# Supplemental Fixation of Inner Graft Limbs in All-Inside, Quadrupled, Single-Tendon Anterior Cruciate Ligament Reconstruction Graft Construct Yields Improved Biomechanical Properties

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Purpose: To compare the time-zero load to failure of a quadrupled, single-tendon, all-inside anterior cruciate ligament (ACL) reconstruction graft construct with (supplemented) and without the incorporation of inner-limb whipstitch sutures (control) into a tibial suspensory fixation button. Methods: Eight matched pairs of peroneus longus tendons were prepared according to a quadrupled, all-inside ACL soft-tissue graft technique with 1 side serving as a control and the contralateral side supplemented. The constructs were biomechanically tested for strain in the inner and outer limbs during a preconditioning protocol, single-cycle load to failure, and elongation of the whole construct. Results: Ultimate load to failure was significantly higher in the supplemented group:  $797.5 \pm 49.6 \text{ N}$  (95% confidence interval [CI], 763.13-831.87 N) versus 719.6  $\pm$  69.6 N (95% CI, 671.38-767.82 N; P = .044). Less graft elongation at failure was observed in the supplemented group (3.1  $\pm$  1.5 mm; 95% CI, 2.07-4.17 mm) versus the control group (21.0  $\pm$  21.2 mm; 95% CI, 6.31-35.69 mm; P = .052). The number of grafts undergoing a 5-mm or greater change in length at failure was 1 of 8 in the supplemented group versus 5 of 8 in the control group (P = .038). Conclusions: Inner-limb supplemental tibial fixation results in higher time-zero load to failure and decreased graft elongation in a quadrupled, single-tendon, all-inside ACL reconstruction graft construct. Clinical Relevance: The weak point of a single-tendon, quadrupled, all-inside ACL graft construct is the tendon-to-tendon suturing to secure the inner limbs of the graft. Adding supplemental fixation by incorporating the sutures from the inner limb to the tibial suspensory fixation button leads to a higher time-zero load to failure and decreased graft elongation.

Gracilis and semitendinosus tendons have been commonly used as autograft tissue in the setting of anterior cruciate ligament (ACL) reconstruction. In their most common preparation, both tendons are doubled over (or folded in half), yielding the final graft construct used for reconstruction. The optimal strength

of this construct has been established at 4,090 N, occurring with symmetrical tensioning across all individual limbs, whereas asymmetrical tensioning has shown lower ultimate loads to failure (ULFs). Although the ULF of this construct exceeds that of the native ACL, studies have shown that a resultant graft

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diameter of 8 mm or less may lead to a greater risk of graft failure.<sup>2,3</sup> This has led to the development of innovative graft preparation techniques that aim to use the same tissue but are configured in a way to obtain a greater graft diameter. One of the more commonly used techniques was described by Lubowitz.<sup>4</sup> This technique involves quadrupling a single tendon by whipstitching the 2 free ends together and securing them inside 1 of the looped ends of the graft with 4 cerclage sutures, allowing for the use of cortical suspensory fixation on either end for an all-inside technique.

Although these techniques do provide a greater graft diameter, biomechanical studies have identified the tendon-to-tendon suturing of the inner graft limbs as the potential weak link of the construct, as well as differences in strain across the limbs of the graft, suggesting asymmetrical tensioning in the construct. <sup>5,6</sup> A recent modification has been made incorporating the inner-limb whipstitch sutures into the tibial fixation, <sup>7,8</sup> foreseeably supplementing the construct. This has been achieved through additional suture anchor fixation into the tibia or simply tying these sutures over the tibial suspensory button.

The purpose of this study was to compare the time-zero load to failure of a quadrupled, single-tendon, all-inside ACL reconstruction graft construct with (supplemented) and without the incorporation of inner-limb whipstitch sutures (control) into a tibial suspensory fixation button (TightRope ABS; Arthrex, Naples FL). Our hypothesis was that supplemental fixation of the inner graft limbs would improve the biomechanical properties of the construct, specifically load distribution within the limbs during graft preparation, time-zero load to failure, and graft elongation.

### Methods

Eight same-donor matched pairs of peroneus longus tendons were acquired from UMTB-Vivex (Miami, FL) (Table 1). Peroneus longus tendons have been shown to have biomechanical properties equivalent to semitendinosus tendons, making them a suitable replacement. All tendons were procured and sterilized in a similar manner per UMTB-Vivex protocol. All grafts were kept in a freezer until preparation and testing were to be performed, at which point they were thawed at room temperature. All graft preparation and testing were carried out over 2 consecutive days, and no freeze-thaw cycles were carried out. All grafts were kept moist with saline solution and refrigerated between preparation and testing. The grafts were prepared using the technique described by Lubowitz<sup>4</sup> by a sports medicine fellowship-trained orthopaedic surgeon (M.G.B.) and were randomly assigned to the control or experimental group after preparation. Cortical suspensory fixation devices were used on both ends to be tested on a traditional load frame; TightRope

RT (Arthrex) was used for the femoral suspensory fixation, and TightRope ABS was used for the tibial suspensory fixation. For all constructs, the end at which the free limbs were secured was referenced as the tibial fixation side. Tendons were passed through the tibial and femoral TightRope loops into a 4-strand configuration. The free ends of the graft were whipstitched with No. 2 FiberWire suture (Arthrex) (Fig 1). This portion is referred to as the "inner limb." This was pulled inside the looped end of the graft predetermined to be the tibial side of the construct. While 20 N of tension was held through these sutures, 2 cerclage stitches were placed using No. 0 FiberWire suture, ensuring passage through all 4 limbs of the tendon, and were wrapped circumferentially. This was repeated on the femoral side. All tendons were prepared whole, without trimming or altering the structure of the matched pair. Matched pairs were identically prepared, with the exception of the supplemented group maintaining the No. 2 FiberWire sutures from the inner-limb whipstitch to be tied over the tibial suspensory fixation button. These sutures were routed around the tibial looped end of the graft to be tied over the ABS button in the experimental group. The control group did not have these incorporated as per the referenced technique.

The diameter of each graft was measured with a slotted graft-sizing tool (Arthrex). This measurement was then verified using a caliper. Initial graft length, as well as graft length after load-to-failure testing, was also measured using a caliper. Each construct was suspended by its buttons from custom-slotted plates gripped in an Instron E3000 servo-electric testing system (Instron, Norwood, MA). The custom-slotted plates allowed placement of the cortical suspensory buttons for the corresponding femoral and tibial fixation ends of the grafts. The suspensory devices were left at base length and not cinched to decrease variability in potential slippage<sup>10</sup> (Fig 2).

We applied 3 digital variable reluctance transducers (DVRTs; MicroStrain, Williston, VT) separately to the graft limbs-1 to the first outer graft limb, 1 to the second outer graft limb, and 1 to the inner graft limb—to measure the mechanical strain in each graft limb (Fig 3). This was measured during a preconditioning protocol including values used commonly during graft preparation and preconditioning to verify whether there was a particular preconditioning load level that might evenly distribute the loads between the limbs of the construct. A 20-N tensile force was then applied to the construct in load-control mode to establish a baseline strain level. Once at a steady state, a computer started recording the voltages from the 3 DVRTs. For the supplemented group, the sutures from the inner-limb whipstitch were tied over the tibial suspensory fixation button using alternating halfhitches, whereas they were left untied and cut for the

Table 1. Graft Characteristics

	Tendon	Construct	Construct			
Graft	Length,	Diameter,	Length,		Donor	Donor
No.	mm	mm	mm	Supplemented	Age, yr	Sex
la	310	11.5	75	Yes	62	M
1b	310	11.5	78	No		
2a	300	12	76	Yes	25	M
2b	300	12	76	No		
3a	270	11	71	Yes	27	M
3b	270	11.5	70	No		
4a	310	11	77	No	55	M
4b	310	11	74	Yes		
5a	300	10.5	80	Yes	23	F
5b	300	11	75	No		
6a	310	10	80	Yes	23	M
6b	310	10	78	No		
7a	310	11.5	83	Yes	55	M
7b	310	12	85	No		
8a	330	12.5	86	Yes	61	M
8b	330	12.5	86	No		

F, female; M, male.

nonsupplemented group. The force on the construct was increased to 40 N at a rate of 4 N/s and held there for 10 seconds. The force was increased to 60 N and held for 10 seconds and subsequently increased to 80 N and held for 10 seconds. The DVRT recording was then stopped, and the DVRTs were removed from the specimens.

The specimens were loaded to failure in displacement-control mode. The load and displacement were both recorded for the single cycle to failure. From the load-displacement curve, the peak load was identified along with any evidence of yield load. The slope of the load-displacement curve was measured to evaluate the structural stiffness of the construct. Each specimen test was recorded on video for subsequent visual analysis to correlate with mechanical measures. The specimens were evaluated for location of failure, as well as final graft construct length.

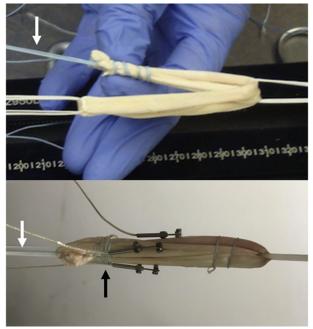
## **Statistical Analysis**

A power analysis was performed to determine the sample size, based on the studies by Petre et al. 11 and Noonan et al., 12 who found that a sample size of 4 was sufficient to detect significant differences in cyclic elongation and load to failure between suspensory fixation constructs at 80% power. We included 8 matched pairs, yielding 16 total samples. Our results were then analyzed using a 2-tailed paired t test to evaluate the differences between matched pairs. Statistical analysis was performed using the TTEST function in Microsoft Excel (Microsoft, Redmond, WA). Analyzed variables included ULF between matched pairs with and without supplemental fixation, final length after load-to-failure testing, and change in length from initial graft length. P < .05 was considered significant.

## Results

The mean graft diameter was  $11.25 \pm 0.80 \text{ mm}$  (95%) confidence interval [CI], 10.69-11.84 mm) for the supplemented constructs versus  $11.43 \pm 0.77$  mm (95% CI, 10.89-11.96 mm) for the control grafts (P = .0549), whereas the mean initial graft length for both supplemented and control grafts was 78.1 mm (78.1  $\pm$  5.0 mm in supplemented group and 78.1  $\pm$ 5.2 mm in control group). Strain values during the initial preconditioning protocol in the outer and inner graft limbs between the supplemented and control grafts did not show any statistically significant differences between groups (Table 2). There were at least 2 easily identifiable slopes on the load-displacement curves, representing a composite of resistance from the sutures, graft stretching, and any slippage in the graft construct (Figs 4 and 5).

Load to failure was higher for the supplemented grafts, at 797.5  $\pm$  49.6 N (95% CI, 763.13-831.87 N), versus 719.6  $\pm$  69.6 N (95% CI, 671.38-767.82 N) for the control grafts (P = .0442) (Table 3). The mean graft lengths after load-to-failure testing were 81.3  $\pm$  5.4 mm (95% CI, 77.56-85.04 mm) for the supplemented grafts versus 99.1  $\pm$  18.6 mm (95% CI, 86.22-111.98 mm) for



**Fig 1.** The blue No. 2 FiberWire sutures from the inner-limb whipstitch (white arrows) link the 2 free ends of the graft. These are used to pull tension through the construct for application of the tendon-to-tendon No. 0 FiberWire cerclage sutures (black arrow) that link all 4 strands. Two cerclage sutures are placed on the tibial and femoral sides. The No. 2 FiberWire sutures are tied into the tibial TightRope ABS button (supplemented group) or cut after placement of tendon-to-tendon No. 0 FiberWire cerclage sutures (control group).



**Fig 2.** Testing was performed on an Instron E3000 servo-electric testing system using custom-slotted plates (arrows) to allow placement of tibial and femoral suspensory buttons.

the control grafts (P=.041). This represented mean graft elongation of 3.1  $\pm$  1.5 mm (95% CI, 2.07-4.17 mm) for the supplemented grafts versus 21.0  $\pm$  21.2 mm (95% CI, 6.31-35.69 mm) for the control grafts (P=.052). When we considered a minimum 5-mm increase in construct elongation (or greater) after load-to-failure testing, 1 of 8 supplemented grafts and 5 of 8 control grafts exceeded this cutoff (P<.04,  $\chi$ -square test).

The observed location of failure varied between groups. In the supplemented group, 5 of 8 grafts failed by breakage of the femoral TightRope sutures whereas the rest failed by breakage of the tibial TightRope sutures. Tibial TightRope breakage was the mode of failure in 6 of 8 control grafts. The remaining 2 failures were due to graft pullout in which the inner limb of the graft pulled out from the construct after failure of the cerclage sutures whereas the suspensory fixation did not fail. There were no failures at the femoral fixation in the control group.

## **Discussion**

Our study shows that tying the inner-limb whipstitch sutures over the tibial suspensory fixation button in quadrupled, single-tendon, all-inside ACL reconstruction graft constructs increases the time-zero load to failure of

the construct and reduces elongation of the graft. The mean load to failure was  $797.7 \pm 49.6 \text{ N}$  (95% CI, 763.13-831.87 N) for the supplemented group versus  $719.6 \pm 69.6 \text{ N}$  (95% CI, 671.38-767.82 N, P = .0442) for their control pairs, which corresponds to a 10.85% increase. The potential clinical application of this information is that the initial strength of any graft construct is dictated by its weakest link, which lies in the strength of initial fixation. Graft constructs work as a temporary scaffold to support the integrity of the repair while the graft-tunnel remodeling process occurs. It is therefore beneficial to optimize construct strength until graft-tunnel remodeling finalizes. Our values are consistent with recent biomechanical studies that have found load-tofailure values for similar adjustable cortical suspensory device constructs ranging from 790.2  $\pm$  100.0 N to  $800.9 \pm 112.5 \text{ N.}^{13,14}$ 

As expected with our testing protocol, most failures occurred by breakage of the suspensory fixation sutures, yielding our ultimate load values. What we observed on video review in some grafts, however, was a change in graft length that occurred before failure of the TightRope sutures. This change in graft length was observed to occur from slippage of the inner limb of the graft. Although slippage between the inner limb and the cerclage sutures had to occur for the graft construct to lengthen, it was only classified as a failure of the



**Fig 3.** Prepared specimen with digital variable reluctance transducers attached to 2 outer limbs and 1 inner limb.

#### SUPPLEMENTAL INNER GRAFT LIMB FIXATION

Table 2. DVRT Strain Data

		Strain Value, %							
		Supplemented Group			Control Group				
	Inner Limb	First Outer Limb	Second Outer Limb	Inner Limb	First Outer Limb	Second Outer Limb			
Mean	2.96	48.23	113.80	3.31	84.36	78.50			
SD	2.97	56.35	160.60	8.05	119.84	118.07			
95% CI	0.91 to 5.01	9.19 to 87.27	2.52 to 225.08	-2.26 to 8.88	1.31 to 167.41	-3.31 to 160.31			

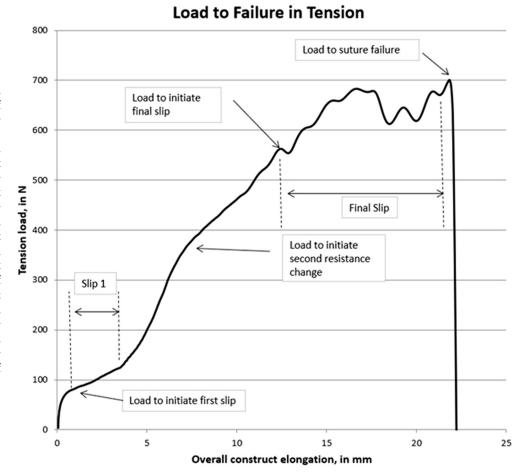
NOTE. The paired t test was performed between matched pairs and showed no significant differences in inner-limb strain (P = .921), first outer-limb strain (P = .669), and second outer-limb strain (P = .332).

cerclage sutures or graft slippage if the TightRope sutures remained intact. This occurred in 2 instances in the control group (Fig 6), whereas in the supplemented group, the graft was observed to recoil to nearly the original length after initial stretching and subsequent failure of suspensory fixation sutures (Fig 7). Whereas both groups had an initial mean length of 78.1 mm, the mean construct length after load-to-failure testing was  $81.3 \pm 5.4$  mm (95% CI, 77.56-85.04 mm) for the supplemented group versus  $99.1 \pm 18.6$  mm (95% CI, 86.22-111.98 mm) for the control group. This represents a change of  $3.1 \pm 1.5$  mm (95% CI,

2.07-4.17 mm) in the supplemented group versus  $21.0 \pm 21.2$  mm (95% CI, 6.31-35.69 mm) in the control group (P = .052). The location of failure also varied between supplemented and control grafts. Whereas 5 of 8 supplemented grafts failed by breakage of the femoral TightRope sutures, none of the control grafts failed at this location. Although the purpose of this study was not to test the adjustable cortical suspensory devices and hence these were left out to full length, this difference in the location of failure may indicate that the addition of the inner-limb sutures to the tibial fixation could improve the fixation strength at this site.

Fig 4. Graft tensile test in specimen 7a, without tying of the inner-limb sutures to the tibial suspensory button (control). Initial slippage occurs in the first 4 mm, beginning at approximately 80 N. The construct stabilizes for the next 4 mm and then begins the second resistance change, with visible inner band slippage at approximately 560 N. Load slippage continues to about 21 mm; then, final failure of the suture occurs after almost 23 mm of construct elongation at approximately 700 N. The final measured change in construct length, from before testing to after completion of

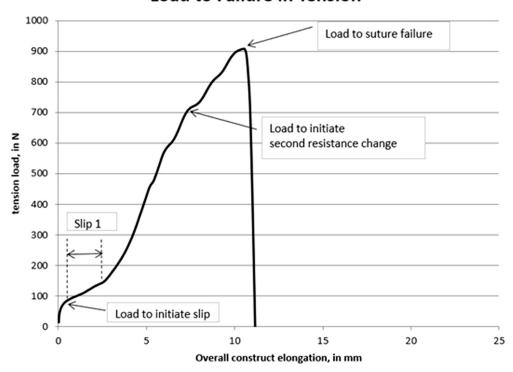
testing, is 12 mm.



CI, confidence interval; DVRT, digital variable reluctance transducer; SD, standard deviation.

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# **Load to Failure in Tension**



**Fig 5.** Graft tensile test in specimen 7b, with the inner-limb sutures tied over the tibial suspensory button. This graft and the control graft represented in Figure 4 comprise a matched pair. There is similarity in the behavior of the constructs before the major slip occurred. At 1 mm of elongation, at a load of approximately 80 N, a change in slope or resistance to load occurs. This is followed by a relatively linear region of resistance with a very similar slope up to 300 N in both constructs. Beyond 300 N, the control construct shows a shift in resistance, whereas the supplemented graft, depicted here, maintains a similar resistance to load and shows only minor evidence of slippage before suture failure at approximately 900 N. The final measured change in construct length, from before testing to after completion of testing, was 4 mm.

Considering that clinical failure of ACL reconstruction corresponds to a side-to-side difference of 5 mm on instrumented laxity testing, a graft elongation of this magnitude, even without a frank disruption of the tendon proper, could be detrimental. Thus, analyzing the data for a 5-mm or greater increase in construct length after load-to-failure testing found that 5 of 8 samples in the control group achieved or exceeded this length change. In the supplemented group, we found that only 1 of 8 samples had a 5-mm or greater change

in length. This change in length was seen in the analysis of the videos as slippage of the inner limb from the circumferential sutures holding it in place. In 2 grafts in the control group, failure occurred exclusively at this site without disrupting the suspensory fixation.

Unique to this particular graft construct is the addition of a potential additional weak link, which is the tendonto-tendon suturing to secure the inner limb of the graft. This does not fall clearly into the category of outright fixation failure, given that the elements holding the

Table 3. Composite Data per Group

	Supplemented Group	Control Group	P Value
Graft diameter, mean $\pm$ SD (95% CI), mm	$11.25 \pm 0.80 \; (10.69 \text{-} 11.84)$	$11.43 \pm 0.77 \ (10.89 - 11.96)$	P = .549
Load to failure, mean $\pm$ SD (95% CI), N	$797.5 \pm 49.6 \ (763.13-831.87)$	$719.6 \pm 69.6 \; (671.38-767.82)$	P = .0442
Initial graft length, mean $\pm$ SD (95% CI), mm	$78.1 \pm 5.0 \ (74.64-81.56)$	$78.1 \pm 5.2 \; (74.5 \text{-} 81.7)$	P > .999
Final graft length, mean $\pm$ SD (95% CI), mm	$81.3 \pm 5.4 \ (77.56-85.04)$	$99.1 \pm 18.6 \ (86.22 \text{-} 111.98)$	P = .041
Change in graft length, mean $\pm$ SD (95% CI), mm	$3.1 \pm 1.5 \ (2.07 - 4.17)$	$21.0 \pm 21.2 \ (6.31-35.69)$	P = .052
No. of grafts with $\geq$ 5 mm of elongation	1 of 8 (12.5%)	5 of 8 (62.5%)	P = .038
Mode of failure	Femoral TightRope breakage: 5	Femoral TightRope breakage: 0	P = .0007
	Tibial TightRope breakage: 3	Tibial TightRope breakage: 6	
	Inner-limb pullout: 0	Inner-limb pullout and/or cerclage	
		suture failure: 2	

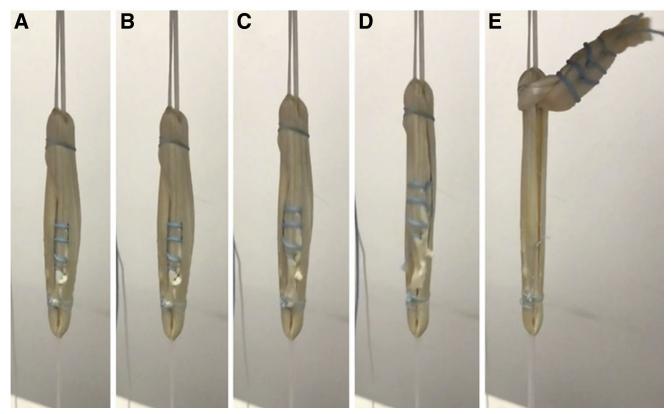
graft to the tibia and femur may remain intact despite failure at this site, nor may it be classified as outright graft failure, given that the tendon proper may remain completely intact. Slippage of the inner limb could foreseeably go undetected on imaging because the overall structure of the graft remains (Fig 8). The clinical importance of this information is that the weak point of an ACL reconstruction immediately after surgery is the fixation strength and not the graft itself. Although quadrupled hamstring grafts have been shown to have an ultimate tensile strength of 4,090 N, it is ultimately the graft construct and fixation strength that come into play early after reconstruction.

A point of interest in our study was to evaluate the behavior of the limbs of the construct under early loading conditions because asymmetrical tensioning in hamstring grafts has been shown to lead to inferior biomechanical properties. Although no differences were obtained on comparison of the strain data of each limb between the supplemented and control groups at load levels expected during pre-tensioning, further evaluation of the strain data did show some findings of interest. An analysis of strain values within the groups comparing the outer versus inner limbs showed a difference in the control group (P = .017), whereas no difference was present in the supplemented group (P = .18). This finding may indicate that the addition of

the supplemental fixation allows for better force distribution in the limbs of the construct. However, even though the difference between the inner- and outer-limb strains in the supplemented group was not statistically significant, there was still much less strain measured in the inner limbs of both constructs, indicating that the outer limbs carry most of the load in this construct regardless of supplemental fixation. In addition, the average strain values measured in the inner limb of the supplemented group increased at each increment of load from 20 to 80 N, suggesting better load-sharing properties. This was not the case in the control group.

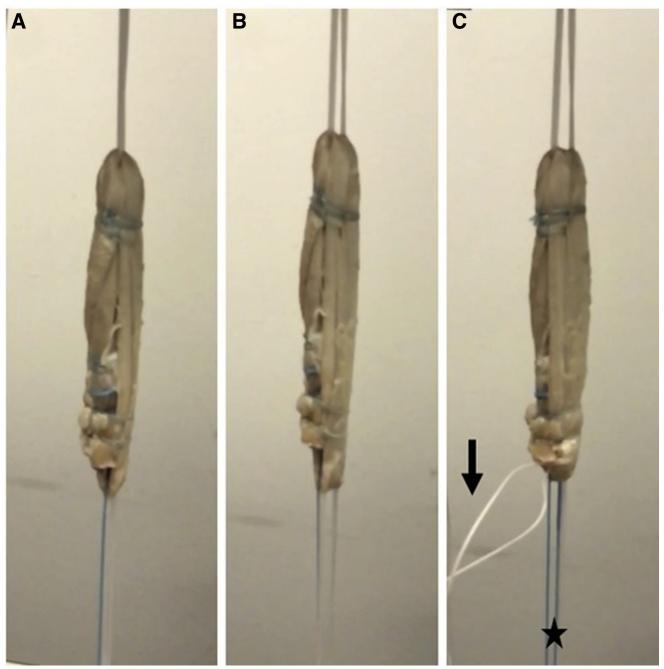
## Limitations

Our study is not without limitations. No cyclic loading was performed in our study. Although cyclic loading would add information as to the behavior of the graft construct under early loading conditions seen in rehabilitation, the purpose of our study was to evaluate the graft construct under preconditioning loads and load to failure at time zero and determine whether these could be improved by supplementing the inner limbs. The goal was not to evaluate in vivo loading conditions; therefore, this was not assessed. Our load-to-failure testing also differed from conventional ligament testing methods, which are performed at 100% strain/s. <sup>17</sup> Load to failure



**Fig 6.** Graft failure from start of loading (A), slippage of inner limb (B-D), and subsequent failure (E) in specimen without supplemental tibial fixation (control). Tibial and femoral suspensory fixation remain intact.

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**Fig 7.** Load to failure of specimen with supplemental inner-limb fixation from start of loading (A), initial elongation (B), and recoil without plastic deformity after failure of tibial suspensory fixation (arrow) and preservation of tibial supplemental fixation sutures (star) at conclusion of failure test (C).

was carried out in displacement-control mode. This does limit our ability to compare our study with other studies using load to failure performed at 100% strain/s, which is meant to simulate the fast strain-rate conditions in traumatic injuries. The testing being performed was essentially on the sutures, given that these are the weak link in our testing configuration and the primary site of failure in the referenced biomechanical studies on similar graft constructs, 5.6.18 with loads to failure at or below 1,000 N compared with 2,294 N for peroneus

longus allografts. Because the sutures are not strain rate sensitive or dependent, we believe this was an adequate method by which to perform our test. Despite this difference, our results are similar to those seen in recent studies evaluating all-inside graft constructs regarding their load-to-failure values and locations and/or modes of graft failure. Tiefenboeck et al. Peported on the biomechanical properties of all-inside, quadrupled, single-tendon ACL reconstruction grafts in the setting of increasing the strength of the cerclage sutures from No. 0



**Fig 8.** Specimen with failure of femoral suspensory device (arrow) showing overall preservation of construct shape despite slippage of inner limb (star).

FiberWire to No. 2 FiberWire. Their experiment involved cyclic loading as well as strain-controlled load-to-failure testing. They reported a mean ULF value of 730.67  $\pm$  94.52 N (range, 619.27-935.4 N), with all failure methods being related to the sutures. Fabbri et al.  $^{19}$  evaluated the effects of tripling, quadrupling, or half quadrupling single tendons for all-inside ACL reconstruction. They did not perform cyclic testing, and the method of ULF testing was not specified. They reported a ULF value of 767.02  $\pm$  53.19 N for their quadrupled grafts, with failures in all tested groups occurring by slippage of the tendon from sutures.

The graft preparation technique for single-tendon constructs is complex and does carry a learning curve. Although our results may be influenced by the graft preparation technique, we believe they are valid because this represents a real clinical scenario in which a surgeon or assistant would be preparing the grafts and thus would be at risk of the same potential errors during graft preparation. We did vary the technique by not adhering to the initial tendon length restrictions of the described technique. We believed that, as a true test of the construct itself, it would create less variability to leave matched pairs without any modification and potential alteration that may result from trimming the tissue. Although the original technique does not

describe the use of peroneus longus allografts, biomechanical studies have shown their equivalence to other commonly used soft-tissue grafts for this technique.

## **Conclusions**

Inner-limb supplemental tibial fixation results in higher time-zero load to failure and decreased graft elongation in a quadrupled, single-tendon, all-inside ACL reconstruction graft construct.

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