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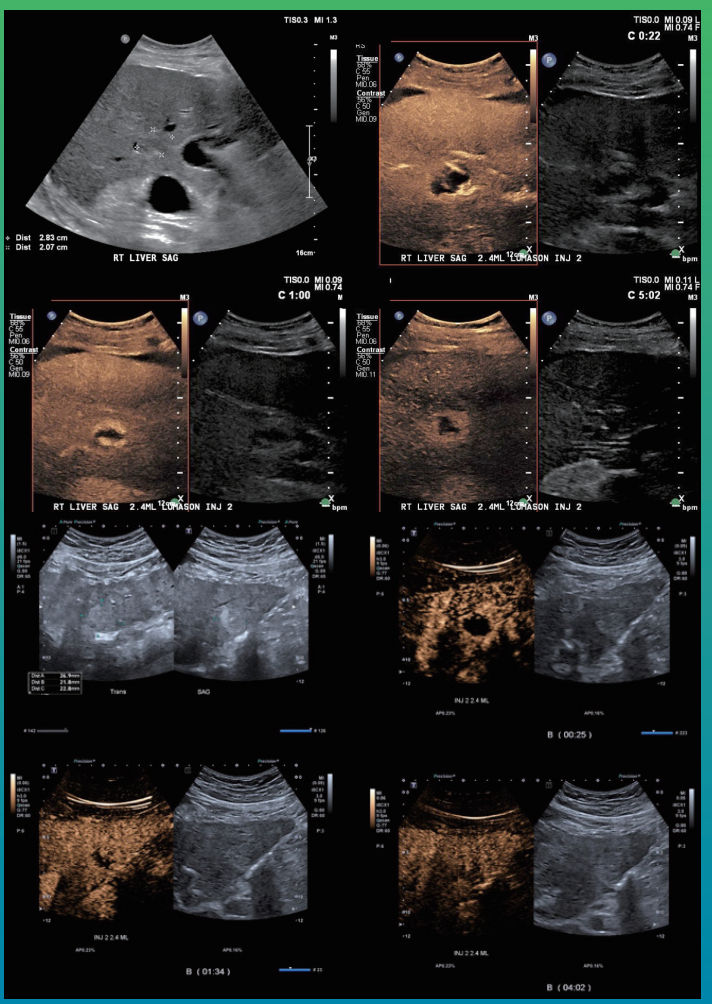

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CONTENTS

Case Reports

ORIGINAL RESEARCH

Ultrasonographic Identification of Muscle Atrophy in Hamstring Muscles after Anterior Cruciate Ligament Repair among Soccer Players: A Case-control Study

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Objectives: To measure the CSA of the HST musculature measured with ultrasonography in soccer players undergoing ACLR and compare limb differences with healthy controls.

Methods: A case-control study was performed with patients after anterior cruciate ligament repair (ACLR) and healthy controls in which cross-sectional areas (CSA) obtained using a model TE7 ultrasound machine (MINDRAY ®, USA) in B mode (4.2 to 13 MHz) with a multifrequency linear array transducer (L12-4S). Three CSA images were taken of the semitendinosus muscle (ST) and the long head of the biceps femoris (BFlh), at a distance of 30% and 70% of the ischial tuberosity insertion. Mean differences between groups were analyzed using SPSS v.20 (IBM®, USA), and statistical analyses were performed using non-parametric techniques to determine differences between groups (Student's t-test) and Cohen's correlation coefficient to quantify effect size.

Results: 14 ACLR operated 17 ± 5.4 months ago and 12 healthy controls (W = 6; M = 20M; 24.5 ± 3.92 years; BMI = 25.1 ± 2.32 kg/m^2) were recruited. There were differences between groups in CSA-ST70 (Post-ACLR = 1.43 ± 1.029 cm^2 vs Control 2.65 ± 0.664 cm^2, T Student = -3.68, 95% CI [-Inf, -0.648], $P < 0.001$, ES = -1.418), but not in CSA-ST30 (Post-ACLR = 8.42 ± 1.596 cm^2 vs Control 9.16 ± 0.945 cm^2, T Student = -1.535; 95% CI [-Inf, -0.0793], $P = 0.068$, ES = -0.5607), CSA-BFlh30 (Post-ACLR = 8.79 ± 1.47 cm^2 vs Control 8.87 ± 2.312 cm^2, T Student = -0.123; 95% CI [-Inf, 1.1049], $P = 0.452$, ES = -0. 049) or CSA-BFlh70 (Post-ACLR = 6.91 ± 1.011 cm^2 vs Control 7.01 ± 1.453 cm^2, T Student = -0.214; 95% CI [-Inf, 0.6795], $P = 0.416$, ES = -0.0783).

Conclusion: Ultrasound measurement of the CSA can be an image marker to identify muscle weakness or atrophy that predicts functional loss early.

Key words: ACLR; Ultrasonography; Atrophy; Hamstring

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The Anterior Cruciate Ligament (ACL) repairing is a procedure to correct the partial or total rupture of ligament fibers as consequence of an excessive tension or torsion of the knee [1,2]. ACL repair (ACLR) consist of the extraction of a hamstring (HST) tendon autograft provide from m. Semitendinosus (ST) and/or the m. Gracilis (GT) which is obtained from the leg on the same side as the injury [3].

There are uncommon side effects of this surgical method. Acute complications may involve bruising, infection or stiffness of the knee. Chronic problems are associated with ACL reinjury, with the surgical procedure itself or osteoarthritis or with chondral damage [4]. Chronic problems can also adversely affect the recovery rate following an ACL reconstruction [5,6].

Severe to moderate loss of function of the knee is very common. Extraction of a harvested graft of this HST muscles or postoperative immobilization of knee in flexion with a limited mobilization cast (LMC) are the most common causes of loss of knee functionality [7-9].

The shortening of HST muscle due to LMC can result in muscle atrophy in both the short term (up to 9 months) and long term (over 4 years) [10].

As a result of the atrophy, the decrease of the muscle thickness measured by the maximum cross-sectional area (CSA) of the ST muscle is an accurate and reliable measure for monitoring athlete's muscle volume [11] and predicting functional recovery after ACLR [12].

Ultrasonography (US) has been proven to be a trustworthy and accurate technique for measuring the CSA of HST muscles, making it an effective tool for monitoring and assessing patients during and after ACLR surgery, as well as throughout their recovery period [13,14].

Although there is a lack of previous literature on this topic, Hjaltadóttir et al. have recently utilized US to measure changes in the muscle area in a group of healthy athletes. Their study focuses on correlating ST atrophy and hypertrophic BFlh compensation on the injured side after repair [15].

While other observational studies failed to quantify the differences in CSA between individuals who underwent ACLR versus healthy through US, this observational cross-sectional study aims to provide comprehensive and current insights into this area of research.

Objective

The main objective of this study was to evaluate the difference in CSA, as measured with US, between the m. Biceps Femoralis long head and m. Semitendinosus in soccer players who have undergone ACLR and healthy controls in the long term.

Methodology

Study design

A cross-sectional case-control study was carried out according to the STROBE criteria (Statement for Reporting Observational Studies) [16], using non-probability convenience sampling to quantify the CSA of the HST musculature measured with US in subjects undergoing surgical ACLR of the knee compared to asymptomatic subjects.

The study was carried out between March 13, 2023, and April 1, 2023 at the European University of the Canary Islands (Campus Casa Salazar, La Orotava) and had previously the approval of the internal university committee. RHG obtained participants written informed consent to participate and registered in an anonymous database prior to inclusion in the study. Finally, CSC was responsible for collecting and storing the data obtained.

Participants

After signing the written informed consent, RHG and ABL conducted a medical history and clinical examination to assess compliance with previously established criteria.

Inclusion criteria

(1) Male and female soccer player over the age of 18 years; (2) suffering from complete ACL tear; (3) undergone to ACLR with ST tendon autograft within 12 months; (4) or healthy without background of capsular-ligament injury; (5) not being treated with analgesic or NSAID drug therapies for pain relief; (6) not having hearing limitations and reading-writing abilities in Spanish; (7) and having signed the informed consent for participation in the study.

Exclusion criteria

(1) Male and female children under the age of 18 years; (2) suffering from partial ACL tear combined or not with another capsular-ligament injury of the knee; (3) undergone to ACLR with m. Gracilis (GT) or patellar tendons autograft within or over 12 months; (4) being treated with analgesic or NSAID drug therapies for pain relief; (5) having hearing limitations and reading-writing abilities in Spanish; (6) and having not signed the written informed consent for participation in the study.

Data collection and measuring instruments

After identifying a total of 87 candidates, 31 participants who did not give their written informed consent were eliminated. 56 subjects were finally interviewed, of which 26 were eliminated for not meeting the eligibility criteria. A total of 30 subjects were included, of which $n = 15$ were soccer players underwent ACLR (Post-ACLR) and $n = 15$ healthy controls (Fig. 1).

Study variables

Anthropometric Measurements Anthropometric measurements were taken regarding weight, height, body mass index, as well as the study of body composition through a calibrated bioimpedance device (Silvercrest®, Deutschland) that allowed the measurement of the percentage of fat, muscle, bone and water mass.

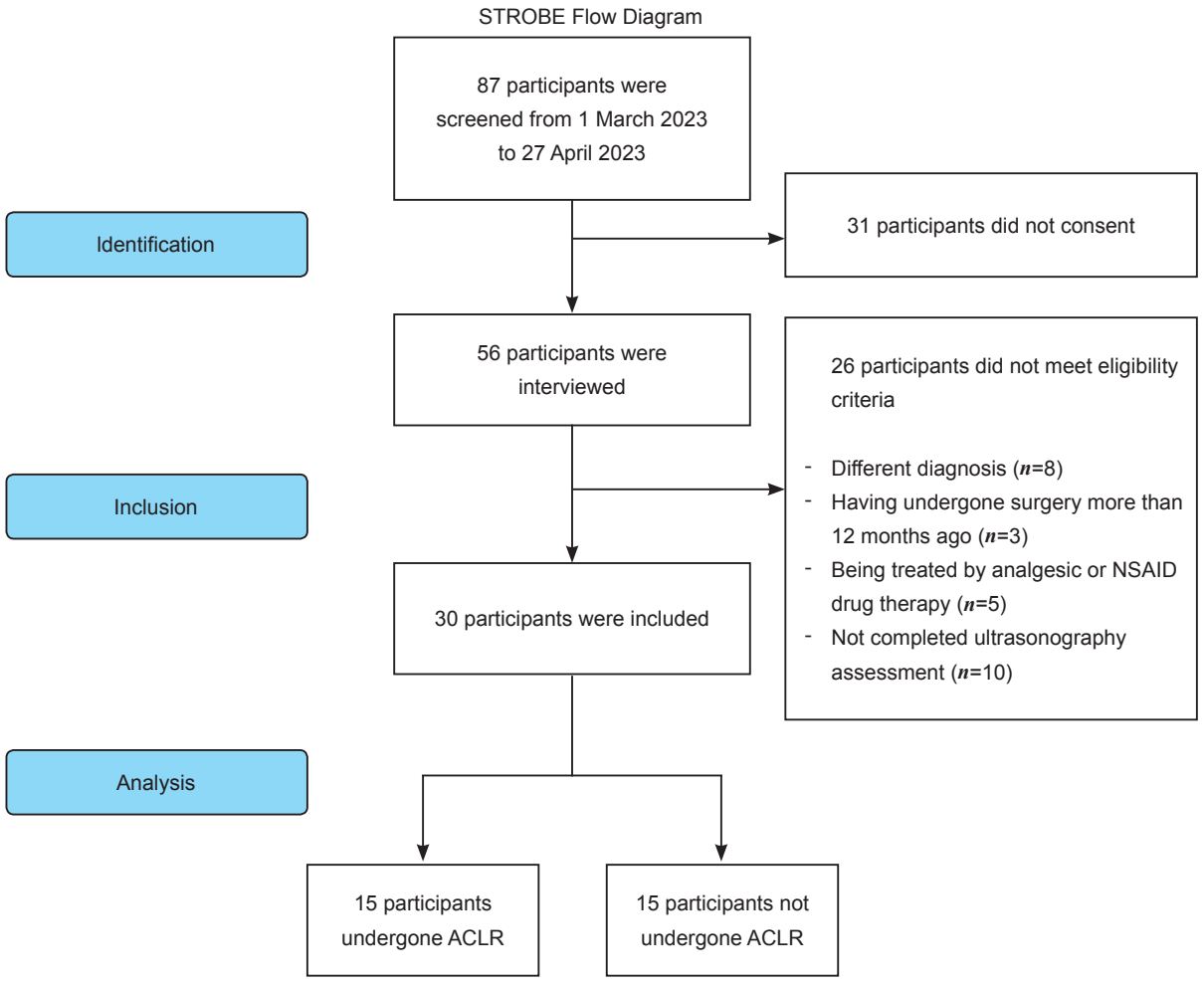

Figure 1 Participant selection process.

Ultrasonography Measurements Imaging data was collected by a physical therapist (SM) with 10 years of experience with an ultrasound machine TE7 (MINDRAY ®, USA) along with a multi-frequency linear array transducer (L12-4S) that captures cross-plane images. Ultrasound measurements was carried out following Kositsky et al. (2020) [17] procedure in which participants rested for approximately 10 minutes in a prone position to identify a distance of 30 and 70% of the distance between the palpated location of the ischial tuberosity (IT) and the popliteal fold (PF), which were marked in the operated leg of the Post-ACLR and the dominant leg of the controls.

The procedure and locations were chosen to provide a simple, standardized method for obtaining a proximal and distal representation of the CSA of HST muscles. The US technique began distally and medially by identifying the ST tendon and following it proximally to the distal imaging site (CSA-ST70), where images were obtained and stored, and then the muscle was followed to the proximal imaging site (CSA-ST30) for further imaging. The same procedure, distal (CSA-BFlh70) to proximal (CSA-BFlh30), was performed laterally for the BF muscle. Furthermore, a panoramic view at each site were obtained to have a wider field of view US imaging.

The images obtained were stored on a removable memory device and sent to another researcher (MHP) who was blinded of participants allocation in charge of performing the calculation of CSA through Area Tool drawing a circumference of muscle cross-plane image. Figure 2 shows Ultrasound measurements process.

Statistical Analysis

Statistical analysis was carried out using SPSS v.20 software (IBM®, USA) for data analysis and graphical

representation. First, researcher ABL recorded in an electronic database the results of the assessment instruments used to measure each study variable while CSC verified the accuracy of the data by completing double data entry.

Secondly, the researcher RHG and FHR performed the calculations of the descriptive statistics of centralization (mean and median), dispersion (standard deviation) and position (minimum, maximum) to describe the study variables. Thirdly, the Shapiro-Wilk test for quantitative variables was performed to determine the normality of the data obtained.

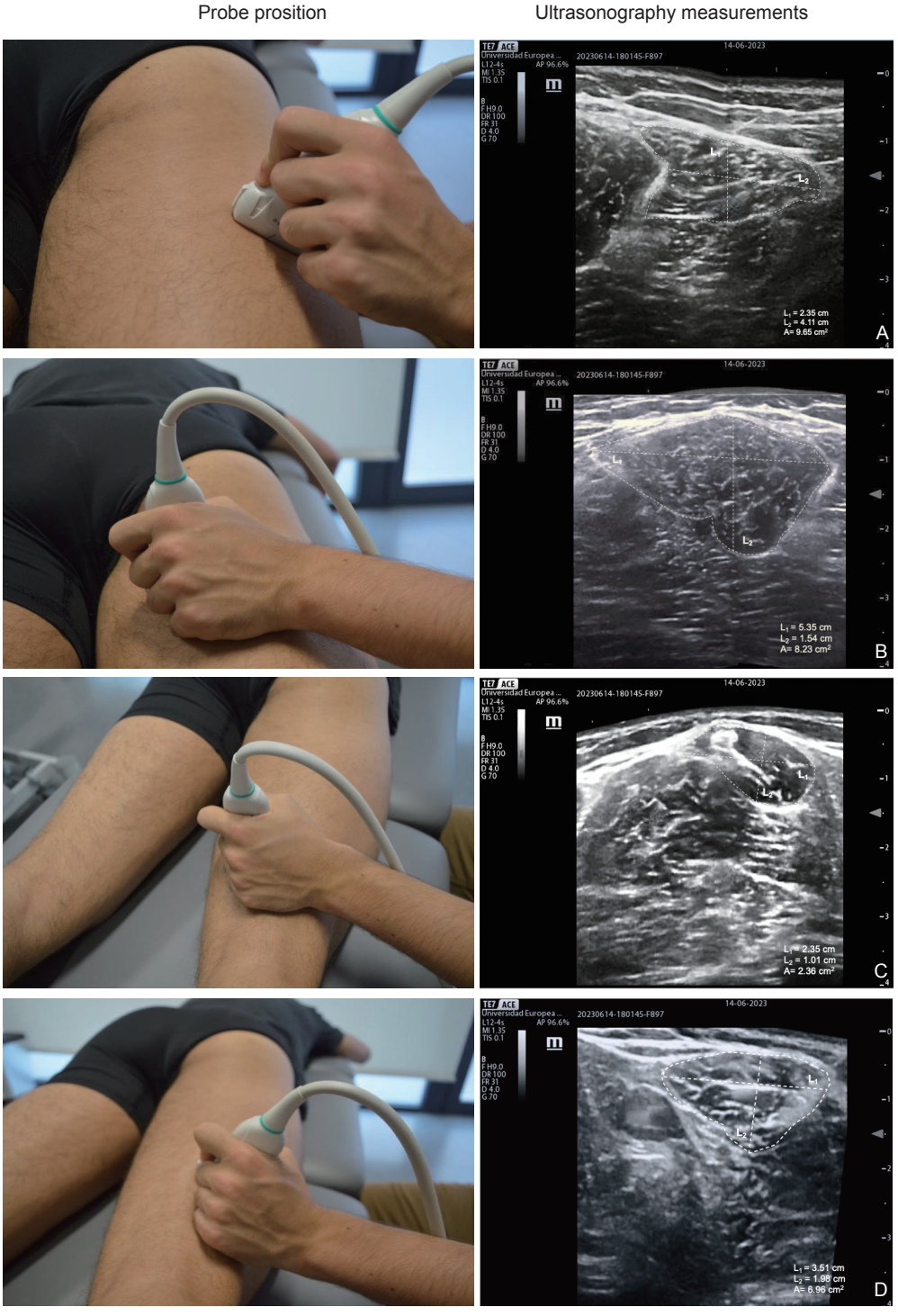

Figure 2 Ultrasonography measurements process. Ultrasonography measurements through L12-4S probe with TE7 device (MINDRAY ®, USA). (A) CSA-BFlh30 measured by ultrasonography 30% away from its origin; (B) CSA-ST30 measured by ultrasonography 30% away from its origin; (C) CSA-BFlh70 measured by ultrasonography 70% away from its origin; (D) CSA-ST70 measured by ultrasonography 70% away from its origin.

The variables that met the assumptions of normality ($P > 0.05$) were studied with an analysis of intergroup mean differences (T-student for independent means). In case of not complying with the assumptions of normality, it was decided to perform an analysis with nonparametric techniques to determine the intergroup differences (Mann-Whitney U test). Finally, we calculated the effect size (Cohen's d or biserial correlation coefficient) to quantify the size of the difference between two groups. Statistical significance was set at a value of $P < 0.05$.

Results

Description of the sample

Subjects were divided into post-ACL group (n = 15, W = 5, M = 10) and control group (n = 15, W = 3, M = 12). The mean age of participants in the case group was 25.13 ± 4.13 years while the control group 24.00 ± 4.08 years.

On the one hand, an average height of 1.76 ± 0.09 m in the control group and 1.78 ± 0.08 m in the Post-ACLR group intervened stands out. In reference to weight, the control group has a mean average of 78.95 ± 15.02 Kg, compared to the Post-ACLR group intervened that maintains an average of 82.39 ± 9.72 Kg. Regarding BMI, the control group had a mean of 25.19 ± 3,487 Kg/m^2 while the Post-ACLR had an average of 25.85 ± 2,096 Kg/m^2.

The percentage in fat in the control group has an average value of 22.14 ± 2.61 %, while the Post-ACLR group is 25.85 ± 1.69 %. The percentage of muscle mass in the control group shows a mean average of 42.74 ± 1.49%, and in the Post-ACLR group 41.59 ± 1.49%.

The percentage of bone mass in the control group 11.67 ± 0.71 % and in the Post-ACLR group is 11.42 ± 0.772 % while the percentage of water in the control group was 53.86 ± 1.979 % and in the Post-ACLR group 53.13 ±1.97% (Table 1).

Table 1 Demographic description of the sample

Item	Group	N	Mean	SD	Min.	Max.	Shapiro-Wilk	
							W	*P*
Age	*Post-ACLR*	15	25.13	4.13	21	33	0.85	0.023
	Control	15	24.00	4.08	19	33	0.85	0.018
Height (m)	*Post- ACLR*	15	1.78	0.07	1.68	1.95	0.94	0.425
	Control	15	1.76	0.08	1.59	1.89	0.94	0.513
Weight (Kg)	*Post- ACLR*	15	82.39	9.71	68.90	99.30	0.91	0.170
	Control	15	78.95	15.01	52.30	103.60	0.95	0.612
BMI (Kg/m^2)	*Post- ACLR*	15	25.85	2.09	22.25	30.58	0.98	0.986
	Control	15	25.19	3.48	19.89	30.24	0.92	0.223
% Fat mass	*Post-ACLR*	15	22.14	1.69	19.70	24.60	0.93	0.300
	Control	15	23.43	2.61	19.00	27.90	0.94	0.460
% Muscle mass	*Post-ACLR*	15	41.59	1.48	39.70	44.30	0.93	0.354
	Control	15	42.74	1.70	40.10	45.10	0.90	0.131
% Bone Mass	*Post-ACLR*	15	11.42	0.77	10.10	12.50	0.92	0.260
	Control	15	11.67	0.70	10.30	12.70	0.95	0.590
% Water	*Post-ACLR*	15	52.13	1.97	49.60	56.10	0.92	0.251
	Control	15	53.86	2.76	50.80	58.50	0.88	0.047

Description of study variables

Cross-sectional Area of m. Biceps Femoris Long head (CSA-BFlh) The mean average CSA by 30% from the insertion of CSA-BFlh30 was 8.79 ± 1.4 cm^2 in the ALCR group and 8.87 ± 2.31 cm^2 in the control group. Moreover, the average cross-sectional area by 70% from

the insertion of the long head of CSA-BFlh70 was 6.91 ± 5.05 cm^2 in the operated group and 7.01 ± 1.45 cm^2 in the control group.

Cross-sectional Area of m. Semitendinosus (CSA-ST) The mean cross-sectional area in 30% since the insertion of CSA-ST30 was 8.42 ± 1.60 cm^2 in the ACLR and 9.16 ± 0.95 cm^2 in the control group. Moreover, the mean cross-sectional area by 70% since the insertion of the CSA-ST70 was 1.43 ± 1.03 cm^2 in the ACLR and 2.65 ± 0.66 cm^2 in the control group (Table 2).

Table 2 Description of the study variables of the sample

Item	Group	N	Mean	SEM	SD	Min.	Max.	Shapiro-Wilk	
								W	*P*
CSA-BFlh30	*Post-ACLR*	15	8.79	0.36	1.41	6.61	11.66	0.96	0.69
	Control	15	8.87	0.59	2.31	4.29	14.36	0.95	0.65
CSA-BFlh70	*Post-ACLR*	15	6.91	0.26	1.01	5.05	8.45	0.94	0.49
	Control	15	7.01	0.37	1.45	4.33	9.11	0.94	0.43
CSA-ST30	*Post-ACLR*	15	8.42	0.41	1.59	5.48	11.22	0.98	0.96
	Control	15	9.16	0.24	0.94	7.56	10.93	0.95	0.64
CSA-ST70	*Post-ACL*	15	1.43	0.26	1.02	0.28	3.34	0.90	0.10
	Control	15	2.65	0.17	0.66	1.82	4.44	0.83	0.01

Main findings

Analysis of Intergroup Differences In relation to the cross-sectional area at 70% distance from the ischial tuberosity (CSA-ST70) there was statistically significant differences between the Post-ACLR group and the control group (t Student = -3.864, $P < 0.001$, ES = -1.41).

On the contrary, the cross-sectional area of BFlh at 30% distal with respect to ischial tuberosity (CSA-BFlh30), no statistically significant differences were identified (t Student = -0.123, $P = 0.903$, ES = -0.04) between patients in the Post-ACL group versus the control.

In relation to the cross-sectional area of BFlh at 70% (CSA-BFlh70) no statistically significant differences were observed (t Student = -0.214, $P = 0.832$, ES = -0.07). In relation to the cross-sectional area of ST at 30% distal (CSA-ST30) no differences were statistically observed between the two groups (t Student = -1.535, $P = 0.136$, ES = -0.56) (Table 3).

Table 3 Analysis of intergroup differences

Item		Statistical	df	*P*	MD	SEM	Effect Size
CSA-BFlh30	*Test Student*	-0.12	28.0	0.903	-0.08	0.70	-0.04
CSA-BFlh70	*Test Student*	-0.21	28.0	0.832	-0.09	0.45	-0.07
CSA-ST30	*Test Student*	-1.53	28.0	0.136	-0.73	0.47	-0.56
CSA-ST70	*Test Student*	-3.86	28.0	<.001	-1.22	0.31	1.41

Discussion

The aim of this study was to evaluate the difference in CSA, as measured with US, between the BFlh and ST in soccer players who have undergone ACLR and healthy controls in the long term.

According to our results, there were not statically significative differences in CSA of the distal and proximal regions of the BFlh and the proximal ST. However, respect to CSA in distal ST, differences were only found in the area being smaller in soccer player undergone ACLR than healthy subjects.

Regarding this last finding, it seems that the decreasing of cross-plane area is only located near the site where the muscle graft for ACLR was removed. Our findings are consistent with those of other studies that have identified muscle atrophy in the neighboring region

of the surgical site.

According to a review conducted by Sherman et al. [18], individuals who undergo ACLR tend to experience muscle atrophy, observed as a decreased CSA and muscle volume of the ST and GT, preferentially on the affected limb when compared to the other side. In contrast, no significant differences were observed in BF neither m. semimembranosus (SM).

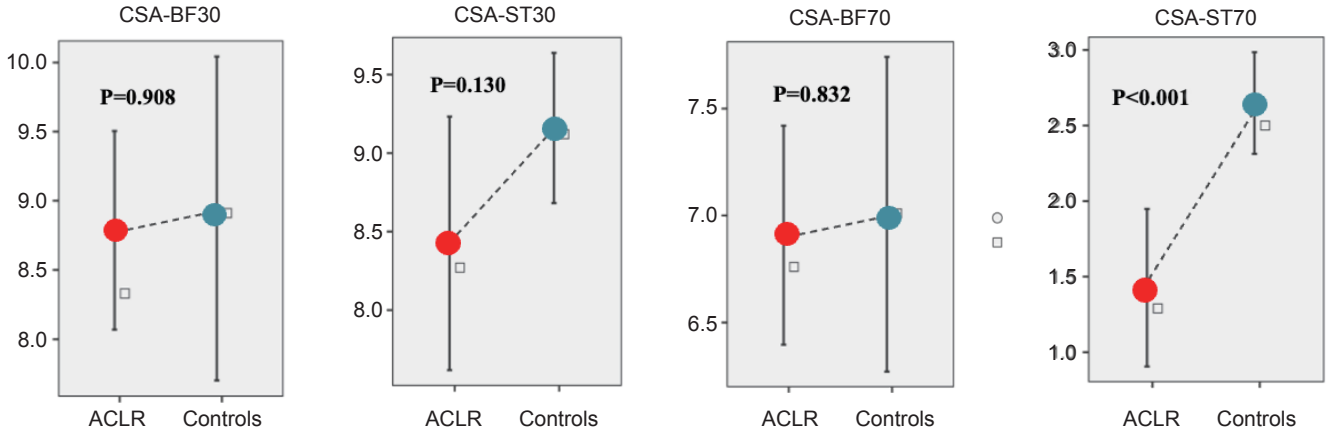

Figure 4 Analysis of intergroup differences. CSA-ST70 has shown statistical differences between the anterior cruciate ligament (ACLR) operated groups and healthy controls ($P > 0.001$).

Another retrospective cohort study carried out by Horteur et al. [19], pointed out that the utilization of HST grafts results in a decrease their contractile capacity as the diminishment observed the quadricipital musculature. Besides, Balki et al. [20] also linked a reduction in HST motor strength following ACL reconstruction with a less favorable prognosis in terms of knee functional recovery.

Moreover, Morrissey et al. [21] confirmed that an increasing of weakness of the HST muscle after ACLR results in a diminishment of the knee flexion torque. Additionally, Gould et al. [22] also found a significant decrease in isokinetic knee flexion strength during knee surgery.

There are several possible explanations for the findings that have been observed, one of which is that inflammation after surgery can lead to muscle atrophy by direct or indirect mechanisms.

The first of these is that the release of IL-1 and IL-6 can activate intramuscular signaling pathways, such as NF-κB, P38 MAPK and JAK/STAT, leading to metabolic changes in muscle tissue [23].

Postoperative inflammation also indirectly affects the HPA axis and leads to elevated ACTH levels. This, in turn, causes an increase in serum glucocorticoids, which accelerates protein degradation through several catabolism-related transcriptional factors, such as FoxOs and KLF15, and the Ubiquitine Proteosome System (UPS) [23].

As a result, this process provokes muscle atrophy in the medium term. This breakdown triggers the weakening and shrinking of muscles, which, in consequence, diminishes functionality among patients undergone ACLR. This phenomenon has been well documented by other researchers [24].

An alternative explanation for the observed results is that prolonged postoperative immobilization due to lack of physical activity leads to muscle atrophy. It is thought that it is caused by disuse which would be responsible for an imbalance in both protein synthesis and degradation.

With respect to the first mechanism, proteogenesis, it has been showed that, under conditions of disuse, there is a decrease in the activation of the Akt-mTOR pathway that is involved in the attenuation of protein synthesis. Furthermore, this proteogenesis imbalance can also be explained by insulin resistance, focal adhesion kinase (FAK) or even eukaryotic elongation factor 2 kinase (eEF2K) that acts at the level of mRNA translation [25].

Regarding proteolysis produced by postoperative immobilization essentially depends on UPS, especially the E3 signaling pathway. Additionally, calpains, caspase-3 and autophagy-lysosomal systems can also contribute to the protein degradation resulting from muscle disuse in surgical patients. There are also other potential mechanisms that are responsible for muscle atrophy associated with disuse, such as the ROS pathway and calcium overload [25].

The muscle atrophy we observed in HST after ACLR is also associated with a longer recovery period and, in the presence of knee pain, lead to a delay in the return to play, ultimately affecting the soccer player's quality of life. This is consistent with previous research on this topic [26,27].

For the reasons above, monitoring muscle's structural transformations can offer important insights into the loss of function due to atrophy. Thus, US measurement of HST muscles CSA arises as imaging marker for quantifying muscle changes after ACLR.

Limitations

Although our study provides valuable information, certain limitations should be considered.

First of all, we have to warn that included participants were at different stages of injury, so a categorical analysis based on time elapsed after surgery was not considered.

Another aspect to consider is the absence of cofactors affecting the process of protein turnover, such as age and gender, that could provide a more precise characterization of the group's clinical features.

We also did not perform an assessment of confounding variables such as metabolic diseases, polypharmacy and diet style before inclusion in the study.

Furthermore, we also did not carry out a biotype secondary study, which is undoubtedly crucial to improve the contextualization and clinical interpretation of the obtained results.

Conclusion

Ultrasound measurement of the CSA can be an image marker to identify muscle weakness or atrophy that predicts functional loss early.

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Conflict of Interest

The authors certify that they have no affiliations or financial involvement in any organization or entity with a direct financial interest in the topic or materials discussed in the article.

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