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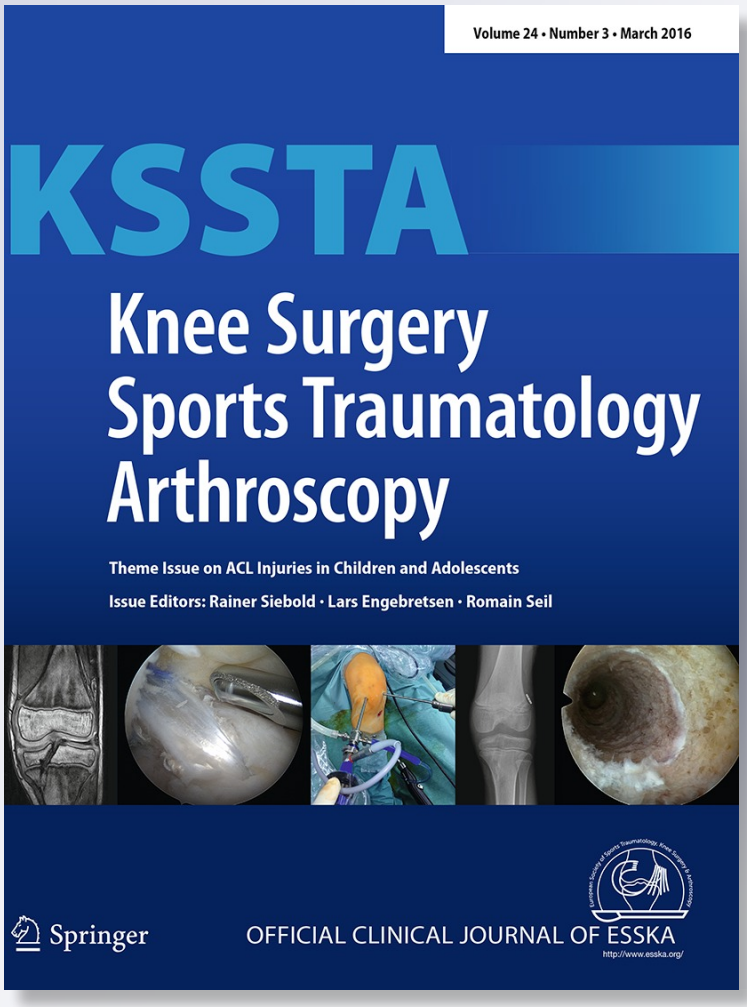

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High-load preconditioning of soft tissue grafts: an in vitro biomechanical bovine tendon model

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Abstract
Purpose No consensus exists regarding the optimal preconditioning protocol that will minimize postoperative elongation while creating a graft that is biomechanically equivalent to the native anterior cruciate ligament (ACL). It was hypothesized that a preconditioning protocol of specific mode and magnitude would create a graft with equivalent stiffness to the native ACL.
Methods Thirty-six bovine extensor tendon grafts were randomly allocated among six preconditioning groups ($n = 6$ per group) including three cyclic (10 cycles at 0.5 Hz between 10–80, 100–300, and 300–600 N) and three static loading protocols (20 s at 80, 300, and 600 N). Grafts were then cyclically loaded between 50 and 250 N at 0.5 Hz for 500 cycles to simulate an early rehabilitation protocol.
Results Cyclic 300–600 N and static 600 N loading protocols both demonstrated significantly less elongation during simulated rehabilitation when compared to lower, current clinical standard preconditioning levels of 10–80 N (–62 % Δ) and 80 N (–69 % Δ). The same high-load preconditioning protocols demonstrated statistical equivalence in stiffness when compared to the previously reported stiffness of the native ACL.
Conclusions In this experimental model, increased force applied to soft tissue grafts during preconditioning significantly decreased the subsequent elongation experienced during simulated early rehabilitation. A static load of 600 N removed the most graft elongation during preconditioning, had the least amount of cyclic displacement during simulated early rehabilitation, and was statistically equivalent to the native ACL stiffness. Implementation of high-load preconditioning of soft tissue grafts may help improve outcomes following ACL reconstruction by reducing residual knee laxity resulting from postoperative graft elongation and the intrinsic viscoelastic properties of the graft tissue while imparting biomechanical characteristics (e.g. stiffness) equivalent to the native ACL.

Keywords ACL reconstruction · Preconditioning · Soft tissue grafts · Hamstring grafts

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Introduction

Many factors play important roles in determining the success of an anterior cruciate ligament (ACL) reconstruction including graft selection, biology and biomechanics of graft tissue, construct design, graft fixation, graft tension at the time of implantation, tunnel placement, graft positioning, and rehabilitation protocols [1, 16–18, 21, 29, 32, 34, 36, 40, 42]. Additionally, tendon grafts used for ACL reconstruction are viscoelastic in behaviour which can lead to intra-articular stress relaxation, elongation, laxity, and failure of the reconstructed ACL postoperatively [5, 14, 15, 19, 35, 38, 41, 42, 44].

Clinically, soft tissue grafts have become increasingly popular for ACL reconstructions [16, 17, 21, 37]. Biomechanically, however, there is still a significant amount

unknown regarding the short- and long-term effects of graft viscoelastic properties and pre-implantation protocols. Current graft preparation protocols, including graft pretensioning and preconditioning, are surgical techniques used to reduce graft viscoelasticity and thus decrease the susceptibility to knee laxity in the initial postoperative period [5, 14, 15, 35]. For consistency in this study, all of the aspects that would occur prior to final fixation were combined under the single heading of "preconditioning." Previous investigations have cited irrecoverable graft lengthening following current preconditioning protocols as a contributing factor to residual knee laxity and cause for further investigation [5]. This, in combination with additional in vitro preconditioning studies [14, 35], suggests that investigation of novel, higher load preconditioning protocols are warranted. Therefore, the purpose of the present study was to analyse the biomechanical consequences of high-load preconditioning protocols with the goal of optimizing time zero soft tissue ACL graft properties by minimizing graft elongation during a simulated immediate postoperative rehabilitation period and conferring biomechanical characteristics (e.g. stiffness) equivalent to the native ACL.

Since numerous factors may contribute to postoperative laxity of soft tissue grafts, viscoelastic effects associated with the inherent biological characteristics of the graft tissue itself were isolated in this study, as the viscoelastic contribution could be independent of the optimal solution of other variables (e.g. fixation method). Native ACL stiffness characteristics [43] were used as control values in order to refrain from creating a graft that was too stiff, which could potentially lead to increased re-rupture or failure rates. It was hypothesized that a preconditioning protocol of specific mode and magnitude would create a graft with equivalent stiffness to the native ACL and minimize laxity resulting from graft elongation during a simulated early rehabilitation protocol.

Materials and methods

Specimen preparation

Thirty-six bovine extensor tendon grafts (age 18–30 months) were used for testing (Innovative Medical Device Solutions, Logan, Utah). Double loop bovine extensor tendon grafts and quadruple loop human hamstring grafts demonstrate similar biomechanical and viscoelastic properties making them a suitable and more readily available model for in vitro testing [12]. All grafts were wrapped in 0.9 % saline-soaked gauze and stored at −20 °C in sealed plastic bags to preserve the biological properties of the tissue. The grafts were thawed to room temperature in a 0.9 % physiological saline solution for 15 min prior to testing. All grafts were prepared by the same investigator (JRJ), sized to 9 mm in diameter (Graft Sizing Block, Arthrex Inc., Naples, Florida), and trimmed to be 180 mm in length [36]. Thirty millimetres of each free end was whipstitched using No. 2 polyester/polyethylene suture (FiberLoop, Arthrex Inc., Naples, Florida) as previously described [8, 36]. Doubled over, this created a graft 60 mm in length which approximated the amount of free graft (graft subject to elongation) in a routine soft tissue reconstruction [36]. All specimens were kept moist with saline solution (0.9 %) during preparation, fixation, and testing to prevent desiccation [23].

Testing groups

Six preconditioning study groups were defined to test different forces as well as examine static versus cyclic loading (Table 1). This study defined 80 N (cyclic or static) as the current "standard of care" [11]. The testing machine was controlled via WaveMatrix software (Instron Systems, Norwood, Massachusetts), which was used to program each of the six different protocols. Grafts were randomized to each of these randomized-order testing protocols via Excel software (Microsoft, Seattle, Washington).

Biomechanical testing

Each graft was secured within a dynamic tensile testing machine (Instron ElectroPuls E10000, Instron Systems, Norwood, Massachusetts) for in vitro testing. Measurement error of the testing machine was certified by Instron to be less than or equal to ±0.01 mm and ±0.3 % of the indicated force. Grafts were looped through a 5-mm-thick, 19-mm-inner-diameter custom steel eyebolt fixture which was rigidly clamped to the base of the testing machine. The rigid eyebolt fixture simulated a continuous loop cortical suspension device while eliminating displacement that would otherwise have been associated with a cortical suspension device (Fig. 1). The free whipstitched ends of the prepared grafts were subsequently wired together with multiple (~10) helical wraps of 24 gauge wire to provide additional interfacial surface area and points for mechanical interlock when secured to the actuator of the testing

Table 1 Testing protocols

Cyclic preconditioning (10 cycles over 20 s, 0.5 Hz)	10–80 N
	100–300 N
	300–600 N
Static preconditioning (20 s)	80 N
	300 N
	600 N

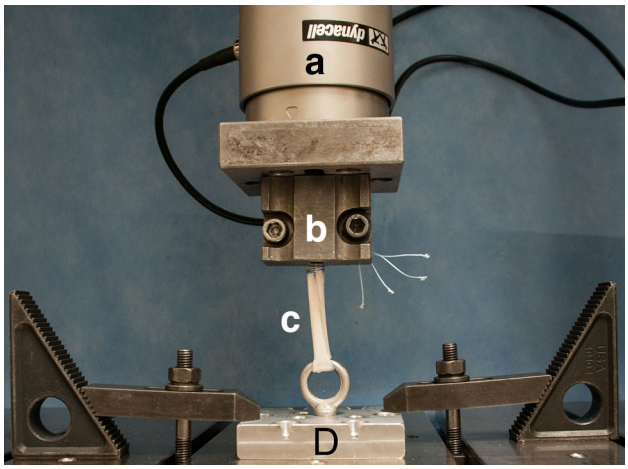

Fig. 1 Previously prepared bovine extensor tendon grafts were clamped to the actuator after being looped through an eyebolt, which was securely fastened to the base of the tensile testing machine. **a** Load cell, **b** custom clamp, **c** prepared graft, **d** custom eyebolt fixture

machine via a custom steel clamp. Visual inspection of the clamped graft construct revealed that all suture and wiring remained in place within the clamp throughout testing and confirmed that elongation measured by the testing machine actuator was only a result of that which occurred within the tendon and not from potential slippage within the clamp.

Cyclic preconditioning was performed for a total of 10 cycles over 20 s (0.5 Hz). Static preconditioning groups were held at a constant load for a total of 20 s. Following preconditioning, the grafts were immediately subjected to cyclic loading [42] to simulate forces of an early rehabilitation protocol of passive flexion–extension loading of the ACL [33] at a frequency approximating that of walking (Fig. 2) [24]. Graft displacement (elongation) was recorded separately during both preconditioning and cyclic loading. The initial (cycle 1) and final (cycle 500) stiffness during cyclic loading were calculated as the slope of the load versus displacement curve between 50 and 250 N at the beginning of the respective cycles.

Statistical analysis

Sample size calculations were made a priori with the goal of powering the statistical equivalence test for comparison with previously published native ACL stiffness data [43]. Assuming an observed standard deviation of 45 N, and defining the threshold of irrelevant difference (delta) as the standard deviation from Woo et al. [43] (90 N), six specimens per group were required to provide 80 % power. Statistical analysis was performed using IBM SPSS Statistics, version 20 (Armonk, New York). One-way analysis of variance (ANOVA) with Games-Howell post hoc tests

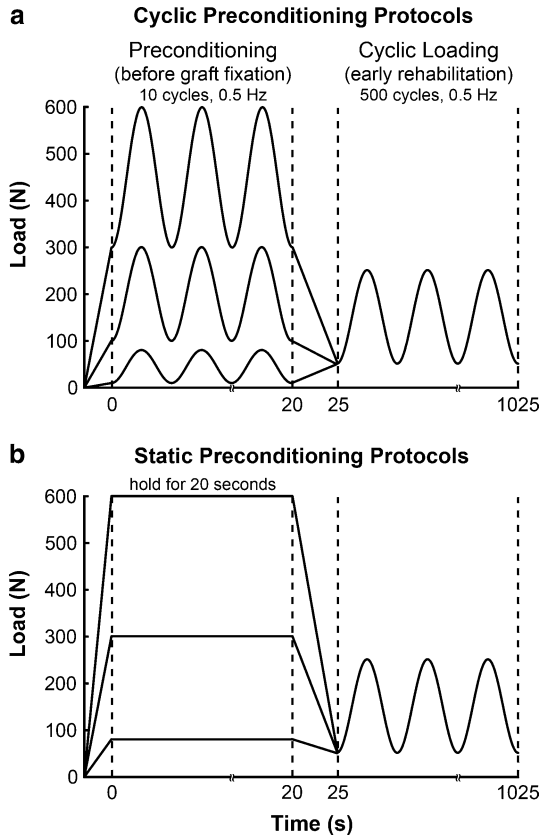

Fig. 2 **a** Graphical representation of *cyclic preconditioning* groups with subsequent cyclic loading to simulate early rehabilitation. **b** Graphical representation of *static preconditioning* groups with subsequent cyclic loading to simulate early rehabilitation

were made between the different preconditioning protocols. Significant differences were determined to be present for $P < 0.05$.

Equivalence tests determined whether cyclic stiffness values could be considered equivalent to previously published data for young adult (22–35 years of age) ACL stiffness [43]. As described by Harris et al. [22] 90 % confidence intervals were constructed for each difference between measured stiffness and previously published stiffness values to achieve a type I error rate of $\alpha = 0.05$. This calculated confidence interval (CI) was compared with a minimal level of distinguishable differences [22]. If the CI fell completely below the threshold for the minimal level of clinically important difference, then the protocols were considered equivalent. The clinical threshold for equivalence was set at the upper limit of half of one standard deviation from prior data by Woo et al. [43]. Equivalence calculations were performed with the statistical computing software R (R version 2.15.2, R Foundation for Statistical Computing, Vienna, Austria) using the equivalence package (R package version 0.5.6, Andrew Robinson, 2010).

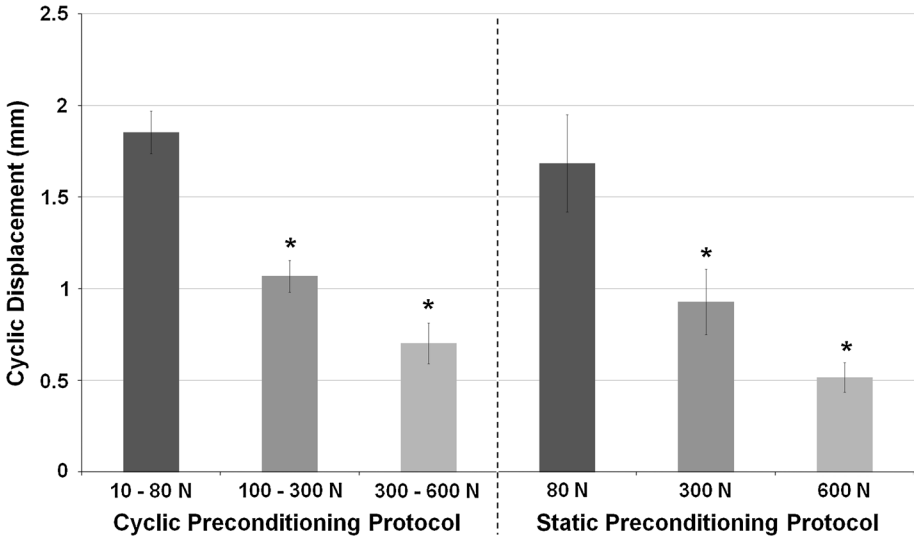

Fig. 3 Amount of displacement following cyclic loading (50–250 N at 0.5 Hz for 500 cycles) for each preconditioning protocol. *Represents statistical difference ($P < 0.005$) from 10 to 80 (*cyclic*) and 80 N (*static*), respectively

Results

Displacement

Displacement (elongation) observed during both preconditioning and cyclic loading is presented as mean values [±SD and (95 % confidence intervals)] and the percent change from the current clinical standard, defined to be either cyclic loading between 10 and 80 N or static loading of 80 N (Table 2). Significant differences ($P < 0.005$) were observed for both preconditioning and cyclic loading displacement as preconditioning force increased regardless of cyclic or static force application (Fig. 3).

Stiffness

Graft stiffness values were calculated following preconditioning (first cycle of cyclic loading) as well as during the final cycle of cyclic loading (Table 3). Stiffness values resulting from cyclic 300 to 600 N and static 600 N load preconditioning protocols demonstrated statistical equivalence ($P = 0.042$ and 0.024, respectively) when compared to the stiffness of the native ACL [43] (Fig. 4). Moreover, cyclic 300–600 N and static 600 N loading groups demonstrated the highest stiffness values following preconditioning and the smallest increase in stiffness values (34 and 28 %, respectively) over the course of cyclic loading.

Discussion

The most important finding of this study was that current preconditioning protocols for soft tissue grafts are not optimized to minimize time zero cyclic displacement nor create a graft biomechanically equivalent to the native ACL. This study demonstrated that increased force applied to soft tissue grafts during preconditioning significantly decreased the subsequent elongation during simulated early rehabilitation. Furthermore, a static load of 600 N removed the most graft elongation during preconditioning, had the least amount of cyclic displacement, and was statistically equivalent to the previously reported [43] native ACL stiffness.

The need for preconditioning of soft tissue grafts has been well established [15, 29, 30]; however, optimal levels have not been defined. Currently, hamstring autografts are typically statically preconditioned on a graft preparation and tensioning board with 20 pounds of force (approximately 89 N) for several minutes [4, 14, 17, 24, 35]. A 50 % increase in postoperative tension was observed when such preconditioning forces were doubled from 80 to 160 N; yet, significant graft relaxation was still observed in vitro [14, 15]. Although this suggests higher preconditioning forces may positively affect graft properties, there is a paucity of data for substantially higher preconditioning loads. Instead, recent studies have continued to focus on the current 80 N standard that has been previously described as both optimal and reproducible in the surgical setting [11, 35, 44]. Such studies continue to confirm that the current standard, whether applied cyclically or statically, does not adequately precondition the graft to eliminate significant graft stress relaxation after fixation [6, 35, 41]. Furthermore, debate exists regarding the most effective preconditioning modality (e.g. cyclic vs. static) [19, 35, 38]. With regard to the debate of cyclic versus static preconditioning, this study demonstrated that stiffness values increase and graft elongation decreases as the amount of force increased for both the cyclic and static preconditioning protocols. However, a static pull would likely be more feasible in the clinical setting. Therefore, a static 600 N load preconditioning protocol may be optimal as this protocol removed

Table 2 Graft cyclic displacement (mm) values

Group	Protocol (N)	Preconditioning displacement	% Increase from current standard[a]	% Increase significance (*P* value)	Cyclic displacement	% Decrease from current standard[a]	% Decrease significance (*P* value)
Cyclic	10–80	1.11 ± 0.22 [0.88, 1.34]	–	–	1.85 ± 0.12 [1.73, 1.98]	–	–
	100–300	3.99 ± 0.69 [3.27, 4.72]	260 %	<0.001	1.07 ± 0.09 [0.98, 1.16]	42 %	<0.001
	300–600	4.49 ± 0.53 [3.94, 5.04]	305 %	<0.001	0.70 ± 0.11 [0.59, 0.82]	62 %	<0.001
Static	80	2.29 ± 0.35 [1.92, 2.66]	–	–	1.68 ± 0.27 [1.40, 1.96]	–	–
	300	4.36 ± 0.82 [3.50, 5.21]	91 %	0.002	0.93 ± 0.18 [0.74, 1.12]	45 %	0.001
	600	5.44 ± 1.28 [4.10, 6.78]	138 %	0.003	0.52 ± 0.08 [0.43, 0.60]	69 %	<0.001

Data reported as mean ± standard deviation [95 % confidence interval]

[a] Current standard is defined as current clinical protocol; either 10–80 N (*cyclic*) or 80 N (*static*)

the most graft elongation during preconditioning, had the least amount of cyclic displacement, and was statistically equivalent to the native ACL stiffness [43].

The authors theorized that a graft which approximates the native ACL stiffness may be clinically superior. Similarly, Burks et al. [7] argued that a "functional stiffness" must be restored by an ACL graft to approximate the stiffness of an intact ACL for proper stability to be regained. Furthermore, studies have shown that a high-stiffness construct implanted with less initial tension at final fixation improved anterior knee laxity and restored the kinematics of a normal knee while avoiding complications such as ongoing knee laxity, over-constraint, decreased range of motion, and early arthrosis [13, 27]. However, the benefits of high-stiffness constructs have yet to be demonstrated in short- and long-term clinical studies. The authors believe that the native ACL stiffness is an important control to avoid creating an overly stiff graft that may be susceptible to rupture.

However, in addition to time zero biomechanical graft characteristics, biological contributions including collagen structure, histology, and their influence on the downstream ligamentization process must be considered. The process of graft remodeling, incorporation, and maturation, collectively referred to as "ligamentization", contributes significantly to the final biomechanical properties and ultimate success or failure of an ACL reconstruction [2, 3, 10, 39]. In addition to the revascularization of graft tissue, significant graft remodeling and changes in the number and diameter of collagen fibres are observed as the graft is incorporated. In patients undergoing quadrupled semitendinosus/gracilis ACL reconstruction, the majority of these changes were observed to occur in the first 2 years postoperatively, with tissues failing to completely mimic native ACL collagen ultrastructure 10 years after surgery [46]. This process has been shown to be significantly influenced by initial graft tension at the time of fixation. In vivo animal models have demonstrated that some level of tension is beneficial to the remodeling process, while overtensioning may over-constrain the knee, impede revascularization, and result in degenerative graft changes [31, 45]. To date, research has primarily focused on the tension at the time of fixation, while the effects of pretensioning and preconditioning magnitudes on subsequent ligamentization are limited. Guillard et al. [20] demonstrated that high-load preconditioning (500 N) can significantly decrease cohesion, integrity, and parallelism of collagen fibrillar ultrastructure, particularly when applied for periods longer than 30 s. However, the effects of these changes on the downstream ligamentization process and ultimate biomechanical properties of the graft are not currently well understood. Furthermore, it is not known whether the time zero biomechanical graft properties imparted by high-load

Table 3 Graft stiffness (N/mm) values

Group	Protocol (N)	Cycle 1 of 500	Cycle 500 of 500	% Increase between cycle 1 and 500	% Increase significance (*P* value)
Cyclic	10–80	145 ± 11 [133, 157]	286 ± 33 [252, 320]	97 %	–
	100–300	207 ± 11 [196, 219]	283 ± 16 [266, 300]	36 %	0.001
	300–600	238 ± 43 [193, 282]	317 ± 44 [271, 363]	34 %	0.002
Static	80	183 ± 27 [155, 211]	334 ± 32 [301, 367]	82 %	–
	300	216 ± 30 [185, 248]	293 ± 43 [248, 338]	35 %	0.001
	600	235 ± 33 [200, 269]	300 ± 45 [254, 347]	28 %	0.001

Data reported as mean ± standard deviation [95 % confidence interval]

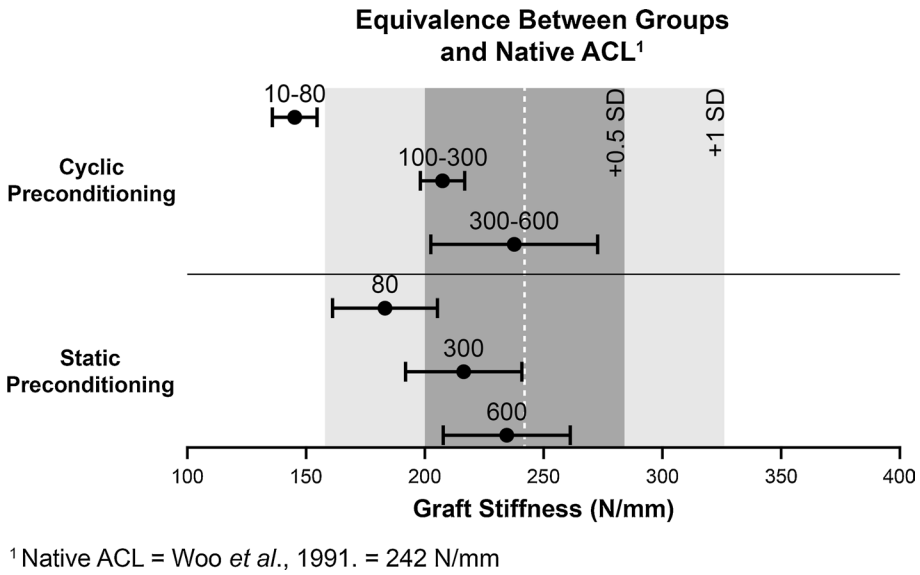

Fig. 4 Equivalence test for graft stiffness. The threshold for equivalence was set at ½ standard deviation (SD) from reported stiffness values of the native ACL stiffness (Woo et al. [43])

preconditioning will be retained throughout the ligamentization process.

An in vitro investigation of a complex biomechanical system carries some inherent limitations. The present study used bovine extensor tendons, and data were extrapolated to human hamstring tendons; however, a previous study demonstrated no statistical difference between the biomechanical properties of bovine extensor and human hamstring tendons [12]. Previous studies have demonstrated that an increase in graft temperature after implantation can also decrease graft tension; however, this study was performed at room temperature [9, 15]. The results of this study are not representative of the potential elongation of the entire ACL reconstruction construct (e.g. femoral cortical suspension device, graft, and tibial fixation) and instead include only the displacement contribution from the graft. Nevertheless, the goal was to isolate and optimally minimize the viscoelastic elongation effects of grafts through preconditioning as this contribution could be independent of the optimal solution of other variables (e.g. fixation method). Furthermore, high forces and graft preparation may permanently alter the basic structural characteristics of the graft and change the physiological remodeling process [25, 26, 28, 45]. However, it is unlikely that a 600 N force would exhibit this effect given that the ultimate failure strength of such grafts has been reported to be approximately 2,900 N [12]. Regardless, further histological studies are warranted. Lastly, in quantifying displacement as changes in actuator position, the observed displacement is representative of the potential displacement of the full testing construct, including the steel fixtures rigidly attached and connected in series from the actuator to the testing machine base. However, visual inspection, the strong and rigid attachments, and their inherent stiffness (steel), suggest their contributions to the measured graft displacement were negligible.

Independent of these limitations, the present study provides valuable and clinically relevant information regarding the viscoelastic behaviour of soft tissue grafts and the potentially beneficial effects of high-load preconditioning protocols. Specifically, the present study suggests high-load preconditioning may aid in the time

zero optimization of soft tissue ACL reconstruction by obviating the intrinsic viscoelastic properties of graft tissue, reducing downstream residual knee laxity resulting from graft elongation during the immediate postoperative period, while simultaneously imparting biomechanical characteristics (e.g. stiffness) equivalent to the native ACL [43].

Conclusion

In conclusion, increased force applied to soft tissue grafts during preconditioning significantly decreased the subsequent elongation experienced during cyclic loading representative of an early rehabilitation protocol as a result of the significantly increased stiffness, which was statistically equivalent to that of an intact ACL [43]. A static preconditioning load of 600 N removed the most amount of preconditioning displacement, had the least amount of cyclic displacement, and was statistically equivalent to the stiffness of a native ACL at time zero.

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