

# Remnant Muscle Preservation on Hamstring Tendon Autograft During ACL Reconstruction Promotes Volumetric Increase With Biological and Regenerative Potential

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**Background:** The removal of all adherent muscle tissue from the hamstring graft during anterior cruciate ligament reconstruction (ACLR) is common practice. However, there is a paucity of research to justify this removal or evaluate its biological implications.

**Purpose/Hypothesis:** The purpose of this study was to (1) evaluate the histological characteristics of the myotendinous muscle tissue harvested from hamstring tendons, (2) compare the final diameter of the prepared graft before and after the removal of the remnant musculature, and (3) evaluate patients who were treated with and without preservation of the graft-adhered muscle. The hypothesis was that the adherent musculature of the graft would have cells that could contribute to graft incorporation and revascularization, assist in the proprioceptive capacity of the neoligament, and increase the graft's diameter.

**Study Design:** Cohort study; Level of evidence, 3.

**Methods:** We divided 84 patients into 2 groups: group 1 underwent ACLR using hamstring tendon autograft with adherent musculature, and group 2 underwent ACLR using hamstring tendon autograft stripped of its remnant muscle. All patients had minimum 2-year follow-up. The muscle harvested from the graft in group 2 was submitted for histological examination, and the graft diameter before and after muscle removal was compared. The Tegner activity scale and Lysholm scores were determined preoperatively and at 12 and 24 months postoperatively.

**Results:** There was a significant difference in graft diameter between groups. The evaluation of the graft diameter in group 2 showed a decrease of 11.52% after removal of muscle tissue from the tendon graft. Patients from group 1 had better Tegner and Lysholm scores (mean  $\pm$  SD) after 12 months (Tegner, 8.03 vs 7 [ $P = .004$ ]; Lysholm,  $95.48 \pm 1.2$  vs  $87.54 \pm 3.21$  [ $P = .002$ ]) and better Lysholm scores after 24 months ( $95.76 \pm 2.1$  vs  $89.32 \pm 2.47$ ;  $P = .002$ ). The muscle tissue of the analyzed fragments presented a pattern with fibrous tissue beams, invaginating regularly and sequentially from the myotendinous junction into the muscles.

**Conclusion:** Preserving the muscle tissue on tendon grafts promoted a volumetric increase in the final autograft diameter and demonstrated biological and regenerative potential. Patients who underwent ACLR using the tendon with the muscle attached had better functional scores at 2-year follow-up as compared with patients treated using the tendon with the muscle removed.

**Keywords:** anterior cruciate ligament; hamstring grafts; biologic treatment; graft diameter; muscle tendon graft.

Graft selection is a key factor for anterior cruciate ligament reconstruction (ACLR). Important characteristics include size (diameter and length), harvest technique, and graft preparation (factors that influence the way that the graft will be fixed and integrated into the bone).<sup>2,5,16,17,24</sup> Also relevant is the ability of the graft to facilitate tendon-bone

incorporation, the biological remodeling of the tendon, and integration into bone tunnels (osteointegration).<sup>11,19</sup>

Grafts with smaller diameters are more susceptible to rerupture.<sup>3,5,27</sup> However, grafts should also be evaluated from a biological perspective. Several techniques for the preservation of the tibial stump of the anterior cruciate ligament (ACL) or an ACL repair are biological strategies to be considered for the religamentization process.<sup>1,30</sup>

During hamstring tendon graft preparation, the removal of all adherent muscle tissue is common practice.<sup>3,12,14,29</sup>

The Orthopaedic Journal of Sports Medicine, 9(3), 2325967121990016  
DOI: 10.1177/2325967121990016  
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There is a paucity of literature to justify its removal or its biological implications.<sup>25</sup>

The main purposes of this study were to (1) evaluate the histological characteristics of the muscle tendon junction (MTJ) tissue harvested from the hamstring tendons, (2) compare the final diameter of the prepared graft before and after the removal of the remnant musculature, and (3) evaluate patients who were treated with and without preservation of the graft-adhered muscle with at least 2-year follow-up. The hypothesis was that the adherent musculature of the graft would have cells that could contribute to graft incorporation and revascularization, assist in the proprioceptive capacity of the neoligament, and increase the graft's diameter.

## METHODS

This research was approved by the research ethics committee of our institution and the participants agreed to the terms of informed consent. A total of 84 patients underwent ACLR between February 2016 and January 2017 and had a minimum 2-year follow-up. Criteria for inclusion were as follows: age between 16 and 55 years, isolated ACL injury, and use of hamstring autograft with semitendinosus tendons at least 30 cm long and gracilis tendons at least 28 cm long. Exclusion criteria were the presence of other lesions or surgery in the same knee, semitendinosus tendon length <30 cm, gracilis tendon length <28 cm, or previous pes anserinus tendinopathy (Figure 1).

The patients were randomly divided into 2 groups using sealed envelopes. Group 1 underwent ACLR using hamstring tendon autograft with adherent musculature, and group 2 underwent ACLR using hamstring tendon autograft stripped of its remnant muscle. The MTJ harvested from the graft in group 2 was also submitted to histological examination.

All patients were diagnosed according to physical examination (pivot-shift, Lachman, and anterior drawer tests) and magnetic resonance imaging (MRI). Only isolated ACLR was performed in all selected patients.

### Autograft Harvesting

The autografts were harvested through a 3-cm longitudinal incision centered 4 cm below the medial joint line, 2.5 cm medial to the tibial tubercle, and along the distal insertion of the pes anserinus.

### Autograft Preparation

The gracilis and semitendinosus autografts were divided into 3 portions: tendon, MTJ, and muscle. First, the musculature of the gracilis and semitendinosus muscles was preserved, and a quintupled hamstring autograft was made (tripled semitendinosus graft and doubled gracilis graft) for all patients (groups 1 and 2).

A standard ruler was used to measure tendon length. To measure tendon diameter, a tendon-measuring block was utilized. The measuring tools were available during the surgical procedure (Figure 2).

### Sample Collection

For the patients in group 2, the autograft preparation was undone, and muscle was stripped from the tendon. A standard 3 cm of tendon was cut from the proximal end of the tendon (gracilis and semitendinosus) (Figure 3A) and analyzed microscopically. Then, 1.5 cm of the tendon was stripped (Figure 3B), and a 1.5-cm area was left with intact musculature (Figure 3, C and D). The sample was pinned onto cardboard paper to prevent deformation. Finally, after removal of the entire muscle, a new quintupled hamstring graft was made (tripled semitendinosus graft and doubled gracilis graft).<sup>27</sup> The graft diameter was compared before and after muscle removal in group 2 and between groups after ACL graft preparation.

### Histological Analysis

The specimens from group 2 were then fixed in 10% buffered formalin and embedded in paraffin blocks. Next, these specimens were cut into 4- $\mu$ m sections. The histological sections were stained with hematoxylin and eosin and Masson trichrome. Immunohistochemistry was also used to detect the S-100 protein for better visualization and characterization of the nerve endings. Imaging and analysis were performed using an optical microscope to determine the damage caused by curettage in the prepared area and the characteristics of the muscle tissues that were not stripped from the tendon. In addition, morphologic characterization was performed, and tissue distribution was documented for different types of nerve endings.

### Surgery and Postoperative Evaluation

All patients underwent ACLR using an adjustable loop femoral cortical suspension device (Ultragraft; Smith &

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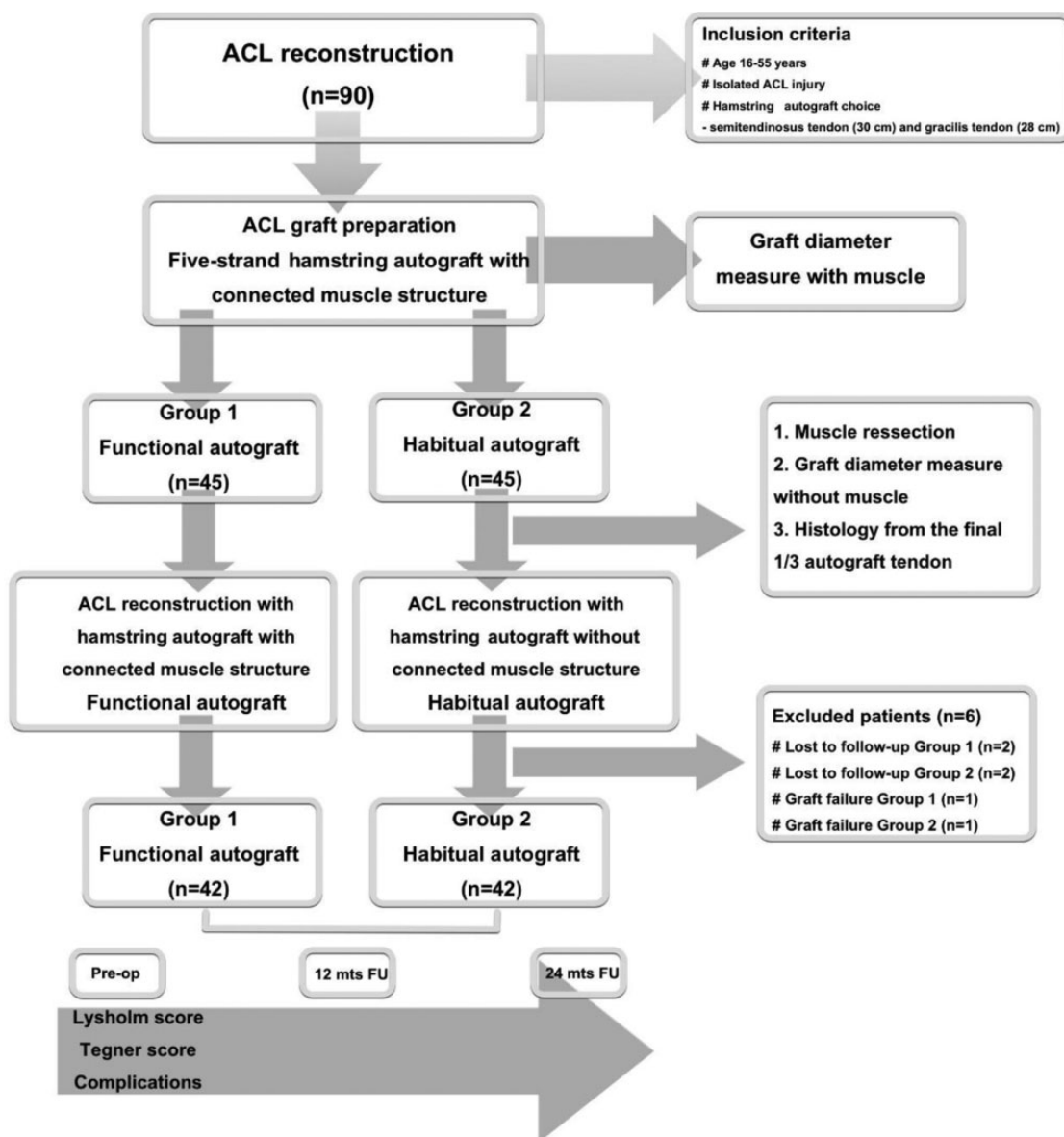
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Final revision submitted September 14, 2020; accepted October 16, 2020.

The authors have declared that there are no conflicts of interest in the authorship and publication of this contribution. AOSSM checks author disclosures against the Open Payments Database (OPD). AOSSM has not conducted an independent investigation on the OPD and disclaims any liability or responsibility relating thereto.

Ethical approval for this study was obtained from Hospital Governador Celso Ramos (No. 26821019.2.0000.5360).



**Figure 1.** Flowchart for the selection of patients included in the evaluation. ACL, anterior cruciate ligament; FU, follow-up; mts, months; Pre-op, preoperatively.

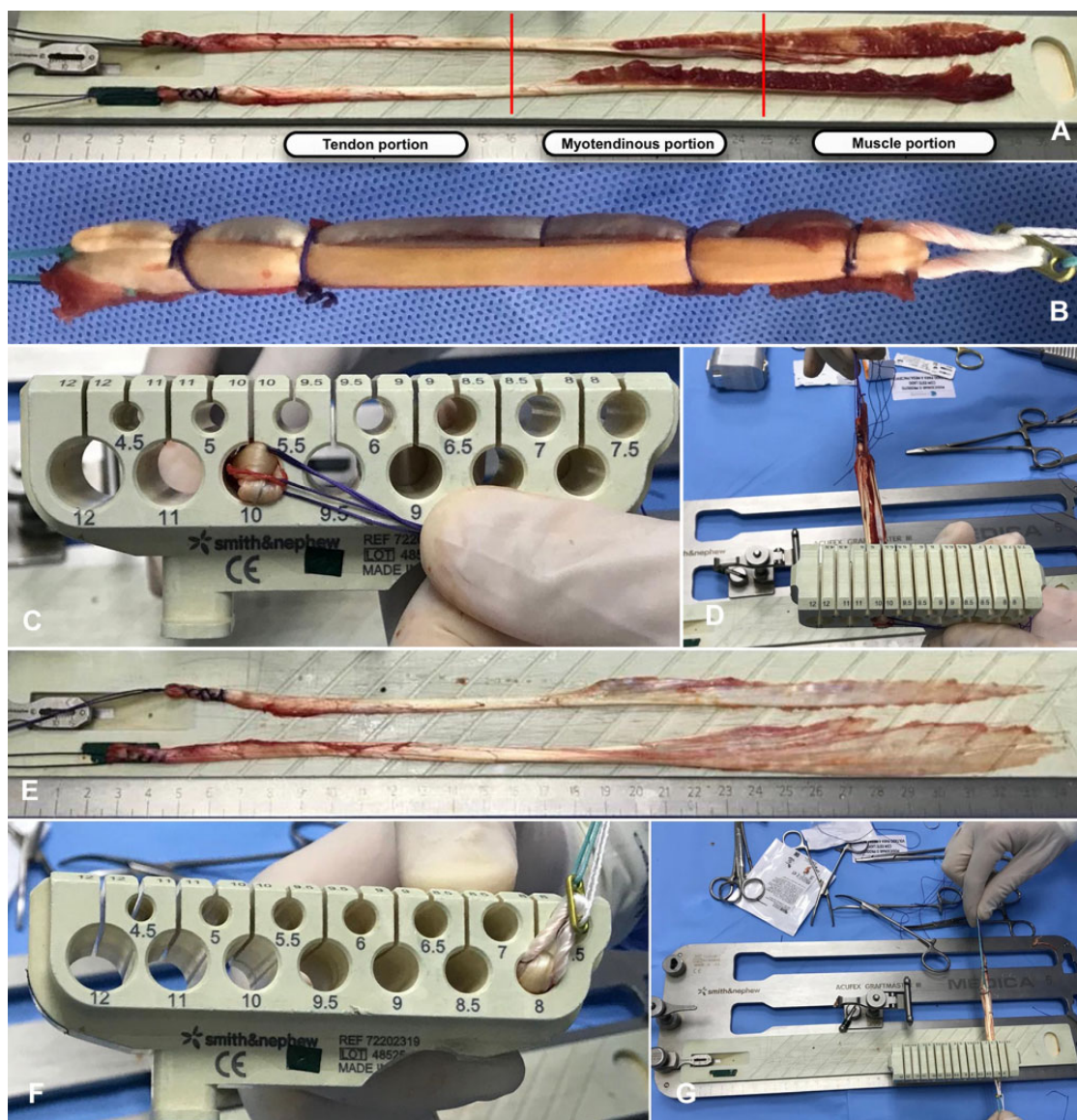
Nephew) and tibial fixation with bioabsorbable interference screws (Biosure; Smith & Nephew). Patients were discharged on the day after surgery, with no range of motion restrictions and with initial partial weightbearing. Patients were provided with an ACLR rehabilitation protocol for 6 to 9 months.

Postoperatively, patients were evaluated for 2 years. Evaluations occurred weekly during the first month, monthly up to 12 months, and finally twice a year. Tegner activity scale and Lysholm knee scores were determined, and complications were evaluated before surgery and at 12 and 24 months postoperatively. The results were compared between groups. Physical examination was performed at 6

months and 1 and 2 years postoperatively. Patients and examiners were blinded to the group.

### Statistical Analysis

A descriptive analysis was carried out (mean  $\pm$  SD and range) for each variable. For the qualitative variables, the absolute and relative frequencies were calculated. The Mann-Whitney test was used to compare all variables between groups and to compare graft diameter before and after muscle resection in group 2. The statistical analysis was carried out using SPSS Version 17.0 for Windows (IBM



**Figure 2.** (A) The graft was divided into 3 portions: tendon, myotendinous junction, and muscle. (B) A quintupled hamstring graft was made (tripled semitendinosus graft and doubled gracilis graft), preserving the muscle. (C) The graft tendon diameters in group 1 group were measured, and the configuration was maintained. (D) In group 2, the tripled semitendinosus graft and doubled gracilis graft demonstrate a smaller final diameter. (E) The semitendinosus and gracilis tendons were cleared of all musculature.

Corp). To obtain a sample power of 80% and a significance level of 95%, a sample size of at least 36 patients was required. The Lysholm and Tegner scores were considered the primary outcomes.

## RESULTS

Table 1 shows the comparison between group 1 (ACLR with preservation of the muscle tissue) and group 2 (ACLR without the muscle tissue). The mean age was  $26.39 \pm 0.78$  years (range, 16-54 years). There were no statistically significant differences between the groups with respect to age, height,

sex, and side. There was no difference between groups for physical examination tests (Lachman, anterior drawer, and pivot shift). However, there was a statistically significant difference regarding the graft diameter ( $P = .01$ ).

## Graft Size

The evaluation of the graft diameter in group 2 showed a decrease of 11.52% after removal of muscle tissue from the tendon graft (Table 2). In all cases, there was a decrease in the final graft diameter after muscular attachments were stripped ( $P < .004$ ).





**Figure 3.** (A) For group 2 patients, 1.5 cm of tendon was left with intact musculature, and the remainder was stripped. (B) Conventional preparation of hamstring tendon autograft with harvested muscle tissue. (C) Sample pinned on cardboard paper to prevent deformation. (D) The 1.5 cm of tendon fixed in 10% formalin for 24 hours and 1.5 cm of tendon-scraped material.

TABLE 1  
Patient Characteristics<sup>a</sup>

	Group 1 (n = 42)	Group 2 (n = 42)	P Value
Age, y	25.7 ± 0.82 (16-54)	27.09 ± 8.67 (19-50)	.06
Height, m	1.76 ± 0.12 (1.56 -1.91)	1.79 ± 0.19 (1.59 -1.90)	.08
Sex			.07
Male	88	85	
Female	12	15	
Side			.06
Right	68	65	
Left	32	35	
Graft diameter, mm	8.76 ± 0.89 (8-11)	8.14 ± 0.66 (7-9)	.01

<sup>a</sup> Data are reported as mean ± SD (range) or percentage.

### Histological Analysis

From the MTJ tissue of the analyzed fragments from group 2, it was possible to identify a structural projection of the tendon proximal to the muscle from the lower portion of the tendon tissue to the end of the macroscopic section (Figure 4A). The histological section is an important factor to confirm and help in understanding the aforementioned

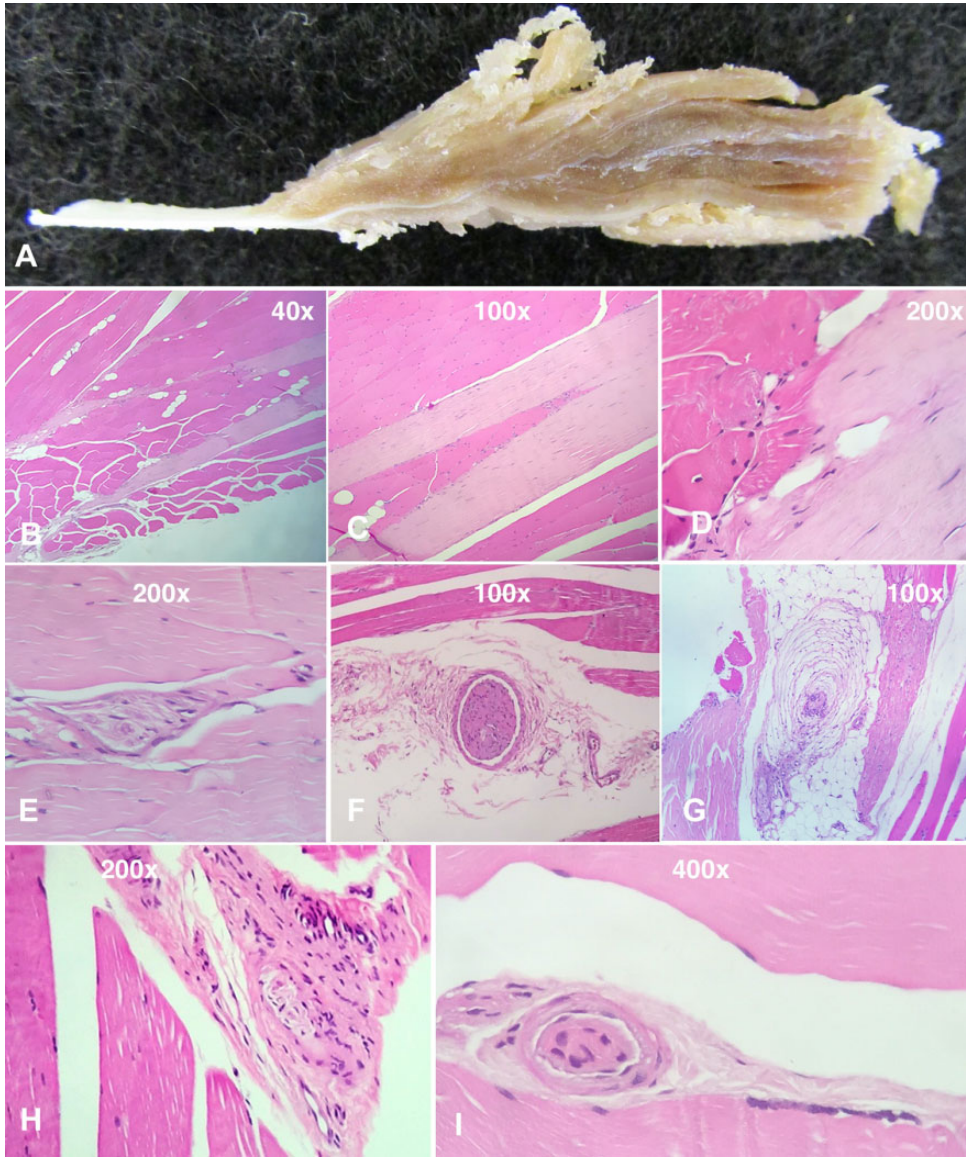
observations. The microscopic analysis of the section (Figure 4B) clearly shows the movement of this projection between muscle beams. A close, practically indivisible relationship between the tendon and the muscle is visible (Figure 4C). The photomicrograph shows the triangle-shaped muscle infiltrated in the tendon tissue. It was not possible to identify any type of cleavage between the tendon and muscle tissue in its transition area (Figure 4, D and

TABLE 2  
Graft Diameter in Group 2 Patients Before and After Stripping the Tissue<sup>a</sup>

	Graft Diameter, mm		P Value
	Graft With Muscle (n = 42)	Graft Without Muscle (n = 42)	
Midpoint	9.20 ± 1.05 (9-11)	8.14 ± 0.66 (7-9)	.002
Femoral portion	9.32 ± 0.93 (9-11)	8.31 ± 0.97 (7-10)	<.001
Tibial portion	9.51 ± 0.76 (8-11)	8.40 ± 1.04 (7-10)	.004

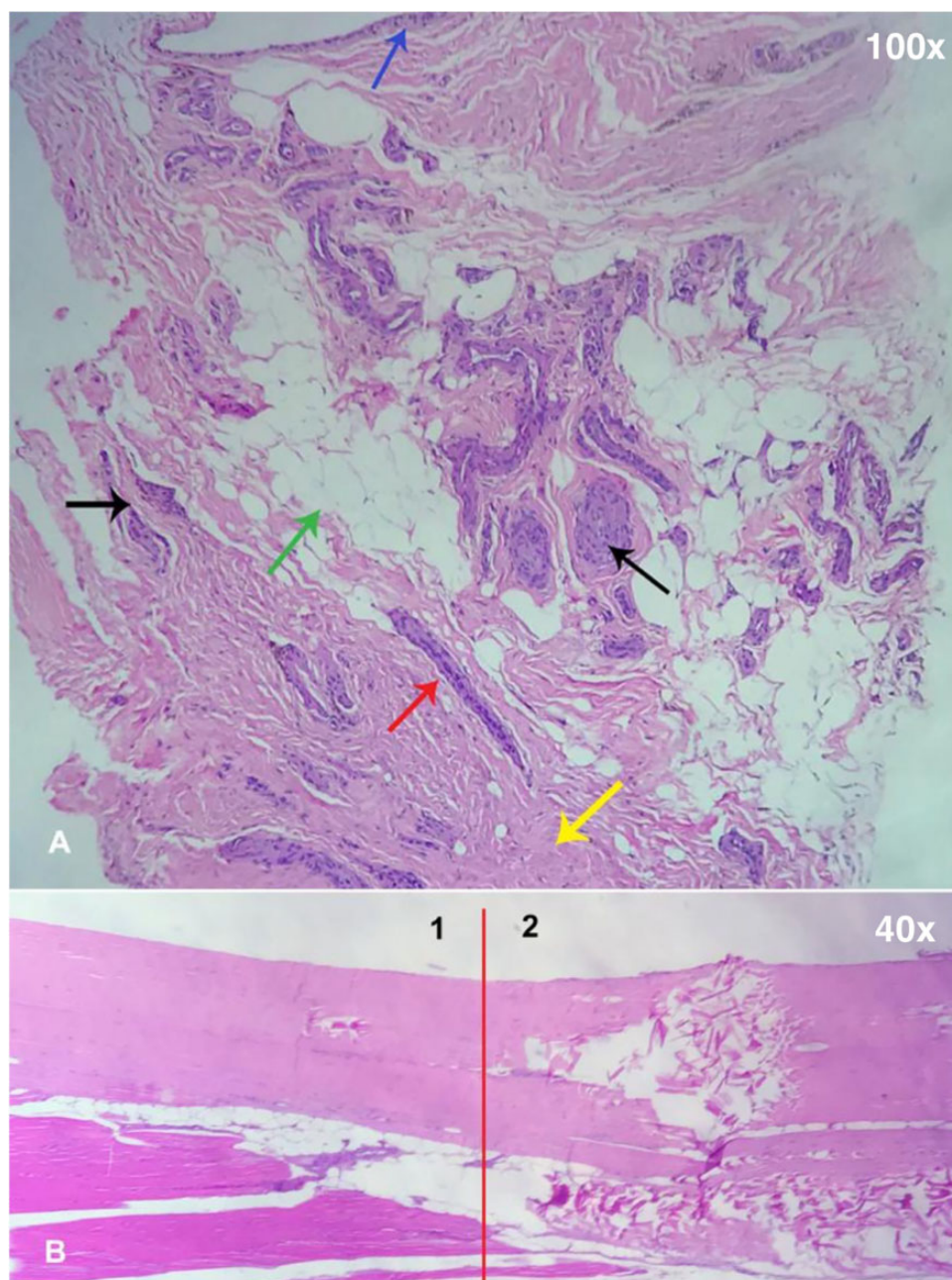
<sup>a</sup> Data are reported as mean ± SD (range).

E). With regard to the qualification of the resident cells, some islands composed of blood vessels and fat deposits can be visualized (Figure 4B). Numerous types of biologically active cells were identified in all samples, determining an important part of the muscle volume attached to the tendon. Within the cell content, we identified mechanoreceptors, such as free nerve endings, Pacinian-like corpuscles, Ruffini-like corpuscles, Golgi tendon organs, and satellite cells (Figure 4, E-I). It is worth mentioning that the same types of cells in native ACL fragments were found in the ACL remnants from patients who underwent surgery (Figure 5).



**Figure 4.** (A) A macroscopic section of the tendon infiltrating the muscle, revealing its clear structural framework. (B) The macroscopic section demonstrates exactly the same aspects; however, the muscle and tendon were analyzed using electron microscopy. (C) There is a clear connection between muscle and tendon. The triangle-shaped muscle infiltrates the tendon tissue. (D) There is no cleavage in these tissues; there is actually a modification: a specialization of the tendon to the muscle or vice versa. The nuclei are transiting simultaneously between the tissues. Mechanoreceptors found in the histological sections: (E) Ruffini-like corpuscles, (F) Golgi tendon organs, (G) Pacinian-like corpuscles, (H) free nerve endings with atypical corpuscles, and (I) possible satellite cells.





**Figure 5.** (A) Aspect of dry anterior cruciate ligament in one of the patients shows the native ligament with nerve endings in its structure, demonstrating the importance of maintaining its original configuration. It is possible to identify synovial lining tissue (blue arrow), blood vessels (red arrow), adipose tissue (green arrow), ligament tissue (yellow arrow), and mechanoreceptors (black arrows). Two distinct aspects: (B1) the intact tendon tissue attached to the muscle tissue and (B2) the cracked aspect of tendon tissue after removal of the muscle tissue by curettage, promoting a microscopic disorganization in its structure.

### Clinical and Functional Assessment

The comparison between groups 1 and 2 showed that patients who had undergone ACLR with preserved muscle had better Tegner and Lysholm scores 12 months postoperatively (Tegner,  $8.03$  vs  $7$  [ $P = .004$ ]; Lysholm,  $95.48 \pm 1.2$  vs  $87.54 \pm 3.21$  [ $P = .002$ ]) and better Lysholm scores 24

months postoperatively ( $95.76 \pm 2.1$  vs  $89.32 \pm 2.47$ ;  $P = .002$ ) (Table 3).

### Complications

Overall, 2 patients in group 1 and 1 patient in group 2 had a new episode of knee injury during the study at 8, 23, and 14

TABLE 3  
Comparison of Tegner Activity Scale and Lysholm Scores  
Between the Study Groups

	Group 1	Group 2	P Value
Tegner score, mean (range; median)			
Before injury	9.2 (8-10; 10)	9.1 (8-10; 10)	.08
Before surgery	3.83 (0-4; 3)	3.9 (0-4; 3)	.09
12 mo	8.03 (6-10; 8)	7 (5-10; 7)	.004
postoperatively			
24 mo	8.36 (7-10; 8)	8.5 (7-10; 8)	.06
postoperatively			
Lysholm score, mean $\pm$ SD			
Before surgery	58.09 $\pm$ 6.97	59.98 $\pm$ 5.87	.09
12 mo	95.48 $\pm$ 1.2	87.54 $\pm$ 3.21	.002
postoperatively			
24 mo	95.76 $\pm$ 2.1	89.32 $\pm$ 2.47	.002
postoperatively			

months, respectively. A histological evaluation of the failed graft was performed in the group 1 patient at 8 months after ACLR and in the group 2 patient at 14 months after ACLR. This revealed full integration and differentiation of tendon and muscle tissues into ligament in these 2 patients (Figure 6). The other group 1 patient sustained a medial meniscal tear 23 months after his ACLR and underwent an arthroscopic partial meniscectomy. During that surgery, we were able to evaluate the reconstructed ACL using MRI scans and compare those scans with those of the 2 failed ACLRs (Figure 7).

All other patients underwent MRI examinations 2 years postoperatively, but it was not possible to differentiate muscle from tendon tissue with this imaging modality.

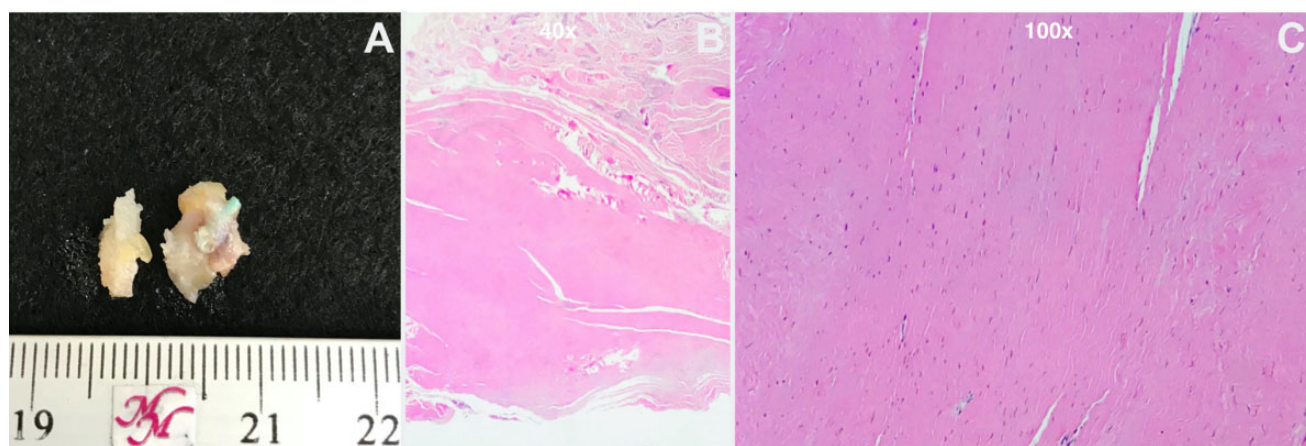
There was no difference in MRI appearance between groups.

## DISCUSSION

The most important finding of this study was that the presence of the muscle in the hamstring tendon used in ACLR resulted in an mean diameter increase of 11.52%. In addition, the histological characteristics of the evaluated muscle tissue allowed us to infer that its preservation is more consistent with current graft preparation techniques. Finally, the group with grafts with preserved muscle tissue achieved better Lysholm scores after 12 and 24 months and better Tegner scores after 12 months.

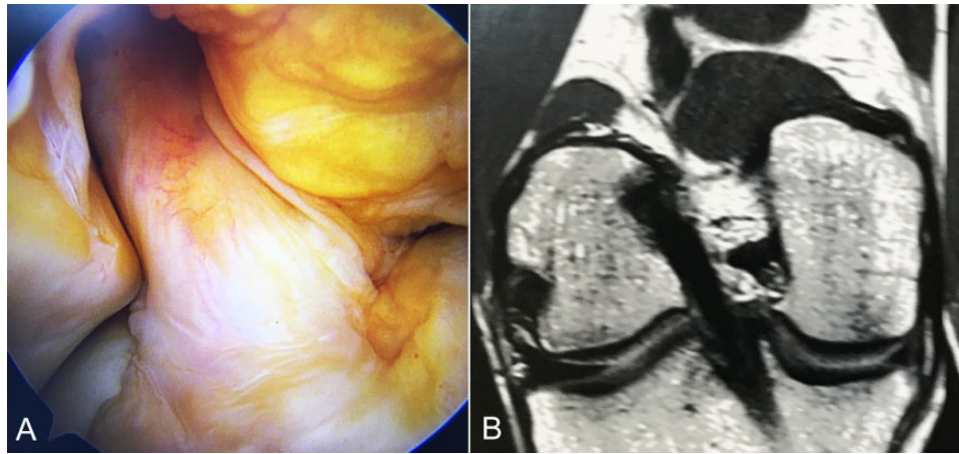
Achieving a minimum graft size has been shown to have an important role in ACLR and may have an effect on the graft choice in ACLR.<sup>3,6</sup> Magnussen et al<sup>15</sup> reported that a graft size <8 mm increases the chance of graft failure rates.<sup>26</sup> The preserved muscle tissue on tendon graft resulted in larger graft diameter. Muscle tissues are a better source of immature mesenchymal cells than are tendon tissues,<sup>7</sup> and in the right microenvironment, these cells are able to differentiate into tendon tissue.<sup>4,23</sup> Given this information, we can infer that increasing the graft diameter using muscle tissue not only serves to increase the diameter but also offers biological potential to differentiate into tendon.

The capacity of muscle tissue to enhance the healing process, aid with the differentiation of tendon tissue, and potentiate the biological activity of the graft makes its use even more relevant.<sup>7</sup> Histological analysis in this study showed the beam-like projection of fibrous tissue derived from the tendon tissues and invagination into the muscle mass, with no signs of cleavage between the tissues. Proximal to the MTJ, the distal tendon portion of the graft



**Figure 6.** (A) Macroscopic aspect of a group 1 patient's right knee graft (tendon + muscle) 8 months after ACL reconstruction. (B, C) In this sample, the histological aspect is completely similar to native ACL, and any muscle remnant is absent. ACL, anterior cruciate ligament.





**Figure 7.** (A) A 23-month postoperative right knee anterior cruciate ligament reconstruction using hamstring tendon autograft with preserved muscle tissue visualized during a partial medial meniscectomy, and (B) the same knee shown on a coronal view of the magnetic resonance imaging scan.

extended its projection to the proximal segment with united beams.

Tendon depends on its adherent musculature for development.<sup>4</sup> The biological potential of the remnant musculature to help the healing of the tendon graft has already been elucidated by Ghebes et al.<sup>10</sup> The muscle tissue contains neurotrophic factors that assist in the survival and growth of proprioceptive neurons.<sup>8</sup> Skeletal muscle tissue also has a regulatory effect on tendon development through the paracrine secretion of key factors related to tendon development.<sup>18,22</sup> The preservation of adherent musculature allows the slow release of substances that are capable of improving ligamentization and tendon-bone integration after ACLR.<sup>7</sup> However, the molecule involved in this process still needs to be better defined.<sup>28</sup>

The macro- and microscopic features suggest that muscle and tendon tissues are indivisible and symbiotic, with the simultaneous presence of the same cell in both tissues. Knudsen et al<sup>13</sup> demonstrated that 3-dimensional reconstruction of the MTJ revealed that the tendon made ridge-like protrusions, which interdigitated with groove-like indentations in the muscle cell. Finally, we have observed that the myofilaments merge with the tendon tissue to form a joint structure. These findings demonstrate a harmonic and natural transition between tissues. It can be inferred that the tendon undergoes specialization and transformation into muscle in this area. Sun et al<sup>25</sup> demonstrated that preserved muscle remnant enhanced ACL graft healing and remodeling in the rabbit model. Chen et al<sup>4</sup> explored the possibility of engineering stronger tendons using mouse skeletal muscle-derived cells (MDCs) with more mature collagen structure and thicker collagen fibrils as opposed to tenocyte-engineered tendons. In addition, MDCs have greater proliferation potential than do tenocytes and may contribute to the clinical translation. Collectively, these results indicated that MDCs may serve as a desirable alternative cell source for engineering functional tendon tissue.<sup>4</sup>

In the histological evaluation of the muscle tissue, it was possible to observe the fragmentation of the surface that resulted in microruptures in the tissue. In these cases, it became evident that the removal of the muscle tissue via curettage promoted a microscopic disorganization of its structure. This finding may be associated with the weakening of the graft's mechanical properties.<sup>31</sup>

In the present study, the presence of numerous islands of fat cells, biologically active tissue, and sites of pluripotent cells was identified in the musculotendinous tissue of the graft, which may strongly contribute to bone-graft integration.<sup>9,21</sup> Moreover, we identified a large amount of blood vessels, which may promote acceleration of the graft revascularization process by offering a greater supply of biologically active cells.<sup>7,14,20,22</sup>

The significant differences in the functional testing using Lysholm and Tegner scores after 12 and 24 months in group 1 may be the result of biological changes promoted by the preserved muscle tissue. Although the results obtained in group 2 were good to excellent, the group with the preserved muscle grafts presented even better results.

### Limitations

A limitation of this study is that it was not possible to evaluate the histological aspect of all patients after the clinical evaluation period, given that it could be done only in the case of ACLR failure (1 patient in each group had ACLR failure). Another limitation is that the static histological analysis allows for the evaluation of tissues at a single point in time. Additionally, we did not evaluate patients using an objective measurement of laxity or other scores (eg, International Knee Documentation Committee and Knee injury and Osteoarthritis Outcome Score). Although we found a statistically significant difference, the Lysholm score did not exceed the minimal clinically important difference. Furthermore, we do not know if the muscle tissue would

remain viable or revascularize after ACLR, and we are unsure about myocyte and Golgi tendon organ survival. Finally, most patients were male, with larger muscles and tendons than those in typical female patients.

## CONCLUSION

Preserving muscle tissue on hamstrings autografts promoted a volumetric increase in the final autograft diameter and may have improved biological and regenerative potential for ACLR. The patients who underwent ACL surgery using the tendon with muscle attached had better functional scores at 2-year follow-up when compared with a control group treated using the graft stripped of muscle.

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