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Objective: To investigate if harvesting of semitendinosus (ST) and gracilis for anterior cruciate ligament (ACL) reconstruction will have an effect in coordinative firing pattern of the hamstrings under fatigue. We hypothesized that fatigue will increase the electromechanical delay (EMD) of the hamstrings on the harvested site and impair the synchronization between the medial and lateral hamstrings, in terms of muscle activity onsets.

Design: Prospective nonrandomized study. Setting: Institutional. Patients: Twelve ACL reconstructed patients with hamstrings, 2 years postoperatively.

Interventions: The patients performed a fatigue protocol with 25 continuous maximal isometric voluntary contractions of 8-second duration with 2-second intervals. Main Outcome Measures: The electromyography activity of biceps femoris (BF) and ST was recorded bilaterally and simultaneously with the torque measurements. The dependent variable examined was the EMD difference between BF and ST (muscle activation pattern).

Results: The fatigue protocol caused significant differences for the EMD values for both the intact and the reconstructed leg, demonstrating the influence of fatigue in EMD. However, the synchronization pattern between the medial and lateral hamstrings did not change significantly throughout the fatiguing protocol, revealing a balanced effect of fatigue.

Conclusions: Although the EMD of ST and BF was significantly increased due to fatigue, as expected, their synchronization pattern as identified by the difference in their EMDs remained the same. Thus, the reconstructed knee responded in a balanced manner and the hamstrings firing pattern remained the same, despite the intervention to the ST tendon.

Key Words: electromechanical delay, ACL reconstruction, hamstrings, electromyography, fatigue, muscle synchronization

Introduction

The use of hamstrings for anterior cruciate ligament (ACL) reconstruction has increased in recent years. Biomechanical studies have demonstrated that this graft exhibits comparable strength and stiffness to the native ACL.[1,2] However, many surgeons are still skeptical about postoperative functionality because some research has demonstrated impaired recovery in extended follow-ups.[3] The majority of studies have focused on evaluating strength of the hamstrings, after harvesting of their tendons.[4] However, some investigators have suggested that the actual effectiveness of the muscles to provide appropriate mechanical response and protection under real-life situations can be revealed only with the measurement of the time delay between the onset of muscle stimulation by the alpha motoneuron and the development of the corresponding torque at the joint.[5–7] This is referred to as the electromechanical delay (EMD).[5] The measurement of the EMD is of great functional importance because regardless of the contractile ability of the muscles, alterations in the EMD of the hamstrings muscle-tendon unit could compromise knee integrity or impair performance by modifying the transfer time of muscle tension to the bones.[5–7]

Factors related to the EMD include the mechanical properties of the in-series elastic components of the muscle, the size and length of the muscle, as well as its fiber type composition, and the presence of fatigue.[8–11] Muscle fatigue, which has been defined as any reduction in the force-generating capacity of the entire neuromuscular system regardless of the force expected, [12] is common in sports activities. Fatigue can affect not only the force-generating capacities but also the temporal characteristics of the neuromuscular mechanism, and especially the EMD.[10] Therefore, changes in the EMD should be expected under muscle fatigue conditions.

In the present study, we tried to identify how fatigue impairs the knee flexor mechanism in terms of the EMD, after harvesting the semitendinosus (ST) and gracilis (G) muscle tendons for ACL reconstruction. Our methodology (surface electromyography) allowed us to evaluate only the ST muscle and not the G muscle. In addition, we decided to investigate the biceps femoris (BF) to ensure a more comprehensive evaluation of the hamstrings. Although BF is not anatomically involved in the operation, investigation of its EMD could be useful because synchronization between the medial and lateral hamstrings is important for knee rotational stability.13 Our rationale for this inclusion was that if changes exist in the EMD of the ST, then changes in the BF may also appear, due to the fact that these 2 muscles act as a coordinated unit when the knee flexor mechanism is initiated.[14] If not, then this could generate a major synchronization issue on the hamstrings muscle response firing pattern.

Therefore, the purpose of this study was to investigate whether muscle fatigue actually affected the EMD of the hamstrings 2 years after ACL reconstruction and if the coordination between the medial and the lateral hamstrings was disturbed. We hypothesized that fatigue of the knee flexors after harvesting of the ST/G tendons for ACL surgery will (1) increase the EMD of the hamstrings on the harvested site and (2) impair the synchronization between the medial and lateral hamstrings, in terms of muscle activation as evaluated with the EMD.

Methods

Subjects

We examined 12 ACL reconstructed patients with a quadrupled hamstrings (ST/G) graft (men: mean age, 26 ± 8 years; mean mass, 74 ±14 kg; mean height, 1.72 ± 0.10 m), approximately 2 years (range, 24-26 months) after the operation. They all followed the same postoperative rehabilitation program. Return to sports-related activities was permitted 24 weeks after reconstruction, provided that the patients had regained full strength and stability. Their strength at that time was determined with the BIODEX (System-3; Biodex, Corp, Shirley, New York) isokinetic dynamometer, revealing acceptable symmetry in quadriceps and hamstrings strength, as well as satisfactory agonist to antagonist ratios. At the time of data collection, no clinical evidence of knee pain was found.

Surgical Reconstruction With a Quadrupled ST/G Graft

Through a 4 to 5 cm longitudinal skin incision over the pes anserinus, a typical harvesting of both the ST and the G tendons was performed in all patients. The tibial tunnel was prepared with the knee in 90° flexion. The hole in the tibial plateau was placed approximately 5 mm anterior and medial to the anatomic center of the natural ACL attachment.[15] Subsequently, the femoral tunnel was drilled with the knee flexed in 120°, through the anteromedial portal at the 10-o'clock position (for a right knee). The graft was secured at the distal femur with an EndoButton (Smith & Nephew Endoscopy, Andover, Massachusetts) and fixated at the tibial tunnel with a bioabsorbable screw.

Clinical Evaluation

Before any data collection, a clinical evaluation was performed for all subjects. During this evaluation, the Lysholm functional score, the Tegner activity scale, and the International Knee Documentation Score (IKDC) Subjective Knee Evaluation Form were also obtained.[16] Anterior tibial translation was evaluated using the KT-1000 knee arthrometer (MEDmetric Corp, San Diego, California) for both limbs of the ACL reconstructed group.[17] The measurements were performed using 134N posterior-anterior external force at the tibia, and maximum posterior-anterior external force until heel clearance. Repeated anterior tractions were performed until a constant reading on the dial was registered.

EMD Measurement

The method used to measure the EMD has been described in detail elsewhere.[18, 19] Briefly, the patients sat on the testing chair of the dynamometer, with the knee and hip joint flexed at 30°. Torque measurements were performed for both knees using the Biodex isokinetic dynamometer. All the subjects were instructed to exert a maximum knee flexion as fast and as hard as possible, after hearing a specific sound generated by the dynamometer. The subject held the maximal force, until the sound stopped. Each subject performed 1-leg fatiguing exercise, which consisted of 25 such maximally explosive isometric voluntary contractions. Each contraction lasted 8 seconds and was followed by 2-second relaxation between each contraction, according to the fatigue protocol developed from Zhou.[20]

Electromyography (EMG) traces were recorded from both the ACL reconstructed and the intact contralateral leg simultaneously with the torque measurements, with a wireless 8-channel EMG system (Telemyo 2400T; Noraxon, Scottsdale, Arizona), and were displayed real time on a computer using dedicated software (MyoResearchXP; Noraxon). Surface electromyography was obtained from the ST and BF muscles bilaterally using bipolar, circular, preamplified, Ag/AgCl electrodes with 10 mm diameter and fixed interelectrode spacing of 20mm(Noraxon). The electrodes were attached parallel to the muscle fibers and over the dorsomedial muscle bulge at two-thirds of the proximodistal thigh length for the ST and at the dorsolateral side of the thigh at half of the proximodistal thigh length for the BF. The subjects were instructed to relax the muscles completely before a contraction trial.

Before the test, all subjects were instructed to stay completely relaxed in the Biodex testing chair, while the EMG signal was calibrated with the ‘‘zero offset’’ function to establish a zero baseline from each of the EMG channels. The EMG signals were acquired at a sampling rate of 1000 Hz. The root-mean-square (RMS) amplitude for each muscle burst was calculated as follows: the raw EMG signals were measured in a band of 10 to 500 Hz, full-wave rectified, high-pass filtered with a Butterworth filter to remove movement artifacts with a cutoff frequency of 20 Hz, and smoothed with a 100-millisecond RMS algorithm. Measurements of the EMD were performed using the isokinetic dynamometer and the surface EMG unit, according to the protocol developed by Zhou et al.11 Based on this protocol, the onset of torque development is defined as a 3.6-Nm deviation above the baseline level and 615 mV deviation from the baseline for the EMG signal.

Statistical Analysis

To address the hypothesis that fatigue of the knee flexors after harvesting of the ST/G tendons for ACL surgery will increase the EMD of the hamstrings on the harvested site, we performed paired t tests on the actual values of the EMD of BF and the EMD of ST for each leg between the first 5 and the last 5 trials of the fatigue protocol. To address the second hypothesis that fatigue of the knee flexors after harvesting of the ST/G tendons for ACL surgery will impair the synchronization between the medial and lateral hamstrings, we examined the coordination of the hamstrings in terms of muscle activation onsets by subjecting the difference in the EMDs between the BF and ST (Figure 1) in a 2-way fully repeated analysis of variance (ANOVA).

The ANOVA factors were identified as Leg (Intact Contralateral vs ACL Reconstructed) and Fatigue (Trial 1 vs. vs Trial 25). Practically, the factor Leg had 2 levels and the factor Fatigue had 25 levels. Tukey post hoc comparisons were used to locate differences when significance was identified. An additional post hoc evaluation was performed to verify the ANOVA outcomes and to explore if grouping the trials would affect these results. Thus, we also performed paired t tests on the same dependent variable (the difference in the EMDs between the BF and ST) between the first 5 trials and the last 5 trials for both the intact and the ACL reconstructed leg and for both investigated muscles. The statistical significance was set at 0.05. All the statistical comparisons were performed with the Statistica (v.8 software; StatSoft, Inc, Tulsa, Oklahoma).

Ethical Considerations

All subjects agreed with the testing protocol and gave their consent to participate in accordance with the Institutional Review Board policies of the University of Ioannina Medical School.

Results

Negative Lachman and pivot-shift tests indicated that the knee joint stability was regained clinically for all ACL reconstructed subjects. The median Lysholm score was 92 (range, 87-95), the Tegner score was 7 (range, 6-8), and the IKDC score was scaled as normal (A) at the time of examination. KT-1000 results revealed that the mean difference between the anterior tibial translation of the reconstructed and intact sides in the ACL reconstructed group was 1.1 mm (range, 0.5-2 mm) for the 134N test and 1.3 mm (range, 1-2 mm) for the maximum manual test, respectively. No significant differences were found for the KT-1000 results between the limbs.

With respect to our first hypothesis, the *t* test comparisons between the first 5 and the last 5 trials of the fatigue protocol showed significant increases for the actual EMD values for both the intact ($P = 0.002$ for BF and $P = 0.008$ for ST) and the reconstructed leg ($P = 0.023$ for BF and $P = 0.025$ for ST), revealing the effect of fatigue on EMD clearly (Figure 2). However, when we evaluated the EMD difference between the BF and ST, we found no significant interaction for the coordination of the hamstrings muscle firing pattern for both legs ($F = 0.67$; $P = 0.878$) (Figure 3). We also found no significant differences for the Leg factor ($F = 1.027$; $P = 0.335$) and the Fatigue factor ($F = 1.061$; $P = 0.390$). In addition, the paired t test comparison for the same parameter (the EMD difference between the BF and ST) between the first 5 and the last 5 trials of the fatigue protocol showed no significant differences for both the intact and the ACL reconstructed leg ($P = 0.108$ and $P = 0.398$, respectively), verifying our ANOVA results and indicating that fatigue does not affect this coordination pattern.

Discussion

Based on the results from our previous work,[18] we hypothesized in the present study that fatigue of the knee flexors after harvesting of the ST/G tendons for ACL surgery will (1) increase the EMD of the hamstrings on the harvested site and (2) impair their coordination, in terms of muscle activation, as evaluated with EMD. Our results supported our first hypothesis and refuted our second. Specifically, we found that although fatigue affected both medial and lateral hamstrings EMD, their coordinated firing pattern remained the same for the 2 legs throughout the fatiguing protocol. This finding is of great importance because it demonstrates that although we had harvested the ST tendon (resulting in postoperative alterations in the muscle's size, length, and fiber type composition), the coordinative firing pattern of the investigated muscles remained the same, continuing to synchronize in the same way as the intact, thus achieving balance between and within legs.

In our protocol, fatigue was induced in the form of repetitive isometric contractions, which is a protocol that is well established in the literature.[8, 11] Fatigue depresses force generation capacity during either static or dynamic muscle contractions.[21] Despite the possible effects of central fatigue, the decreased muscle contractile performance during fatigue has been related to impairment of membrane excitability, reduction in titanic cytosolic calcium (Ca^{+2}) concentration, reduced myofibril Ca^{+2} sensitivity, and the direct inhibitory effects of phosphate and hydrogen ions on force generation.[21,22] Because EMD measures the time lapse from muscle activation until a certain threshold of muscle tension is developed, muscle fatigue, which affects the aforementioned processes, is expected to prolong the EMD.

However, despite the numerous investigations in the area of muscle fatigue, its effect on EMD remains controversial. There have been reports that the EMD increased after fatiguing dynamic exercise,[23] whereas other studies have showed no significant change in EMD after repeated dynamic or isometric contractions.[7] Kroll[24] had shown no significant changes of EMD after a fatigue protocol that involved bench stepping exercise and a plantar flexor fatigue. Vos et al[7] also found no significant change in EMD of the rectus femoris muscle after 150 repetitions of 50% isometric maximal voluntary contraction. On the other hand, Nilsson et al[25] have shown a significant lengthening of EMD after a fatigue protocol.

Similarly, Zhou[20] studied the effects of fatigue on the EMD of the knee extensor muscles and showed a significant increase in EMD after a fatigue protocol that included 4 periods of 30 seconds of an all-out sprint cycling exercise. Horita and Ishiko[23] also reported that the EMD was affected by repeated maximal isokinetic knee extensions. In our study, we also found significantly increased EMD values for BF and ST muscles at the end of the fatigue protocol. The discrepancies between the above studies are probably related to the different types of muscle contractions used and the variability in the fatigue protocols.

Our methodology allowed us to evaluate only the ST muscle and not the G muscle. In addition, we decided to investigate the BF to ensure a more comprehensive evaluation of the hamstrings. Rotatory stability of the knee is a major concern for knee protection and because it is impaired after ACL injury, in the current study, we focused on investigating if the EMD of the BF shows a different pattern than the EMD of the lateral hamstrings. A possible difference would question knee stability under fatigue, after using the hamstrings tendons as a graft. Our results showed that the BF-ST firing pattern remained the same for both legs of the ACL reconstructed individuals throughout the fatigue protocol, even though the EMD values for each muscle separately were affected by the fatigue protocol, as expected. A possible explanation for the lack of changes of the BF-ST firing pattern is that the BF works synergistically with the ST during knee flexion to provide functional balance to the knee. Changes in the EMD of the ST may also be accompanied by changes in the BF, due to the fact that these 2 muscles act as a unit when the knee flexor mechanism is initiated.[14,26]

Several studies have emphasized the importance of muscular alterations after injury to achieve joint stability.[14,26] Johansson et al[26] suggested that joint stability is achieved by the continuous adjustment of muscle activity around the joint (cocontraction). The ACL is loaded and potentially injured via anterior tibial translation.27 The hamstrings are synergistic to the ACL, providing posterior tibial shear force, which limits ACL loading attributable to anterior tibial translation.[28] Noncontact ACL injury typically occurs during landing and gait activities,[29] which incur rapid changes in the forces applied to the knee joint. As such, timely dynamic response from the hamstrings seems to be essential for ensuring knee joint stability and limiting the load imparted to the ACL or its substitute graft in reconstructed patients. A change in the hamstrings muscle activation pattern could actually prove detrimental for knee joint stability and protection of the graft. This is why the evaluation of the EMD measurement is of great functional importance. Regardless of the contractile ability of the muscles, which is depicted usually by measuring knee flexion peak torque, alterations in the EMD of the donor-site muscle-tendon unit could compromise knee integrity or impair performance by modifying the transfer time of muscle tension to the bones.

Limitations in this study include the absence of a specific methodology in the literature for determining EMD and defining specific threshold levels of both signals, onset of EMG, and force generation. Reported values for EMD differ substantially across muscles and investigations due to differences in operational definitions,[30] characteristics of the muscles being tested,[31] contraction type,[6,11] and data processing techniques.[32] Corcos et al[32] evaluated biceps brachii EMD under various experimental conditions, including differences in hardware sensitivity, characteristics of the subject-force sensor interface, and time-scale resolution, and reported significantly different EMD values as functions of the various experimental characteristics. Another limitation of our study is the use of surface electrodes to acquire the EMD measurements. However, similar procedures have been used in previous studies and are considered reliable.[7,23,33]

In conclusion, we found that although the EMD of ST and BF was significantly increased due to fatigue protocol as expected, their synchronization pattern remained the same, as the reconstructed knee responded in a balanced manner. The hamstrings coordinative firing pattern remained the same, despite the intervention to the ST tendon. Therefore, harvesting the ST/G tendon for ACL reconstruction seems to have no effect on the coordinative firing pattern of knee flexors. This may be an important preventive mechanism for the anterior cruciate ligament reconstructed athlete because possible modifications in hamstrings response under fatigue conditions could have endangered knee balance and increased the potential for reinjury.

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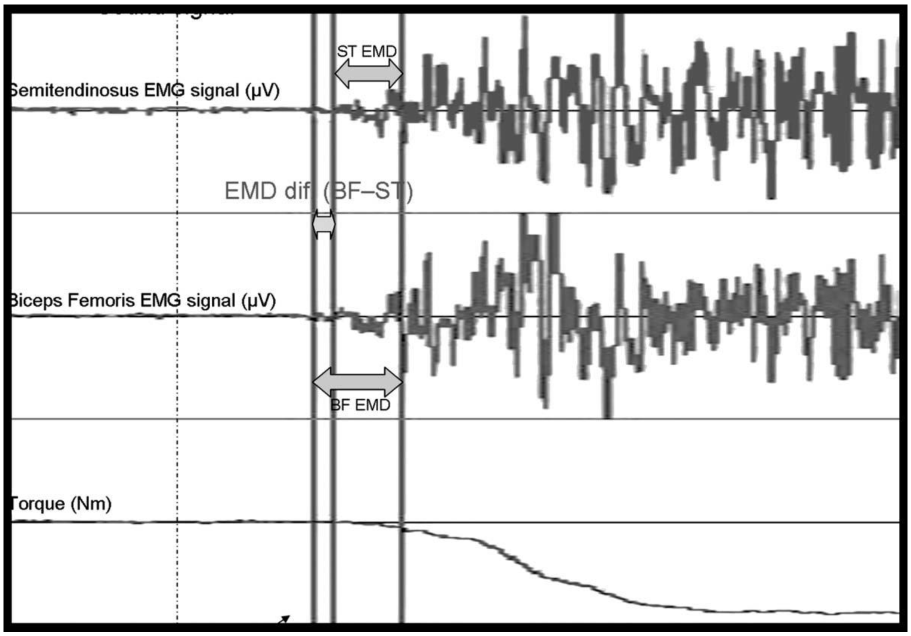

Figure 1. A typical time plot of a single trial on the presentation of the stimulus (sound signal) to the onset of the EMG signal for both muscles (ST and BF) and force generation (torque). The onset of torque development is defined as a 3.6 Nm deviation above the baseline level and the onset of EMG signal as ±15 μV deviation above the baseline. The EMD difference (muscle sequence firing pattern) is indicated with small arrow (EMD of BF 2 EMD of ST).

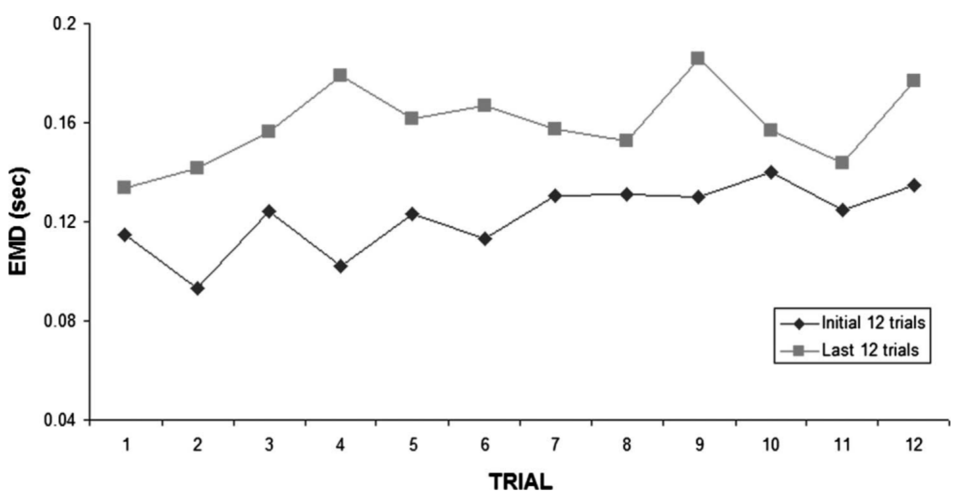

Figure 2. A line graph indicating the influence of fatigue in EMD values of a separate muscle (ST) of the intact leg in a randomly selected patient from the examined group. The black line represents the 12 first trials, whereas the gray line represents the last 12 trials of the fatigue protocol.

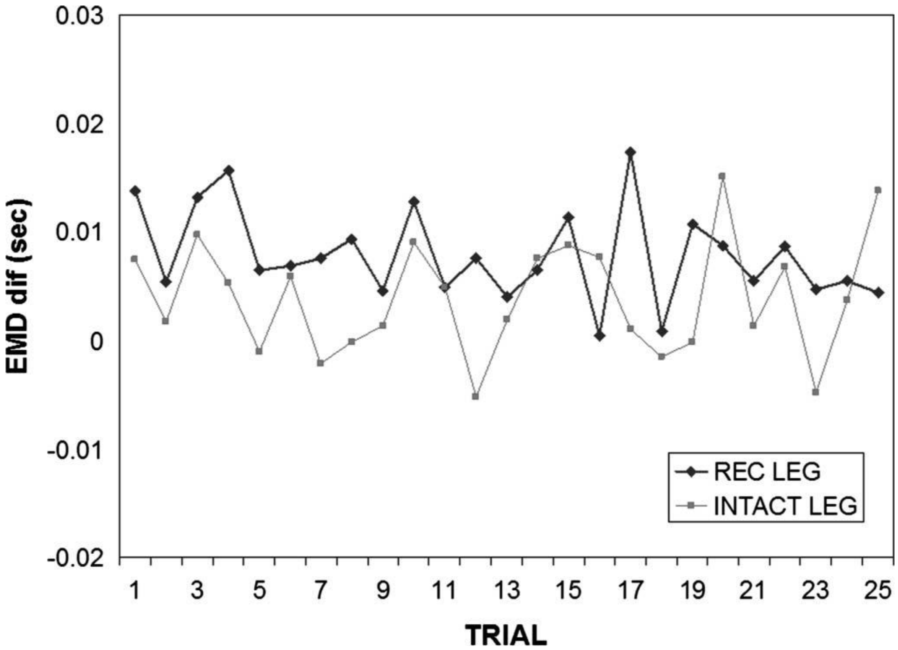

Figure 3. A line graph indicating the hamstrings muscle firing pattern for the intact (gray line) and the reconstructed leg (black line) in a randomly selected patient from the examined group. Although the EMDof ST and BF significantly increased during the fatigue protocol, we found that their coordination firing pattern remained the same during the fatigue protocol.

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