Research Square

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Four-strand Semitendinosus with Three- vs Two-Strand Gracilis Grafts for ACL reconstruction: A Biomechanical Study in a Porcine model

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Abstract

Purpose

To compare six-strand (quadrupled semitendinosus, doubled gracilis; 4ST+2GR) and seven-strand (quadrupled semitendinosus, tripled gracilis; 4ST+3GR) all-inside hamstring anterior cruciate ligament (ACL) grafts biomechanically.

Methods

Thirty-six grafts were prepared from porcine extensor tendons (wider tendon as semitendinosus substitute, narrower tendon as gracilis substitute –two pairs from one leg) and divided equally to two groups (n = 18 each).

In the 4ST+2GR group, the gracilis was cut to 50% of the semitendinosus length and doubled.

In the 4ST+3GR group, it was cut to 75% of the semitendinosus length and tripled alongside the quadrupled semitendinosus.

Diameter was measured under an 80 N preload. Grafts underwent incremental cyclic loading (25 N per cycle, 20 mm/min) until failure.

Elongation was measured with a video extensometer, and stiffness was calculated for the last two cycles.

Results

Maximum load at failure was higher in the 4ST+3GR group (967.4 ± 192.1 N vs. 777.02 ± 152.6 N; p = 0.002; Cohen's d = 1.10).

Diameter did not differ significantly (8.09 ± 0.81 mm vs. 7.59 ± 1.03 mm; p = 0.110).

Elongation and penultimate-cycle stiffness did not differ, whereas final-cycle stiffness was higher in the 4ST+3GR group (p = 0.010).

Conclusions

In this porcine model, tripling rather than doubling the gracilis increased graft load at failure by approximately 25% without compromising elongation or stiffness. When gracilis length allows, we advocate to consider the seven-strand configuration.

Introduction

Anterior cruciate ligament (ACL) rupture is one of the most common ligamentous knee injuries, with a reported annual incidence of 68.6 per 100,000 person-years, and the number of ACL reconstructions

(ACLR) performed has increased in recent years [1]. ACL reconstruction surgery can be performed using a wide range of graft types, including autografts, allografts, and synthetic grafts. The selection of grafts remains a subject of discussion within the field of orthopaedic sports medicine, with each method having its own advantages and disadvantages [2]. Bone–patellar tendon–bone (BPTB) autograft has historically been regarded as the "gold standard", however, hamstring tendon (HT) autograft has become one of the most frequently used options, accounting for almost half (48%) of primary ACLR graft choices among surgeons surveyed by the ACL Study Group in 2023 [3]. A Numerous HT graft preparation techniques have been described, using either the semitendinosus (ST) tendon alone or both the ST and gracilis (GR) tendons [4,5]. During the graft preparation process, harvested tendon is usually folded multiple times to obtain desired size. In instances where the Semitendinosus tendon alone fails to produce an adequate graft diameter, even when quadrupled, addition of gracilis tendon can be considered [6].

Obtaining a sufficient graft diameter is considered vital for successful, long term ACL reconstruction. Biomechanical data show that tensile strength increased with graft diameter across 6-,7-, 8- and 9-mm grafts (up to 6-stranded grafts) [7]. Revision rate after using a graft diameter of less than 8mm has been reported to be up to 7.2 times higher [8]. A minimum tendon length of 24 cm is recommended before quadrupling. The ST usually meets this requirement, whereas the shorter GR frequently cannot be quadrupled [9]. In such cases, the GR is commonly doubled and its surplus length discarded, producing a six-strand (4ST+2GR) graft. If the GR is at least approximately 75% as long as the ST, it can instead be tripled, producing a seven-strand (4ST+3GR) graft that uses tendon tissue which would otherwise be discarded and that could increase graft diameter and tensile strength. To our knowledge, this configuration has not previously been evaluated biomechanically.

The aim of this study was to compare the biomechanical properties of the 4ST+3GR graft with those of the standard 4ST+2GR graft in a porcine model. We hypothesised that the 4ST+3GR graft would have a larger diameter and a higher load at failure, without inferior elongation or stiffness.

Methods

1. Materials

Porcine forelimbs (n=9) were obtained from local slaughter house. Four extensor tendons from each leg were harvested (Figure 1), cleared from all adherent tissue, covered in a sterile, saline-soaked gauze and then vacuum sealed, and stored at -20°C until graft preparation. On the day of testing, the tendons were thawed to room temperature and used immediately.

2. Graft Preparation

Each forelimb provided two wider and two narrower extensor tendons. For each graft, one wider tendon (ST substitute) and one narrower tendon (GR substitute) were paired, creating one 6-strand and one 7-

strand graft from single forelimb. Each group consisted of 18 tendon pairs, producing 18 grafts per group (Fig. 2).

Graft configurations:

Control Group (4ST + 2GR) – Semitendinosus tendon substitute was folded over first metal loop simulating the use of a suspensory device for an all-inside ACL reconstruction technique. The GR substitute was cut to half the length of the ST (the excess was discarded) and laid alongside the doubled ST. The doubled ST and the single GR strand were then folded together over a second metal loop, producing a six-strand graft with a quadrupled ST and a doubled GR. Both ends of the graft were whipstitched 4 times using PremiCron N.0 suture (B. Braun Surgical, S.A., Rubi. Spain) passing through all strands (Fig. 3).

Test Group (4ST + 3GR) – Gracilis tendon substitute was cut to approximately 75% of the length of the ST substitute. Both tendons were laid together and passed back and forth between two metal loops, simulating the two suspensory devices of an all-inside ACLR technique, producing a single graft with a quadrupled ST and tripled GR. Both ends of the graft were whipstitched 4 times using PremiCron N.0 suture (B. Braun Surgical, S.A., Rubi. Spain) passing through all strands, creating a 7-strand final graft (Fig. 3).

3. Biomechanical Testing

Metal loops of both graft ends were securely fastened into the grips of Zwick-Roell Z020 (Zwick-Roell Gmbh Co. Kg, Ulm, Germany) Universal Testing Machine. Two points were marked on both ends of the graft at the level of the stitches for elongation measurements. This ensured that deformation of the metal loops was not taken into the account. During testing elongation was measured using videoXtensometer (Zwick-Roell Gmbh Co. Kg, Ulm, Germany – a dedicated high-speed camera calculating the change in distance between two markers (Figure 4). Each graft was preloaded to 80 newtons (N), and the diameter was subsequently measured at the midpoint of the graft using an electronic caliper. Each specimen was cyclic loaded with force incrementally increased by 25 N per cycle. During each cycle, the specimen was loaded to the peak force of a given cycle and subsequently returned to its initial position, constituting one complete cycle. A dwell time of 1 second was applied at both the maximum load and the unloaded state to ensure stabilization of the specimen before the subsequent phase. Crosshead displacement rate was 20 mm/min. Test was run until graft failure which resulted in no possible increase in max load. The data was collected using TestExpert III – V1.7 (Zwick-Roell Gmbh Co. Kg, Ulm, Germany).

4. Statistics and data analysis

The data were collected using Excel 2016 (Microsoft Corporation, Redmond, WA, USA). All statistical analysis was performed using Jamovi (Version 2.6) [Computer Software] (Sydney, Australia).

Max load was reported as the highest load in newtons, reached by a sample during all cycles prior to breakage, as read from the load/elongation curve.

Elongation was measured as the percentage difference between the length of the sample when preloaded with a force of 80 N and the maximum length of the sample reached during the test. The diameter of the sample was measured and subsequently, the surface area was calculated under the assumption that each sample possesses a circular cross-section. The pull-out stiffness, corresponding to the value of load at its inflection point, was calculated for the penultimate (last cycle without the graft breakage) and the final cycle (cycle which resulted with the graft breakage). The position of the inflection point was estimated as follows. First, to reduce the influence of sharp noise and missing values, the load (N) versus elongation (%) curve was smoothed using a Savitzky–Golay filter with an 11-point window length and a third-order polynomial. The position of the maximum gradient of the smoothed curve (d[Load]/d[Elongation]) was then taken as the position of the inflection point.

Prior to inferential testing, all continuous variables were assessed for normality using the Shapiro-Wilk test applied independently to each group. The equality of variances was evaluated using Levene's test for variables that met the assumption of normality. For variables demonstrating a normal distribution with equal variances, between-group comparisons were performed using the independent samples Student's t-test. For variables violating the normality assumption, the non-parametric Mann-Whitney U test was applied. The level of statistical significance was set at $\alpha = 0.05$ for all analyses. The effect size was reported as Cohen's d for parametric comparisons and as rank-biserial correlation (r) for non-parametric comparisons.

Results

Maximum load at failure was significantly lower in Group A (777.02 ± 152.58 N) compared to Group B (967.36 ± 192.12 N), with a mean difference of 190.34 N (Fig. 5). Equality of variances was confirmed prior to analysis (Levene’s test, $p = 0.357$). An independent samples Student’s t-test demonstrated a statistically significant difference between groups ($t(34) = -3.29$, $p = 0.002$), with a large effect size (Cohen’s d = -1,10).

No statistically significant difference in graft diameter was observed between Group A (7.59 ± 1.03 mm) and Group B (8.09 ± 0.81 mm; $t(34) = -1.63$, $p = 0.110$) (Fig. 6).

No statistically significant difference in elongation at maximum load was observed between Group A (median 3.27% [IQR 2.18–7.10]) and Group B (median 4.27% [IQR 3.11–6.31]; $U = 158.00$, $p = 0.913$, $r = 0.02$) (Fig. 7). The rank-biserial correlation indicated a negligible effect size, suggesting the two preparation methods produced near-identical elongation behaviour at failure.

No statistically significant difference in pull-out stiffness at the penultimate loading cycle was observed between Group A (median 41163.49 N/ratio [IQR 34790.82–85602.23]) and Group B (median 64403.14 N/ratio [IQR 44899.27–93422.30]; $U = 119.00$, $p = 0.181$, $r = 0.27$) (Fig. 8). Although statistical

significance was not reached, the rank-biserial correlation indicated a small-to-medium effect size, with Group B demonstrating consistently higher pull-out stiffness values. The considerable variability observed within Group B (SD = 73048.13) compared to Group A (SD = 31692.16), supported by unequal variances between groups (Levene's test, p = 0.035), likely contributed to the reduced statistical power of this comparison.

Pull-out stiffness at the final loading cycle was significantly greater in Group B (median 81117.40 N/ratio [IQR 39497.02–129757.72]) compared to Group A (median 37220.68 N/ratio [IQR 25301.19–48618.19]; U = 81.00, p = 0.010, r = 0.50) (Fig. 9). The rank-biserial correlation indicated a large effect size, with Group B demonstrating a median pull-out stiffness approximately 118% greater than Group A at the final cycle. The substantial variability observed within Group B (SD = 135700.43) relative to Group A (SD = 32060.50), corroborated by unequal variances between groups (Levene's test, p = 0.015), further supports the use of non-parametric analysis for this parameter.

Discussion

The main finding of this study is that tripling, rather than doubling, the gracilis increased the load at failure of an all-inside hamstring graft by approximately 25% (190 N), with a large effect size, without compromising elongation or stiffness. Contrary to our hypothesis, the 0.5-mm increase in mean graft diameter was not statistically significant.

A minimum graft diameter of 8 mm has been commonly mentioned in the orthopaedic community as a critical threshold, below which the risk of reconstruction failure increases substantially. Indeed, published data indicate a significantly higher failure rate associated with graft measuring less than 8 mm in diameter, underscoring the clinical importance of achieving adequate graft size prior to implantation [10,11,12]. In contrast, Mirzayan et al., in an analysis of 5972 primary ACLRs, found a 32% higher risk of revision for smaller grafts but could not identify a definitive diameter threshold [13]. A meta-analysis by Itoh et al. showed that each 0.5-mm increase in graft diameter was associated with a measurable improvement in graft survival [14] — a difference of the same magnitude as that observed between our groups. However, larger is not always better: grafts larger than 9 mm have been associated with a higher incidence of postoperative cyclops lesions (7.7% vs. 4.2%; p = 0.042) [15], and in smaller, lighter female athletes, grafts of 8 mm or less were not associated with higher failure rates than larger grafts [16]. These findings suggest that graft diameter should be individualised rather than uniformly maximised.

When graft diameter is insufficient, adding strands — most commonly by including the gracilis — is a common solution in ACLR. We hypothesised that the addition of another strand would result in an increased graft diameter, which was not By contrast, Wan et al. reported a significantly larger diameter with a five-strand (3ST+2GR) than with a four-strand (2ST+2GR) hamstring graft [17]; however, theirs was a clinical study, in which tensile strength could not be assessed. A likely explanation for this discrepancy is the smaller relative gain in cross-sectional area in our model. Converting a four-strand graft into a five-strand graft increases the number of strands by 25%, whereas adding one strand to a six-strand graft

increases it by only about 17%. Because diameter scales with the square root of cross-sectional area, the expected increase in diameter in our model is only about 5–8% (approximately 0.4–0.6 mm for a 7.6-mm graft). The observed difference of 0.5 mm is consistent with this estimate, suggesting that the lack of statistical significance reflects limited power rather than the absence of an effect.

A number of biomechanical studies have previously been conducted on the load-to-failure of graft preparation methods. In a particular study, 4- (4ST) and 6- (4ST + 2G) grafts were compared. The use of additional ripstop suturing in the 6-stranded group was found to result in a significantly higher tensile strength [18]. In contrast to the findings of the present study stands Broadhead et al. animal model study comparing 4- vs 5-strand grafts [19]. In their aforementioned study, no statistical significance was demonstrated in load to failure, and stiffness between both groups. Contrary to this results, in our study, pull out stiffness in the final elongation cycle has been statistically significantly higher in favour of the 3-stranded gracilis group. However, no statistically significant difference has been shown in the stiffness calculated from the penultimate cycle. This progressive divergence in stiffness as loading approached failure may suggest that 4ST+3G grafts maintain superior structural integrity under near-failure loading conditions, a finding that is consistent with the significantly greater maximum load at failure observed in its group. However, stiffness measured during the failure cycle partly reflects progressive strand damage and should be interpreted with caution.

In our intraoperative experience, the harvested gracilis is frequently long enough to be tripled for an all-inside graft. A minimum tendon length of 24 cm has been recommended for a tripled hamstring graft, resulting in an 8-cm construct [4]. In the 4ST+3GR configuration, however, the graft length is determined by the quadrupled ST, so the GR needs to be only three times the final graft length (approximately 75% of the ST length); for example, a 6-cm graft requires a GR of at least 18 cm. Pereira et al. reported a mean intraoperative GR length of 25.28 ± 3.81 cm (range 14–34 cm) [20]. Ilahi et al. compared preoperative magnetic resonance imaging estimates with ex vivo measurements and reported a mean harvested GR length of 22.7 ± 2.8 cm [21], and Dietvorst et al. reported a median intraoperative GR length of 27.0 cm (IQR 25.0–29.0) in adolescents [22]. These data suggest that tripling the GR is technically feasible in most patients.

Limitations

The primary constraint of this study is that it was conducted using an animal model. Due to their immediate availability and lower cost biomechanical research is often performed on porcine tendons as a human tendons substitute [23,24,25]. Research by Domnick et al., demonstrated similar values of stiffness and high failure loads of porcine flexor tendons compared with human cadaveric semitendinosus tendons, deeming them suitable as substitute material [26]. Wichern et al. conducted a similar study comparing all-inside ACL reconstruction graft preparation techniques utilizing porcine extensor tendons, like in our research [27]. Since the primary objective of this study was to compare the mechanical performance of the two graft preparation methods, relative to one another, rather than to establish absolute load-to-failure benchmark, usage of the same substitute material for both groups was

justified. Nevertheless, our use of an porcine model is a limitation that must be noted. A further limitation of the present study is the relatively modest sample size of 18 specimens per group. This may have been insufficient to detect statistically significant differences in pull-out stiffness across all loading cycles, and could have inflated the true magnitude of maximum load advantage of group B. This is most evident in the penultimate cycle stiffness analysis, where a small-to-medium effect size ($r = 0.27$) was observed in the absence of statistical significance ($p = 0.181$), a pattern consistent with an underpowered comparison rather than a true absence of effect. While the final cycle stiffness analysis did achieve statistical significance ($p = 0.010$, $r = 0.50$), the progressive nature of this finding across final cycles raises the possibility that difference in the penultimate cycle may equally reach significance with a higher number of samples.

Conclusions

In this porcine model, increasing the number of gracilis strands from two to three in an all-inside semitendinosus–gracilis ACL graft model significantly increased the load at failure, without inferior elongation or stiffness. The 0.5-mm increase in mean graft diameter was not statistically significant. We advocate that when the gracilis is long enough, tripling it rather than doubling it and discarding the remainder may be considered to obtain a stronger graft.

Declarations

Author Contribution

"M.D. wrote the main manuscript text and prepared figures. M.C prepared the study plan and supervised the process. Authors M.D., K.G. contributed in preparing the specimens. Authors P.K. and A.Z. conducted the testing and contributed in data analisys. All authors reviewed and accepted the final version of the manuscript.

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Data Availability

The datasets generated and analysed during this study are not publicly available because this was a biomechanical laboratory study on porcine tissue and not a clinical study, and no patient data were collected. The data are available from the corresponding author upon reasonable request.

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Figures

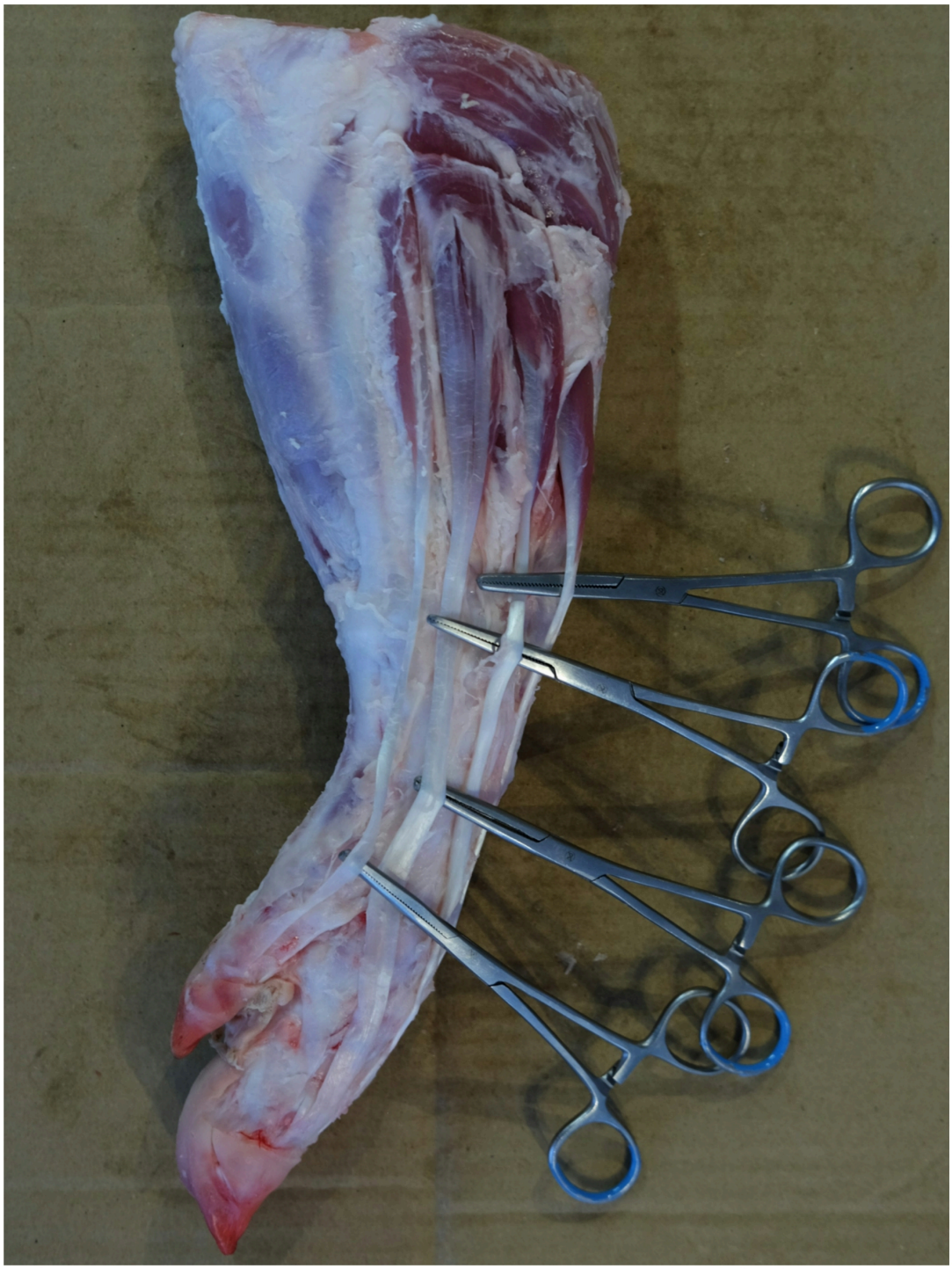


Figure 1

Porcine forelimb with extensor tendons prepared for harvesting.

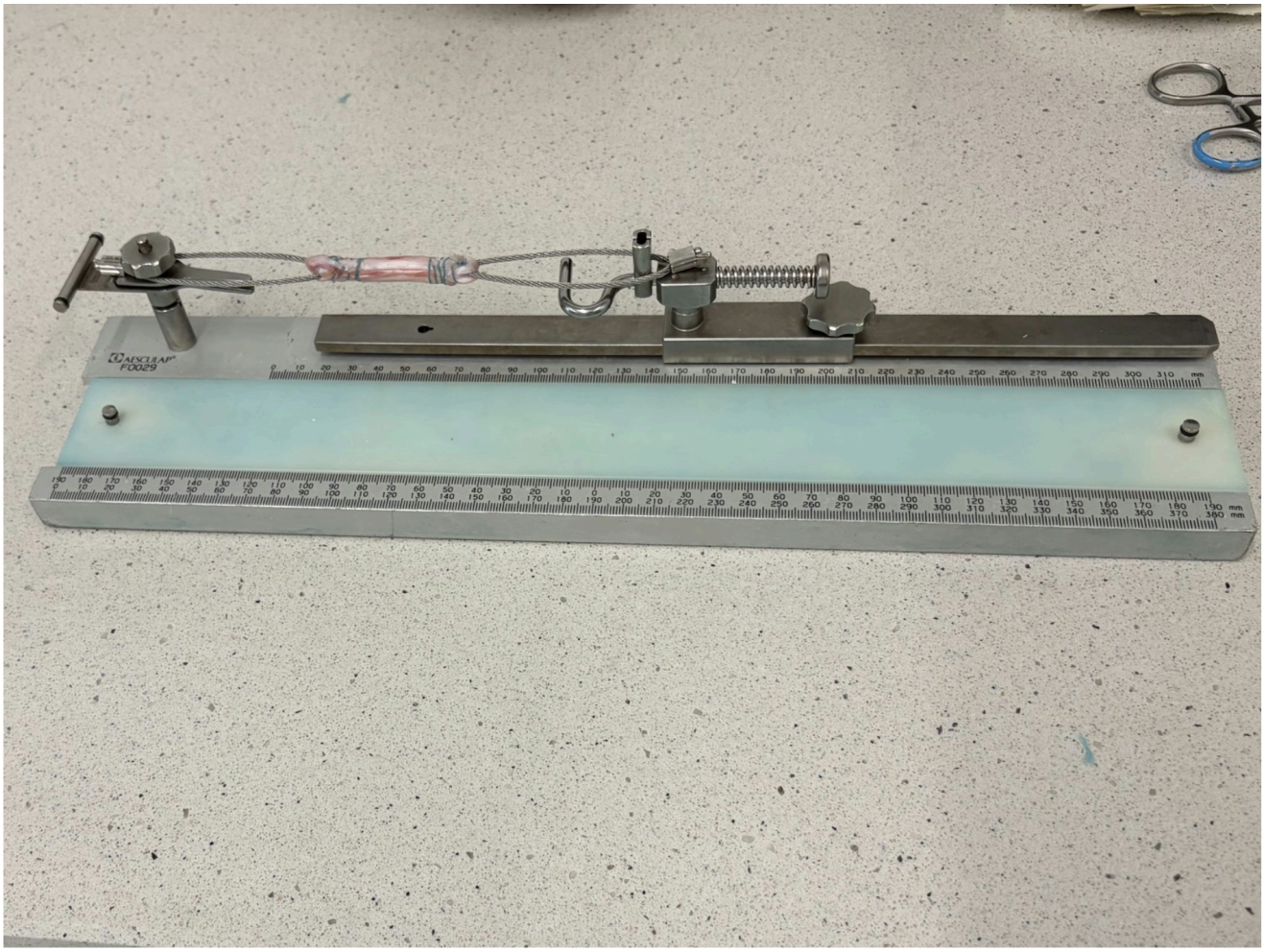


Figure 2

Finished graft with metal loops simulating suspensory devices, secured on the ACL graft preparation board

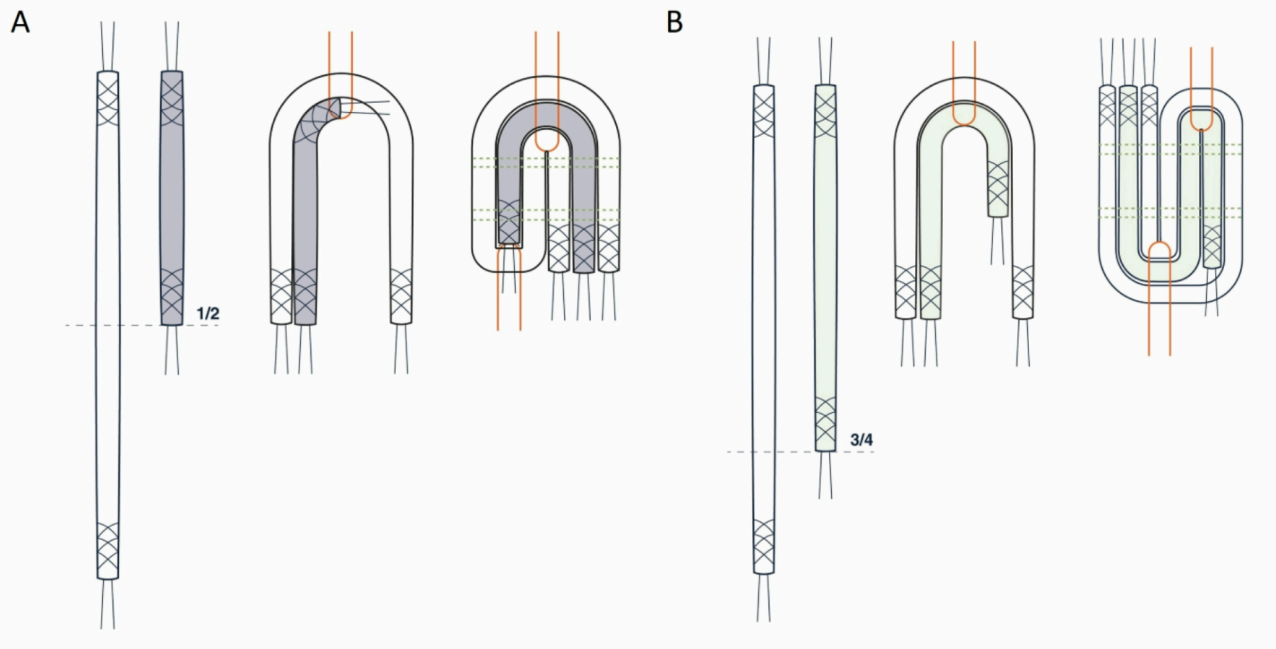


Figure 3

Graft preparation methods diagram. Tendon ends are whipstitched to allow for proper graft tensioning. Graft A – quadrupled Semitendinosus and doubled Gracilis tendons. Gracilis tendon of ½ length of the Semitendinosus tendon. Tendons are folded over two suspensory devices. Graft B - quadrupled Semitendinosus and tripled Gracilis tendons. Gracilis tendon of ¾ length of the Semitendinosus tendon. Tendons are folded over two suspensory devices. Additional whipsitch sutures passing through all the strands of the grafts at both ends.

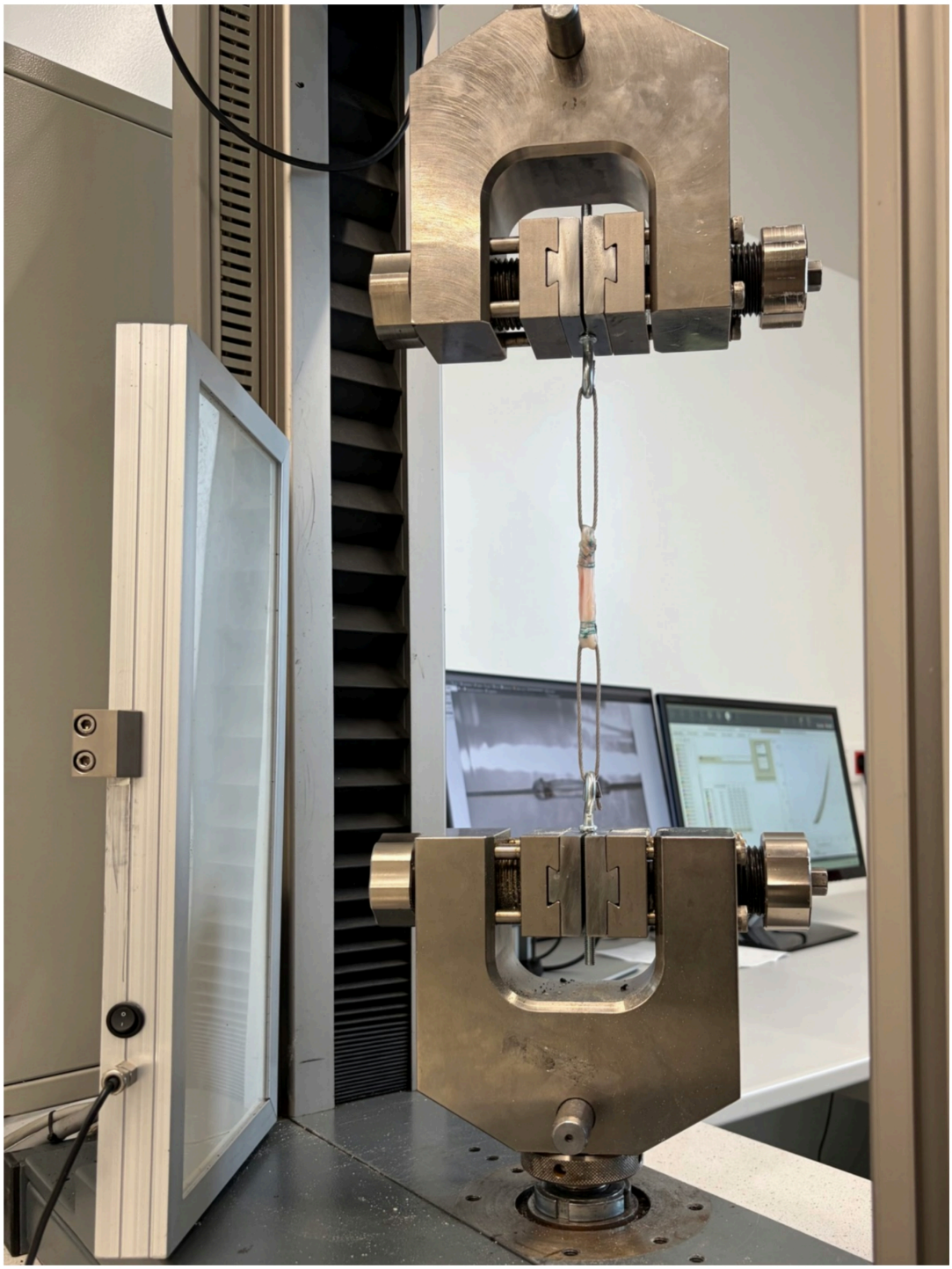


Figure 4

Graft during testing, with the metal loops securely fastened into the grips of Zwick-Roell Z020 Universal Testing Machine. High speed camera recording the graft elongation.

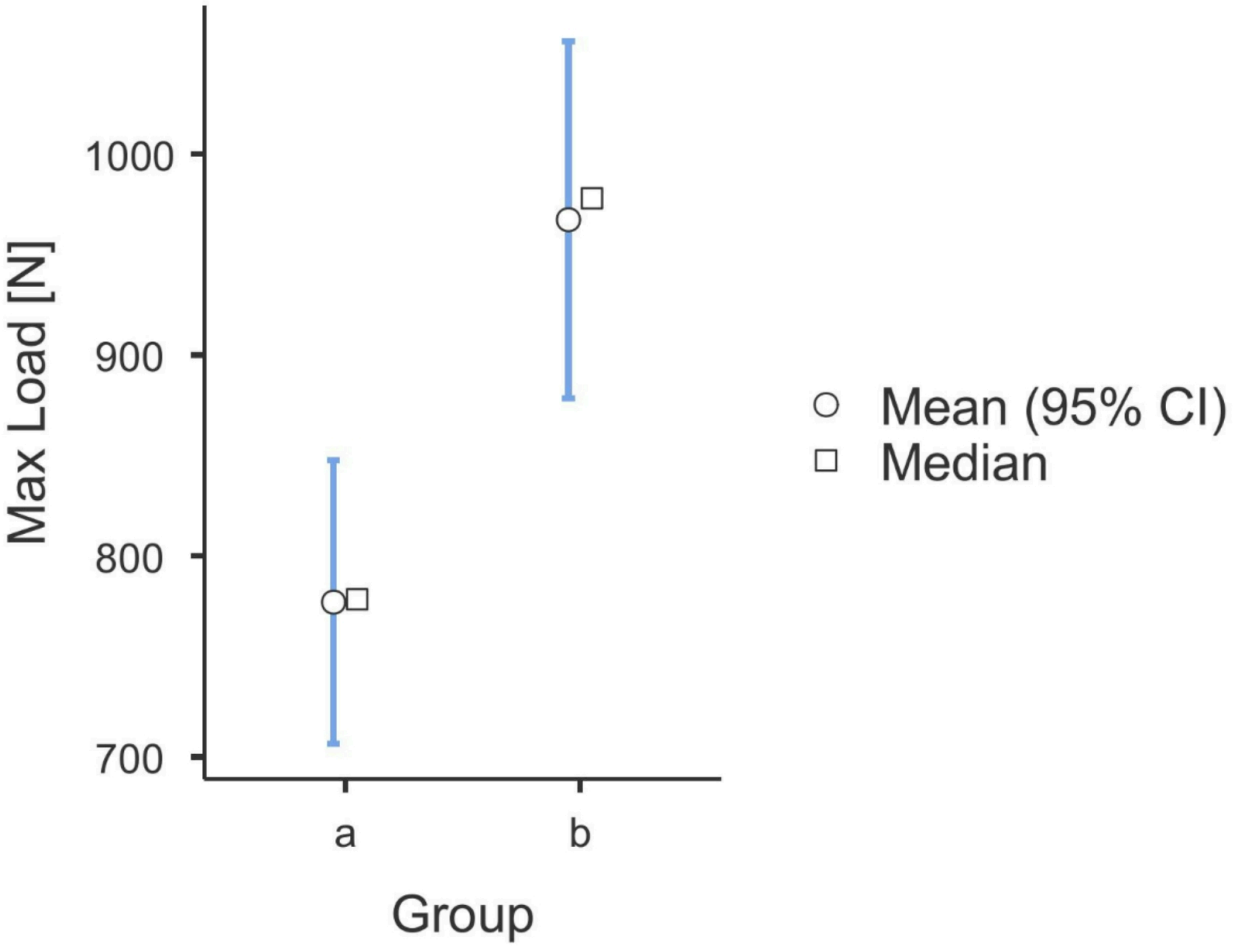


Figure 5

Maximum load achieved during all testing cycles in newtons. Group A – 4ST + 2G, Group B – 4ST + 3 .

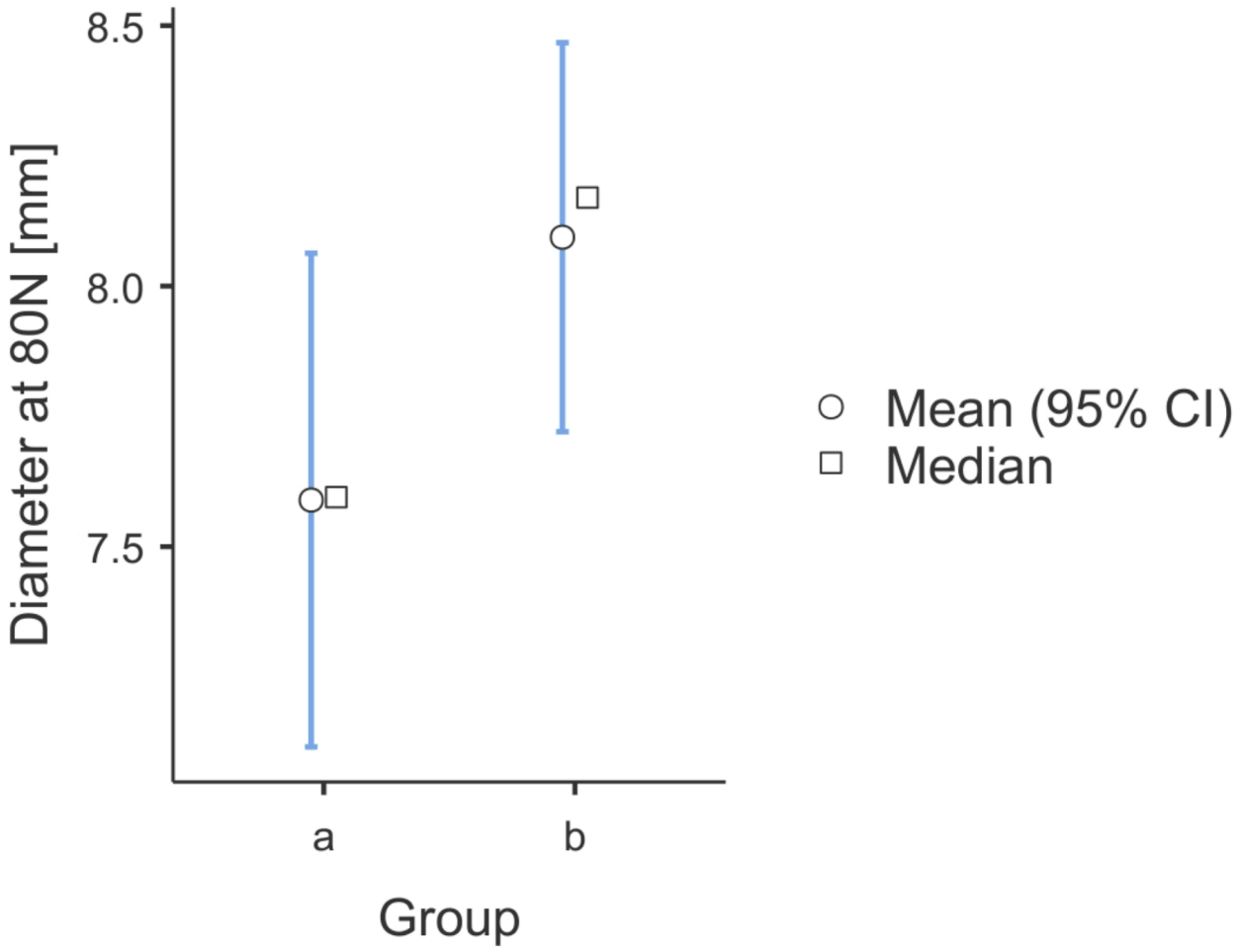


Figure 6

Diameter of the final graft (in mm), measured at the midpoint of the graft at 80N of load. Group A – 4ST + 2G, Group B – 4ST + 3.

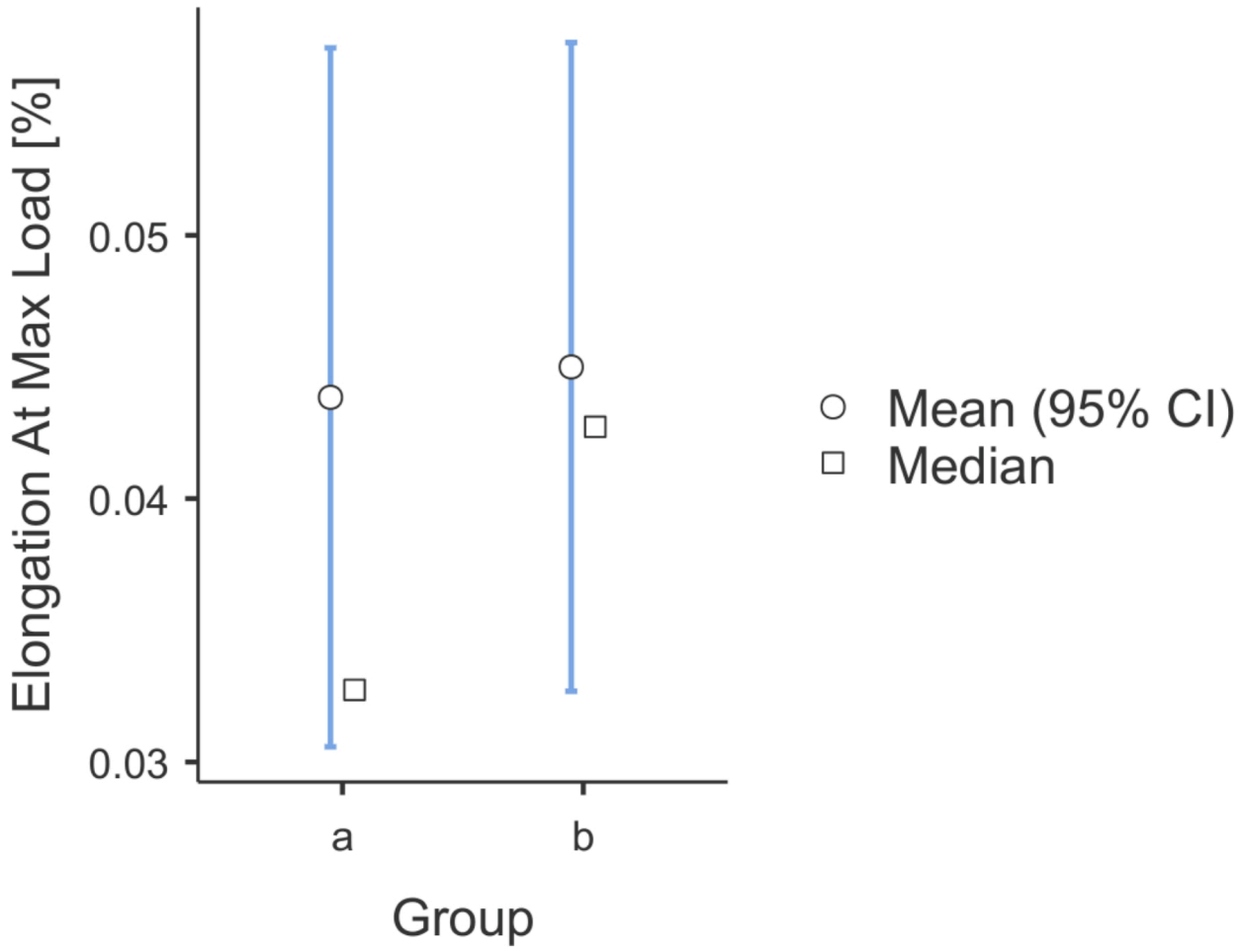


Figure 7

Elongation, in percent of elongation of the midportion of the graft observed at maximum load achieved

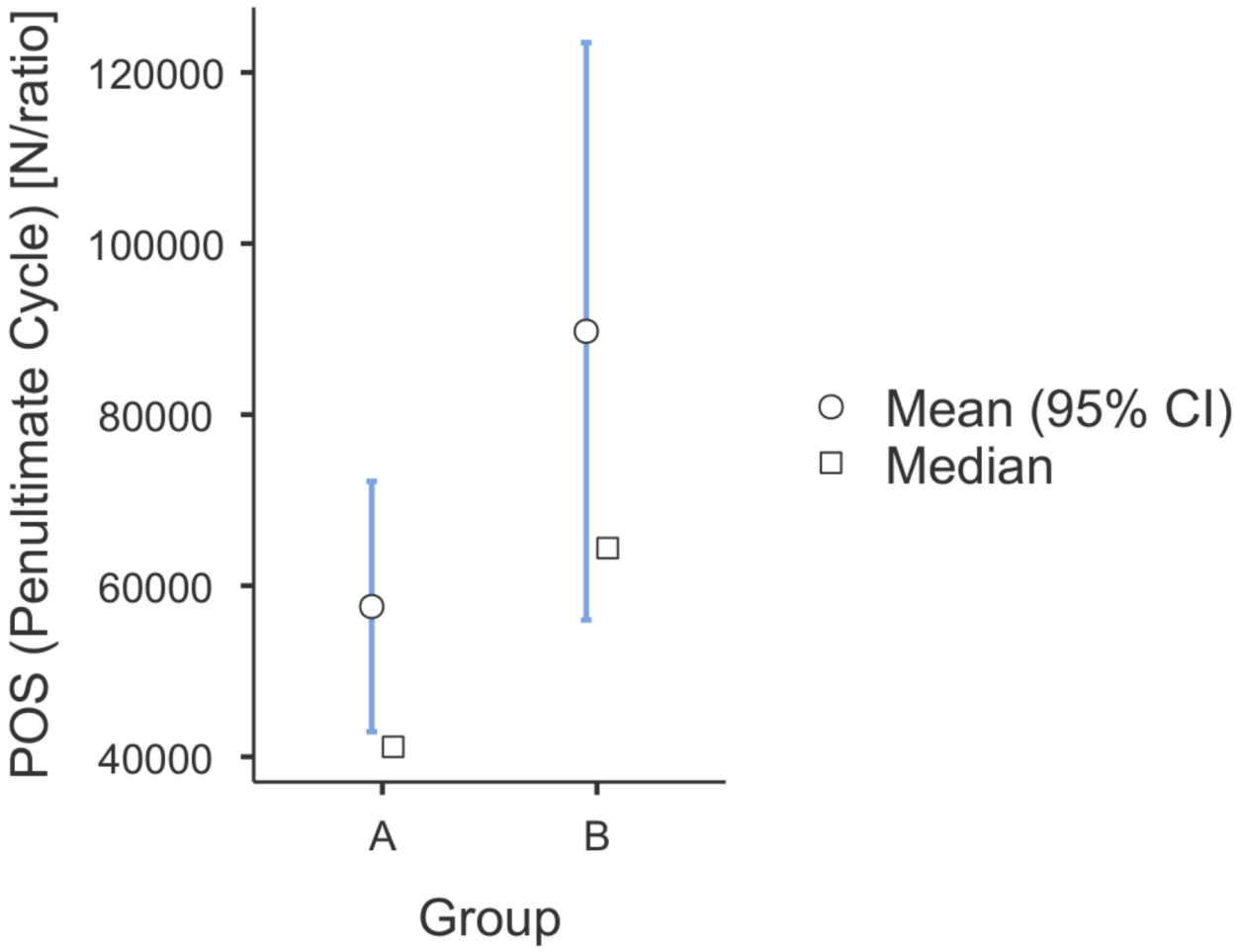


Figure 8

Pull-out stiffness (POS) [N/ratio], corresponding to the value of load at its inflection point, of the penultimate cycle.

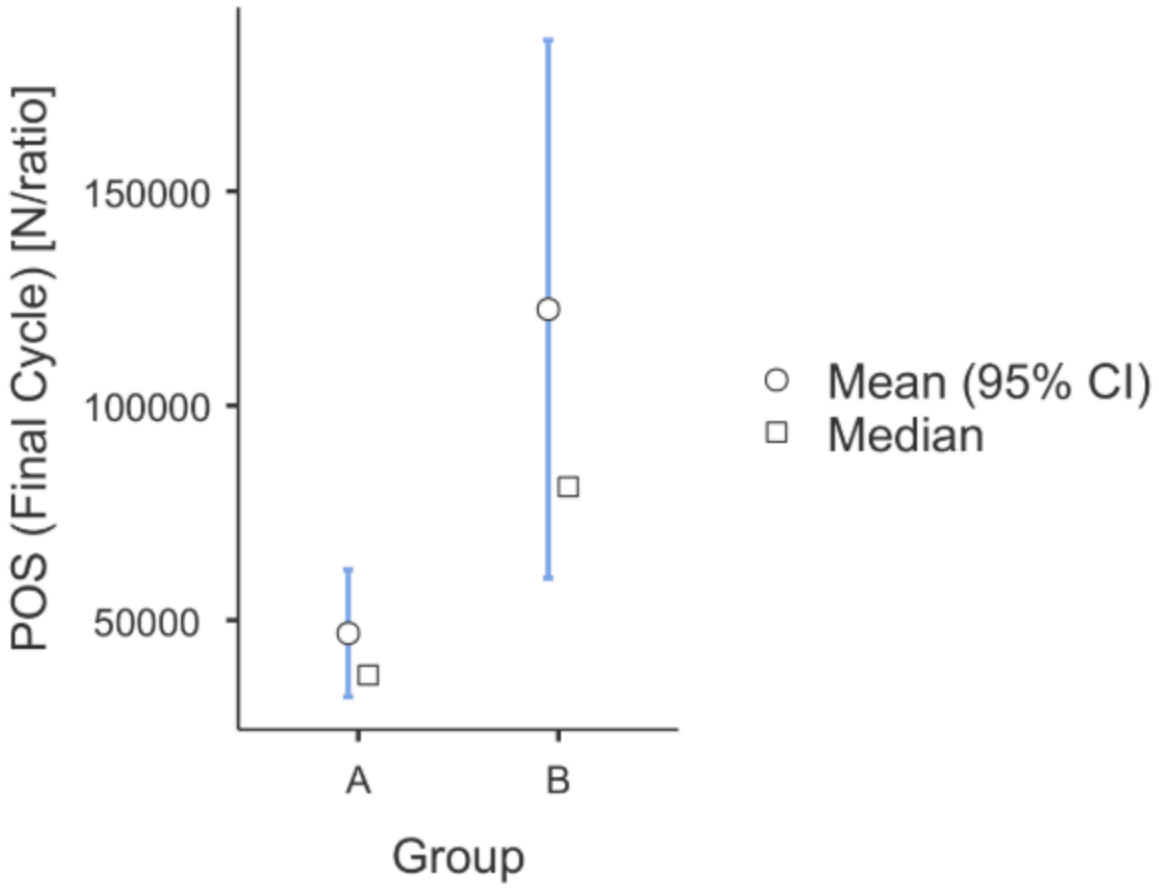


Figure 9

Pull-out stiffness (POS) [N/ratio], corresponding to the value of load at its inflection point, of the final cycle