



Article

How Do the Biomechanical Properties of Four-Strand Semitendinosus Grafts Compare to Those of Four-Strand Semitendinosus + Gracilis Combination Grafts in ACL Reconstruction?

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Abstract

Background/Objectives: Hamstring tendon (HT) autografts are commonly used for anterior cruciate ligament reconstruction (ACLR), traditionally combining semitendinosus and gracilis (ST + GR) tendons to achieve adequate graft length and diameter. More recently, four-strand semitendinosus (ST-only) constructs have been adopted to avoid gracilis harvest and reduce donor-site morbidity when sufficient ST tendon length is available. However, to our knowledge, the intrinsic biomechanical properties of four-strand ST-only and ST + GR grafts have not been directly compared. **Methods:** Twenty-five hamstring allografts (17 ST, 8 GR) from a single provider were prepared into two four-strand configurations: ST-only ($n = 9$) and ST + GR ($n = 8$). Manufacturer-reported folded tendon diameters were used to estimate four-strand graft diameters. Grafts underwent preconditioning, cyclic loading (80–250 N), and load-to-failure testing using a servomechanical testing apparatus. Ultimate failure load, stiffness, cyclic elongation, and elongation at failure were recorded. Groups were compared using the Mann–Whitney U test. **Results:** Mean donor age was 55.8 years (range, 27–72 years). Estimated mean graft diameter was significantly greater for ST-only constructs compared with ST + GR constructs (8.88 ± 0.62 mm vs. 7.70 ± 0.38 mm; $p < 0.001$). ST-only constructs demonstrated greater stiffness than ST + GR constructs (458.99 ± 54.51 vs. 364.67 ± 57.18 N/mm; $p = 0.007$) and lower elongation at failure (5.67 ± 1.25 vs. 7.42 ± 0.86 mm; $p = 0.01$). Cyclic elongation (0.259 ± 0.045 vs. 0.256 ± 0.049 mm; $p = 0.89$) and ultimate load to failure (1689.84 ± 121.36 vs. 1626.59 ± 324.92 N; $p = 0.64$) did not differ between groups. **Conclusions:** When tested in isolation, four-strand ST-only grafts are not biomechanically inferior to ST + GR grafts and demonstrate greater stiffness and larger estimated diameter. When sufficient semitendinosus length is available

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ble, ST-only grafts represent a biomechanically viable option for ACLR without the need for gracilis harvest.

Keywords: ACL reconstruction; hamstring grafts; graft biomechanics

1. Introduction

Anterior cruciate ligament (ACL) reconstruction is one of the most common orthopedic operations, with over \$1 billion spent on procedures annually [1]. ACL injuries are frequently a result of noncontact injury events [2].

The choice of grafts for ACL reconstruction (ACLR) is a critical factor and can be individualized based on patient characteristics and goals [3–6]. Allografts reduce operation time and preserve native biomechanics by avoiding graft harvest-site morbidity but they greatly increase total procedure cost and have shown higher failure rates than autografts, especially in young patients [4,6–12]. Additionally, the Multicenter ACL Revision Study (MARS) showed that the use of an autograft for ACL reconstruction improved functional sports outcomes over a two-year follow-up [6]. Based on extensive biomechanical analysis of ACL grafts, a minimum preferred graft diameter of 7–8 mm [4,13–17] is recommended to reduce the risk of graft revision [6,18].

Popular autografts for ACLR include Bone–Patellar Tendon–Bone (BTB), quadriceps tendon (QT), and hamstring tendon (HT) grafts, the latter including both the semitendinosus (ST) and gracilis (GR) tendons [3,6,19]. BTB autografts have excellent strength, the fastest recovery time in some studies, and the lowest revision rate. However, the required osseous harvest causes significant donor-site morbidity, leading to frequent complications including recurring anterior knee pain. Hamstring autografts have become a common choice in ACLR to avoid the donor-site morbidity associated with BTB autografts and the hesitancy of athletes to risk the diminished quadriceps strength associated with QT harvest [8,20,21]. However, while the use of HT autografts keeps the quads intact, it similarly may impact donor hamstring strength.

Despite the HT graft's common usage, nearly half of all HT grafts used do not meet the 8 mm diameter threshold for sufficient graft size [6,18]. To increase HT graft diameter, recent research has evaluated different HT constructs including the ST graft folded into four strands [22–25]. However, HTs have higher variability and unpredictability in size compared to other tendons [6]. While four-strand ST grafts allow for larger diameter, in some patients, four-stranded ST-only grafts may be too short for implantation. Consequently, in cases of inadequate ST length for a four-strand ST reconstruction, the addition of the GR harvest may be essential to optimize graft dimensions [6]. This construct combines a folded ST tendon with a folded GR tendon to create a combined four-strand HT autograft. While this creates a HT autograft with increased diameter, this technique requires harvesting two tendons which may increase donor-site morbidity and rehabilitation time. To our knowledge, no prior study has directly compared the intrinsic biomechanical properties of four-strand ST-only and ST + GR graft constructs in isolation, without the influence of suspensory fixation. Although the ST + GR has traditionally been used, it remains important to determine whether the newer four-strand ST-only construct has different biomechanical properties compared with the ST + GR construct and whether it confers biomechanical advantages beyond reduced donor-site morbidity.

The purpose of this study was to compare the biomechanical properties of four-strand ST-only grafts with those of four-strand combination ST + GR grafts. We hypothesized that the four-strand ST-only graft would show no difference in ultimate failure load,

stiffness, cyclic elongation, or elongation at failure compared to the four-strand ST + GR graft.

2. Materials and Methods

2.1. Sample Preparation

Twenty-five HT allografts, comprising 17 ST and 8 GR tendons, were obtained from a single allograft provider, with all grafts undergoing an identical proprietary cleaning process. During graft preparation, the manufacturer measured the folded tendon diameter under standardized tension using cylindrical sizing blocks. IRB approval was not necessary since the tissues utilized in the study were donated for research purposes, de-identified, no genetic information was used, and there was no contact with family members.

Two graft configurations were prepared for biomechanical testing. The first group (ST-only) consisted of bisected STs which were folded in half and combined to form a four-strand construct ($n = 9$). The second group (ST + GR) consisted of combined ST and GR tendons, both folded then combined to make a four-stranded construct ($n = 8$). ST tendons were randomized to one of the two groups, whereas all GR tendons were assigned to the ST + GR group. Manufacturer-reported folded tendon diameters were used to estimate four-strand graft diameters for both ST-only and combined ST + GR constructs. Folded diameter measurements were first converted to cross-sectional area assuming an equivalent circular geometry. For ST-only constructs, the semitendinosus tendon was bisected transversely at its midpoint, and each segment was folded to create a four-strand graft. This configuration was approximated as two identical two-strand bundles with each bundle assigned the manufacturer-reported folded ST diameter. For both the ST-only and ST + GR four-strand constructs, cross-sectional areas of the component two-strand bundles were summed, and the equivalent four-strand graft diameter was then calculated under the assumption that total cross-sectional area was preserved under tension (Figure 1).

Two-Strand Graft Two-Strand Graft Four-Strand Graft

(a) $\frac{\pi * d_1^2}{4} = A_1$ $\frac{\pi * d_2^2}{4} = A_2$ $CA \approx A_1 + A_2 = \frac{\pi * d_1^2}{4} + \frac{\pi * d_1^2}{4}$

(b) $\frac{\pi * d_{four_strand}^2}{4} = CA$

(c) $d_{four_strand} = \sqrt{\frac{4 * CA}{\pi}}$

(d) $d_{four_strand} = \sqrt{\frac{4 * \frac{\pi * d_1^2}{4} + \frac{\pi * d_1^2}{4}}{\pi}}$

(e) $d_{four_strand} = \sqrt{d_1^2 + d_2^2}$

Figure 1. Estimation of four-strand graft diameter from manufacturer-reported folded tendon diameters. (a) Manufacturer-reported folded (two-strand) tendon diameters were used to calculate individual cross-sectional areas assuming circular geometry ($A = \pi d^2/4$). For four-strand constructs, total cross-sectional area was estimated as the sum of the component two-strand areas, assuming preservation of cross-sectional area under tension and an equivalent circular graft shape. (b) The area of a circle was then used to relate total cross-sectional area to the estimated diameter of the four-strand graft. (c) Cross-sectional area equation was rearranged to solve for estimated four-strand diameter. (d) Substitution of the summed two-strand areas into the diameter equation yields the estimated four-strand diameter. (e) Simplification of this relationship results in an approximate expression for four-strand graft diameter.

2.2. Biomechanical Testing

To prepare the grafts for loading into the tensile apparatus, each specimen was covered with abrasive coated material and liquid adhesives to form unyielding polyurethane polymers upon water contact (Figure 2). The biomechanical testing protocol followed similar pediatric cadaveric tissue testing previously established by our laboratory in the literature [26,27]. Samples were separately clamped into an Instron 5944 machine (Instron, Norwood, MA, USA), and subject to 20 cycles of preconditioning between 0 and 80 N at a rate of 10 mm per minute; no biomechanical data was collected during preconditioning. After preconditioning, the constructs underwent a cyclic loading protocol of 80 and 250 N at a rate of 10 mm/min. A tissue recovery period of 30 min was followed by testing for load to failure at 10 mm/min.

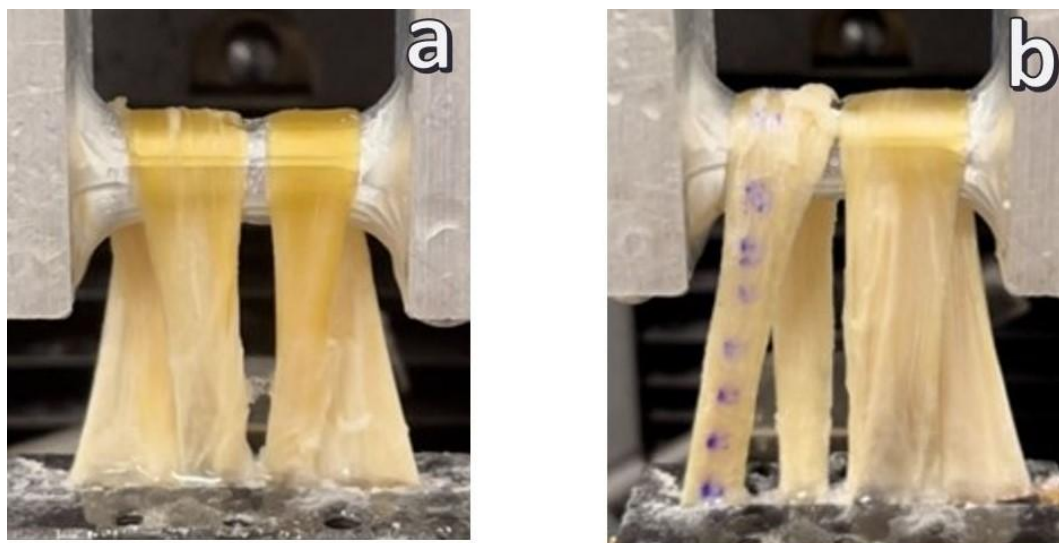


Figure 2. Mounting of constructs on Instron machine during cyclic loading protocol. (a) The 4-strand Semitendinosus only construct. (b) 4-strand Semitendinosus + Gracilis (purple dotted fibers) construct.

2.3. Data Recording and Statistical Analysis

Load displacement curves were generated for all cyclic loading and load-to-failure tests (Bluehill; Instron, Norwood, MA, USA). The primary outcome was ultimate failure load (N), defined as the maximum load recorded on the load–displacement curve. Secondary outcomes included stiffness, cyclic elongation, and elongation at failure. Stiffness (N/mm) was calculated as the slope of the best-fit linear regression line applied to the load–displacement curve during load-to-failure testing, excluding the cyclic loading phase; the linear region was identified as the segment demonstrating the highest R^2 prior

to ultimate failure. Cyclic elongation (mm) was defined as the change in construct displacement between the 5th and 500th cycles at a matched load of 75 N, representing the total accumulated elongation over cyclic loading. Elongation at failure (mm) was defined as the total extension from the initiation to the completion of load-to-failure testing. The failure modes observed during testing were noted qualitatively.

All statistical analyses were performed in python. Given the small sample sizes, a nonparametric Mann–Whitney U test was used to compare groups regardless of distributional normality. The threshold for statistical significance was set at $p < 0.05$.

3. Results

Grafts were obtained from donors (23 male; 2 female) with a mean age of 55.8 years (range: 27–72). The estimated mean diameter of four-strand ST-only grafts was 8.88 ± 0.62 mm, compared with 7.70 ± 0.38 mm for ST + GR grafts, with a statistically significant difference between groups ($p < 0.001$). ST-only constructs demonstrated significantly greater stiffness than ST + GR constructs (458.99 ± 54.51 N/mm vs. 364.67 ± 57.18 N/mm; $p = 0.007$) and significantly lower elongation at failure (5.67 ± 1.25 mm vs. 7.42 ± 0.86 mm; $p = 0.01$). Ultimate load to failure did not differ between groups (1689.84 ± 121.36 N vs. 1626.59 ± 324.92 N; $p = 0.64$), nor did cyclic elongation (0.259 ± 0.045 mm vs. 0.256 ± 0.049 mm; $p = 0.89$). Cyclic elongation increased approximately linearly across the 500 loading cycles in both groups, with no evidence of accelerating elongation indicative of progressive damage accumulation (Figure 3). All specimens completed the full 500-cycle protocol without failure. Full biomechanical results are detailed in Table 1. Observed modes of failure included rupture, slippage, and fiber tearing (Figure 4).

Table 1. Comparison of biomechanical properties.

Biomechanical Property	Semitendinosus	Semitendinosus + Gracilis	<i>p</i> -Value
Maximum Load at Failure (N)	1689.84 ± 121.36	1626.59 ± 324.92	0.64
Elongation at Failure (mm)	5.67 ± 1.25	7.42 ± 0.86	0.01
Cyclic Elongation (mm)	0.259 ± 0.045	0.256 ± 0.049	0.89
Stiffness (N/mm)	458.99 ± 54.51	364.67 ± 57.18	0.007

All data is presented as mean $\pm$ SD.

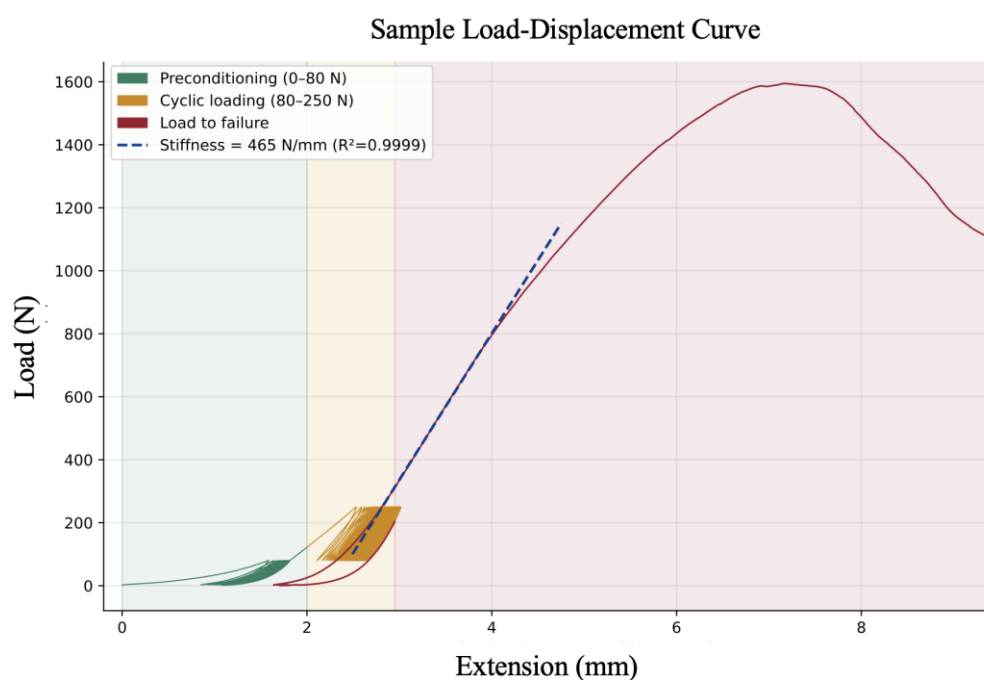


Figure 3. Representative load–displacement curve illustrating the preconditioning, cyclic loading, and load-to-failure phases. Stiffness was calculated as the slope of the best-fit linear regression applied to the linear region of the load-to-failure phase (the segment with the highest R^2), and maximum load was defined as the highest load recorded prior to failure. Data are from a single ST-only specimen and are shown for illustration only; they are not representative of the full dataset.

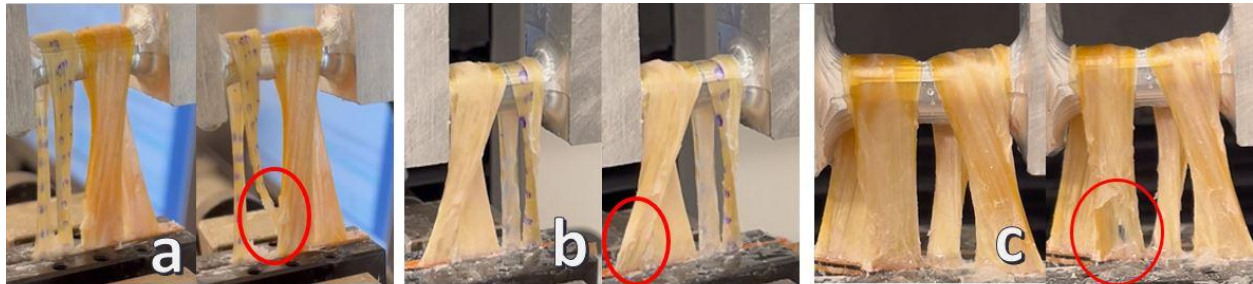


Figure 4. Red circles indicate the sequence of failure modes during maximum load at failure. (a) Rupture of fibers. (b) Slippage. (c) Tearing of fibers.

4. Discussion

The most important finding of this study was that when tested in isolation without additional fixation, ST-only and ST + GR constructs did not differ in the most significant biomechanical metric of ultimate failure load nor in cyclic elongation. However, differences were observed in construct behavior under load, with the ST-only graft demonstrating significantly greater stiffness and lower elongation at failure compared with the ST + GR graft. In addition, the average four-strand ST-only construct exhibited a significantly larger calculated diameter than the average ST + GR construct, exceeding the commonly cited 8 mm threshold for adequate graft sizing (8.88 mm vs. 7.70 mm) [4,13–17].

Given the widespread use of HT grafts in ACLR, there is a significant amount of published literature covering their biomechanics [22,23,28]. However, many prior studies evaluate HT grafts as part of a reconstruction construct, incorporating specific fixation devices or surgical techniques, or focus exclusively on either ST-only or ST + GR graft testing without comparison between the two. As a result, direct comparisons of the intrinsic biomechanical properties of ST-only and ST + GR grafts themselves remain limited. Isolating graft behavior is critical for understanding differences attributable to graft options rather than fixation method, particularly given the evolving nature of fixation techniques over time. The present study addresses this gap by directly comparing ST-only and ST + GR graft constructs tested in isolation, thereby providing insight into graft-specific biomechanical behavior independent of fixation.

One comparative study conducted by Höher et al. evaluated a four-strand ST construct fixed with a titanium button, polyester tape, and screw against a four-strand ST + GR construct fixed with a cross-pin and interference screws [23]. In that study, the ST + GR construct demonstrated significantly lower cyclic elongation (0.3 ± 0.2 mm vs. 3.8 ± 1.2 mm), greater stiffness (119 ± 19 N/mm vs. 32 ± 1 N/mm), and higher ultimate failure load (658 ± 128 N vs. 415 ± 35 N) compared with the ST-only construct [23]. These findings contrast with the results of the present study, which demonstrated no differences in ultimate failure load or cyclic elongation when grafts were tested in isolation. Importantly, Höher et al. reported that failure of the ST-only construct occurred at the polyester tape interface, indicating that the observed differences were driven by fixation-related limitations rather than intrinsic graft properties [23]. This distinction highlights the importance of isolating graft constructs when comparing ST-only and ST + GR configurations, as fixation interfaces may substantially influence measured biomechanical outcomes and potentially confound graft-level comparisons.

Similarly, Vertullo et al. compared four-strand ST-only and ST + GR constructs using differing tibial fixation methods, including polyethylene terephthalate tape tied over a screw for ST-only grafts and interference screw fixation for ST + GR grafts [25]. While no significant differences were identified between constructs, the primary focus of that study was fixation strategy rather than graft-specific biomechanics. Consequently, although informative with respect to tibial fixation, that work does not directly address intrinsic differences between ST-only and ST + GR grafts themselves.

A landmark paper in HT ACLR by Hamner et al. demonstrated that increasing the number of strands in HT grafts significantly improves both ultimate failure load and stiffness [22]. This study included testing of four-strand ST + GR constructs, which exhibited ultimate failure loads of 2831 ± 538 N and stiffness values of 456 ± 97.7 N/mm [22]. Although these values exceed those observed in the present study, differences in graft preparation and testing methodology likely contribute to these discrepancies. Notably, Hamner et al. did not evaluate four-strand ST-only grafts, limiting the ability to draw conclusions regarding biomechanical equivalence between ST-only and ST + GR configurations [22]. More recently, Madañ et al. evaluated the biomechanical performance of a four-strand ST-only graft using cortical button fixation with adjustable loops, reporting a mean ultimate failure load of 849 ± 115 N and stiffness of 221 ± 25 N/mm [28]. However, similar to the aforementioned studies, this work focused on the performance of the graft–fixation construct rather than providing a direct comparison between ST-only and ST + GR grafts or isolating intrinsic graft behavior.

Taken together, despite the extensive literature on HT graft biomechanics, direct comparisons of the intrinsic biomechanical properties of ST-only and ST + GR graft constructs remain limited. By testing grafts in isolation and using identical methodologies across constructs, the present study contributes novel data clarifying graft-specific biomechanical differences between ST-only and ST + GR configurations. These findings may help inform graft selection decisions based on intrinsic graft behavior rather than fixation-dependent performance.

Importantly, both four-strand ST-only and ST + GR grafts demonstrated biomechanical properties that meet or exceed reported values for the native anterior cruciate ligament. Prior studies have reported native ACL ultimate failure loads ranging from approximately 658 to 2160 N [4,29,30] and stiffness values between 180 and 242 N/mm [4,29], depending on age of patient and testing methodology. In the present study, ultimate failure loads for ST-only and ST + GR grafts (1689.84 N and 1626.59 N, respectively) fell within this reported range. In addition, both graft constructs demonstrated stiffness values (458.99 N/mm for ST-only and 364.67 N/mm for ST + GR) exceeding those commonly reported for the native ACL. Although graft stiffness exceeded reported native ACL values, stiffness alone does not solely increase the risk of over constraint. It is additionally influenced by multiple factors including tunnel placement, initial graft tension and fixation method [31,32]. Together, these findings suggest that both graft configurations provide sufficient time-zero biomechanical strength to serve as viable options for ACL reconstruction.

The greater stiffness and lower elongation at failure observed in the ST-only constructs compared with the ST + GR constructs (458.99 ± 54.51 vs. 364.67 ± 57.18 N/mm, $p = 0.007$; and 5.67 ± 1.25 vs. 7.42 ± 0.86 mm, $p = 0.01$, respectively) may be partially explained by differences in graft diameter. The four-strand ST-only constructs demonstrated a significantly larger estimated diameter than the ST + GR constructs (8.88 mm vs. 7.70 mm). Prior biomechanical work by Hamner et al. demonstrated that increasing the number of strands in hamstring tendon grafts results in improvements in stiffness and load-to-failure properties [22]. A similar mechanism may be present in this study, whereby greater effective graft cross-sectional area in the ST-only construct contributes to increased stiffness

and resistance to elongation under load. In addition to graft size, differential tendon properties between the semitendinosus and gracilis tendon including collagen fibril diameter, density, and alignment are known to influence tendon stiffness and may also contribute to the observed differences between the constructs [30,33].

Beyond biomechanical considerations, differences in graft diameter may also carry clinical relevance. Larger hamstring graft diameters have been associated with lower graft failure rates, with an 8 mm threshold commonly cited in the literature for appropriate sizing [4,6,13–18]. In the present study, the average ST-only construct exceeded this threshold, whereas the average ST + GR construct did not. Although graft diameter alone does not determine clinical outcomes, this finding suggests that ST-only constructs may offer a theoretical advantage with respect to graft size when sufficient tendon length is available.

From a surgical perspective, these findings support consideration of an ST-only graft when adequate length permits usage of a four-strand graft. Compared to the ST + GR graft, the similarities in ultimate failure load and cyclic elongation, coupled with greater stiffness and larger diameter of the ST-only graft, suggests that ST-only grafts do not confer biomechanical disadvantage and may even offer mechanical advantages. Preservation of the gracilis tendon may also reduce donor-site morbidity, postoperative hamstring weakness, and maintain the gracilis as a potential autograft source in cases of multiligament reconstruction or revision surgery [28]. Importantly, when semitendinosus length is insufficient for a four-strand construct, the use of an ST + GR graft remains appropriate. Despite lower stiffness and greater elongation at failure, ST + GR constructs demonstrated adequate ultimate failure loads in the present study. Clinical data comparing ST-only and ST + GR grafts remain limited. A randomized controlled trial by Martínez-Cano et al. reported no differences in strength, patient-reported outcomes, return to sport, or failure rates between four-strand ST-only and ST + GR grafts at short-term follow-up [24]. However, the relatively small cohort size and limited follow-up duration warrant cautious interpretation and further clinical research investigating the comparison between graft options.

Limitations

This study has several limitations. First, four-strand graft diameter was not measured directly; instead, graft diameter was estimated from manufacturer-reported folded (two-strand) diameters. This approach may introduce imprecision in absolute graft sizing. However, folded diameters were measured using cylindrical sizing blocks under standardized tension, and the assumption of preserved cross-sectional area when combining strands reasonably reflects graft behavior under load. Second, this study represents time-zero biomechanical testing and does not account for biological processes such as graft incorporation or remodeling following implantation. Although this approach isolates intrinsic graft behavior, additional studies are required to determine whether biological healing differentially affects ST-only and ST + GR grafts *in vivo*.

Third, fixation was intentionally excluded from testing. Because grafts must be secured during reconstruction and fixation methods can substantially influence construct stiffness and elongation, this study cannot comment on specific techniques used in HT ACLR. However, this design was deliberate to isolate graft-specific biomechanical properties and provide a baseline for comparison independent of evolving fixation technologies. Fourth, ST-only and ST + GR grafts were not derived from matched specimens. In clinical autograft reconstruction, these tendons typically originate from the same patient; however, in the present study, grafts were obtained from different donors with varying ages, which may contribute to inter-graft variability. However, ST grafts were randomized to either ST-only or ST + GR groups to minimize systematic biases between groups

and reduce the likelihood that donor-related factors such as age disproportionately favored one group over the other.

An additional limitation relates to the preparation of the four-strand ST-only constructs. Clinically, a four-strand semitendinosus graft is created from a single, intact tendon folded twice upon itself, with collagen fibers running continuously around each fold. In the present study, the tendon was instead bisected at its midpoint and the two segments folded and combined into a four-strand construct. This approach was chosen to standardize construct length and geometry and ensure consistent, reproducible mounting within the testing apparatus. While it improved experimental standardization, the resulting transverse cut ends do not replicate the continuous fiber architecture of an intact graft and may influence load transmission, strand-to-strand load sharing, and measured stiffness and elongation. These structural differences should be considered when extrapolating to intact clinical grafts, and future studies using continuously folded constructs may help confirm these findings.

Finally, the sample size was relatively small. Despite this limitation, statistically significant differences were identified, suggesting that the observed effects are robust; nonetheless, larger studies are warranted to confirm these findings and improve generalizability.

5. Conclusions

This study demonstrates that when tested in isolation, four-strand ST-only grafts are not biomechanically inferior to ST + GR grafts, exhibiting comparable ultimate failure load and cyclic elongation, with greater stiffness and larger estimated diameter. Both grafts are viable candidates for ACLR as they demonstrate failure loads and stiffness values meeting or exceeding that of the native ACL. Ultimately, graft selection in ACL reconstruction should be individualized, incorporating considerations of graft length, diameter, donor-site morbidity, and biomechanical performance. Both ST-only and ST + GR grafts appear to be reasonable options, with optimal choice dependent on patient-specific anatomy and surgical goals.

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Informed Consent Statement: Institutional review board (IRB) approval was not required, as this study was conducted on cadaveric tissue without patient identifiers, no genetic information was analyzed, and familial consent was obtained for research purposes at the time of tissue donation.

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