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Quadriceps Force Fluctuation During Maximal Isometric Contraction is Altered in ACL Injury and is Associated with Lower Limb Functional Performance: A Cross-Sectional Study with Athletes

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SUMMARY

Objective. This study aims to investigate the effects of ACL injury on force fluctuation measures (stability and complexity) of the quadriceps muscle, and how these measures relate to functional performance.

Methods. Forty-two athletes (14 females) with unilateral ACL injury were assessed. Quadriceps maximal isometric contraction was performed for both limbs before any surgical intervention. A 1.5s window from maximal torque signal was used to compute average torque (AT), force stability (coefficient of variation, CV), and complexity (sample entropy, SE), from which the limb symmetry index (LSI, % of contralateral limb) was estimated. The single hop test (SHT) was also performed and expressed as LSI. A nonparametric approach was used for between-limb comparison (Wilcoxon test) and to assess the relationship between force variables and SHT (Spearman correlation).

Results. Significant limb asymmetry was found, with the injured limb exhibiting smaller AT ($p < 0.001$) and higher SE ($p = 0.044$) than the contralateral limb. A significant, moderate association was found only for LSI-SE and LSI-SHT (rho = -0.535, $p = 0.002$).

Conclusions. In summary, along with the average torque, force complexity was altered in ACL-injured limbs of professional athletes, and was associated with larger asymmetries in functional performance, corroborating its relevance as a marker of ACL injury-related neuromuscular deficits.

KEY WORDS

Athletic injuries; muscle strength; musculoskeletal diseases; neuromuscular function.

INTRODUCTION

Anterior cruciate ligament (ACL) injury is a common orthopedic condition in athletes and leisure-sports participants, with an overall rate of 1.44 to 6.1 per 10,000 athletes-exposures (1, 2). The negative impact of ACL injuries, such as delayed return-to-play, early retirement, and economic burden (3-5), along with structural and functional joint and muscular impairments (6), makes the clinical and functional assessment of knee function a priority in sports medicine practice. Recently, changes in force fluctuation control – a general term referring both to force stability (levels of force variation around a target) and complexity (temporal regularity) – have been used to characterize neuromuscular deficits in a variety of contexts (7-11).

Briefly, force stability refers to the variability in the force signal observed during voluntary contractions (11), reflecting the neural drive to the muscle (*e.g.*, common synaptic input) and the properties of the motor unit population (*e.g.*, its recruitment and contractile features; (12)), and is expressed in statistical terms (*e.g.*, coefficient of variation). Force stability has already been assessed in athletes with ACL injuries, both before and after surgical interventions. In individuals with ACL-injured deficits (before reconstruction), a reduction in force stability of quadriceps muscle (*i.e.*, increased variability) was observed during maximal anisometric contractions (9), and it was associated to performance in the single hop test. Remarkably, the decrease in quadriceps force stability appears to persist even after ACL reconstructions (7, 10).

On the other hand, force complexity is a measure of the interaction among neuromuscular structures (*e.g.*, motor units and muscle fibers) and regulatory feedback loops (such as proprioceptive afferents) across various temporal-spatial scales (13). Force complexity is usually presented in terms of temporal regularity (*e.g.*, entropy analysis (14, 15)). So far, no investigation on force complexity in ACL-injured athletes has been conducted. However, it has been demonstrated that quadriceps force complexity is reduced (*i.e.*, regularity is increased) in elderly individuals compared to young people (8, 16, 17) and in healthy young individuals submitted to fatigue-inducing protocols (18, 19).

A clear understanding of the role of force fluctuations measures in ACL injury-related deficits in the neuromuscular system is hindered by the lack of studies in ACL-injured athletes, as well as the use of different contractions regimens (7, 9, 10) and subjective assessment of knee function (7) in the existing investigations. Given the prevalence of ACL lesions in sports- and exercise-related activities and their impacts on the quality-of-life of the physically active individuals, a thorough assessment of neuromuscular deficits is crucial for the proper design of therapeutic interventions.

This study aimed to determine whether and how force fluctuation measures taken from quadriceps maximal isometric voluntary contraction are related to ACL injury-related neuromuscular deficits and to functional performance in professional athletes. We hypothesize that force stability and complexity will be both reduced in ACL injured knee, compared with contralateral (non-injured) one, reflecting quadriceps neuromuscular deficits. In addition, a negative association between force fluctuations measures and functional performance is expected. If confirmed, this information could justify the inclusion of such measurements in routine neuromuscular assessment in medical and rehabilitation contexts.

METHODS

Participants and ethical concerns

For this cross-sectional study, several clinical and functional tests of knee-injured athletes were acquired between October 2017 and August 2020. Data collection was approved by the institutional ethical committee (process number 15653519.2.0000.5273 – date of approval: July 4, 2019), and all participants signed informed consent before test participation. The inclusion criteria for the present analysis were: 1) being a professional, active athlete; 2) having complete unilateral anterior cruciate ligament (ACL) injury (confirmed through physical and imaging assessment), accompanied or not by another structural damage (*e.g.*, meniscal); 3) had performed the maximal isometric voluntary contraction test; and 4) not having undergone any surgical intervention at the time of the assessment.

The flowchart of data selection is presented in **figure 1**; from 112 athletes assessed, data from 42 (from which 14 were females) were used; *a priori* analysis provided a sample estimative of n = 35 athletes (nonparametric paired test, moderate effect size = 0.5, $\alpha = 0.05$ and $1\text{-}\beta = 80\%$).

The participants' characteristics were described in **table I**. Briefly, the ACL injury was similarly distributed between the limbs. Most of the athletes (69%) experienced isolated ACL injury. Among those with additional structural damages (31% of the athletes), there were 11 cases of meniscal tears (6 medial, 2 lateral and 3 unidentified), along with single cases of patellar fracture, osteoarthritis, medial collateral ligament injury, posterior cruciate ligament injury, and osteochondral injury. The main sports practiced by the participants were soccer (n = 13) and martial arts (n = 12; 5 from MMA, 4 from judo, 2 from jiu-jitsu, and 1 from kickboxing).

Data acquisition and analysis

For the evaluation of the quadriceps function, a maximal voluntary isometric test was performed in an isokinetic dynamometer (HUMAC NORM II®, CSMi, USA). The torque signals were acquired through an external analog/digital converter (EMG830c®, EMG System do Brasil, Brazil) with a 1 kHz sampling rate. The participant was positioned sitting on the device with the hip positioned at 85 degrees and the knee positioned at the athlete's individual peak torque angle, to provide an optimal condition for quadriceps force production (20). Inelastic bands were used to stabilize the trunk and lower limbs. After familiarization and warm-up, the participant was asked to perform a knee extension movement "as quickly and strongly as possible", after an auditory command. Three contractions

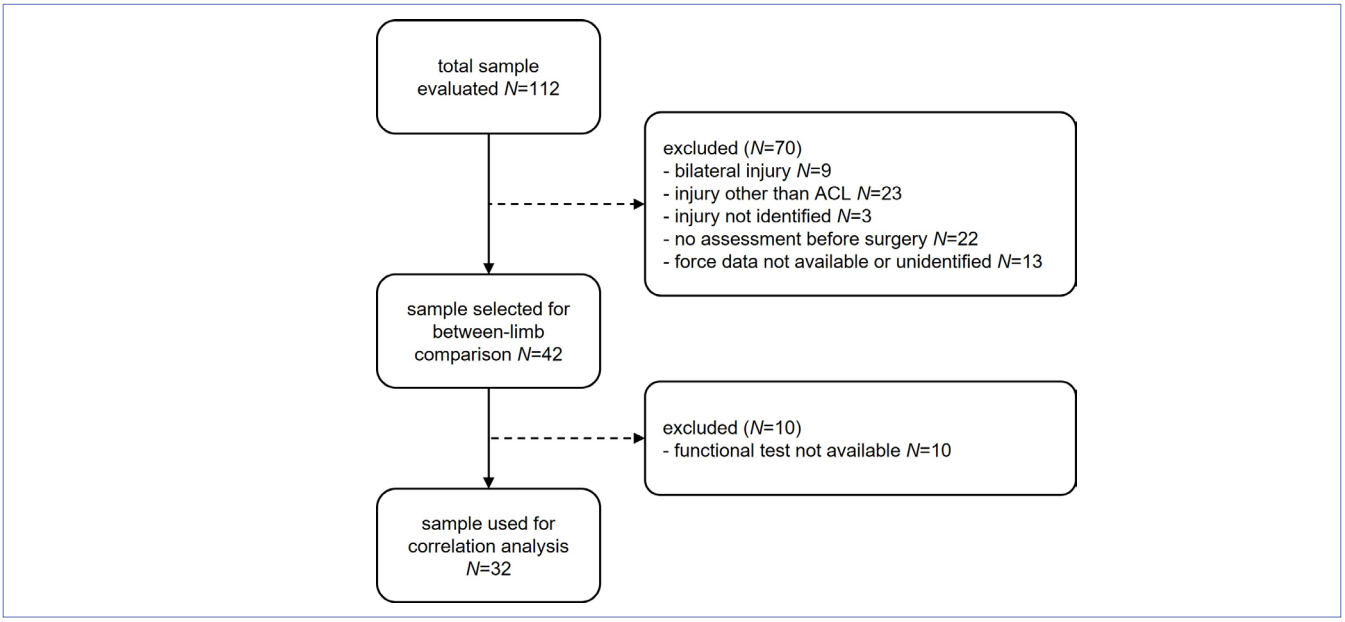

Figure 1. Flowchart of data selection.

Table I. Demographic, anthropometric, and clinical description of the sample.

Variable	Statistics
Age (years)	23 (20-27)
Height (cm)	173 (169-177)
Weight (kg)	70 (64-78)
BMI (kg/m^2)	24 (22-25)
Footedness (% of total, L/R)	17 / 83
ACL injury (% of total)	
Isolated injury	69
Additional structural injuries	31
Side of ACL injury (% of total, L/R)	52 / 48
Sports practiced (N)	
Soccer	13
Handball	6
Martial arts (MMA, judo, jiu-jitsu, and kickboxing)	12
Rugy	2
Volleyball	1
Unidentified	8

Data expressed as median (lower-upper quartile), percentage of total, or as absolute frequency (N).

with 3-5s (min.-max.) duration were executed, and the one in which the average maximal torque (see below) was found was used for further analysis (**figure 2**). Data processing was run in Python environment (version 3.10.9).

Torque data were filtered (low-pass Butterworth 4th order filter, 50 Hz cutoff (20)) and a region-of-interest of 1.5s (1,500 points) in the middle of the torque curve was defined (**figure 2**). From the region-of-interest the following variables were calculated: the average maximal torque (AT, N × m); the coefficient of variation of the torque (standard deviation divided by mean; CV, %), as a measure of force stability (21); and the sample entropy (SE, au,), a measure of

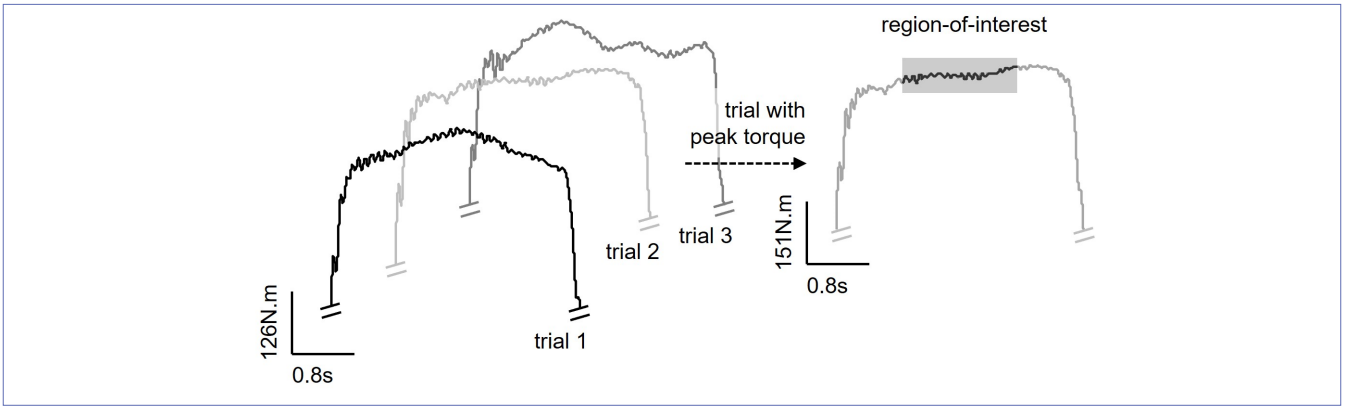

Figure 2. Representative acquisition of maximal isometric voluntary torque from one participant (female, 35 years old).
From the three contractions performed (1-3 trials, left panel), the one that showed peak torque was selected. A 1.5s window (1,500 data points; gray "region-of-interest" in the right panel) was used to compute force descriptors. See the Methods section for further details.

force complexity (18), corresponding to the temporal regularity of the torque time-series. The SE was computed using a tolerance r of 0.1, and embedded dimension m of 2 (18).
The limb symmetry index (LSI) was calculated for each neuromuscular variable, based on the ratio between the injured and contralateral limb, multiplied by 100: LSI > 100% refers to higher values in the injured limb, while LSI < 100% indicates higher values for the contralateral limb.

Single hop test

The single hop test (SHT) is a functional assessment that measures the individual's lower limb strength and knee stability and is largely used for functional assessment after ACL injuries and surgical interventions (22-24). To perform the SHT, participants were asked to stand on one leg and jump forward as far as possible, landing on the same leg. The distance from the starting point to the tip of the toe was marked with chalk and recorded. The test was repeated three times, and the best distance was considered. The single hop test's LSI was computed as described above (24).

Statistical analysis

As most of the data showed non-Gaussian distribution (Shapiro-Wilk's P-values < 0.032), a nonparametric approach was applied. Preliminary analysis (Wilcoxon test) reveals that male athletes had higher levels of AT and SE absolute values than females (all P-values above 0.001), but there were no differences in LSI values ($p > 0.224$), indicating that, besides sex differences in muscle function and knee joint anatomy (*e.g.*, (25)), the overall effect on the injured knee is similar. For simplification purposes, data were pooled, and its subsequent analysis was shown.

First, the Wilcoxon paired test was used to check for limb differences (injured *vs* contralateral), and the rank-biserial correlation coefficient (r_{BS}) was used as an effect size measure (26). Additionally, data from third-two participants (single hop tests data from ten athletes were not available; **figure 1**) was used to compute the Spearman correlation coefficient, together with its 95% confidence interval, to check for significant associations between LSI-SHT and LSI from force measures. The statistical threshold was set at 5%. All analyses were run in the JASP environment (version 0.18.1, The JASP Team 2023, Netherlands).

RESULTS

Both group (boxplot) and individual data (circles) were presented in **figure 3**; since a nonparametric approach was applied, outliers (crosses) were not removed. There was a significant limb effect for average maximal torque ($p < 0.001$, $r_{BS} = 0.721$; **figure 3A**), and sample entropy ($p = 0.044$, $r_{BS} = -0.362$; **figure 3B**), but not for the coefficient of variation ($p = 0.783$, $r_{BS} = 0.050$; **figure 3C**). These results reflect the smaller average maximal torque and higher sample entropy produced by the injured limb.
Group results for LSIs were median (quartiles): LSI-AT 86 (76-92) %; LSI-SE 130 (67-250) %, LSI-CV 99 (56-137) %. Overall, these data corroborate the reduced average torque and increased force complexity in ACL-injured limbs. Furthermore, SHT results indicates the lower functional performance of the ACL-injured limb (injured limb 136 (126-153) cm; contralateral limb 162 (147-188) cm; LSI-SHT 86 (76-92) %). Results from the correlation anal-

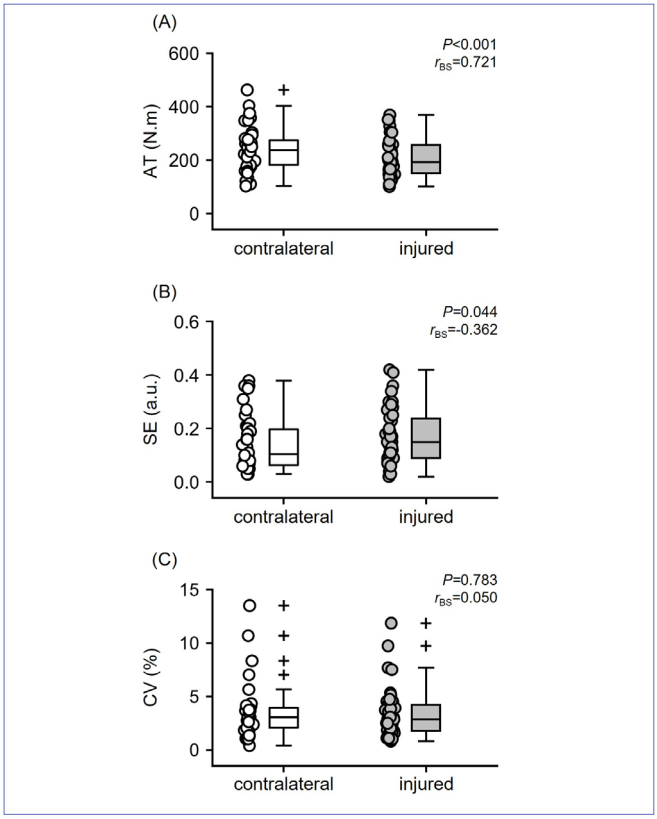

Figure 3. Boxplot of group and individual data (circles) showing **(A)** average torque (AT); **(B)** Sample entropy; **(C)** Coefficient of variation from the contralateral (white markers) and ACL injured limb (gray markers).

Cross markers indicate outliers; P-value and effect size were shown as inset.

ysis (rho, 95%CI, and the corresponding P-values) are shown in **figure 4A**. There was a significant correlation only between LSI-SHT and LSI-SE (**figure 4B**), with a marginal result being achieved for the association between LSI-SHT and LSI-CV (**figure 4C**), but no significant association for LSI-AT (**figure 4D**).

DISCUSSION

In this study we analyze quadriceps force fluctuations measures and lower limb functional performance of professional athletes, to investigate whether and how they were affected by ACL injury-related deficits. As expected, quadriceps maximal torque was smaller in the ACL injured limb (27-29), indicating the marked effected of the ligament injury and related deficits on overall muscle force production capacity. The assessment of another neuromuscular property, nevertheless, revealed that force complexity, but not force stability, was altered in the ACL injured limb, and is negatively associated with single-hop test performance. The physiological and practical utility of these findings are discussed below.

Changes in maximal force complexity in ACL injuries

The main finding of this study was the increase in force complexity, computed as sample entropy, on the ACL injured side during quadriceps maximal isometric contraction (**figure 3B**). Previous investigations on the subject, assessing maximal and submaximal force, consistently showed decreases in force complexity (*i.e.*, reduction in entropy measures) as a function of aging or muscle fatigue (16-19). This reduction in complexity, reflecting increases in temporal regularity of the signal, is related to a reduction in either the number of anatomical/physiological elements engaged in the execution of the task or the number of elements' interactions (13, 30). Although not fully understood, the observed decreases in force complexity are attributed to changes in a varied of neuromuscular features, such as the number of available motor units, its force output, and the responsiveness to excitatory inputs (8, 16).

Nevertheless, it is recognized that the direction of the change in complexity (*i.e.*, increases or decreases) depends on the nature of the observed phenomenon, its dynamics, and the specific demands of the task (31). For example, elderly persons exhibit a higher degree of postural sway complexity than their young counterparts during prolonged standing tasks (32). Additionally, force complexity is higher in older than in young persons during index abduction sine wave task (31). In this context, increases in complexity could be attributed either to increases in the number of elements contributing to force production or in the level of its interactions (33); in the case of ACL-injured quadriceps activation, this could be related to additional recruitment of motor units or muscle groups and increases in common inputs or timing of firing rates. Several changes in neuromuscular properties were found in those with ACL injury-related deficits, including higher hamstring-to-quadriceps asymmetry in motor units firing rate, changes in recruitment and de-recruitment thresholds, and loss of mechanoreceptor afferents (34, 35). Altogether, these changes seem to interfere with force production control during maximal contractions, leading to the observed increases in force complexity. Which is the mechanism responsible for this, however, is a matter of debate.

Maximal force stability is not affected by ACL injury

Although we know much about force stability achieved at submaximal contractions in different clinical and sporting contexts (10-12), the measurement of force stability obtained during maximal voluntary isometric contraction has been previously investigated only in a few studies (7, 36, 37).

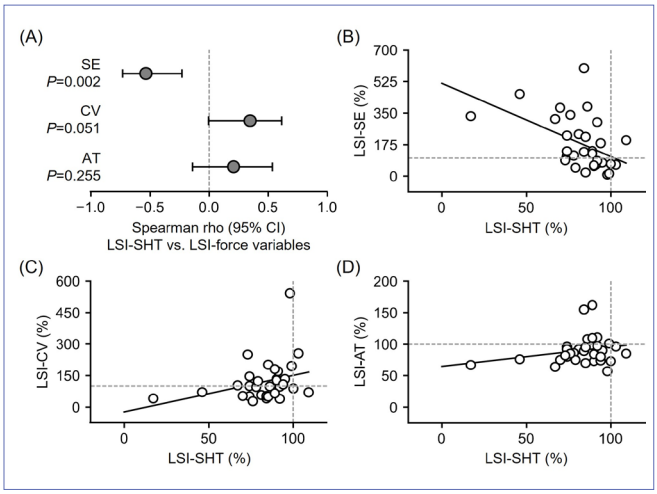

Figure 4. The Spearman coefficient of correlation (rho), its corresponding 95% confidence interval (CI), and the related P-values for each association are shown in **(A)**. The scatter plots illustrate the relationship between LSI-SHT and LSI-SE **(B)**, LSI-CV **(C)** and LSI-AT **(D)**. The regression lines were shown to indicate the overall trend of the association. The dotted lines refer to zero-value **(A)**, and to 100% **(B-D)**.

Although not frequently applied for force fluctuations analysis, maximal contraction protocols could be less time-consuming and provide, with a few trials, a variety of measures related to neuromuscular features.

Force stability obtained in maximal contractions of knee extensors is lower (*i.e.*, variability is higher) in hemophilic patients compared to healthy controls (36), and it had a positive association with quality-of-life scores in individuals with Parkinson's disease (37). Only one study has investigated maximal force stability in patients undergoing ACL reconstruction surgery (7) and found lower quadriceps force stability (*i.e.*, increased force fluctuations; CV of circa 1.2%) compared to controls (CV ~0.8%). In the present study, no significant between-limbs differences regarding force stability were observed (**figure 3C**). The median force stability obtained was around 3% for both lower limbs, higher than the values reported by González *et al.* (36) and Goetschius *et al.* (7) (approximately 1-2%), but close to those observed by Sá *et al.* (37) (circa 3-5%). These differences are probably related to the range of temporal windows used for CV computation – 100-500 ms (7, 36) and 2s (37). As a larger temporal window ensures greater reproducibility of the isometric force signal (38), the one used in the present study (1.5s) appears to be adequate. Therefore, the lack of asymmetry in force stability between limbs with and without ACL injury seems to reflect the absence of alterations in this neuromuscular feature in this clinical condition.

Associations among maximal force measures and SHT

In this study, athletes demonstrated lower limb functional asymmetry, as reflected by LSI-SHT values below the recommended 85-90% for return to sports (24, 39). This asymmetry was moderately associated with neuromuscular function measures, particularly force complexity (**figure 4B**). SHT asymmetry is common in ACL injuries (24, 40), even after surgical reconstruction (24), and is strongly association with measures of knee extensor strength (40-42). On the other hand, the relationship between ACL-injured individuals' functional performance and neuromuscular properties like force stability or complexity has been less explored.

Previous studies have investigated quadriceps force stability during maximal isometric contraction and its association with functional outcomes (7, 9). Our study partially supports these findings and introduces force complexity as a closely related measure to the functional performance of athletes with ACL injuries (**figure 4C**). In our investigation, the non-significant or marginally significant associations observed for peak torque and force stability with SHT performance may be related to the use of an asymmetric index. Nevertheless, the positive spectrum of correlation coefficients variability (**figure 4A**) suggests a potential association between these measures. Overall, our results imply that neuromuscular properties, particularly force complexity, play a crucial role in influencing functional performance in athletes recovering from ACL injuries beyond the capacity for maximal force generation.

Study limitations

While the study presents significant findings, it is essential to acknowledge some of its limitations. Mainly, specific participant demographics and the nature and the time from injuries may impact the generalizability of the results. Furthermore, the diversity of sports practiced among the professional athletes could introduce a higher risk of bias. Future research should include more detailed clinical information and focus on sport-specific populations to strengthen the findings. Additionally, exploring longitudinal changes in LSI-SHT and LSI-force measures during the rehabilitation process could provide insights into the effectiveness of various interventions over time.

CONCLUSIONS

This study demonstrated, for the first time, that the quadriceps muscle of athletes with ACL-injured knee exhibits decreased force production and force complexity during maximal isometric contraction, with no observed change in force stability. Moreover, quadriceps force complexity was associated with poor performance in lower limb functional tests. The neurophysiological basis of these changes and how they can be reverted, however, remains subjects of debate.

These information regarding the changes in neuromuscular function in ACL-injured athletes could provide valuable insights for sports medicine practitioners. For instance, designing interventions to enhance not only overall muscle force but also specific aspects like quadriceps force fluctuations control could be crucial in the rehabilitation process. By incorporating targeted approaches (*e.g.*, neurofeedback) to address these deficits, rehabilitation practices can be refined and tailored to better meet the unique needs of individuals recovering from ACL injuries.

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DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

TL, JCSA, SCS, APM: investigation, writing – original draft. TL, JCSA: formal analysis. SCS, APM: writing – review & editing.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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