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Muscle fiber conduction velocity in the vastus lateralis and medialis muscles of soccer players after ACL reconstruction

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The neural factors underlying the persistency of quadriceps weakness after anterior cruciate ligament reconstruction (ACLR) have been only partially explained. This study examined muscle fiber conduction velocity (MFCV) as an indirect parameter of motor unit recruitment strategies in the vastus lateralis (VL) and medialis (VM) muscles of soccer players with ACLR. High-density surface electromyography (HDsEMG) was acquired from VL and VM in nine soccer players (22.7 ± 2.9 years; BMI: 22.08 ± 1.72 kg·m^{-2}; 7.7 ± 2.2 months post-surgery). Voluntary muscle force and the relative myoelectrical activity from the reconstructed and contralateral sides were recorded during linearly increasing isometric knee extension contractions up to 70% of maximal voluntary isometric force (MVIF). The relation of MFCV and force was examined by linear regression analysis at the individual subject level. The initial (intercept), peak ($MFCV_{70}$), and rate of change (slope) of MFCV related to force were compared between limbs and muscles. The MVIF was lower in the reconstructed side than in the contralateral side (−%20.5; $P < .05$). MFCV intercept was similar among limbs and muscles ($P > .05$). $MFCV_{70}$ and MFCV slope were lower in the reconstructed side compared to the contralateral for both VL (−28.5% and −10.1%, respectively; $P < .001$) and VM (−22.6% and −8.1%, respectively; $P < .001$). The slope of MFCV was lower in the VL than VM, but only in the reconstructed side (−12.4%; $P < .001$). These results suggest possible impairments in recruitment strategies of high-threshold motor units (HTMUs) as well as deficits in sarcolemmal excitability, fiber diameter, and discharge rate of knee extensor muscles following ACLR.

KEYWORDS

anterior cruciate ligament, high-density surface EMG, motor unit recruitment, neural alterations, persistent quadriceps weakness, sports injury

1 | INTRODUCTION

Anterior cruciate ligament reconstruction (ACLR) represents the current clinical standard to ensure a successful return to sport after unilateral ACL injury.[1] Nevertheless, athletes cleared to return to high-level sports (ie, soccer) after ACLR have a 15 times higher risk of sustaining a second ACL injury than an uninjured equivalent cohort.[2]

One possible factor contributing to recurrent ACL injury is the persistency of quadriceps weakness (PQW) which is typically observed months or even years after ACLR,[3,4] despite best efforts to recover both quadriceps strength and size during post-surgery rehabilitation. PQW is independently associated with severe short and long-term consequences including higher risk of a second ACL injury,[5,6] early onset of knee osteoarthritis,[7] and asymmetries in knee joint biomechanics.[4]

The mechanisms underlying PQW have not been fully explained. Some studies suggest that chronic morphological and cellular alterations in both vastus lateralis (VL) and medialis (VM) muscles may be associated with persistent weakness of the knee extensors after ACLR.[8,9] Other studies showed that arthrogenic muscle inhibition (AMI) may be a key contributor to PQW.[10,11] AMI is a reflexive and protective phenomenon elicited by joint trauma, characterized by an ongoing inhibition that prevents the surrounding musculature to be fully activated.[10] It is caused by the combination of altered afferent feedback from damaged mechanoreceptors and abnormal excitability of both spinal-reflexive and corticospinal pathways[3,10,12] which, collectively, affect the efferent neural drive from the α motoneuron pool to the muscle.[7,10] Specifically, higher active motor threshold (AMT), reduced motor evoked potentials (MEP), brain plasticity,[3,13] and lower Hoffman reflex values,[3] with respect to both the contralateral and healthy control limbs, were found in the first months as well as years after ACLR, attesting the existence of short and long-term quadriceps neural impairment. Interestingly, despite several studies evaluating the neural alterations occurring at the spinal and supraspinal levels in ACLR individuals, the potential changes in neural control strategies in vasti muscles have been poorly explored in individuals post-ACLR, either directly or indirectly.

Muscle fiber conduction velocity (MFCV) has been extensively used to indirectly infer the progressive recruitment of MUs,[14] due to the linear relation between fiber conduction velocity and fiber diameter.[14-17] MFCV refers to the average propagation velocity of action potentials along the muscle fibers of the active MUs and can be estimated non-invasively through multiple EMG electrodes placed over the muscle in the direction of the muscle fibers.[18] Additionally, MFCV may reflect the electrophysiological attributes of the sarcolemma, such as the activity of the Na+-K+-ATPase pump, a protein that plays a key role in maintaining the excitability of the muscle membrane.[19,20]

This study investigates MFCV of the VL and VM muscles following ACLR in a cohort of soccer players. Because of the documented morphological[8,9,19] and neural alterations occurring after ACLR,[3,4] we hypothesized that participants would present with reduced MFCV in both vasti muscles on the reconstructed side with respect to the contralateral limb.

2 | METHODS

2.1 | Participants

Nine male soccer players (22.7 ± 2.9 years; BMI: 22.08 ± 1.72 $kg \cdot m^{-2}$; Tegner score ≥ 7) took part in this observational study. Individual demographics and clinical characteristics are reported in the Table S1. All participants underwent a unilateral arthroscopic ACLR within the preceding 12 months of the study (time post-surgery: 7.7 ± 2.2 months). This threshold was chosen as the ACL reinjury rate is particularly high during the first year after ACLR and return to sport.[2,6] Additionally, asymmetries in quadriceps strength prior to return to sport contribute to increase significantly the knee reinjury rate.[6] ACLR was performed by the same surgeon within 30 days from a non-contact ACL injury, using either an ipsilateral bone-patellar tendon-bone (BPTB) or a semitendinosus and gracilis tendon (STGR) graft. The same standardized rehabilitation protocol was followed by all patients.[21] Those with previous knee joint injury or multi-ligament knee surgery, as well as those reporting anterior knee pain during open kinetic chain exercises, were excluded.

The study procedures were approved by the institutional review board of the University of Rome "Foro Italico" (*CARD 2018/07*) and conducted in accordance with the Declaration of Helsinki. A signed informed consent was obtained from all participants prior their involvement in the study.

2.2 | Study overview

The study was conducted at the laboratory of human and exercise physiology of the University of Rome "Foro Italico." Participants attended two laboratory sessions separated by one day. During the first session, participants familiarized with the testing procedures by performing a series of submaximal ramp contractions. Both self-reported knee function[22] and physical activity level rates[23] were also evaluated during this session.

The second session started with the anthropometric data collection that was included to estimate the anatomical quadriceps cross-sectional area (QuadCSA). Afterward, muscle force and high-density surface electromyography (HDsEMG) signals were recorded during maximal and submaximal voluntary contractions, to examine the neuromuscular function of

the knee extensors. All trials were performed in isometric conditions with the knee positioned in 45° of flexion since quadriceps activation is selectively impaired at this angle and the association between functional performance and quadriceps strength is stronger at 45° of knee flexion compared to 90°.[11]

2.3 | Protocol

All participants were instructed to refrain from any kind of physical activity in the 48 hours prior the test and to avoid caffeine intake in the 24 hours preceding both sessions. In the second session, the participants firstly warmed up for 5 minutes on a treadmill (stride walking at 5-7 Km h^{-1}) and performed 3-5 seconds contractions at 30% (×2), 50% (×2), 70% (×2), and 90% (×1) of their perceived MVIF. After 5 minutes of rest, they performed three maximal voluntary contractions (MVCs) during which they were verbally encouraged to "push as hard as possible" and attempt to keep their maximum for at least three seconds. The maximal voluntary isometric force (MVIF) obtained was set as reference for the submaximal trapezoidal ramps. Two trapezoidal contractions up to 70% of MVIF were then performed, each separated by three minutes of rest. The trapezoidal force profile consisted in an ascending phase (linear increase of force at 5% MVIF s^{-1} rate), a plateau phase (steady state at the target force, 70% of MVIF), and a descending phase (linear decrease of force at 5%MVIF·s^{-1} rate) (Figure 1A). Only the ascending phase was used for subsequent analysis (*see EMG processing*). The protocol was carried out bilaterally and the first limb tested was randomly chosen.

2.4 | Quadriceps anatomical CSA estimation

QuadCSA was estimated using a non-invasive anthropometric method described and validated by Housh and colleagues[24] that involved the measurements of the mid-thigh circumference and skinfold thickness (Harpenden caliper; Milan, Italy). The estimation of QuadCSA was strongly correlated with MRI assessment ($r = .85$) with a standard error of estimate of 5.4 cm^2.[24]

2.5 | Force assessment

Force measurements were carried out on a Kin-Com isokinetic dynamometer. The trunk was reclined to 10° whereas hip and knee angles were standardized at 90° (full extension at 180°) and 45° of flexion, respectively. The rotational axis of the dynamometer was aligned to the lateral epicondyle of the femur. Three adjustable straps (chest, pelvis, and proximal thigh) were used to secure participants to the dynamometer and hence avoid unnecessary movements. An ankle strap was fastened at a standardized distance above the lateral malleolus (25% of the tibial length), in series with a calibrated load cell placed perpendicularly along the tibial axis. The analog force signal from the transducer was amplified (×200) and sampled at 2048 Hz with an external analog-to-digital converter (EMG-Quattrocento, OT Bioelettronica). A custom labVIEW software (LabVIEW 8.0; National Instruments) was used to provide a real time feedback of the force exerted during each trial. The force signal was displayed at a constant visual gain on a screen placed 1 m in front of the participant (Figure 1B).

2.6 | High-Density Surface Electromyography (HDsEMG) recording

Surface EMG was recorded by means of two-dimensional high-density grids (matrices) of 64 electrodes each (5 columns × 13 rows; inter-electrode distance of 8 mm; gold-coated; diameter of 1 mm; OT Bioelettronica). The use of multichannel surface EMG provides a more reliable estimation of MFCV than unidimensional arrays.[25] The skin overlying both the VL and VM muscles was marked in accordance

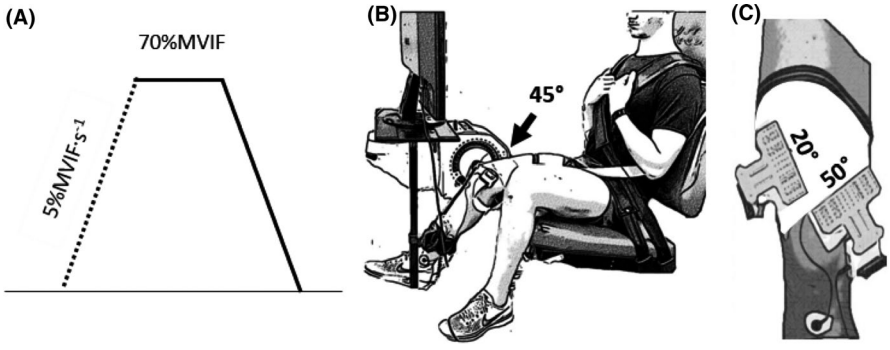

FIGURE 1 Experimental setup. A, Trapezoidal ramp contraction up to 70% of MVIF; MFCV was assessed only during the ascending phase represented by the dotted line. B, Schematic representation of a participant's limb during the test. Knee extension force was exerted isometrically at 45° of knee flexion. C, High-density surface electromyogram (HDsEMG) signals were recorded by two grids of 64 electrodes each, placed over the vastus lateralis (20° with respect to the reference line) and vastus medialis (50° with respect to the reference line)

with the guidelines provided by Barbero and colleagues [26] (Figure 1C).

The orientation of the grids was firstly adjusted based on brief recordings carried out with a non-adhesive array of 16 electrodes (silver bars; 1 mm thick; 5 mm of inter-electrode distance; OT Bioelettronica). Specifically, the main innervation zone (IZ), defined as the point where the propagation of motor unit action potentials (MUAPs) is reversed, was firstly identified.[15,27] Then, the anatomical direction of the muscle fibers was estimated through the visual inspection of MUAP's propagations. Once no notable changes in MUAP's waveforms from IZ to distal tendon region were observed, the electrode matrixes were applied. The same procedure was adopted for both muscles.

The skin was then shaved, gently abraded and cleansed with 70% ethanol. Thereafter, the two electrode grids were fixed between the proximal and distal tendon regions of both VL and VM with the five columns of the grid aligned longitudinally with respect to the muscle fiber orientation, as previously done.[27] The attachment to the skin was permitted via disposable bi-adhesive foam layers (Spes Medica). To ensure a proper skin-electrode contact, the electrode cavities were subsequently filled with conductive paste (SPES Medica). Reference electrodes were placed on the patella and on medial malleolus for the VL and VM grids, respectively. A ground electrode was additionally attached on the contralateral wrist. The HDsEMG signals were acquired in monopolar derivation through a multichannel amplifier (EMG-Quattrocento, 400 channel EMG amplifier; 3 dB, bandwidth 10-500 Hz; OT Bioelettronica), amplified, A/D converted on 16 bits, and sampled at 2048 Hz. Force and HDsEMG signals were synchronized at source by the same amplifier. All data were stored on a computer hard disk for offline analysis.

2.7 | Data analysis

2.7.1 | Force signal

The force signal was first converted in Newton (N) and then low-pass filtered using a cutoff frequency of 15 Hz (4th order, zero-lag Butterworth filter). The offset was removed by gravity correction, and all the trapezoidal contractions showing pre-activation or countermovement were excluded.[28] In order to assess the quadriceps force-generating capacity, the ratio between the MVIF and the anatomical QuadCSA (MVIF/QuadCSA) was computed.

2.7.2 | EMG processing

Estimates of MFCV were obtained only during the ascending phase of the ramp contraction, in non-overlapping time windows of 250 ms, through a reliable multichannel maximum-likelihood algorithm.[18] The monopolar HDsEMG signals were band-pass filtered using a 2nd order 20-500 Butterworth filter. Subsequently, double-differential HDsEMG signals were computed and visually inspected by the same investigator (SN). To estimate MFCV, the five channels with highest signal quality were selected. In particular, the criterion adopted for selection was the minimal change of MUAP shapes throughout their propagation to the distal tendon and a correlation coefficient higher than 0.8.[18]

Participant-specific linear regressions between force (%MVIF) and MFCV were computed to indirectly infer the progressive recruitment of MUs, due to the strong relationship between a) the rate of MFCV change with respect to force and b) the rate of MUCV change with respect to recruitment thresholds.[14] The initial MFCV value (intercept), the rate of change of MFCV with respect to force (slope), and the greatest MFCV value ($MFCV_{70}$) obtained during the ascending portion of the ramp (ie, at 70% MVIF) were then extracted for comparison. MFCVs exceeding the physiological range (2-6 m s^{-1}) were excluded.[15]

2.8 | Statistical analysis

The normality of the distribution of data was tested with the Shapiro-Wilk test. MVIF, QuadCSA, and MVIF/QuadCSA were compared using multiple paired t tests. To detect between-limb differences in slope, intercept, and $MFCV_{70}$, a series of two-way repeated measures analysis of variance (ANOVA) were carried out, with limb (reconstructed vs contralateral) and muscle (VL vs VM) set as within factors. In case of significant interaction effects, separate paired t tests were used to detect differences during the post-hoc analysis. The significance level was set at $P < .05$. A Bonferroni correction was applied when appropriate to account for multiple comparisons. Effect size from the ANOVA was computed as partial eta squared.[29]

Three distinct stepwise multiple regression analysis was performed to identify significant predictors of the absolute differences in MVIF, QuadCSA, and MVIF/QuadCSA between the reconstructed and contralateral side. When two or more dependent variables were significantly predicted by the same independent variable, a subsequent stepwise regression analysis was conducted to examine the individual contribution of Δ MVIF and/or Δ QuadCSA and/or Δ MVIF/QuadCSA, in predicting MFCV changes. Lastly, separate Pearson's product-moment of correlation was run to determine the relationship between self-reported knee function (sCKRS) and Δ MVIF, Δ QuadCSA, and Δ MVIF/QuadCSA. The following classification was adopted to interpret the correlation coefficient (r): weak (0-0.4), moderate (0.4-0.7), and strong (0.7-1.0).[29] All analyses were carried out with SPSS (version 22; IBM Corporation).

3 | RESULTS

3.1 | MVIF, estimated QuadCSA, and specific strength

The MVIF was lower on the reconstructed side with respect to the contralateral one (655.6 ± 176.2 N vs 824.7 ± 137.2 N; $P = .001$; −20.5%). Similarly, a smaller estimated QuadCSA (62.3 ± 7.3 cm^2 vs 71.3 ± 6.0 cm^2; $P = .001$; −12.7%) and a lower specific strength (10.4 ± 1.7 N cm^{-2} vs 11.5 ± 1.2 N·cm^{-2}; $P < .05$; - 9.6%) were observed on the reconstructed side with respect to the contralateral side (Figure 2).

3.2 | MFCV regressions

MFCV estimated from VL and VM muscles was linearly correlated with force among all contractions and participants ($P < .001$) with a mean R^2 of .90 ± .40 and R^2 of .90 ± .50, respectively.

Individual values of coefficient of determination (R^2), intercept, slope, and $MFCV_{70}$ are reported in the Table S2. An example of the bilateral relationship between MFCV and force of both VL and VM, for one representative individual, is shown in Figure 3.

The repeated measures analysis revealed a significant main limb effect on MFCV slope ($F_{1,8} = 31.411$; $\eta_p^2 = 0.797$; $P = .001$) and $MFCV_{70}$ ($F_{1,8} = 125.924$; $\eta_p^2 = 0.940$; $P < .001$). Similarly, a significant main muscle effect was found for MFCV slope ($F_{1,8} = 8.878$; $\eta_p^2 = 0.526$; $P = .018$) and $MFCV_{70}$ ($F_{1,8} = 5.460$; $\eta_p^2 = 0.406$; $P < .05$). The post-hoc pairwise comparisons revealed that the mean MFCV slope was steeper in the contralateral side with respect to the reconstructed side, for both the VL (0.0218 ± 0.003 m s^{-1}·%MVF vs 0.0156 ± 0.003 m·s^{-1}·%MVF; $P < .001$; +28.5%; Figure 4A) and VM (0.0230 ± 0.002 m·s^{-1}·%MVF vs 0.0178 ± 0.003 m·s^{-1}·%MVF; $P < .001$; +22.6%; Figure 4D). Moreover, the contralateral side showed greater $MFCV_{70}$ compared to the reconstructed one, for both the VL (5.26 ± 0.23 m·s^{-1} vs 4.73 ± 0.23 m·s^{-1}; $P < .001$; +10.1%; Figure 4B) and VM (5.51 ± 0.26 m·s^{-1} vs 5.06 ± 0.35 m·s^{-1}; $P = .001$; +8.1%; Figure 4E).

The initial MFCV was similar among limbs and muscles ($P > .05$; Figure 4C,F). Interestingly, MFCV increased at a lower rate in VL compared to the VM only in the reconstructed side (0.0156 ± 0.003 m·s^{-1}·%MVF vs 0.0178 ± 0.003 m·s^{-1}·%MVF; $P < .001$; −12.4%). No further differences were found among limbs and muscles.

The regression analysis revealed that Δ $MFCV_{70}$ of the VL explained the 60.1% of the variance for Δ MVIF ($F_{1,7} = 10.528$; $P < .05$; Figure 5). Similarly, Δ $MFCV_{70}$ of the VL explained the 57% of the variance for Δ QuadCSA ($F_{1,7} = 9{,}519$; $P < .05$). The subsequent stepwise regression model showed that Δ MVIF and Δ QuadCSA together predicted 64.1% of the variance in Δ $MFCV_{70}$ ($F_{2,6} = 5{,}365$; $P < .05$). However, Δ QuadCSA had a non-significant contribution (Δ $R^2 = .04$; $P = .44$) to the regression model. Finally, we found negative correlations between sCKRS and Δ MVIF ($r = -.78$, n = 9, $P = .013$), Δ QuadCSA ($r = -.81$, n = 9, $P = .007$), and Δ MVIF/QuadCSA ($r = -0.71$, n = 9, $P = .03$).

4 | DISCUSSION

This study demonstrated alterations in MFCV of the quadriceps in soccer players within 12 months after ACLR. Lower slopes and maximal values of MFCV were observed on the reconstructed side compared to the contralateral side. In contrast, no differences were detected for initial MFCV values. Furthermore, ACLR-related clinical deficits in MVIF, QuadCSA, and specific strength were found. Notably, the deficit in $MFCV_{70}$ of the VL represented the sole predictor of the asymmetry in MVIF, accounting for about the 60% of its variance.

As expected, the reconstructed side showed lower MVIF (−20.5%), QuadCSA (−12.7%), and specific strength (−9.6%)

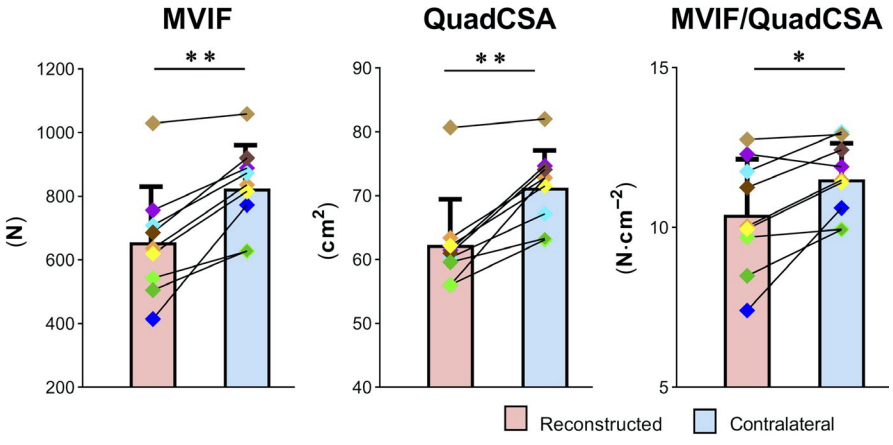

FIGURE 2 Differences in maximal voluntary isometric force (MVIF), estimated quadriceps cross-sectional area (QuadCSA), and specific strength (MVIF/QuadCSA); *$P < .05$; **$P < .001$

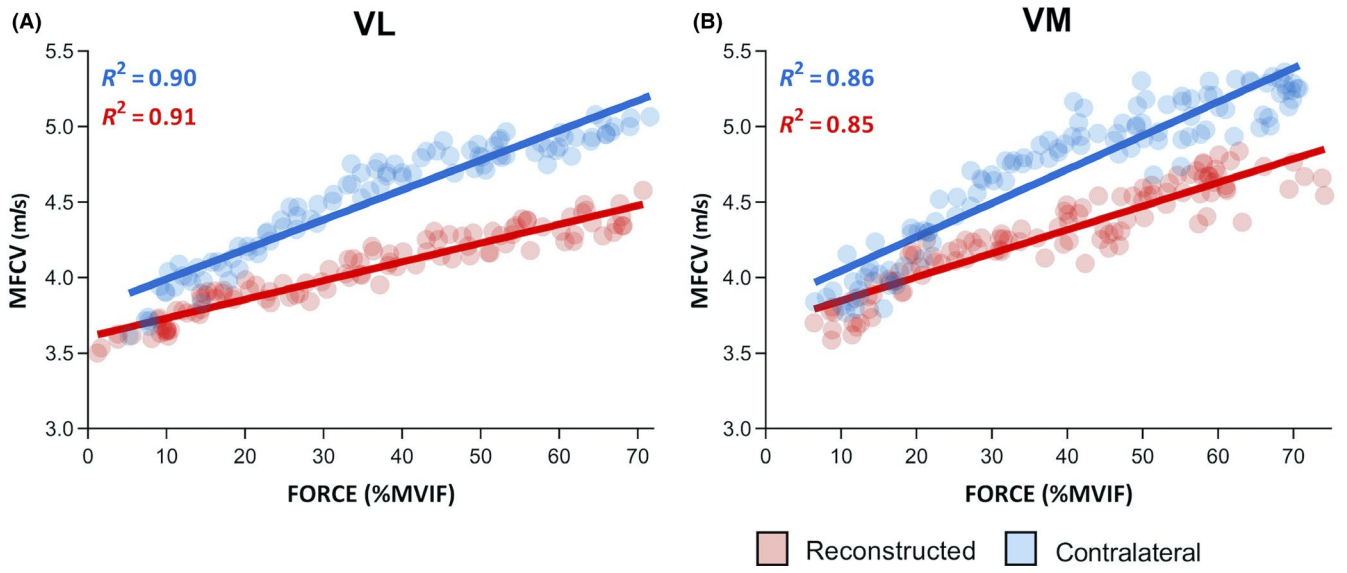

FIGURE 3 Muscle fiber conduction velocity (MFCV) plotted as a function of voluntary force. Bilateral regression lines are outlined for both vastus lateralis (A) and medialis (B) in one representative participant; $P < .001$

with respect to the contralateral side. Additionally, a strong negative relationship between these functional measurements and patient self-reported knee function was found, confirming that the recovery of both muscle strength and structure is imperative to ensure a safe return to pre-injury level of sport.[30] In line with our observations, both quadriceps atrophy and strength deficits are common at 6-8 months following ACLR.[5,31] The large force difference found in the current study may be due to factors related to the experimental setup. Indeed, voluntary quadriceps activation is lower when exerting force in 45° of knee flexion compared to 90° in ACLR individuals.[11] In addition, an open kinetic chain exercise promotes larger discharge rate and estimated synaptic input than closed chain exercise, only at high force levels (70% of MVIF),[32] suggesting that such a rise in the synaptic input to both vasti muscles is related to HTMUs, whose activation can be reduced as a result of AMI.[33] Moreover, the lower specific strength found for the reconstructed side with respect to the contralateral side suggests that deficits in muscle strength may exceed that of muscle mass, as previously indicated.[5] However, factors such as reduced muscle fiber pennation angle and increased antagonist co-contraction may contribute to the decreased specific tension observed.[34]

It should be remarked that the strength deficit in the participants could be also the result of adaptations occurring in the uninjured side. Specifically, due to different ACLR-induced neuromuscular alterations occurring at both spinal and supraspinal levels (ie, cross-over inhibition and gamma-loop dysfunction) and/or post-surgery deconditioning, the contralateral side may be weaker when compared with healthy controls.[3,10,35] At the same time, due to the adoption of potential compensation strategies during the early rehabilitation phases (ie, additional weight-loading) a gain in quadriceps strength of the contralateral side cannot be excluded.

The average of the initial MFCV was similar among limbs and muscles. Values of MFCV obtained for the lowest force seem to reflect the CV of the low-threshold motor units (LTMUs).[14] Relevantly, Del Vecchio et al found no difference between the initial CV of MUs and the initial MFCV estimates, confirming experimentally such hypothesis.[14] Accordingly, it seems that LTMUs of both vasti muscles had normal function, reflecting no evident deficits at low force levels.

The analysis of the participant-specific regressions revealed considerable asymmetries in both MFCV slope and $MFCV_{70}$. Only one study assessed the rate of MFCV change in the VM of a population of ACLR individuals.[36] This previous study focused on understanding changes in quadriceps fatigability evaluated by analyzing the decline of MFCV during sustained isometric contractions. The authors found lower MFCV slopes at 12 months post-ACLR with respect to healthy controls, and this change was ascribed to altered recruitment strategies and/or discharge rate of HTMUs. However, direct comparisons are difficult since the slopes obtained in this previous study were due to fatigue and not to increasing force.

Interestingly, Rice and colleagues[33] estimated the greatest MFCV of the VM during MVC, prior and after an experimentally induced knee pain in healthy individuals. They found a significant 15% decrease in maximal MFCV after joint infusion, suggesting that AMI observed acutely after a joint trauma may selectively impair the recruitment and/or firing rate of quadriceps HTMUs. The 10.1% and 8.1% difference in the $MFCV_{70}$ of the VL and VM observed in the current study suggests that AMI may impair HTMUs even after several months following joint trauma, thus contributing to PQW.[3,4]

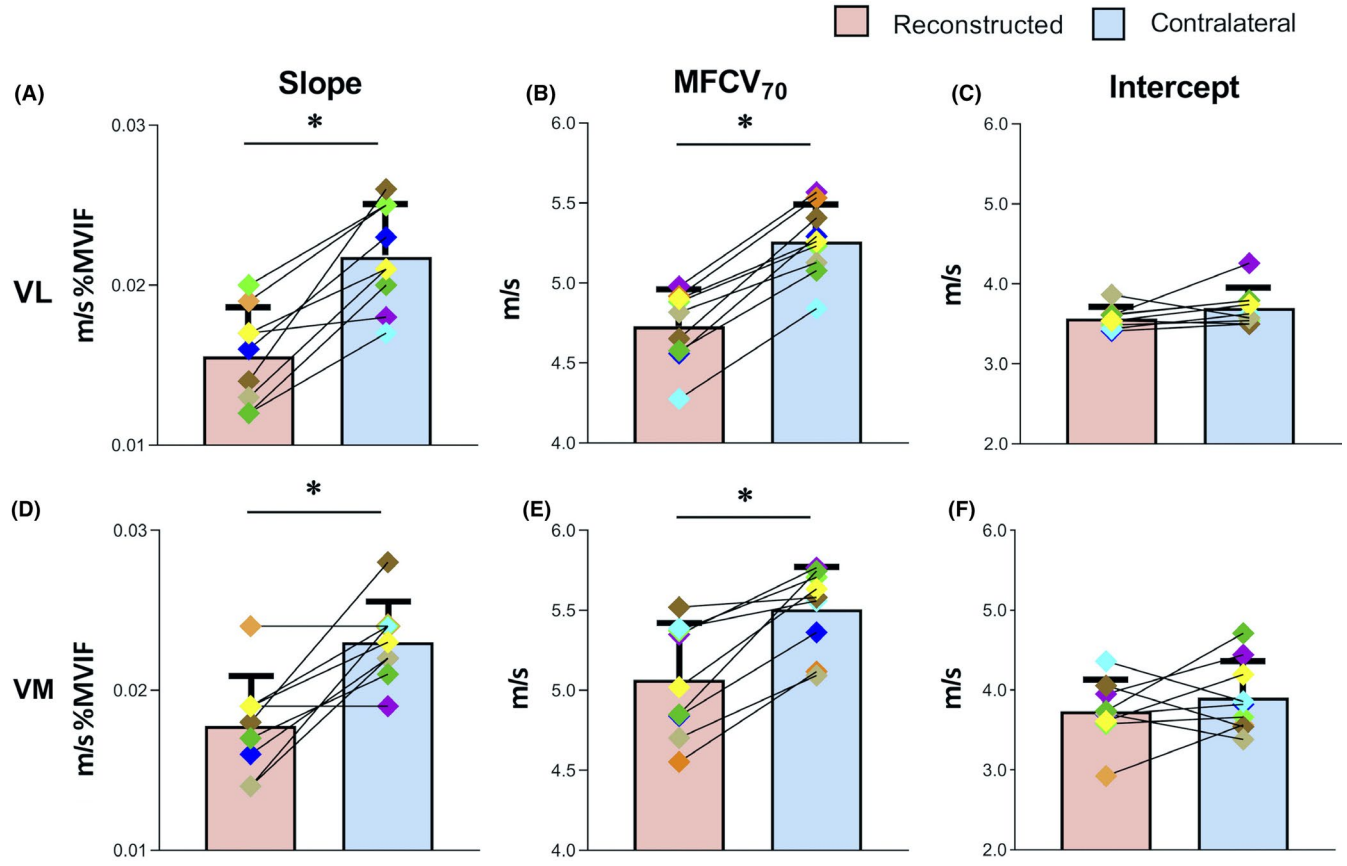

FIGURE 4 Differences in the rate of change (slope), peak ($MFCV_{70}$), and initial (intercept) MFCV values obtained from the ascending phase of the trapezoidal contraction. A-D, Average rate of MFCV change with respect to voluntary force (slope). B-E, Maximal MFCV values obtained during the ramp contraction up to 70% of MVIF ($MFCV_{70}$). E-F, Average values of the initial MFCV (intercept). Subject-specific values are represented by using different colors. Mean and SD are reported for both the reconstructed (red bars) and the contralateral side (blue bars); VL = vastus lateralis; VM = vastus medialis; *$P < .001$

The lower $MFCV_{70}$ and MFCV slope of the VL and VM found in the reconstructed side compared to the contralateral one may indicate different neural changes affecting the HTMUs. First, the results are consistent with an incomplete MU recruitment.[14,15] On the other hand, it may be possible that all the MUs are normally recruited but the discharge rate of the HTMUs is reduced.[37] Alternatively, the results may be explained by peripheral alterations (ie, lower CV of the HTMUs) occurring without any change in neural strategies.

The most plausible peripheral mechanism that explains the observed deficits in MFCV is the reduced function of the Na+-K+-ATP_{ase} pump, due to its role in facilitating the spread of action potentials along the sarcolemma.[20] Physiologically, the Na+-K+-ATP_{ase} pump contributes to maintain and regulate the basal membrane excitability as well as the muscle contractility, strength, and endurance.[19,38,39] Moreover, since the proportion of Na+-K+-ATP_{ase} pump is higher in type II compared to type I muscle fibers,[38] high MFCV values may reflect the increased spread of action potentials along the muscle fibers belonging to HTMUs.[20,38] Downregulation of the Na+-K+-ATP_{ase} has been demonstrated in various adverse conditions.[38] Interestingly, a 63% decline in the Na+-K+-ATP_{ase} pump content was observed in VL biopsies of individuals at an average of 3 ½ months after ACL tear.[19] Hence, the reduced $MFCV_{70}$ that we found in both VL (−10.1%) and VM (−8.1%) might be partially due to biophysical alterations occurring in type I and, to greater extent, type II muscle fiber membranes. Moreover, because of the strong relation between MFCV and muscle fiber diameter,[14-17] a reduction in type II muscle fiber diameter is also reasonable.

A selective decrease in type IIA muscle fiber amount and an increased proportion of type IIX muscle fibers were documented in the VL of ACLR athletes returned to sport.[8] Similar results were found in the reconstructed side after 6 ½ months from ACLR.[9] Additionally, reduced satellite cells distribution, increased extracellular matrix and reduced physiological CSA were reported in the VL 6-to-12 months post-surgery.[8] Collectively, these findings strengthen our hypothesis of a selective neuromuscular impairment occurring in HTMUs.

Interestingly, the Δ $MFCV_{70}$ of the VL was the only variable from the interference EMG that independently explained ~60% of variance in MVIF. Arguably, almost 2/3 of

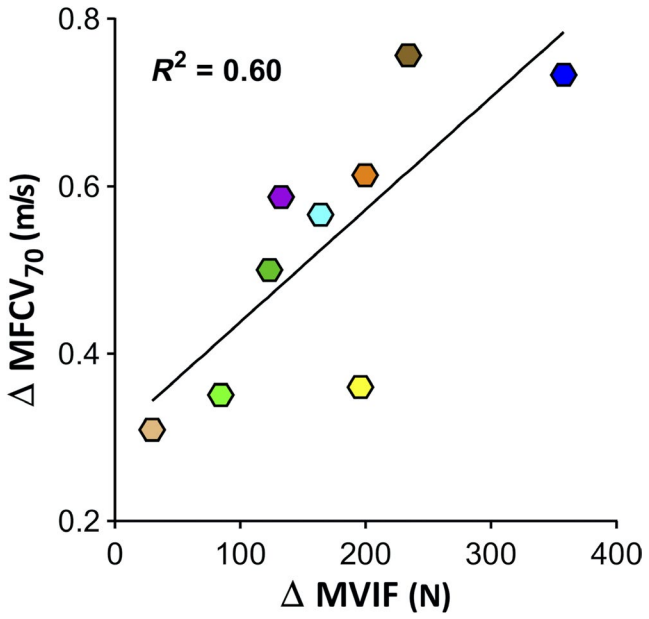

FIGURE 5 Scatter plot with the regression line of the between-limb difference in maximal muscle fiber conduction velocity (Δ $MFCV_{70}$) of the vastus lateralis in function of the difference in maximal voluntary isometric force (Δ MVIF). Individual values are reported using different colors.

the decreased force observed seems to be caused by either the impaired neural strategies (ie, reduced recruitment and/or firing rate) or the reduced excitability of the sarcolemma affecting the HTMUs of the VL. However, it should be remarked that the current study is underpowered for multiple regression analysis. Therefore, although interesting, this finding should be regarded as purely preliminary. Lastly, the rate of MFCV change of the VL was 12.4% lower compared to the VM, only in reconstructed side. Although the estimated neural drive received by the motor neuron pool of both vasti muscles is the same in healthy individuals,[32] an imbalance among synergistic muscles may potentially occur in clinically impaired conditions.[40] It is plausible that the progressive MU recruitment of the VL is selectively altered when increasing levels of knee extensor's force are needed.

This study has some limitations. The absence of healthy controls or pre-injury values represents the main limitation of the present investigation. The use of the uninjured side as a reference implies potential biases. Specifically, increased or decreased quadriceps strength of the uninjured side may reflect into either an overestimated or an underestimated quadriceps weakness of the reconstructed side.[3,10,35] Therefore, despite an unequivocal neuromuscular deficit of the reconstructed side has been found, caution is needed when interpreting the results of the current study. Moreover, although the estimation methods used are valid[23] and reliable,[24] both MFCV and QuadCSA were not directly measured.

In conclusion, we observed changes in MFCV which may be ascribed to altered recruitment of quadriceps HTMUs, biophysical modifications of the Na+-K+-ATPase pump, and deficits of motor unit discharge rate. Moreover, quadriceps strength asymmetries are largely explained by deficits in MFCV of the vastus lateralis.

4.1 | Perspectives

A full recovery of quadriceps strength and function is crucial to ensure a safe return to sport and a reduced risk of ACL reinjury following ACL reconstruction.[4-6] The results of this study suggest a selective impairment in the HTMUs of the quadriceps following ACL reconstruction. However, an analysis at motor unit level is required to experimentally confirm this hypothesis.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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