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Can the gracilis replace the anterior cruciate ligament in the knee? A biomechanical study

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Abstract

Purpose The purpose of this study was to determine whether a four-strand gracilis-only construct possesses the biomechanical properties needed to act as an anterior cruciate ligament (ACL) reconstruction graft.

Methods This was a pilot study with 32 cadaver specimens. The biomechanical properties of three types of grafts were determined using validated tensile testing methods: patellar tendon (BTB), both hamstring tendons together (GST4) and gracilis alone (G4).

Results The maximum load at failure of the G4 was 416.4 N (± 187.7). The GST4 and BTB had a maximum load at failure of 473.5 N (± 176.9) and 413.3 N (± 120.4), respectively. The three groups had similar mean maximum load and stiffness values. The patellar tendon had significantly less elongation at failure than the other two graft types.

Conclusions The biomechanical properties of a four-strand gracilis construct are comparable to the ones of standard grafts. This type of graft would be useful in the reconstruction of the anteromedial bundle in patients with partial ACL ruptures.

Keywords ACL reconstruction · Gracilis · Short Graft · Experimental study

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Introduction

The choice of grafts for anterior cruciate ligament (ACL) reconstruction is not without consequences. One of the advantages of using the pes anserinus tendons—gracilis and semitendinosus—is that harvesting these tendons leads to lower morbidity than harvesting a bone–patellar tendon–bone (BTB) graft. Using these tendons provides sufficient strength, limits extensor mechanism weakening and lessens anterior knee pain [1–3].

However, harvesting the gracilis and semitendinosus tendons has its own issues, namely reduction in flexion strength and lack of control over internal rotation [4]. Recent studies have shown the semitendinosus alone can be used as a graft [5]. However, the semitendinosus muscle–tendon unit controls knee rotation in full extension [6]. Using the gracilis tendon alone should reduce the morbidity induced when both hamstring tendons are harvested and should preserve the semitendinosus.

We have recently shown that a four-strand gracilis construct (G4) meets the anatomical specifications for use as an ACL reconstruction graft [7]. The next logical step is to determine whether the G4 has the biomechanical properties needed to act as an ACL graft. Zamarra et al. [8] evaluated the potential use of the gracilis alone or semitendinosus alone to reconstruct the ACL. The relative laxity obtained with G4, four-strand semitendinosus (ST4) and four-strand gracilis–semitendinosus (GST4) grafts was evaluated through biomechanical testing. The results were similar for all three graft types and each graft was able to restore normal knee kinematics. These results were not unexpected because these tendons all have similar biomechanical properties [9]. Doubling the tendons appears to more than double their failure strength. A single-strand gracilis construct has a maximum strength of 925 ± 127 N, while a two-strand construct has a maximum

strength of $2,573 \pm 496$ N [10]. This same study found that the maximum strength was $1,246 \pm 243$ N for the native ACL and was $3,855 \pm 592$ N for the patellar tendon [9].

There are no published data on the strength of a G4 construct. There is also no information on the maximum load that a graft in its surgical configuration can withstand before failing. Several studies have reported on the strength of each individual tendon [9], but none has determined the strength of the graft in the configuration used for ACL ligament reconstruction. This led us to ask whether a G4 construct has suitable properties to be used as the sole replacement for a ruptured ACL.

Our null hypothesis is that the biomechanical properties of a four-strand gracilis graft are equal to those of standard ACL reconstruction grafts. The primary objective was to measure the maximum load that the G4 could withstand before failing. The secondary objective was to compare the biomechanical properties (maximum load and elongation at failure, stiffness) of three types of ACL graft: patellar tendon (BTB), combined hamstring graft (GST4) and the G4.

Materials and methods

Materials

This was a comparative biomechanical study using 32 cadaver knees from 16 donors. The donors had a mean age at death of 84 years (range, 77–90). The cadavers were stored at -20 °C and thawed overnight at 2 °C before dissection and subsequent biomechanical analysis. All knees were free of wounds and macroscopic signs of intra-articular lesions (Outbridge > grade 3, no osteophytes in the intercondylar notch). All knees had an intact ACL and the passive joint range of motion measured with a goniometer was always at least 130° .

Graft harvesting

A standard anteromedial incision was performed. The pes anserinus tendons were located at the lower part of the incision, and then harvested with an open-ended tendon stripper. The tendons were cut at the periosteum of their tibial insertions. The 10-mm wide, middle-third patellar tendon graft (BTB) was harvested with patellar and tibial bone blocks as described by Neyret et al. [11]. The cuboid-shaped patellar bone block was 15 mm long, 9 mm wide and 5 mm thick. The cuboid-shaped tibial bone block was 30 mm long, 10 mm wide and 5 mm thick.

Graft preparation

The various grafts were tested in the same configuration as the one used during a surgical procedure. The surgical techniques were reproduced exactly. The hamstring tendons in the left knee

were used together. They were folded in two. This graft was named GST4. The G4 graft was prepared by folding it into four.

Graft preservation

The prepared grafts were stored at -4 °C in a cold freezing solution containing saline and 10 % dimethylsulphoxide. They were removed from the freezer the evening before testing and kept at room temperature (21 °C) for at least 12 hours. This process does not alter the biomechanical properties of tendons [12].

Methods

Graft fixation

The grafts were fixed using validated methods [5, 9, 13]. The distal 15 mm of each graft was compressed between two metal clamps (Fig. 1). As a consequence, the distance between clamps (initial specimen length) varied depending on the graft's length.

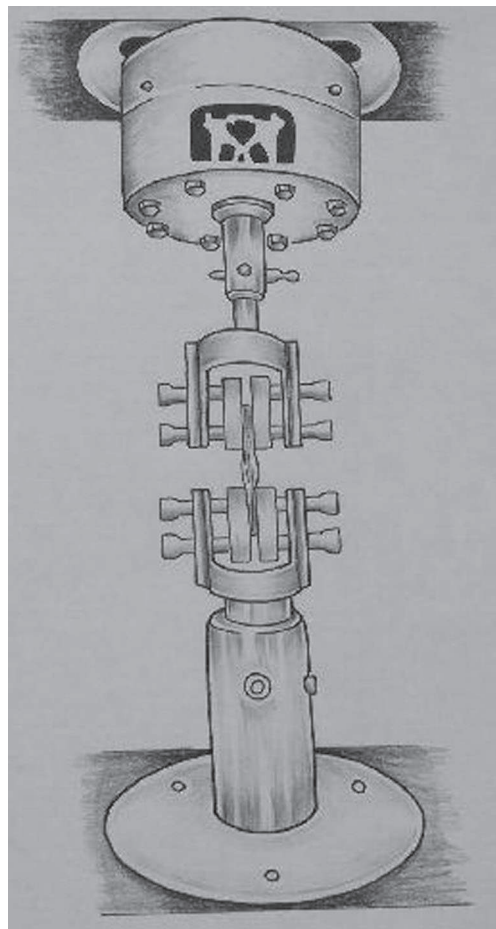
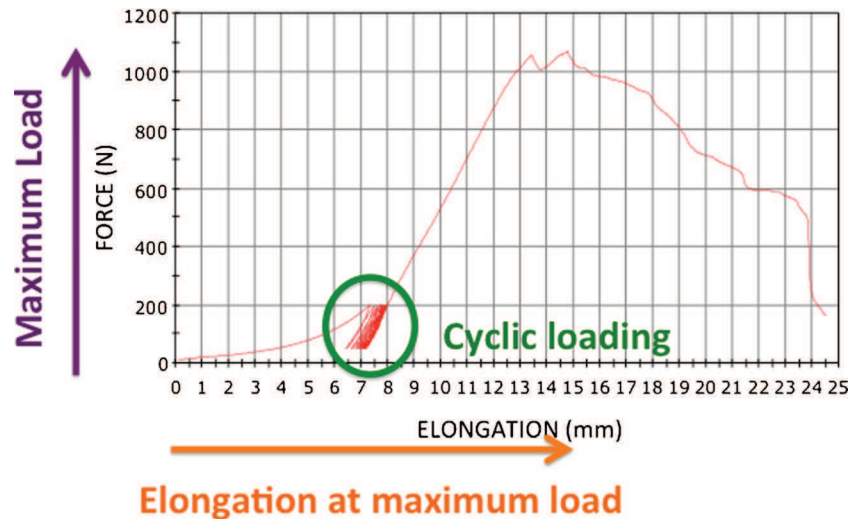


Fig. 1 Drawing of clamps used to grip the tendon specimens based on Shi et al. [13] and Handl et al. [9]

Fig. 2 Typical force–elongation curve generated by the BlueHill® software (Instron SA France, Elancourt, France). A cyclic preconditioning regimen (*green circle*) was performed before the graft was loaded to failure



Measurement protocol

Each set of clamps was attached to a materials testing system (Instron 3300®; Instron, Canton, MA, USA) to execute the tensile testing and measure the biomechanical properties of the graft. The measurements were performed using the system’s software (BlueHill®; Instron SA France, Elancourt, France). Since the initial specimen length varied as a function of graft length, the length measurement sensor was reset before each test. A typical load-elongation curve is shown in Fig. 2. Each graft was preloaded to 10 N, then cycled 100 times between 50 and 200 N at 0.5 Hz. A tensile test was then performed using a 10 mm/min crosshead speed until the graft failed. This sequence is a standard, validated test protocol [14]. The following structural properties were measured: (1) maximum load at failure (N), (2) maximum elongation at failure (mm) and (3) linear stiffness ($\text{N}\cdot\text{mm}^{-1}$).

Statistical analysis

The statistical analysis was performed with the Excel 2011 (Microsoft, Redmond, WA, USA) and XLSTAT 2011 (Addinsoft, Paris, France) software packages. The descriptive analysis consisted of mean, median and standard deviation values. The mean values for maximum load to failure, maximum elongation to failure and linear stiffness were compared between the three groups (G4, GST4 and BTB) using Student’s *t*-test. To ensure the conditions had been met for parametric testing, the normality of the measured variables was verified using the Shapiro-Wilk test and the homogeneity of variances was verified using Fisher’s *f*-test and Levene’s test. The significance threshold was set at $P < 0.05$.

We found no published information regarding the expected maximum load at failure for G4 grafts, which made it difficult to determine how many samples were needed. We decided to

perform a pilot study with at least 30 specimens [15]. Ultimately, 32 specimens were tested.

Results

The maximum load at failure of the G4 was 416.4 N (± 187.7). The GST4 and BTB had a maximum load at failure of 473.5 N (± 176.9) and 413.3 N (± 120.4), respectively. The results for the entire series are summarised in Fig. 3. The maximum elongation at failure of the G4 was 18.0 mm (± 10.6); it was 21.2 mm (± 11.6) for the GST4 and 5.1 mm (± 4.1) for the BTB. The linear stiffness of the G4 construct was

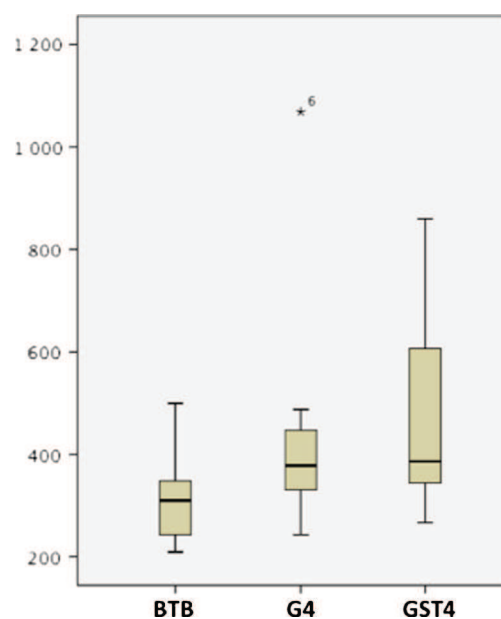


Fig. 3 Box-and-whisker plot of the maximum load at failure for each graft type. *BTB* Bone-patellar tendon-bone, *G4* four-strand gracilis construct, *GST4* four-strand gracilis and semitendinosus construct

192.9 N.mm⁻¹ (± 41); it was 198.5 N.mm⁻¹ (± 44.9) for the GST4 and 164.6 N.mm⁻¹ (± 52.0) for the BTB.

The three groups had similar mean values in terms of the maximum load that they could withstand before failing (Table 1). The BTB had the lowest elongation at failure of the three graft types; this difference was statistically significant. Since the three groups had similar mean stiffness values, no conclusions can be drawn about this comparison.

Discussion

The primary objective was to measure the maximum load that the G4 can withstand at failure to determine if its structural properties are equal to those of standard ACL reconstruction grafts. The maximum load at failure of the G4 construct was 416 N±187 (range, 242–1,069 N), which is equivalent to the reference tendon grafts, namely the patellar tendon and four-strand semitendinosus and gracilis. To our knowledge, this is the only study where the maximum failure load was measured with the grafts in their surgical configuration. Instead of measuring the strength of each tendon making up the graft, the strength of the fully prepared graft was measured. The goal of this study was to measure the mechanical properties of the graft itself. This aspect is novel. Most studies on this topic focus only on graft fixation methods. Few studies have reported the strength of the graft itself, which we felt was important information to have. Since the various grafts used for ACL reconstruction are fixed with different methods, adding the fixation variable in this study would have been a confounding factor.

We have recently shown that a G4 construct meets the anatomical specifications for use as an ACL reconstruction

graft [7]. The available length of G4 and four-strand semitendinosus grafts is always sufficient to place at least 15 mm of graft in the bone tunnels [7]. Using the G4 reduces the risk of oversizing in the middle portion of the graft, which is a problem with other types of graft [16]. An excessively thick graft can get impinged in the notch, which would disrupt its healing [17]. In addition, use of the gracilis only preserves the semitendinosus, which plays an important role in controlling rotational stability when the knee is fully extended [6].

We have recently shown that a four-strand semitendinosus graft has better biomechanical properties (namely failure strength) than standard grafts [6]. For this reason, it is our graft of choice for ACL reconstruction. We think that the G4 is particularly well-suited to being used alone during surgical treatment of partial ACL tears [18]. A G4 graft could be used for the isolated reconstruction of the anteromedial bundle when the posterolateral bundle is intact [19, 20]. Its biomechanical properties are comparable to those of other types of grafts and its volume is lower [7] thus the risk of oversizing is reduced [16]. But these findings must be tempered by this study's limitations. The tensile testing was performed with tissues that had been frozen at -4 °C and then thawed. Several studies have explored the effect of freezing and thawing tendons on their biomechanical properties [21]. Based on the results of these studies, the biomechanical properties of tendons are unaffected when fewer than three gradual freeze-thaw cycles are performed.

Only axial tension tests were performed in this study. Although this testing protocol does not reproduce the multi-axial loads experienced by the ACL in vivo, it is consistent with previous research done into graft strength and fixation [5, 13, 14, 22].

The fixation method is also another basic consideration, as it can affect the results of tensile tests [23]. Novel serrated jaw clamps that allow tendons to be tested in a simple and reproducible manner have recently been described by Shi et al. [13]. Resin-based clamps and cryoclamps are difficult to work with and have not been formally validated [24]. Pap et al. [25] recently validated a fixation method for autografts that used the serrated jaw clamps described by Shi et al. by comparing them with other types of clamps. This is the type of clamp used in the current study.

To ensure quasi-static conditions, the testing was carried out with a slow crosshead speed, so as to not bring the tendon's visco-elastic properties into play. The tensile strength will be lower when slower elongation speeds are used. When ligaments and tendons are loaded more quickly, the risk of damaging these structures increases [26].

The maximum load values in the current study were much lower than published values. The leading studies on this topic reported maximum load values of 1,719 N±1,167.80 (range, 456–4,546 N), which is nearly 3 times higher than the value reported here [27]. It was also surprising to see that this difference did not apply to the stiffness values, which were very

Table 1 Comparison of the maximum load at failure, elongation and stiffness performed with Student's *t*-test

	Mean	SD	SE	<i>P</i>
MAXIMUM LOAD AT FAILURE				
BTB-GST4	25.7	142.3	47.4	0.6
BTB-G4	7	309.8	103.3	0.9
G4-GST4	22.2	266.3	84.2	0.8
ELONGATION AT FAILURE				
BTB-GST4 ^a	16.1	10.6	2.7	<0.01
BTB-G4 ^a	12.9	12.6	3.2	<0.01
G4-GST4	3.2	15.9	3.9	0.4
STIFFNESS				
BTB-GST4	39.7	94	23.5	0.1
BTB-G4	34.0	117.4	29.3	0.3
G4-GST4	5.6	65	16.3	0.7

*P*probability, *SD*standard deviation, *SE*standard error

^aSignificant difference between mean values

similar to ours (Table 2). A review of the literature was performed to better understand the reasons for these differences.

The first reason is related to donor age. The studies reporting the highest failure loads were also the ones with the youngest donors (20–30 years) [9, 27].

A second reason relates to the method used to induce tendon failure. The study with the largest number of ACL grafts and highest published loading values had a significant bias [9]. The tensile testing system consisted of applying tension to the tendon by dropping a weight from a set height. The maximum load and stiffness were measured using a custom, but non-validated accelerometer-based device. These methodological considerations bring the validity of their results into question.

The third aspect relates to the elongation speed used in the various studies (Table 2). Many of these tensile tests used elongation rates greater than 5 mm/s (about 10 %/s). Under these conditions, the tensile tests were not being performed under static conditions, thus bringing the tendon's visco-elastic properties into play. This could explain the higher maximum load values

reported in these studies. We performed an ANOVA test on the published data and found a relationship between the elongation rate and maximum failure load ($P=0.032$). However, this potential tendon stiffening as the elongation rate increases must still be demonstrated with specific biomechanical studies.

This study has a certain number of limitations. The population from which the cadaver donors were taken is not representative of the population in which ACL reconstruction is typically performed. Age, BMI, gender and physical activity levels affect the biomechanical properties of tendons and ligaments [22]. But these limitations are partially overcome by the comparative design of the study. Since all three grafts were harvested from the same individual, all grafts had the same age, BMI, gender and activity level. As a consequence, any confounding factors were evenly distributed between groups. In addition, the biomechanical testing protocol used in this study—elongation speed, grips, measurement methods—was based on published studies [13] (Table 2). There is no way of knowing whether the various types of grafts would react

Table 2 Summary of published biomechanical study describing the structural properties of tendons used for ACL reconstruction

Author	Year	n	Speed	Fixation	Test system	Age	Graft	Max. Load	Stiffness $N.m^{-1}$
Chandrashekar [28]	2007	20	100 %/s	Cerro bend plot	Instron 8500+	38	ACL	1526	250
Elias [29]	2008	6	10 mm/min	custom grip	Mini Bionix II, MTS Systems	77	Hamstring	NA	115
Elias [29]	2008	6	10 mm/min	custom grip	Mini Bionix II, MTS Systems	77	BTB	NA	129
Handl [9]	2011	21	1.5 m/s	custom grip	drop-weight velocity custom system	62	Gracilis	2573	432
Handl [9]	2011	21	1.5 m/s	custom grip	drop-weight velocity custom system	62	Hamstring	4546	490
Handl [9]	2011	21	1.5 m/s	custom grip	drop-weight velocity custom system	62	ST	3395	487
Handl [9]	2011	21	1.5 m/s	custom grip	drop-weight velocity custom system	62	BTB	3850	364
Handl [9]	2011	21	1.5 m/s	custom grip	drop-weight velocity custom system	62	ACL	1246	182
Harner [30]	1995	14	200 mm/min	methyl methacrylate	Motion Analysis	52	PCL	1120	120
Hashemi [31]	2011	20	100 %/s	custom grip	Instron 8500	40	ACL	1500	
Hoher [32]	2013	16	1 mm/min	custom grip	Zwick 1455		Hamstring	634	283
Kennedy [33]	1976	10	500 mm/min		Instron Tension Analyzer		ACL	1051	NA
Meuffel [34]	2008	10	1 mm/s	custom grip	Testometric 250–2.5AX, Instron	80	BTB	456	72
Noyes [27]	1984	18	100 %/s	custom grip	LeBow Load cell	26	Gracilis	838	170
Noyes [27]	1984	18	100 %/s	custom grip	LeBow Load cell	26	ST	1216	186
Noyes [27]	1984	18	100 %/s	custom grip	LeBow Load cell	26	BTB	2900	686
Noyes [35]	1976	26	100 %/s		Instron biaxial		ACL	723	129
Pap [25]	2014	5	20 mm/min	custom grip	Instron 8872		BTB	1542	NA
Prietto [36]	1995	4	100 %/s			20	PCL	1627	204
Race [37]	1994	10	1000 mm/min			75	PCL	739	180
Trent [38]	1976	6	50 mm/min	custom grip		40	PCL	620	145
Yanke [14]	2013	9	10 %/s	methyl methacrylate	Insight 5	46.5	BTB	2293	356

n number of samples, *EP* entrance potential, *Max.* maximum, *ACL* anterior cruciate ligament, *PCL* posterior cruciate ligament, *ST* semitendinosus

differently if one of these parameters was altered. Because the same protocol was used, the various groups could be directly compared to each other.

Conclusions

A G4 construct has the anatomical features needed to serve as an ACL reconstruction graft. Its biomechanical properties are comparable to those of the standard grafts (patellar tendon and hamstring). The G4 is particularly well-suited to serving as an augmentation graft in cases of partial ACL rupture but the four-strand semitendinosus graft remains our first choice for complete ACL reconstruction. Clinical studies of the G4 must be performed to confirm these results.

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Compliance with ethical standards

Conflict of interest No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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