Smeets, A, Malfait, B, Dingenen, B, Robinson, MA, Vanrenterghem, J, Peers, K, Nijs, S, Vereecken, S, Staes, F and Verschueren, S

Is knee neuromuscular activity related to anterior cruciate ligament injury risk? A pilot study.

Is knee neuromuscular activity related to anterior cruciate ligament injury risk? A pilot study

Smeets Annemie [a], Malfait, Bart [a], Dingenen, Bart [a,b], Robinson, Mark A [c], Vanrenterghem, Jos [a], Peers, Koen [d], Nijs Stefaan [e], Vereecken, Styn [e], Staes, Filip [a], Verschueren, Sabine [a]

[a] Musculoskeletal Rehabilitation Research Group, Department of Rehabilitation Sciences and Physiotherapy, Faculty of Kinesiology and Rehabilitation Sciences, KU Leuven, Belgium.

[b] Rehabilitation Research Centre, Biomedical Research Institute, Faculty of Medicine and Life Sciences, UHasselt, Diepenbeek, Belgium.

[c] Research Institute for Sport and Exercise Sciences, Faculty of Science, Liverpool John Moores University, Liverpool, UK.

[d] Physical Medicine and Rehabilitation, University Hospitals Leuven, Campus Pellenberg, Belgium.

[e] Division of Traumatology, Department of Development and Regeneration, University Hospitals Leuven, Belgium.

Corresponding author:

Sabine Verschueren, PT, PhD
KU Leuven, Musculoskeletal Rehabilitation Research Group,
Department of Rehabilitation Sciences, Faculty of Kinesiology and Rehabilitation Sciences,
Tervuursevest 101 bus 1501
3001 Leuven, Belgium
sabine.verschueren@kuleuven.be

ABSTRACT

Background: Limited evidence exists on neuromuscular risk factors for anterior cruciate ligament injuries, with most work mainly focussing on the strength of hamstrings and quadriceps muscles. In this prospective pilot study, we explored if neuromuscular activation patterns of the quadriceps and hamstrings during a drop vertical jump influence anterior cruciate ligament injury risk.

Methods: Forty-six female athletes performed a drop vertical jump at baseline. Injuries were monitored throughout a one year follow-up. Neuromuscular activation patterns of the vastus medialis, vastus lateralis, hamstrings medialis and hamstrings lateralis, and selected landing kinematic and kinetic profiles (knee flexion, knee abduction and hip flexion angles, and knee abduction moments), were compared between athletes who sustained a non-contact anterior cruciate ligament injury and those who remained injury-free. EMG vector fields were created to represent neuromuscular activation patterns of muscle pairs around the knee joint rather than only considering individual muscle activations, and compared using Statistical Parametric Mapping.

Results: Four athletes sustained an anterior cruciate ligament injury. Significantly greater {hamstrings medialis, hamstrings lateralis}, {vastus lateralis, hamstrings lateralis} and {hamstrings lateralis, vastus medialis} activations, mainly due to greater hamstrings lateralis activation, were found in the injured group around peak loading and just before take off ($P < 0.001$). No group differences were found in knee flexion, knee abduction and hip flexion angles, or knee abduction moments.

Conclusions: This pilot study revealed initial evidence that athletes already showed altered neuromuscular activation patterns prior to sustaining an anterior cruciate ligament injury, namely increased lateral and posterior muscle activations.

Key words: ACL injury, neuromuscular activation, risk factor, drop vertical jump, injury prevention, electromyography

1. INTRODUCTION

Anterior cruciate ligament (ACL) injuries are common during dynamic sports activities in the young, active population and often have important short and long-term physical, psychological and professional consequences [1], resulting in a lengthy absence from sports and high economic cost for society [2]. Therefore, establishing neuromuscular and biomechanical risk factors for ACL injury can assist the development of effective prevention programmes [3] as both neuromuscular and biomechanical risk factors are potentially modifiable through interventions [4,5]. To establish the risk factors for a specific injury, prospective studies with injury as primary outcome are needed. So far, many studies assessed neuromuscular and/or biomechanical changes after ACL injury or after intervention programs but very few prospective studies assessed ACL injury risk with ACL injury as primary outcome [6,7]. To date, only 4 prospective studies assessed neuromuscular risk factors for ACL injuries [8–11], and only 4 prospective studies assessed biomechanical risk factors [12–15], delivering contradictory outcomes.

Of the four prospective studies investigating the relationship between neuromuscular factors and ACL injury risk, three studies focussed on muscle strength (isokinetic strength of the quadriceps and hamstrings, and reciprocal muscle strength ratios) [8,9,11]. Myer et al. [11] found that 22 female athletes who went on to sustain an ACL injury demonstrated decreased hamstrings strength and unchanged quadriceps strength during concentric isokinetic testing (300°/s) compared to 88 female controls. Similarly, Söderman et al. [9] found that 5 ACL injured female athletes out of 146 players showed a reduced hamstrings to quadriceps ratio

(H/Q ratio) during concentric isokinetic strength tests (90°/s) prior to injury. However, on the contrary, in the study of Uhorchak [8] no differences were found in H/Q ratios during isokinetic strength tests at 60°/s (concentric action of quadriceps, eccentric action of hamstrings) between 24 ACL injured adolescents and 895 non-injured adolescents (males and females). Only one of the four studies focused on neuromuscular activation patterns [10]. Zebis et al. [10] investigated neuromuscular activation patterns during cutting maneuvers in 55 elite female athletes (handball and soccer). The 5 athletes that sustained an ACL injury during the follow-up period (2 match seasons) showed reduced activity of the semitendinosus and increased activity of the vastus lateralis during the preparatory phase (10ms before initial contact) [10].

Based on the limited evidence, we hypothesize that imbalances in neuromuscular activation do play a role in ACL injury risk. Furthermore, as neuromuscular activation patterns can be modified by prevention programs [16], more prospective studies on neuromuscular risk factors for ACL injury are needed in order to optimize prevention programmes. [17]. However, neuromuscular risk factors first need to be identified, and if possible independently confirmed by further prospective studies. This is very time consuming and often expensive as one has to screen a large number of participants and subsequently follow them over an extensive period of time to ensure that an adequate number of injurious events take place. The search for neuromuscular factors could therefore benefit from pilot work in which a comprehensive exploratory analysis precedes hypothesis testing [18,19]. Therefore, the aim of this pilot study was to help justify future large cohort studies by exploring whether female athletes who went on to sustain a non-contact ACL injury already showed meaningful differences in their knee neuromuscular activation patterns during the landing phase of a bilateral drop vertical jump (DVJ) prior to injury when compared to uninjured controls.

2. METHODS

2.1. Participants

Forty-six female athletes (21 soccer, 9 handball and 16 volleyball) aged between 16 – 28 years participated in this study. All athletes, who were members of a an elite level team (first national division), were tested at the beginning of their respective playing season and injuries were monitored for one year. Participants who had a previous ACL or posterior cruciate ligament injury, a previous lower extremity injury within the three months prior to testing, or another lower extremity injury within the one-year follow-up period, had been excluded. All participants provided informed consent, and the study was approved by the local ethics committee.

2.2. Injury registration

The medical staff of each involved team registered all lower extremity time loss injuries during follow-up. A time loss injury was defined as having occurred during sports participation and resulting in being unable to take a full part in future training or match play [20]. We adapted this definition to include "unable to take a full part in future training or match play for at least two consecutive weeks".

2.3. Test protocol

Each test session started with a standardized warm-up (two series of eight bilateral squats and eight bilateral jumps) [21–23]. Body mass and height were measured before the test session using scales (SECA, Hamburg, Germany) and a portable stadiometer (SECA, Hamburg, Germany). Standardized indoor footwear (Indoor Copa, Kelme, Elche, Spain) was worn and where necessary, long hair was tied up to avoid marker occlusion.

Subsequently, all athletes were asked to perform bilateral DVJ's. Bilateral DVJ's are commonly used for screening in clinical settings to assess injury risk [13,14]. The protocol is briefly summarized below. Participants were instructed to drop off a 0.3 m high box with their feet initially positioned 0.2 m apart on the box, and upon landing to immediately perform a maximum vertical jump. Participants were also instructed to reach upwards with both hands as high as possible, as if performing a block in volleyball [24]. Participants were allowed to familiarize themselves with the tasks by performing three practice repetitions before the start of the tests. Subsequently, a minimum of three valid trials were completed. A trial was excluded if subjects jumped off the box instead of dropping, if both feet did not land on the force plates, if subjects reached upwards with only one hand, or if subjects clearly lost balance upon landing [14]. A short rest period between consecutive trials was permitted to avoid fatigue [21].

2.4. Data collection

A wireless EMG system (Zerowire, Aurion, Milan, Italy) was used to record muscle activity at 1000Hz of the vastus lateralis (VL), vastus medialis (VM), biceps femoris (referred to as hamstring lateralis, HL) and semitendinosus (referred to as hamstring medialis, HM) using surface electrodes positioned according to the SENIAM guidelines [25]. All electrode locations were shaved and gently cleaned with 70% isopropyl alcohol to reduce skin impedance. Silver-silver chloride, pre-gelled bipolar surface EMG electrodes (Ambu Blue Sensor, Ballerup, Denmark) were placed over the muscle belly and aligned with the expected muscle fibre orientations, with 2 cm inter-electrode distance. Fauth et al. [26] observed that surface electromyography (EMG) was reliable for measuring mean muscle activation of HL, HM, VL and VM during the performance of a DVJ (ICC 0.83-0.97). As we were interested in the muscle activation during the entire landing phase (from initial contact until take off), rather than the mean muscle activation, we additionally assessed the reliability of the EMG data in 4 uninjured subjects. These analyses showed both high intra- and inter-session reliability (see Appendix).

Secondary to the neuromuscular activation patterns, we also measured knee and hip flexion angles, knee abduction angles and knee abduction moments as these have previously been identified as potential biomechanical risk factors [12–14]. Therefore, we recorded three-dimensional kinematic data using six MX-T20 optoelectronic cameras (VICON, Oxford, UK) sampling at 100 Hz, synchronized with data recorded from two 0.8 x 0.3 m^2 force plates (AMTI, Watertown, USA) sampling at 1000 Hz.. Each participant had 44 spherical reflective markers positioned according to the eight segment 'Liverpool John Moores University' model including feet, upper and lower legs, pelvis and trunk. This model was previously described in detail and shown to be reliable for measuring kinematics and kinetics during DVJ [21].

2.5. Data analysis

All modelling and data processing were undertaken in Visual 3D (v.4.83, C-Motion, Kingston, ON, Canada). Only the first landing (first contact) within each DVJ trial was used for analysis [21]. Raw EMG signals were high pass filtered using a digital filter at a cut-off frequency of 10 Hz, full wave rectified, and low-pass filtered with a 4^{th} order zero-lag Butterworth filter at a cut-off frequency of 6 Hz. The EMG signal amplitudes were subsequently normalized to the maximum root mean square amplitude (over a period of 100 ms) of 3 isometric maximum voluntary contractions. Marker trajectories and forces were filtered using a 4^{th} order low pass Butterworth filter with a cut-off frequency of 18 Hz [27]. Initial contact and take off events were created when the vertical force crossed a 20 N threshold. Knee and hip flexion angles, knee abduction angles and moments were calculated using inverse dynamics. External joint moments are described in this study; i.e. an external knee abduction moment will abduct the knee (move the distal end of the tibia away from the midline of the participant's body).

Kinetic, kinematic and EMG data were time normalized to 101 data points starting at 100 ms before initial contact until take off (Fig. 1). The short time period prior to initial contact was included based on Zebis et al. [10] who showed that neuromuscular pre-activity might be a risk factor for ACL injury albeit during cutting. Therefore 0% of normalised time corresponds to 100 ms prior to initial contact and 100% corresponds to take off, whereas initial contact is situated around 17% and peak loading around 50%. For all included variables, the average of three trials was calculated for each participant and then means were calculated across the groups.

2.6. Statistical analyses

Participants' baseline characteristics were compared between the control and the ACL-injured group using independent sample Student's t-tests (Table 1).

To compare neuromuscular activation patterns of muscle pairs around the knee joint rather than only considering individual muscle activation patterns as independent observations, time-varying EMG vector fields were created. A major advantage of this technique is that it accounts for inter-muscle covariance. The following vector fields were created to represent the vector magnitude of anatomically relevant muscle groupings: an overall EMG {VM,VL,HM,HL} (time) vector field, an anterior {VM,VL} (time) vector field, a lateral {VL,HL} (time) vector field, a posterior {HM,HL} (time) vector field, a medial {HM,VM} (time) vector field and 2 diagonal vector fields: a {HM, VL} (time) vector field, and a {HL, VM} (time) vector field [28]. To statistically compare these vector fields between groups, seven Hotelling's T^2 tests were conducted (the vector field equivalent of an independent samples Student's t-test) using Statistical Parametric Mapping (SPM) [28,29] for which Alpha was set at 0.05. SPM has become well established in biomechanical research. Basically, first a test statistic is calculated for each time node (e.g., t-values). Second, the threshold problem of repeated comparisons over time is handled by modeling the behavior of random time-varying signals as a random field based on signal smoothness [18,30]. Given the differences in group sizes, equality of variance between groups was not assumed. Post-hoc SPM independent t-tests were used to estimate the contribution of individual muscles to vector field based group differences. Detailed examples, theoretical background and interpretations of vector field and SPM statistics are available elsewhere [28,29]. In summary, if at certain times of the landing the T^2 or t-statistic exceeded the critical threshold then a significant difference between groups was assumed of which the probability (*P*-value) depends on the magnitude and duration of the threshold crossing.

Finally, external knee abduction moments, knee abduction angles, knee flexion and hip flexion angles were compared between the ACL-injured and the control group across the landing phase (excluding the 100ms pre-landing phase), again using SPM independent sample t-tests.

3. RESULTS

During the one year follow-up, four participants sustained a non-contact ACL injury. All injuries occurred during competitive match play (1 in soccer, 2 in handball, 1 in volleyball). These ACL injuries were diagnosed with magnetic resonance imaging (MRI) and all underwent ACL reconstruction. Three out of four ACL injuries affected the non-dominant leg. The dominant leg was defined as the preferred leg to kick a ball [31].

Of the remaining non-ACL-injured participants, five participants sustained an ankle inversion trauma, and two other participants sustained an overuse knee injury during the one year follow-up period (one pes anserinus tendinopathy and one degenerative lateral meniscus lesion with associated cyst). As the purpose of this study was to assess ACL injury risk, the latter seven participants who got injured during the follow-up were excluded, resulting in a control group of 35 participants in total (Fig. 2). No significant differences were found for age, weight, height and body mass index between the ACL-injured and the control group (Table 1).

3.1. Within group analysis

Both in the control group and the ACL-injured group, no significant differences were found between limbs for the amplitude of the overall EMG {VM,VL,HM,HL} (time) vector, for knee abduction moments, knee abduction angles, or for knee or hip flexion angles ($P > 0.05$). As our study was not concerned with leg preference and since there were no significant leg differences we pooled our data across legs resulting in a control sample consisting of 70 legs and an ACL-injured sample consisting of 8 legs.

3.2. Between group analysis

To investigate whether there was any difference in neuromuscular activation between the ACL-injured athletes and the non-injured athletes, the overall multi-muscle EMG vector {VM,VL,HM,HL} was compared between both groups. A significant difference in the overall multi-muscle EMG vector {VM,VL,HM,HL} was found between the ACL-injured group and the control group from peak loading until the last part of the acceleration phase just before take off (34-100% time; $P < 0.001$).

Subsequently, 6 Hotelling's T^2 tests were performed to identify which muscle pairs were significantly different between both groups and were thus responsible for the significant differences in the overall multi-muscle EMG vector. These Hotelling's T^2 tests showed significantly greater amplitudes of the {HL, HM} (t) vector , the {HL, VL} (t) vector and the {HL,VM} (t) vector in the ACL-injured group compared to the control group during the peak loading phase (43-80% time, $P < 0.001$; 55-71% time, $P < 0.001$ and 58-79% time, $P < 0.001$ respectively) and during the last part of the acceleration phase just before take off (93-100% time; $P < 0.001$, 84-100% time; $P < 0.001$ and 90-100% time; $P = 0.002$ respectively) (Fig. 3 and 4). Furthermore significantly greater amplitudes in {HM, VL} (t) vector were found in the ACL-injured group the last part of acceleration phase just before take off (91-100% time, $P < 0.035$).

Finally, post-hoc SPM independent t-tests showed significantly greater HL amplitudes in the ACL-injured group compared to the control group during the peak loading phase (51-78%; $P < 0.001$) and during the last part of the acceleration phase just before take off (92-100% time; $P = 0.006$) (Figure 3). No differences were found between groups for the individual muscle activation amplitudes of VM, VL and HM (Fig. 3 and 5).

Furthermore, no significant differences in external knee abduction moments, knee abduction angles, knee flexion, and hip flexion angles were found between the ACL injured group and the control group (Fig. 6). However, a clear tendency was observed for the ACL-injured group to show larger knee abduction angles and smaller knee flexion angles, seen by the SPM{t}-curve being consistently respectively below and above 0.

4. DISCUSSION

The purpose of this pilot study was to prospectively explore the presence of neuromuscular differences in athletes who would go on to suffer an ACL injury compared to uninjured controls. Distinct muscle activation patterns were found in the ACL injured group compared to controls, primarily showing greater activation levels during peak loading and just before take-off, mostly due to increased lateral hamstring activations.

It is commonly accepted that quadriceps and hamstrings co-contractions are necessary to ensure dynamic knee joint stability during dynamic activities [32]. Balanced hamstrings to quadriceps co-contraction might protect against anterior tibial translation [33,34] and appropriate medial to lateral co-contraction of the hamstrings and quadriceps should protect the knee joint from high knee abduction loads [35]. However, when the lateral hamstrings are favored in this co-contraction, then this may have detrimental consequences leading to increased risk of injury. For example, Serpell et al. [36] have shown that during a step-up task, selectively increasing the lateral hamstrings-quadriceps co-activation results in more ACL elongation, while stronger medial hamstring-quadriceps co-activation reduces ACL elongation and knee joint rotation, abduction, translation and distraction. Furthermore, the prospective study of Zebis et al. [10] demonstrated that a neuromuscular imbalance between lateral and medial activation during a side cutting manoeuvre was associated with increased ACL injury risk. The authors suggested that sufficient HM activity against VL activity is important to compress the medial knee joint and thereby limit the risk of excessive abduction of the knee and minimizing the strain on the ACL [10]. This is in agreement with Palmieri-Smith et al. [37], who showed that increased activation of the vastus lateralis and hamstrings lateralis during the preparatory phase of a 1m forward hop was associated with higher peak knee valgus angles in female athletes.

However, comparing our results with other studies should nonetheless be done with some apprehension, as muscle activations were assessed in a variety of dynamic tasks. For example, Husted et al. [38] showed that neuromuscular pre-activity of the HM, HL and VL was only low to moderately correlated between side cutting manoeuvres and the DVJ. Therefore, we recently investigated the relationship between muscle activation of quadriceps and hamstrings and the kinematics of the knee and hip joint during the performance of a DVJ in female athletes [22]. We found that those athletes who had a more erect landing pattern (less knee and hip flexion), showed an increased {VL, HL} activation.

Thus, we expected that the altered muscle activation patterns in the ACL-injured group would go together with significant differences in the selected landing kinematics or kinetics (knee abduction moment, knee abduction angle, knee flexion angle and hip flexion angle). In this pilot study we did not find significant differences between groups but a clear tendency was observed for the ACL-injured group to show larger knee abduction angles and smaller knee flexion angles. The fact that landing kinematics and kinetics were not significantly different between the ACL-injured group and the control group suggests that neuromuscular activation patterns might be more sensitive for predicting injury risk in well-trained female athletes. Probably, subtle alterations in muscle activation only influence the stability and arthrokinematics of the knee joint and therefore may not necessarily result in significant changes in joint angles and moments.

The risk of ACL injuries is multifactorial and therefore the results of this paper should neither be interpreted in isolation nor prematurely. The aim of this study was not to find an 'ultimate predictive factor' for ACL injury risk, or to criticize existing factors, but was to explore whether neuromuscular activation patterns are worthwhile consideration in the multifactorial approach to prevent ACL injuries. Recently, Bittencourt et al. [39] published a conceptual paper to propose a new framework on sports injury prevention, in which injury prediction is based on risk pattern recognition. They proposed a 'web of determinants' that visualizes the complex interactions between the different risk factors. Based on the results of our pilot study we suggest that also neuromuscular activation patterns may play a meaningful role in this 'web of determinants for ACL injury risk'. In the past, muscle activation could only be measured with laboratory techniques and therefore it was not feasible to implement it in injury prevention programmes. But recent technological developments (e.g. sport clothes with embedded textile electrodes) made it possible to also measure muscle activation in field settings

To our knowledge, this prospective pilot study is the first study that comprehensively investigated the relationship between neuromuscular activation patterns and the incidence of ACL injuries, yet it comes with some limitations. First, we would like to reiterate that the sample size of this pilot study was small and therefore we should be extremely cautious not to generalize the observations as facts. Second, other muscles such as the gastrocnemius and glutei can influence dynamic knee joint stability but these were not measured [40,41]. Third, as we did not measure tibio-femoral contact forces, ACL strain, or actual joint kinematics for example through the use of video fluoroscopy, we cannot describe the underlying mechanics that explain whether a specific neuromuscular activation pattern would result directly in an increased ACL injury strain. The calculation of medial and lateral tibio-femoral contact forces might reveal for example an unloading phenomenon of the medial knee compartment compared to high contact forces at the lateral knee compartment, but this would need to be confirmed.

5. CONCLUSIONS

This pilot study has prospectively demonstrated altered neuromuscular activation patterns in ACL injured elite female team sports athletes. Participants who sustained an ACL injury showed increased lateral hamstring activation during peak loading and the push off phase of a

DVJ. Previously suggested kinematic or kinetic indicators of increased risk of injury were not confirmed, providing a preliminary rationale for including neuromuscular activations in future large scale prospective studies.

CONFLICT OF INTEREST

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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FIGURES

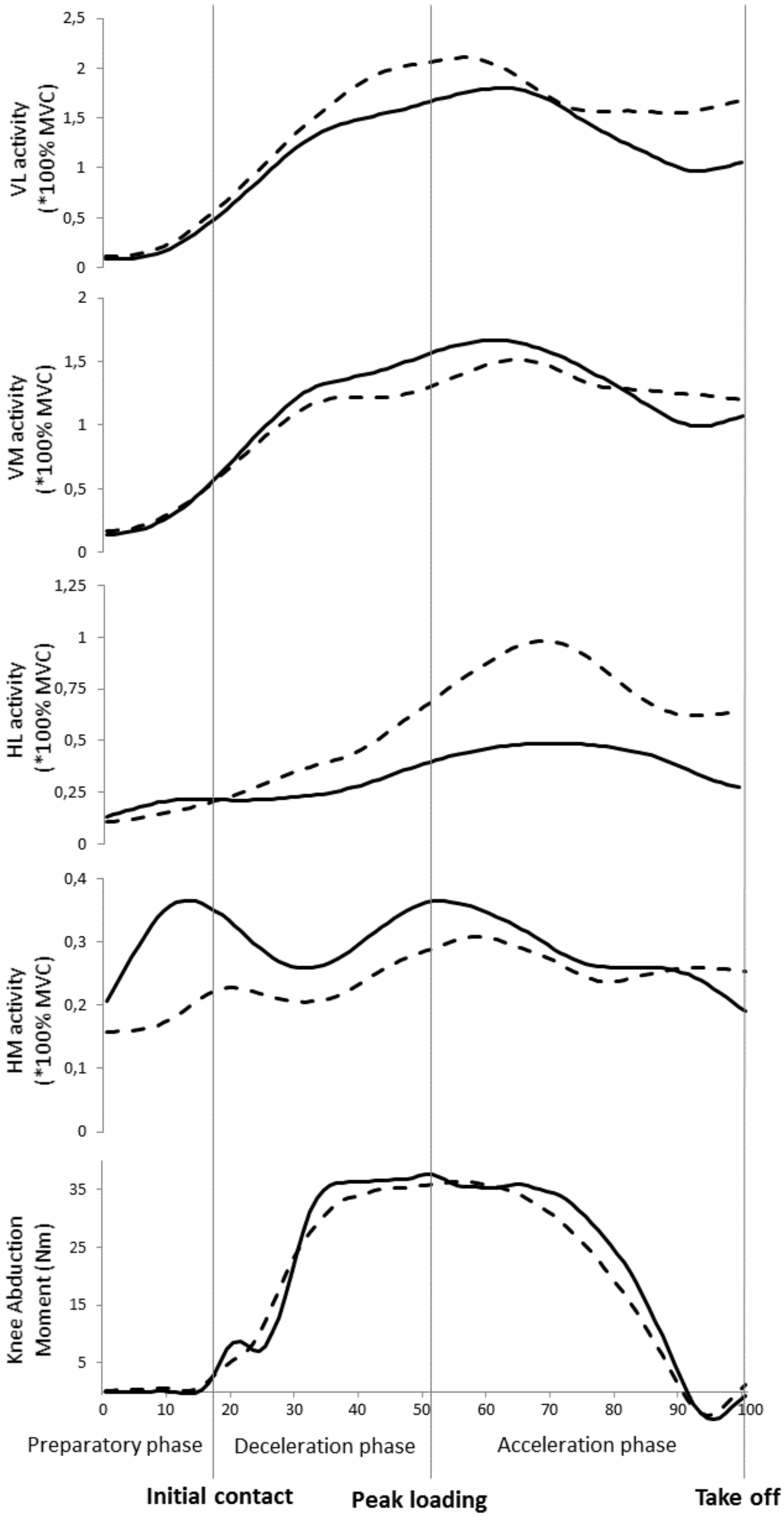
VL activity (*100% MVC)
VM activity (*100% MVC)
HL activity (*100% MVC)
HM activity (*100% MVC)
Knee Abduction Moment (Nm)
2,5
2
1,5
1
0,5
0
1,25
0,75
0,25
0,4
0,3
0,2
0,1
35
25
15
5
0 10 20 30 40 50 60 70 80 90 100
Preparatory phase
Deceleration phase
Acceleration phase
Initial contact
Peak loading
Take off

Fig. 1

Mean data of the ACL-injured group (dotted line) versus the control group (bold line) to illustrate the different time periods during DVJ from 100ms before initial contact until take off.

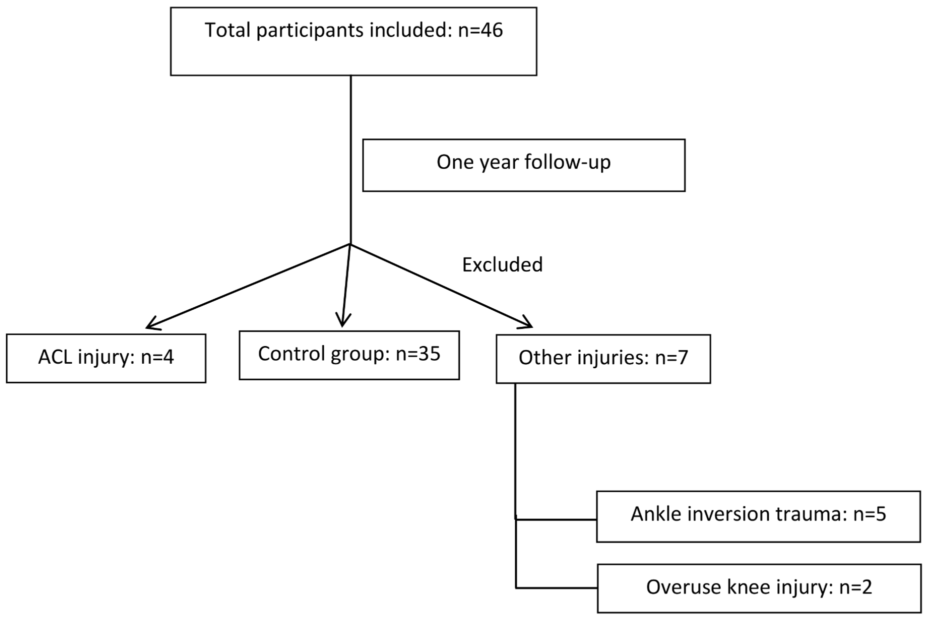

Fig. 2

Flowchart of the participants

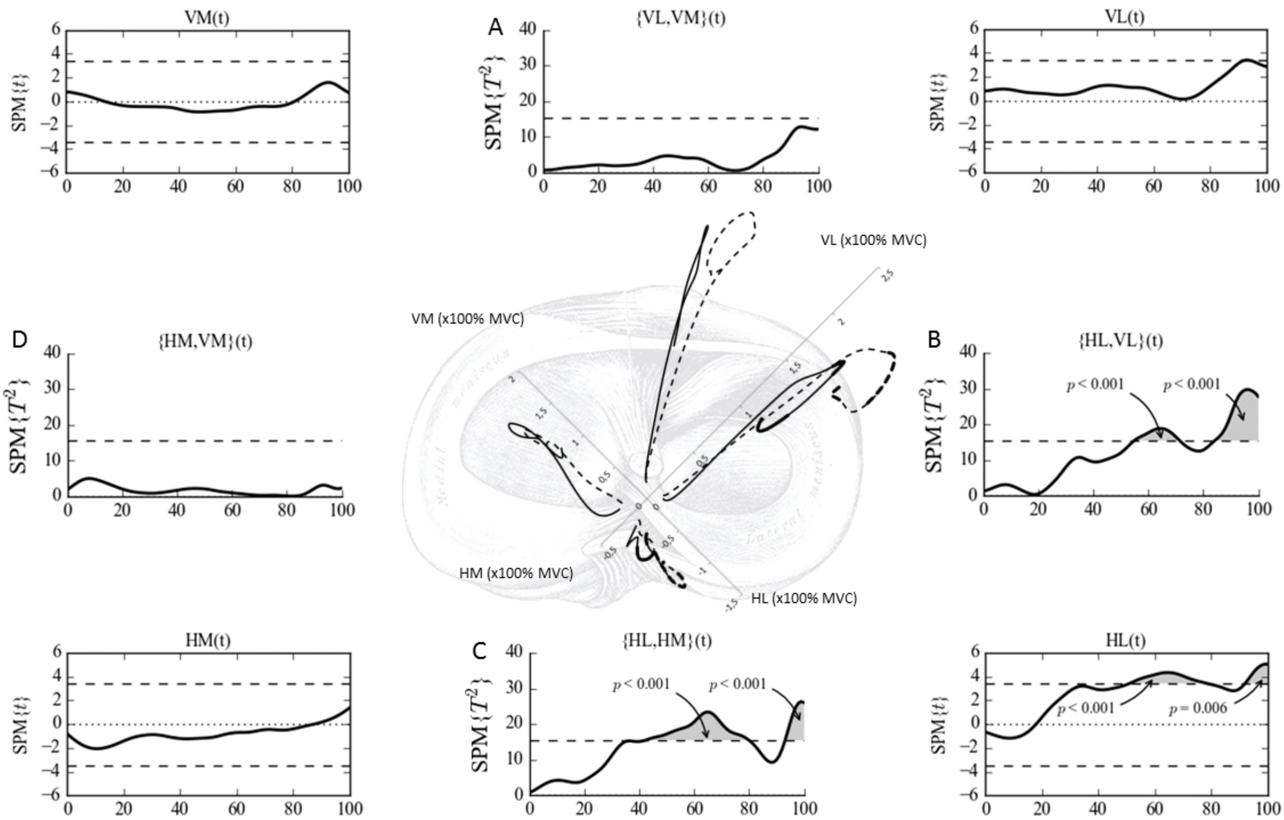

Fig. 3

Top view representation of group differences.

Central figure: Differences in two-muscle activation patterns between the ACL-injured (dotted line) and the control group (solid line) are visualized. The bold parts represent the time periods in which significant differences between groups were found.

A,B,C,D: Trajectory level SPM analyses show the differences between groups for the anterior {VM,VL}, lateral {HL,VL}, posterior {HM,HL} and medial {HM,VM} EMG vector. The horizontal dashed line represents the critical threshold ($P < 0.05$).

Corner figures: Trajectory level SPM analyses show differences between groups for the individual amplitudes of VM, VL, HM and HL. The horizontal dashed line represents the critical threshold ($P < 0.05$).

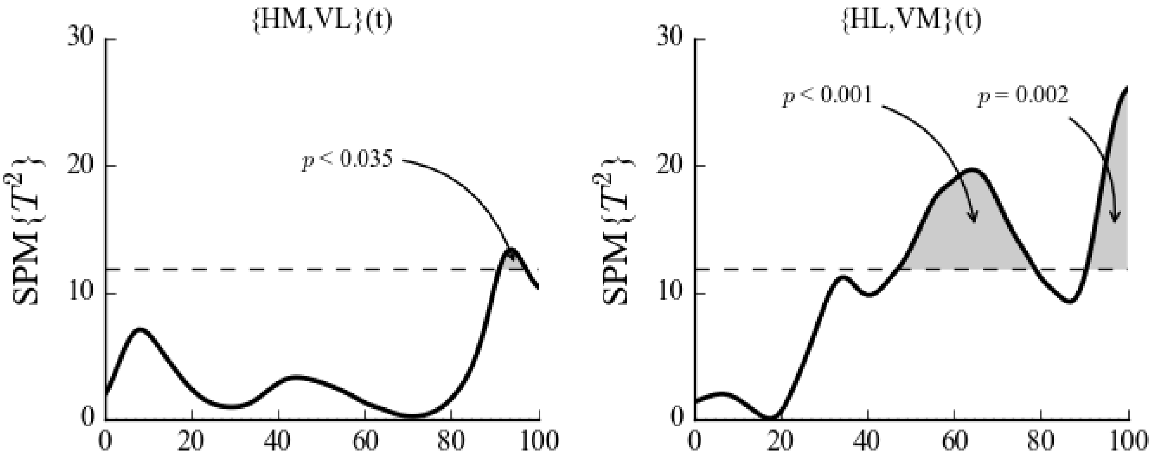

Fig. 4

Trajectory level SPM analyses show the differences between groups for the {HM, VL} and the {HL,VM} EMG vector. The horizontal dashed line represents the critical threshold ($P < 0.05$).

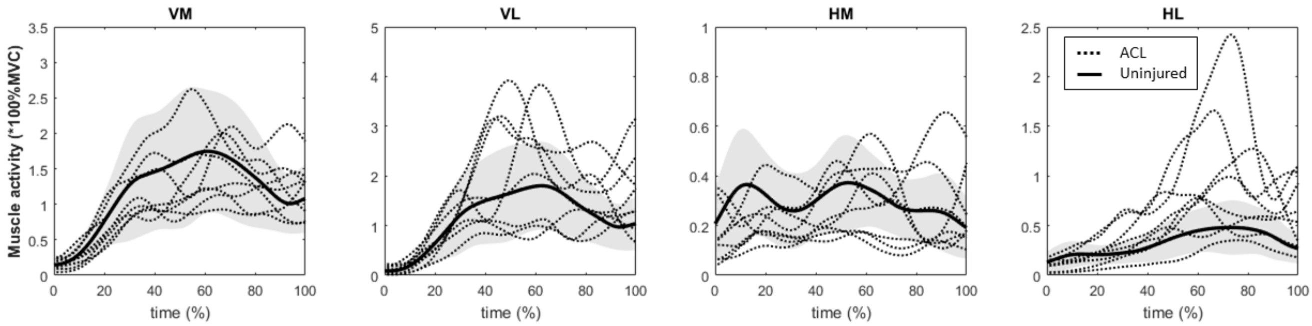

Fig. 5

This figure visualizes the activation of the HL, HM, VL and VM muscles. The 8 dotted lines represents the data of the ACL injured athletes. The solid line represents the average activation of the respective muscles in the control legs and the shaded zone represents the standard deviation.

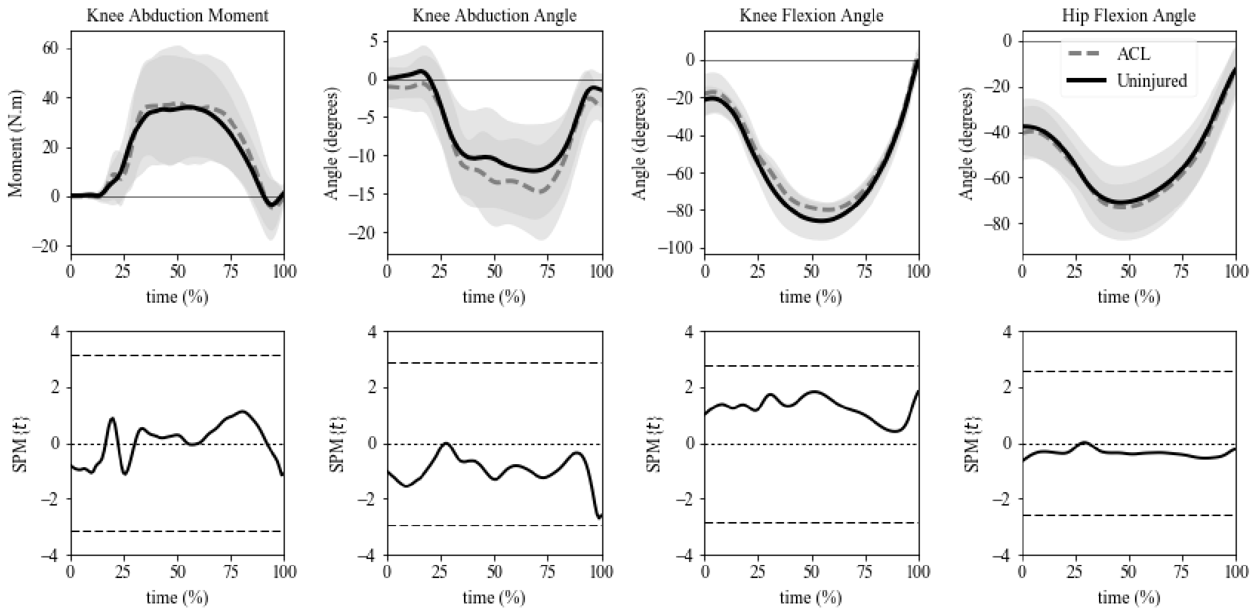

Fig. 6

The upper figures illustrate the mean data for the respective parameters. The dotted line represents the ACL-injured group and the bold line represents the control group. Standard deviation clouds are represented by the shaded areas. The lower figures illustrate the SPM output. No significant differences were found between groups for the external knee abduction moment, knee abduction angle, knee flexion angle and the hip flexion angle over the entire time period during DVJ. However, a clear tendency is observed for the ACL-injured group to show larger knee abduction angles and smaller knee flexion angles, seen by the being consistently respectively below and above 0.

Table 1: Participants characteristics

	Control group	**ACL-injured group**	**P-value**
Participants (n)	35	4	
Age, yrs (Mean ± SD)	20.69 ± 3.19	21.02 ± 2.96	0.85
Body height, m (Mean ± SD)	1.72 ± 0.09	1.72 ± 0.11	0.88
Body weight, kg (Mean ± SD)	64.94 ± 7.45	62.58 ± 6.94	0.44
BMI, kg/m^2 (Mean ± SD)	22.05 ± 1.61	21.21 ± 1.02	0.31

n: number of participants; SD: standard deviation; BMI: body mass index

Appendix – Reliability of time-varying EMG data

To investigate the intra- and inter-session reliability of the time-varying EMG data, we did an additional reliability study in 4 uninjured subjects.

All subjects (1 female, 3 males, age 28.5 ±3.5y height 185.6±9.1cm, weight 76.9±9.6kg) were involved in recreational or competitive team sports (soccer, volleyball, handball and basketball) and performed the same protocol as described in the manuscript on 3 different days. They performed the protocol always at the same time during the day and all sessions were performed in the same week (Monday, Wednesday and Friday). All data was processed in the same way as described in the manuscript.

As our outcome is 1 dimensional (1D) data (e.g. emg curves) and not traditional 0 dimensional (0D) data (e.g. peak/ mean values), we could not calculate traditional 0D ICC's for investigating reliability. There are no explicit tests of reliability for 1D data yet, thus instead we addressed reliability implicitly using ANOVA. In particular, if inter-session reliability is high, then we would expect intra-subject variability to be greater than inter-session variability, and thus produce a small session effect. Conversely, if inter-session reliability is low, then we would expect a large session effect.

To implicitly investigate the inter-session reliability, we used an SPM one-way ANOVA with 1 repeated measure (e.g. session). These analyses showed no significant differences in muscle activation between the 3 different sessions as the F-curve never exceeded the critical threshold (see fig. 1). As there was no session-effect, we can conclude that inter-session reliability is high.

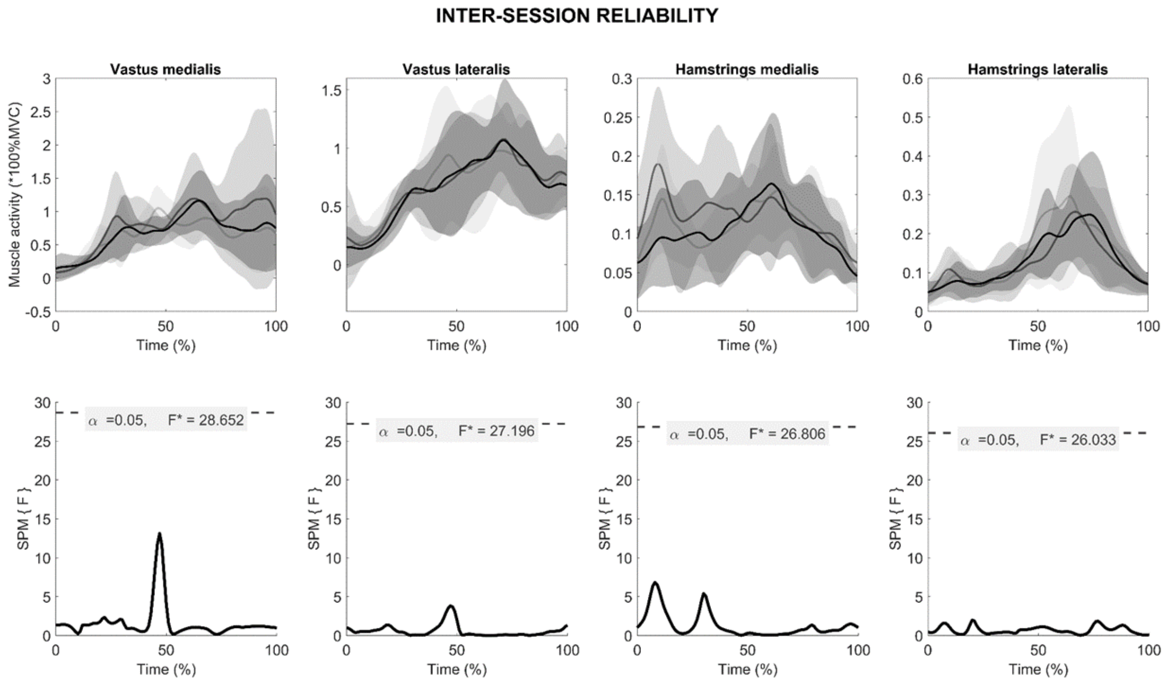

Fig. 1.

The upper figures illustrate the mean data for session 1 (light grey), session 2 (dark grey) and session 3 (black) for the respective muscles. Standard deviation clouds are represented by the shaded areas. The lower figures illustrate the SPM output. No significant differences were found between sessions as the F-curve never exceeds the critical threshold (horizontal red dashed line).

To implicitly investigate intra-session reliability, we performed another SPM one-way ANOVA with 1 repeated measure (e.g. trial) on the data of session 1. These analyses showed no significant differences in muscle activation between the 3 different trials (see fig. 2). As there was no trial-effect we can conclude that intra-session reliability is high.

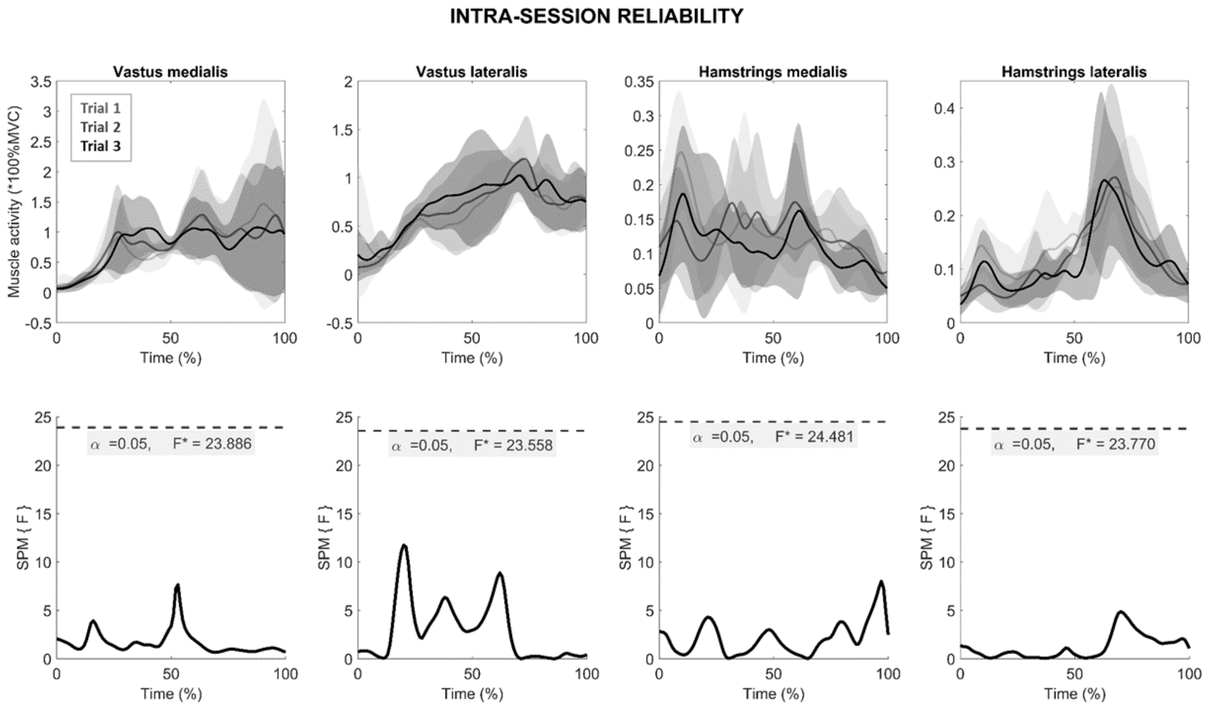

Fig. 2.

The upper figures illustrate the mean data for trial 1 (light grey), trial 2 (dark grey) and trial 3 (black) of session 1 for the resprective muscles. Standard deviation clouds are represented by the shaded areas. The lower figures illustrate the SPM output. No significant differences were found between trials as the F-curve never exceeds the critical threshold (horizontal dashed line).

Below (fig. 3) you can find al the individual emg data of every trial and for every session.

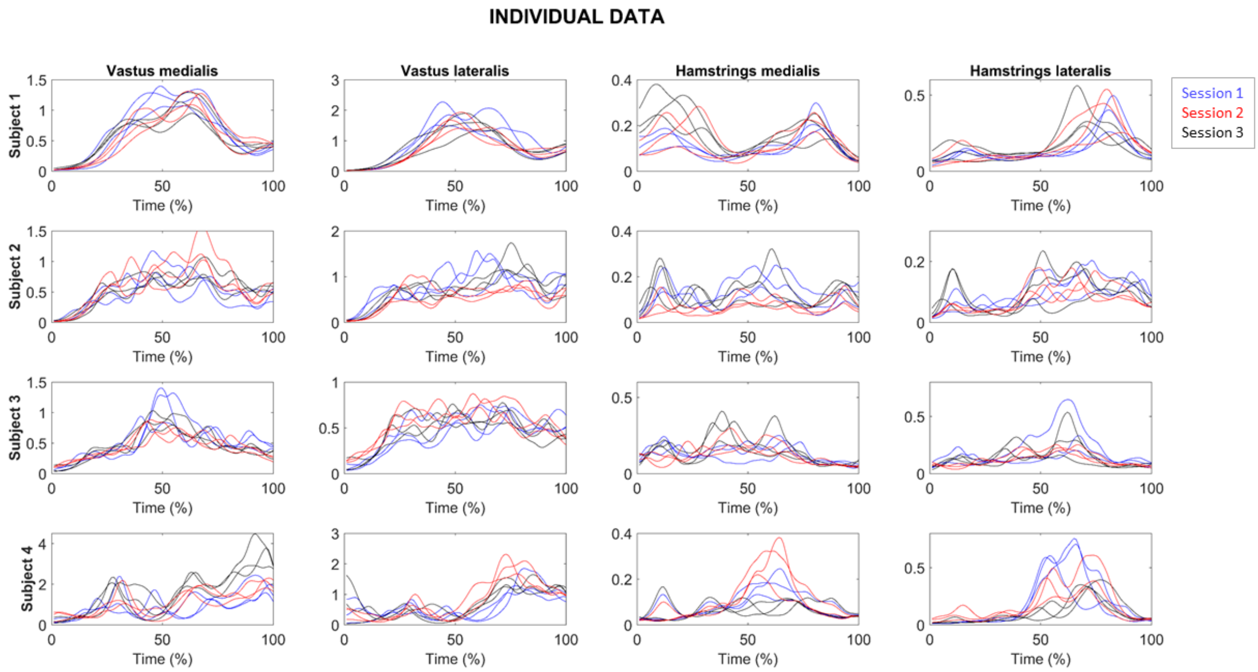

Fig. 3.

Each row represents the emg data of another subject. All subjects performed 3 trials per session (session 1 = blue, session 2= red, session 3 = black).

The figures above confirm that the time-varying data of the individual muscles is highly reliable. As ANOVA testing does not exist for vectors, the same approach as described above could not just be repeated on the vector data of the muscle pairs. However, the reliability of muscle co-activation pairs relies on (1) the reliability of measuring each individual muscle within the pairs and (2) the minimization of cross-talk between muscles. Whilst cross-talk between muscles within the pairs would inflate the amount of co-variance, its impact on the muscle pairs is the same as on the individual muscles. In both cases cross-talk has to be avoided as much as possible, which is established by careful electrode placement.

We did not investigate inter-rater reliability as we only had 1 rater in this study. We can assume that the rater was very consistent in electrode placement as both the quadriceps and hamstrings muscles were very easy to palpate in our athlete population. Furthermore, the rater is a qualified physiotherapist with extensive palpation experience, and used the SENIAM guidelines to standardize his way of electrode placement.