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1	ACL RECONSTRUCTED PATIENTS WITH A BPTB GRAFT PRESENT AN IMPAIRED
2	VASTUS LATERALIS NEUROMUSCULAR RESPONSE DURING HIGH INTENSITY
3	RUNNING.
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1 ABSTRACT

2 The purpose of the present study was to investigate whether the electromyographic response of the 3 vastus lateralis (VL) muscle in the anterior cruciate ligament (ACL) reconstructed leg is similar to that 4 of the intact contralateral leg and healthy controls, during moderate and high intensity running. 5 Fourteen bone-patellar tendon-bone (BPTB) ACL reconstructed amateur soccer players and fourteen 6 healthy control amateur soccer players volunteered to participate in the study. Electromyographic 7 (EMG) traces from the vastus lateralis (VL) muscle were collected bilaterally, as athletes ran on a 8 treadmill for 10 minutes on separate occasions, at moderate and high intensity. The dependent variable 9 examined was the EMG amplitude during stance. During the moderate intensity running, EMG 10 amplitude of the VL did not increase with time for any of the tested legs. During the high intensity 11 running, the EMG amplitude of the VL increased significantly with time for the intact (F=6.747, 12 p=0.001) and the control leg (F=4.258, p=0.008), but remained unchanged for the ACL reconstructed 13 leg. During moderate intensity running, there was no difference in the neuromuscular response of the 14 VL in the reconstructed leg compared to the intact and control leg. High intensity running resulted in 15 an impaired neuromuscular response of the VL in the reconstructed leg compared to the intact and 16 control leg. It seems that potential impairments of the neuromuscular response after ACL 17 reconstruction should be tested under high rather than moderate intensity efforts.

18

1	9 Kev words:	elemetric electromyograph	iv. running. ACI	L reconstruction.	exercise intensitv
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1 INTRODUCTION

Anterior cruciate ligament (ACL) function is closely associated with optimal activation of the muscles surrounding the knee joint [1, 2]. Rupture of the ACL leads to alterations in muscle recruitment patterns [3, 4]. It has been demonstrated that reconstruction of the ACL re-establishes muscle activation levels, as assessed by surface electromyography (EMG) amplitude, towards normative values during moderate intensity activities [5-7]. However, moderate intensity exercises do not resemble situations that are encountered during participation to sports activities.

8 One feature of the neuromuscular response after ACL reconstruction that has not been 9 investigated by previous studies is the progression of EMG activation levels with time during the 10 exercise. It is well established that EMG amplitude time course is greatly influenced by exercise 11 intensity [8, 9, 10]. During moderate intensity, EMG amplitude does not increase with time [9, 11, 12], 12 while during high intensity, it does [9, 11-13]. The increasing EMG amplitude with time, during high 13 intensity exercise is considered to reflect the physiological response that is required for the increasing 14 metabolic demands of the exercise [8, 9, 10].

15 Previous EMG studies reported that ACL reconstructed (ACLR) subjects exhibit neuromuscular 16 alterations, such as selective muscle fiber atrophy in the involved vastus lateralis (VL) [14-16], altered 17 motor unit activation of the quadriceps following surgery and subsequent retraining [17] and loss of 18 joint afferent information which may lead to suboptimal muscle activation [1, 18]. These 19 neuromuscular alterations may therefore impair the proper physiological response required at high 20 exercise intensities. Thus, it is possible that the VL muscle of the operated leg could exhibit impaired 21 neuromuscular response resulting in inability to naturally increase the EMG amplitude with time 22 during high intensity exercise.

Therefore, the purpose of the present study was to investigate the effect of ACL reconstruction with BPTB graft on VL activation levels with time during moderate and high intensity running. It is well established that exercise intensity is best assigned relative to the lactate threshold and VO₂max [8, 19]. We hypothesized that (a) during moderate intensity exercise the EMG amplitude of the VL will not increase with time for any of the ACLR, intact and control legs, (b) during high intensity exercise the EMG amplitude of the VL will increase with time for the control and intact leg but not for the
 ACLR leg.

3

4 METHODS

5 Two groups of athletes participated in the study. Fourteen amateur male soccer players [mean 6 (SD) age, body weight and height, 24.8 (5.3) years, 77.3 (7.5) kg and 177 (5.3) cm] with ACL-7 reconstructed knees and fourteen healthy amateur male soccer layers who had never suffered of any 8 kind of orthopaedic or neurological condition [mean (SD) age, body weight and height, 21.7 (4.4) 9 years, 72.2 (8.3) kg and 180 (9.0) cm]. The operated athletes had undergone ACL reconstruction with 10 bone-patella tendon-bone (BPTB) graft, 18.5 (4.3) months before testing. ACL reconstruction was 11 performed sub-acutely within 6 months after the injury from the same surgeon (A.G). All subjects had 12 a unilateral ACL tear confirmed by MRI and arthroscopy.

13 All operated athletes underwent the same rehabilitation protocol, starting from the first post 14 operative day with the use of passive exercises. Return to sports was permitted 6 months after 15 reconstruction provided that the athletes had regained stability and full functional strength, according 16 to well accepted criteria [20]. All subjects agreed with the testing protocol and gave their consent to 17 participate in accordance with the Institutional Review Board policies of our Medical School. Prior to 18 any data collection, a clinical evaluation was performed in all athletes by the same clinician. During 19 this evaluation, the Tegner and Lysholm scores were obtained, while anterior tibial translation was 20 evaluated using the KT-1000 knee arthrometer (MEDmetric Corp., San Diego, California) [21].

21 The athletes reported to the laboratory on three different occasions, separated by 48 hours, 22 within a two weeks period. For their first visit to the laboratory, athletes performed an incremental 23 treadmill (Technogym Runrace 1200, Italy) running test to volitional exhaustion with 3 minute-stages, 24 to determine VO₂max and lactate threshold (LT) [22]. A computerized system was used for all 25 metabolic measurements (CPX Ultima, Medical Graphics, St Maul, MN, USA). At the end of each 26 stage, capillary blood samples were collected and analyzed for lactate (Accutrend, Roche Diagnostics, 27 Germany). Prior to each test, all analyzers were calibrated according to the manufacturer instructions. 28 Attainment of VO₂max was verified according to criteria established by the American College of 1 Sports Medicine [22]. Lactate threshold was determined according to Cheng et al [23]. The high 2 intensity running was set at 40% of the difference between VO₂max and lactate threshold (40% D) and 3 the moderate intensity running was set at 80% of the lactate threshold [19].

In each of the two subsequent visits to the laboratory, athletes were required to perform a 10minute run at the pre-selected intensities. We tested one intensity at each visit and the order was randomly assigned for every athlete. During running, EMG data were collected for 15 seconds at the 3rd, 5th, 7th and 10th minute. Gas exchange data were recorded simultaneously breath-by-breath, heart rate was measured throughout the test and blood lactate was measured prior to running and immediately after termination of exercise.

10 EMG traces were obtained from the VL muscle bilaterally using bipolar, circular, pre-amplified, 11 pre-gelled Ag/AgCl electrodes with 10 mm diameter and fixed inter-electrode spacing of 20 mm 12 (Noraxon Inc, Scottsdale, AZ, USA). EMG data were recorded with a wireless 8-channel EMG system 13 (Telemyo 2400T, Noraxon Inc, Scottsdale, AZ, USA) and displayed real-time on a personal computer 14 using dedicated software (MyoResearchXP, Noraxon Inc, Scottsdale, AZ, USA). The surface of the 15 skin was prepared by shaving hair, rubbing it with abrasive paper and cleaning it with alcohol. The 16 electrodes were fixed longitudinally over the muscle belly. Electrodes were placed at the antero-lateral 17 muscle bulge at 2/3 of the proximo-distal thigh length [24, 25]. The visually largest area of muscle 18 belly was selected using a contraction against manual resistance. The ground electrode was placed on 19 lateral femoral condoyle of the right leg. Electrodes and cables were secured with surgical tape, in 20 order to avoid any interference with the running pattern of the subjects.

Footswitches (Noraxon Inc, Scottsdale, AZ, USA) placed under the heel and big toes of both legs were used to denote heel-strike and toe-off. EMG was acquired at a sampling rate of 1500 Hz. The raw EMG was measured in a band of 10 to 500 Hz, full-wave rectified, high pass filtered (cut-off frequency at 20 Hz) with an 8th order Butterworth filter to remove movement artifacts and smoothed with a 100 ms RMS algorithm.. Values from 20 strides were averaged to calculate the mean peak amplitude during stance for each of the four time intervals.

Based on our hypotheses, the dependent variable examined in the present study was the mean
peak EMG amplitude during the stance phase. A 2-way fully repeated ANOVA within the control

group, with time (four levels) and leg (two levels) as within-subjects factors, revealed no time*leg
interactions for the EMG amplitude for either the moderate or high intensity running. Thus, the left leg
was selected as the control leg.

The time course of the EMG amplitude during running was compared between the reconstructed and intact leg with a 2-way fully repeated ANOVA, with time (four levels) and leg (two levels) as within-subjects factors. Similarly, the time course of the EMG amplitude during running was compared between the reconstructed and the control leg and between the intact and control leg, with 2way mixed ANOVAs, with time (four levels) as within- and group (two levels) as between-subject factor. Significant main effects and interactions were investigated with Tukey's post hoc analyses. The level of significance was set at a=0.05.

11

12 **RESULTS**

13 At the time of data collection no clinical evidence of knee pain and effusion was found in the 14 ACL reconstructed athletes. All athletes in the ACL-reconstructed group were satisfied with the 15 outcome of the surgery and resumed their pre-injury level of sports participation. Negative Lachman 16 and pivot-shift tests indicated that the knee joint stability was regained clinically for all ACL 17 reconstructed athletes. For the athletes with ACL reconstruction, the median Lysholm score was 95 18 (range 94-100) and the Tegner score was 8 (range 7-9) at the time of examination. KT-1000 results 19 revealed that the mean difference between the anterior tibial translation of the reconstructed and intact 20 sides in the ACL reconstructed group was 1.6 mm (range 1 to 2 mm) for the 134N test and 1.8 mm 21 (range 1-2 mm) for the maximum manual test, respectively.

During the moderate intensity running, EMG amplitude did not increase with time for both the intact and ACL reconstructed leg (F=0.477, p=0.7). Similarly EMG amplitude did not increase with time for both the control and the reconstructed leg (F=0.838, p=0.477). Finally, EMG amplitude remained unchanged with time for both the control and intact leg (F=0.782, p=0.507).

During the high intensity running, the EMG amplitude of the VL increased significantly with time for the intact but not for the reconstructed leg (time*leg interaction: F=6.747, p=0.001, power=0.89). Similarly, the EMG amplitude of the VL increased significantly with time for the control but not for the reconstructed leg (time*group interaction: F=4.258, p=0.008, power=0.85).
Finally, the EMG amplitude of the VL increased significantly with time for both the control and intact
leg (main effect of time: F=11.28, p<0.001, power=0.99) with no difference between legs (time*group
interaction: F=1.23, p=0.303).

All effects for the moderate and high intensity running are presented in Table 1.

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INSERT TABLE 1 ABOUT HERE

7

8 **DISCUSSION**

9 The purpose of the present study was to investigate the effect of ACL reconstruction with BPTB 10 graft on VL activation levels with time, during moderate and high intensity running. We hypothesized 11 that that (a) during moderate intensity running, the EMG amplitude of the VL will not increase with 12 time for any of the ACLR, intact and control legs, (b) during high intensity running, the EMG 13 amplitude of the VL will increase with time for the control and intact leg but not for the ACLR leg.

Our results indicated that during 10 minutes of moderate intensity running, the EMG amplitude of vastus lateralis remained unchanged with time for each of the control, intact and reconstructed legs. Thus, our results supported our first hypothesis. This is in agreement with previous studies indicating that in healthy individuals performing moderate intensity exercise, EMG amplitude of the exercising musculature remains unchanged with time [9, 11, 12]. Thus, our results verify that under low demand activities there are no differences between the reconstructed and either the intact or control leg [5-7].

20 A major finding of the present investigation was that during 10 minutes of high intensity 21 running, EMG amplitude of the VL increased with time for the control and intact leg but not the 22 reconstructed leg. Our results confirmed our second hypothesis. Previous studies have shown that 23 during high intensity exercise, there is a need by the neuromuscular system to compensate for the 24 accumulating physiological stress [8, 9]. This is accomplished by enhanced activation of the 25 exercising muscles as exercise progresses and is detected as increased EMG amplitude with time [9, 26 11-13]. The fact that our two separate groups of athletes responded by increasing EMG amplitude with 27 time is in good agreement with the above studies. However, the results of our study revealed that 28 during high intensity exercise the VL muscle of the reconstructed leg failed to respond in a similar 1 manner and showed no increase in EMG amplitude with time compared to either the intact or the 2 control leg (FIGURE 1). This behaviour may indicate that discrepancies in the neuromuscular 3 response of the reconstructed leg may be revealed under high intensity sustained athletic efforts.

4 INSERT FIGURE 1 ABOUT HERE

5 Several explanations can be given for the lack of increase in EMG amplitude with time for the 6 VL of the reconstructed leg. These may include selective muscle fiber atrophy in the involved 7 quadriceps [14-16], altered motor unit activation following surgery and subsequent retraining [17] and 8 loss of joint afferent information which may lead to suboptimal muscle fiber activation [18]. These 9 neuromuscular alterations following ACL reconstruction may be responsible for the lack of increase in 10 the EMG amplitude with time at high intensities, where there is a need for neuromuscular 11 compensation of the accumulating physiological stress [8, 9, 11-13].

12 To the best of our knowledge this is the first study that investigated EMG activation levels with 13 time during running in ACL reconstructed and healthy athletes. Previous studies on ACL 14 reconstructed athletes have compared EMG levels under moderate intensity activities but no study has 15 investigated the progression of EMG levels with time [5-7]. Furthermore, studies on healthy subjects 16 have investigated neuromuscular response with time but only during high intensity cycling [11, 12, 17 13]. Our approach enabled us to extend our findings to intense running which represents a highly 18 functional activity for the ACL reconstructed athlete. Furthermore, using preliminary VO₂max and 19 lactate threshold testing, we assigned moderate and high intensity exercise according to individual 20 fitness levels. Thus, all subjects exercised under identical controlled conditions.

21 In the present study, we recruited athletes with BPTB autograft. Thus, it is unknown if a similar 22 response pattern is observed in athletes with a different graft, such as hamstrings. EMG signal 23 capturing, recording and processing was performed according to established guidelines [27, 28]. We 24 examined EMG activity developed solely during the stance period, thereby reducing to some extent 25 the role of the signal non-stationarities [10]. Furthermore, the activity of many successive steps was 26 averaged providing a reasonable estimation of peak EMG amplitude. Normalisation of EMG data (for 27 example to maximum voluntary contraction) was not performed due to the additional error introduced 28 by this process and the fact that our study design involved repeated measures, thereby overcoming

1	influences of electrode positioning and inter-electroded distance on the signal value [26]. Additionally,
2	we incorporated 2 different control conditions (the intact leg of the reconstructed athletes and the
3	control leg of a separate group of athletes) to ensure the existence of differences in our dependent
4	variable. We assumed that because the same instrumentation was use for all subjects, the level of
5	measurement noise would be consistent for all subjects and that any differences could be attributed to
6	changes within the system itself.
7	
8	Practical applications
9	• Moderate intensity activities may not reveal differences in the neuromuscular response
10	between the reconstructed and either the intact or the control leg.
11	• High intensity sustained efforts are more likely to expose neuromuscular defects of the ACL
12	reconstructed knee.
13	• Endurance type activity may represent a more functional approach to assess neuromuscular
14	response under fatiguing conditions in ACL reconstructed athletes.
15	
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1 FIGURE LEGENDS

FIGURE 1: (a) EMG amplitude over time for the intact (n=14) and ACL reconstructed leg (n=14) during the high intensity running. EMG amplitude increased compared to initial values only for the control leg. * indicates significantly higher than initial value. Vertical bars represent ±95% confidence intervals. (b) EMG amplitude over time for the control (n=14) and ACL reconstructed leg (n=14) during the high intensity running. EMG amplitude increased compared to initial values only for the intact leg. * indicates significantly higher than initial value. Vertical bars represent ±95% confidence intervals.

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