB, HT, and QT harvested from the same cadaveric knee. The use of grafts harvested from the same knee adds better controls of donor demographics (age, sex, sideto-side differences, etc.) across all study groups.

The purpose of this study was to compare the biomechanical properties and ultimate load to failure of 3 commonly used graft options, BTB, HT, and QT, for which all were prepared from the same cadaveric specimens. Our hypothesis is there will be no difference in ultimate load between graft types.

Methods

Nine fresh-frozen (-20 °C) human cadaveric knee specimens (mid-femur to mid-tibia) were utilized from a single source, Science Care Inc. (Phoenix, AZ). Specimen information included age, height, weight, sex, race, and cause of death. Each specimen was inspected for any signs of bone or soft tissue disorder that would exclude the graft from analysis.

Graft preparation

Hamstring (HT) graft: An open-ended tendon stripper was utilized followed by release of the tendons from their tibial attachment. The remaining muscle was removed and both ends of the tendons were whipped stitched to each other with a No. 2 FiberWire (Arthrex, Naples, FL) and looped to create a 4-strand HT graft.

Quadriceps (QT) graft: A harvest knife was used (10 mm width and 7 mm depth) to incise the central portion of the tendon starting distally at the level of superior pole of patella. A grasping suture was placed in the free end of the QT. Metzenbaum scissors were used to dissect proximally along the tendon trying to ensure a partial thickness graft was taken. A No. 2 FiberWire (Arthrex, Naples, FL) was utilized to whip-stitch each end of the graft.

Bone-patellar tendon-bone (BTB) graft: The central third of the tendon (10 mm) was marked and cut in-line with its fibres. A bone block of 10×25 mm was obtained from the tibia and a 10×20 mm bone block from the patella was obtained with combination of an oscillating saw and osteotome (Fig. 1).

Mechanical loading protocol

A custom soft tissue cryo-clamp was used to secure the grafts to the materials testing machine (ElectroPuls E10000, Instron, Norwood, MA) at both the distal and proximal ends for the QT and BTB grafts. HT grafts were secured to the testing machine using a similar method described previously [21] in which the HT were folded over a pin with the free ends pre-tensioned with a 453.6 g mass on each end. The masses were removed after the graft was secured to the clamp (Fig. 2).

The clamps were tightened with a torque wrench and the grafts were allowed to freeze for 3 min before the testing procedure began [22, 23]. The testing procedure began with a preconditioning step of cyclic loading from 50 to 800 N at 0.5 Hz for 200 cycles. The grafts were then loaded to failure at a rate of 1 mm/s. This loading protocol has been previously described by Staubli et al.[19].



Fig. 1 Examples of each graft harvest from left to right; 4-strand hamstring tendon graft, partial thickness quadriceps tendon graft, and bone–patellar tendon–bone graft



Fig. 2 Fixation of HT graft pre-tensioned and secured to the clamp

To simulate the intra-articular length of the ACL, an initial clamp-to-clamp distance of approximately 30 mm was used for testing [21]. In the case of the HT grafts, the clamp-to-pin distance was approximately 60 mm. Variables analysed included; cyclic elongation (mm), linear stiffness (N/mm), ultimate load (N), ultimate stress (N/ mm²), ultimate strain (%), elastic modulus (MPa) and cross-sectional area (mm²). Cyclic elongation was defined as the change in crosshead position from the bottom of the last cycle relative to the first cycle. Linear stiffness (N/mm) was calculated as the slope of the linear portion of load-displacement plots. Similarly, elastic modulus (MPa) was calculated as the slope of the linear portion of stress-strain plots. Ultimate load (N) was the peak load recorded during the load to fail portion of the loading procedure. Ultimate stress (N/mm²) was the ultimate load divided by cross-sectional area of the graft. Strain was calculated as grip-to-grip strain using the extension of the crosshead of the testing machine divided by initial graft clamp-to-clamp and clamp-to-pin lengths. Cross-sectional area was defined as the product of the thickness and width of the graft measured with a digital caliper while the graft was under 50 N tension (the low end of cyclic loading).

Statistical analysis

Mechanical and structural properties for each graft type were compared with one-way ANOVA and Tukey's honest significant difference using SPSS statistical software version 24 (SPSS Inc., Chicago, IL). Statistical significance was set at p < 0.05. An a priori calculation determined a sample size of 9 specimens in each group was adequate to detect a difference of 500 N while achieving a $1 - \beta$ of 0.83 assuming a standard deviation of 400 N in the primary outcome measure of ultimate load.

Results

Of the 27 grafts that were harvested and prepared, 22 were used for final analysis. Five grafts (HT; 1, QT; 2, BTB; 2) had to be discarded due to an error with the load cell, fixation mechanism during testing, or failure during cyclic loading. Failures during cyclic loading were influenced by small tendon thickness (<5 mm). The mean age of the donors was 75.2 years (range 53–84).

The ultimate load (N) to failure did not demonstrate any significant differences between all 3 grafts. Stiffness (N/mm) of QT (672 ± 210) was significantly greater than HT (397 ± 91) but not BTB (543 ± 73) (p = 0.004). Hamstring tendon (557 ± 305) was noted to have a significantly greater elastic modulus (MPa) than both QT (269 ± 72) and BTB (297 ± 65) (p = 0.016). Stress (N/mm²) at ultimate load was greater in HT (44.3 ± 16.8) than QT (26.5 ± 8.6) but not BTB (34.6 ± 8.2) (p = 0.036). Quadriceps tendon (81.4 ± 19.2) demonstrated a significantly larger crosssectional area (mm²) than both HT (49.1 ± 12.2) and BTB (61.8 ± 8.3) (p = 0.001). Complete breakdown of structural and mechanical properties can be found in Table 1. Averaged load–displacement and stress–strain behaviour are illustrated in Figs. 3 and 4, respectively.

The modes of graft failure observed were tearing initiated in the tendon mid-substance, tearing initiated at the insertion into the clamp, and fracture of the bone end in BTB grafts (Fig. 5). The majority of graft failures within all 3 grafts were noted to be insertion tears. Mid-substance tears occurred in only 2 HT, 1 QT, and no BTB grafts. Fracture of the bone end was noted to occur in 3 of the BTB grafts. No grafts were noted to have slipped out of the clamp during testing. Breakdown of graft failure can be seen in Table 2.

Discussion

QT grafts demonstrated favourable structural properties to HT and BTB grafts in terms of a significantly greater crosssectional area when compared to both HT and BTB and a

| Table 1 | Tensile properties of | of |
|-----------|-----------------------|----|
| graft typ | bes (mean \pm SD) | |

| Property | QT $(n = 7)$ | HT $(n=8)$ | BTB $(n=7)$ | P value |
|-----------------------------------------|---------------------|--------------------------|-----------------|---------|
| Cyclic Elongation (mm) | 1.0 ± 0.6 | 0.6 ± 0.3 | 0.8 ± 0.7 | 0.370 |
| Linear stiffness (N/mm) | $672 \pm 210^{**}$ | 397 ± 91 | 543 ± 73 | 0.004 |
| Ultimate load (N) | $2,097 \pm 567$ | $2,046 \pm 455$ | $2,129 \pm 521$ | 0.951 |
| Ultimate stress (N/mm ²) | 26.5 ± 8.6 | $44.3 \pm 16.8^\ddagger$ | 34.6 ± 8.2 | 0.036 |
| Ultimate strain (%) | 13.7 ± 1.7 | 11.4 ± 3.0 | 15.1 ± 4.9 | 0.139 |
| Elastic modulus (MPa) | 269 ± 72 | $557 \pm 305^{\dagger}$ | 297 ± 65 | 0.016 |
| Cross-sectional area (mm ²) | $81.4 \pm 19.2^{*}$ | 49.1 ± 12.2 | 61.8 ± 8.3 | 0.001 |

BTB Bone-patellar tendon-bone, HT Hamstring tendon, QT Quadriceps tendon

*Denotes statistical significance when compared to HT and BTB

** Denotes statistical significance when compared to HT but not BTB

[†]Denotes statistical significance when compared to both QT and BTB

[‡]Denotes statistical significance when compared to QT but not BTB



Fig. 3 Averaged load–displacement plot from all cadaveric graft specimens in each group. A specimen in the BTB group that failed via bone fracture at low load and displacement caused a deviation in the curve

significantly higher stiffness than HT but not BTB. No difference in ultimate load to failure was noted between grafts and all demonstrated similar load to failure as that of the native ACL $(2,160 \pm 157 \text{ N})$ [24]. Stress was significantly greater in HT than QT in the current study. Clinically, ultimate load to failure is often considered the most important biomechanical property as it represents the ability of a graft to withstand the anticipated load that initially caused injury [17, 25–27].

There has been wide variability in the reported ultimate load to failure of HT that can likely be attributed to variable biomechanical testing protocols as well preparations of HT graft from single to doubled to quadrupled to most recently 6-stranded graft preparations [21, 27–29]. The ultimate load to failure within the current study was 2046 ± 455 N which is

similar to that reported by Wilson et al. [30] using a similar 4-stranded graft. The failure load of a 4-stand graft has also been reported as high as 4590 ± 674 N [21]. What is becoming evident is increasing graft size leads to increased biomechanical properties within HT. Significant differences in ultimate load with increasing graft diameter have been reported [29]. A graft diameter larger than 9 mm demonstrated a load to failure of 4360 ± 606 N [29]. More recent biomechanical studies compared the properties of a 6-stranded HT graft to QT and reported an ultimate load to failure of 2641 ± 662 N and was also found to be significantly stiffer than a soft tissue QT (1,148 ± 339 vs. 809 ± 173 N/mm) [27].

The ultimate load to failure of a 10 mm wide BTB was 2129 ± 521 N in the current study. Similar-sized grafts have been reported to have an ultimate load of 2977 N [31]. The





Fig. 5 Failure modes observed during load-to-fail testing. (A) Tearing of HT graft at the clamp insertion. (B) Fracture of bone end of BTB graft within the clamp. (C) Tearing of QT graft initiated in the mid-substance

Table 2 Breakdown of graft failure mode

| Graft type | Mid-substance tear No. | Insertion tear No. | Bone fracture No. |
|------------|---------------------------|-----------------------|-------------------------|
| QT | 1 | 6 | NA |
| HT | 2 | 6 | NA |
| ВТВ | 0 | 4 | 3 |

BTB Bone-patellar tendon-bone, HT Hamstring tendon, QT Quadriceps tendon

ultimate load of 13 mm wide grafts was found to be 3424 N [32] and 2900 N for 13.8 mm wide grafts [17]. In all 3 previously mentioned studies, the method of fixation was different from the current study as the bone blocks were anchored to the tensile testing machine by encasing them within materials that cured around them. This method of fixation exposes the graft to potentially fail by bony avulsion but does seem to provide strong fixation to the machine as evidenced by the reported ultimate loads. This method also reduces the potential of applying too much pressure to the tissue at the fixation point as is the risk with using clamps. Studies that have used clamps as a means of fixing BTB bone ends to the testing machine have reported ultimate loads of $1,580 \pm 479$ N with a cryo effect [33] and 413.3 ± 120.4 N without a cryo effect [34].

Within this study, the ultimate load to failure of an all soft tissue QT was 2097 ± 567 N. This is consistent with other recently reported values [22, 25, 27]. Proponents of QT graft for ACL reconstruction argue QT provides a thicker graft with more favourable tensile properties when compared to

HT and BTB [18, 19, 25]. Our study reiterates some of these findings as QT was found to be have a significantly larger cross-sectional area than both HT and BTB (81.4 vs. 49.1 vs. 61.8 mm^2 ; p = 0.001). Some argue the larger cross-sectional area increases the collagen content and may help mitigate the windshield wiper and bungee effect as well as tunnelgraft mismatch that may be seen with BTB due to its thin and flat morphologic shape that may result in inflow of synovial fluid and cytokines leading to bone resorption [5, 35, 36]. The ability to predict a graft size is crucial in planning ACL reconstruction. Historically, BTB was considered most predictable when considering graft volume but recently QT has also been shown to demonstrate similar predictability [37].

Stiffness represents the resistance of a structure to deformation [38]. All grafts within this study were considerably stiffer than the reported stiffness of the native ACL [24]. QT demonstrated a significantly increased linear stiffness when compared to HT (672 ± 210 vs. 397 ± 91 N/mm; p = 0.004) in the current study. Conversely, Urchek et al. [27] when testing of a thicker 6-stranded HT graft noted a statistically increased stiffness of HT opposed to QT (1,148 \pm 339 vs. 809 ± 173 N/mm). Shani et al. [25] noted a significant increase in stiffness of QT when compared to BTB (466 ± 133 vs. 278 ± 75 N/mm). While obtaining a stiffer and stronger graft is felt to be advantageous for ACL reconstruction, the clinical implications of a stiffer graft are still not well elucidated.

The mean age (75.2; range 53–84) of the specimens in this study were considerably older than many of the previously mentioned biomechanical studies. The effect of age and orientation of the femur-ACL-tibia complex has been examined and reported significant decreases in load, stiffness, and energy absorbed with increasing age [24]. The effect of age has also been studied on patellar tendons [32, 39]. Higher ultimate tensile stress in younger specimens (29-50 years) has been seen in one study [39] while no difference in failure load, stress, modulus, or elongation for specimens aged 18–55 has been seen in another [32]. Despite the older age of specimens in this study, the ultimate failure loads were similar to that of native ACLs in patients aged 22 to 35 [24]. Moreover, the ultimate failure loads within this study are similar to other biomechanical studies with younger cadaveric specimens [22, 25, 27, 30].

There were several limitations within this study. Sample size within each group is relatively small which may have led some comparisons in this study to be underpowered. In order to try and minimize variability due to fixation method and allow for comparisons across groups, a single fixation device was used for 3 different graft types. Cryogenic fixation for soft tissue grafts is well described but is not as commonly used for grafts with bone blocks which are typically fixated with bone cement. It may be optimal in future studies to use a unique fixation method for different graft types to achieve the greatest potential peak tensile load. Strains were calculated as the gripto-grip elongation and may not be indicative of local strains in the region of rupture of the graft. While age has been discussed previously, the age of our specimens should still be considered with interpretation of our results. Lastly, testing of isolated grafts is more representative of the direct postoperative phase prior to any tissue remodelling that may occur which could affect the mechanical properties.

Conclusion

The ultimate load to failure of BTB, HT, and QT grafts within this study was not significantly different between each other. All three grafts had similar loads to failure with a significant increase in stiffness when compared to the native ACL. Furthermore, QT demonstrated favourable structural properties to HT and BTB with an increased cross-sectional area to both HT and BTB along with increased stiffness compared to HT. All 3 grafts would be viable graft options for ACL reconstruction.

Acknowledgements The authors would like to acknowledge the Orthopaedic Innovation Centre in Winnipeg, Manitoba, Canada, for their assistance with mechanical testing and clamp design and fabrication in this study.

Author contributions All authors contributed substantially to the project. DH, TG-D, JL, and PM designed the study. DH conducted the data acquisition. DH and SM participated in the analysis of the data. DH, TG-D, JL, RL, ASE, SM, and PM conducted the data interpretation. DH, TG-D, JL, RL, ASE, SM, and PM drafted and reviewed the manuscript. All of the authors approved the content of the manuscript before the submission.

Funding This study was funded by the University of Manitoba Alexander Gibson Fund and the Pan Am Clinic Foundation. The institution of authors DH, SM, and PM has received general funds for research and education from Arthrex, CONMED Linvatec, and Zimmer Biomet.

Data availability Data and materials are available upon request.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

Ethical Approval Ethical approval for this study was obtained from the University of Manitoba Research Ethics and Compliance Health Research Ethics Board. Ethics# HS24041(H2020:301).

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