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

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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.

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

72   (H/Q ratio) during concentric isokinetic strength tests (90°/s) prior to injury. However, on the

73   contrary, in the study of Uhorchak [8] no differences were found in H/Q ratios during isokinetic

74   strength tests at 60°/s (concentric action of quadriceps, eccentric action of hamstrings) between

75   24 ACL injured adolescents and 895 non-injured adolescents (males and females). Only one of

76   the four studies focused on neuromuscular activation patterns [10]. Zebis et al. [10] investigated

77   neuromuscular activation patterns during cutting maneuvers in 55 elite female athletes

78   (handball and soccer). The 5 athletes that sustained an ACL injury during the follow-up period

79   (2 match seasons) showed reduced activity of the semitendinosus and increased activity of the

80   vastus lateralis during the preparatory phase (10ms before initial contact) [10].

81   Based on the limited evidence, we hypothesize that imbalances in neuromuscular activation do

82   play a role in ACL injury risk. Furthermore, as neuromuscular activation patterns can be

83   modified by prevention programs [16], more prospective studies on neuromuscular risk factors

84   for ACL injury are needed in order to optimize prevention programmes. [17]. However,

85   neuromuscular risk factors first need to be identified, and if possible independent confirmed by

86   further prospective studies. This is very time consuming and often expensive as one has to

87   screen a large number of participants and subsequently follow these over an extensive period

88   of time to ensure that an adequate number of injurious events take place. The search for

89   neuromuscular factors could therefore benefit from pilot work in which a comprehensive

90   exploratory analysis precedes hypothesis testing [18,19]. Therefore, the aim of this pilot study

91   was to help justify future large cohort studies by exploring whether female athletes who went

92   on to sustain a non-contact ACL injury already showed meaningful differences in their knee

93   neuromuscular activation patterns during the landing phase of a bilateral drop vertical jump

94   (DVJ) prior to injury when compared to uninjured controls.

95

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

120   summarized below. Participants were instructed to drop off a 0.3 m high box with their feet

121   initially positioned 0.2 m apart on the box, and upon landing to immediately perform a

122   maximum vertical jump. Participants were also instructed to reach upwards with both hands as

123   high as possible, as if performing a block in volleyball [24]. Participants were allowed to

124   familiarize themselves with the tasks by performing three practice repetitions before the start

125   of the tests. Subsequently, a minimum of three valid trials were completed. A trial was excluded

126   if subjects jumped off the box instead of dropping, if both feet did not land on the force plates,

127   if subjects reached upwards with only one hand, or if subjects clearly lost balance upon landing

128   [14]. A short rest period between consecutive trials was permitted to avoid fatigue [21].

129   **2.4. Data collection**

130   A wireless EMG system (Zerowire, Aurion, Milan, Italy) was used to record muscle activity at

131   1000Hz of the vastus lateralis (VL), vastus medialis (VM), biceps femoris (referred to as

132   hamstring lateralis, HL) and semitendinosus (referred to as hamstring medialis, HM) using

133   surface electrodes positioned according to the SENIAM guidelines [25]. All electrode locations

134   were shaved and gently cleaned with 70% isopropyl alcohol to reduce skin impedance. Silver-

135   silver chloride, pre-gelled bipolar surface EMG electrodes (Ambu Blue Sensor, Ballerup,

136   Denmark) were placed over the muscle belly and aligned with the expected muscle fibre

137   orientations, with 2 cm inter-electrode distance. Fauth et al. [26] observed that surface

138   electromyography (EMG) was reliable for measuring mean muscle activation of HL, HM, VL

139   and VM during the performance of a DVJ (ICC 0.83-0.97). As we were interested in the muscle

140   activation during the entire landing phase (from initial contact until take off), rather than the

141   mean muscle activation, we additionally assessed the reliability of the EMG data in 4 uninjured

142   subjects. These analyses showed both high intra- and inter-session reliability (see Appendix).

143   Secondary to the neuromuscular activation patterns, we also measured knee and hip flexion

144   angles, knee abduction angles and knee abduction moments as these have previously been

145 identified as potential biomechanical risk factors [12–14]. Therefore, we recorded three-

146 dimensional kinematic data using six MX-T20 optoelectronic cameras (VICON, Oxford, UK)

147 sampling at 100 Hz, synchronized with data recorded from two 0.8 x 0.3 m^2 force plates (AMTI,

148 Watertown, USA) sampling at 1000 Hz.. Each participant had 44 spherical reflective markers

149 positioned according to the eight segment 'Liverpool John Moores University' model including

150 feet, upper and lower legs, pelvis and trunk. This model was previously described in detail and

151 shown to be reliable for measuring kinematics and kinetics during DVJ [21].

152 **2.5. Data analysis**

153 All modelling and data processing were undertaken in Visual 3D (v.4.83, C-Motion, Kingston,

154 ON, Canada). Only the first landing (first contact) within each DVJ trial was used for analysis

155 [21]. Raw EMG signals were high pass filtered using a digital filter at a cut-off frequency of

156 10 Hz, full wave rectified, and low-pass filtered with a 4th order zero-lag Butterworth filter at a

157 cut-off frequency of 6 Hz. The EMG signal amplitudes were subsequently normalized to the

158 maximum root mean square amplitude (over a period of 100 ms) of 3 isometric maximum

159 voluntary contractions. Marker trajectories and forces were filtered using a 4th order low pass

160 Butterworth filter with a cut-off frequency of 18 Hz [27]. Initial contact and take off events

161 were created when the vertical force crossed a 20 N threshold. Knee and hip flexion angles,

162 knee abduction angles and moments were calculated using inverse dynamics. External joint

163 moments are described in this study; i.e. an external knee abduction moment will abduct the

164 knee (move the distal end of the tibia away from the midline of the participant's body).

165 Kinetic, kinematic and EMG data were time normalized to 101 data points starting at 100 ms

166 before initial contact until take off (Fig. 1). The short time period prior to initial contact was

167 included based on Zebis et al. [10] who showed that neuromuscular pre-activity might be a risk

168 factor for ACL injury albeit during cutting. Therefore 0% of normalised time corresponds to

169 100 ms prior to initial contact and 100% corresponds to take off, whereas initial contact is

170　situated around 17% and peak loading around 50%. For all included variables, the average of

171　three trials was calculated for each participant and then means were calculated across the

172　groups.

173　**2.6. Statistical analyses**

174　Participants' baseline characteristics were compared between the control and the ACL-injured

175　group using independent sample Student's t-tests (Table 1).

176　To compare neuromuscular activation patterns of muscle pairs around the knee joint rather than

177　only considering individual muscle activation patterns as independent observations, time-

178　varying EMG vector fields were created. A major advantage of this technique is that it accounts

179　for inter-muscle covariance. The following vector fields were created to represent the vector

180　magnitude of anatomically relevant muscle groupings: an overall EMG {VM,VL,HM,HL}

181　(time) vector field, an anterior {VM,VL} (time) vector field, a lateral {VL,HL} (time) vector

182　field, a posterior {HM,HL} (time) vector field, a medial {HM,VM} (time) vector field and 2

183　diagonal vector fields: a {HM, VL} (time) vector field, and a {HL, VM} (time) vector field

184　[28]. To statistically compare these vector fields between groups, seven Hotelling's T^2 tests

185　were conducted (the vector field equivalent of an independent samples Student's t-test) using

186　Statistical Parametric Mapping (SPM) [28,29] for which Alpha was set at 0.05. SPM has

187　become well established in biomechanical research. Basically, first a test statistic is calculated

188　for each time node (e.g., t-values). Second, the thresholding problem of repeated comparisons

189　over time is handled by modeling the behavior of random time-varying signals as a random

190　field based on signal smoothness [18,30]. Given the differences in group sizes, equality of

191　variance between groups was not assumed. Post-hoc SPM independent t-tests were used to

192　estimate the contribution of individual muscles to vector field based group differences. Detailed

193　examples, theoretical background and interpretations of vector field and SPM statistics are

194　available elsewhere [28,29]. In summary, if at certain times of the landing the T^2 or t-statistic

195   exceeded the critical threshold then a significant difference between groups was assumed of

196   which the probability (P-value) depends on the magnitude and duration of the threshold

197   crossing.

198   Finally, external knee abduction moments, knee abduction angles, knee flexion and hip flexion

199   angles were compared between the ACL-injured and the control group across the landing phase

200   (excluding the 100ms pre-landing phase), again using SPM independent sample t-tests.

201

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.

273 However, comparing our results with other studies should nonetheless be done with some

274 apprehension, as muscle activations were assessed in a variety of dynamic tasks. For example,

275 Husted et al. [38] showed that neuromuscular pre-activity of the HM, HL and VL was only low

276 to moderately correlated between side cutting manoeuvres and the DVJ. Therefore, we recently

277 investigated the relationship between muscle activation of quadriceps and hamstrings and the

278 kinematics of the knee and hip joint during the performance of a DVJ in female athletes [22].

279 We found that those athletes who had a more erect landing pattern (less knee and hip flexion),

280 showed an increased {VL, HL} activation.

281 Thus, we expected that the altered muscle activation patterns in the ACL-injured group would

282 go together with significant differences in the selected landing kinematics or kinetics (knee

283 abduction moment, knee abduction angle, knee flexion angle and hip flexion angle). In this

284 pilot study we did not find significant differences between groups but a clear tendency was

285 observed for the ACL-injured group to show larger knee abduction angles and smaller knee

286 flexion angles. The fact that landing kinematics and kinetics were not significantly different

287 between the ACL-injured group and the control group suggests that neuromuscular activation

288 patterns might be more sensitive for predicting injury risk in well-trained female athletes.

289 Probably, subtle alterations in muscle activation only influence the stability and

290 arthrokinematics of the knee joint and therefore not necessarily result in significant changes in

291 joint angles and moments.

292 The risk of ACL injuries is multifactorial and therefore the results of this paper should neither

293 be interpreted in isolation nor prematurely. The aim of this study was not to find an 'ultimate

294 predictive factor' for ACL injury risk, or to criticize existing factors, but was to explore whether

295 neuromuscular activation patterns are worthwhile consideration in the multifactorial approach

296 to prevent ACL injuries. Recently, Bittencourt et al. [39] published a conceptual paper to

297 propose a new framework on sports injury prevention, in which injury prediction is based on

298 risk pattern recognition. They proposed a 'web of determinants' that visualizes the complex

299 interactions between the different risk factors. Based on the results of our pilot study we suggest

300 that also neuromuscular activation patterns may play a meaningful role in this 'web of

301 determinants for ACL injury risk'. In the past, muscle activation could only be measured with

302 laboratory techniques and therefore it was not feasible to implement it in injury prevention

303 programmes. But recent technological developments (e.g. sport clothes with embedded textile

304 electrodes) made it possible to also measure muscle activation in field settings

305 To our knowledge, this prospective pilot study is the first study that comprehensively

306 investigated the relationship between neuromuscular activation patterns and the incidence of

307 ACL injuries, yet it comes with some limitations. First, we would like to reiterate that the

308 sample size of this pilot study was small and therefore we should be extremely cautious not to

309 generalize the observations as facts. Second, other muscles such as the gastrocnemius and glutei

310 can influence dynamic knee joint stability but these were not measured [40,41]. Third, as we

311 did not measure tibio-femoral contact forces, ACL strain, or actual joint kinematics for example

312 through the use of video fluoroscopy, we cannot describe the underlying mechanics that explain

313 whether a specific neuromuscular activation pattern would result directly in an increased ACL

314 injury strain. The calculation of medial and lateral tibio-femoral contact forces might reveal for

315 example an unloading phenomenon of the medial knee compartment compared to high contact

316 forces at the lateral knee compartment, but this would need to be confirmed.

317

5. CONCLUSIONS

319 This pilot study has prospectively demonstrated altered neuromuscular activation patterns in

320 ACL injured elite female team sports athletes. Participants who sustained an ACL injury

321 showed increased lateral hamstring activation during peak loading and the push off phase of a

322   DVJ. Previously suggested kinematic or kinetic indicators of increased risk of injury were not

323   confirmed, providing a preliminary rationale for including neuromuscular activations in future

324   large scale prospective studies.

325

326   **CONFLICT OF INTEREST**

329

330   **6. REFERENCES**

331 [1]   Noyes FR, Mooar P a, Matthews DS, Butler DL. The symptomatic anterior cruciate-
332   deficient knee. Part I: the long-term functional disability in athletically active
333   individuals. J Bone Joint Surg Am 1983;65:154–62. doi:10.1007/978-1-4471-5451-
334   8_38.

335 [2]   Gottlob CA, Baker CL. Anterior cruciate ligament reconstruction: socioeconomic
336   issues and cost effectiveness. Am J Orthop 2000;29:472–6.

337 [3]   Finch C. A new framework for research leading to sports injury prevention. J Sci Med
338   Sport 2006;9:3–9. doi:10.1016/j.jsams.2006.02.009.

339 [4]   Zebis MK, Bencke J, Andersen LL, Dossing S, Alkjaer T, Magnusson P, et al. The
340   Effects of Neuromuscular Training on Knee Joint Motor Control During Sidecutting in
341   Female Elite Soccer and Handball Players. Clin J Sport Med 2008;18:329–37.
342   doi:10.1097/JSM.0b013e31817f3e35.

343 [5]   Donnell-Fink LA, Klara K, Collins JE, Yang HY, Goczalk MG, Katz JN, et al.
344   Effectiveness of knee injury and anterior cruciate ligament tear prevention programs: A
345   meta-analysis. PLoS One 2015;10:1–17. doi:10.1371/journal.pone.0144063.

346 [6]   Rafeeuddin R, Sharir R, Staes F, Dingenen B, George K, Robinson MA, et al. Mapping
347   current research trends on neuromuscular risk factors of non-contact ACL injury. Phys
348   Ther Sport 2016;22:101–13. doi:10.1016/j.ptsp.2016.06.004.

349 [7]   Sharir R, Rafeeuddin R, Staes F, Dingenen B, George K, Vanrenterghem J, et al.
350   Mapping current research trends on anterior cruciate ligament injury risk against the
351   existing evidence: In vivo biomechanical risk factors. Clin Biomech 2016;37:34–43.
352   doi:10.1016/j.clinbiomech.2016.05.017.

353 [8]   Uhorchak JM, Scoville CR, Williams GN, Arciero RA, St. Pierre P, Taylor DC. Risk
354   Factors Associated with Noncontact Injury of the Anterior Cruciate Ligament. A
355   Prospective Four-Year Evaluation of 859 West Point Cadets. Am J Sports Med
356   2003;31:831–42. doi:0363-5465/103/3131-0831$02.00/0.

357   [9]   Söderman K, Alfredson H, Pietilä T, Werner S. Risk factors for leg injuries in female
358   soccer players: A prospective investigation during one out-door season. Knee Surgery,
359   Sport Traumatol Arthrosc 2001;9:313–21. doi:10.1007/s001670100228.

360   [10]   Zebis MK, Andersen LL, Bencke J, Kjaer M, Aagaard P. Identification of athletes at
361   future risk of anterior cruciate ligament ruptures by neuromuscular screening. Am J
362   Sports Med 2009;37:1967–73. doi:10.1177/0363546509335000.

363   [11]   Myer GD, Ford KR, Barber Foss K, Liu C, Nick T, Hewett TE. The Relationship of
364   hamstrings and quadriceps strength to anterior cruciate ligament injury in female
365   athletes. Clin J Sport Med 2009;19:3–8.

366   [12]   Leppänen M, Pasanen K, Kujala UM, Vasankari T, Kannus P, Äyrämö S, et al. Stiff
367   Landings Are Associated With Increased ACL Injury Risk in Young Female Basketball
368   and Floorball Players. Am J Sports Med 2016:363546516665810.
369   doi:10.1177/0363546516665810.

370   [13]   Krosshaug T, Steffen K, Kristianslund E, Nilstad A, Mok K-M, Myklebust G, et al.
371   The Vertical Drop Jump Is a Poor Screening Test for ACL Injuries in Female Elite
372   Soccer and Handball Players: A Prospective Cohort Study of 710 Athletes. Am J
373   Sports Med 2016;44:874–83. doi:10.1177/0363546515625048.

374   [14]   Hewett TE. Biomechanical Measures of Neuromuscular Control and Valgus Loading
375   of the Knee Predict Anterior Cruciate Ligament Injury Risk in Female Athletes: A
376   Prospective Study. Am J Sports Med 2005;33:492–501.
377   doi:10.1177/0363546504269591.

378   [15]   Leppänen M, Pasanen K, Krosshaug T, Kannus P, Vasankari T, Kujala UM, et al.
379   Sagittal Plane Hip, Knee, and Ankle Biomechanics and the Risk of Anterior Cruciate
380   Ligament Injury: A Prospective Study. Orthop J Sport Med 2017;5:232596711774548.
381   doi:10.1177/2325967117745487.

382   [16]   Zebis MK, Andersen LL, Brandt M, Myklebust G, Bencke J, Lauridsen HB, et al.
383   Effects of evidence-based prevention training on neuromuscular and biomechanical
384   risk factors for ACL injury in adolescent female athletes: a randomised controlled trial.
385   Br J Sports Med 2015:bjsports-2015-094776. doi:10.1136/bjsports-2015-094776.

386   [17]   Finni T, Hu M, Kettunen P, Vilavuo T, Cheng S. Measurement of EMG activity with
387   textile electrodes embedded into clothing. Physiol Meas 2007;28:1405–19.
388   doi:10.1088/0967-3334/28/11/007.

389   [18]   Pataky TC, Vanrenterghem J, Robinson MA. The probability of false positives in zero-
390   dimensional analyses of one-dimensional kinematic, force and EMG trajectories. J
391   Biomech 2016:1–9. doi:10.1016/j.jbiomech.2016.03.032.

392   [19]   Pataky TC, Robinson MA, Vanrenterghem J. Region-of-interest analyses of one-
393   dimensional biomechanical trajectories: bridging 0D and 1D theory, augmenting
394   statistical power. PeerJ 2016;4:e2652. doi:10.7717/peerj.2652.

395   [20]   Fuller CW, Ekstrand J, Junge a., Andersen TE, Bahr R, Dvorak J, et al. Consensus
396   statement on injury definitions and data collection procedures in studies of football
397   (soccer) injuries. Scand J Med Sci Sport 2006;16:83–92. doi:10.1111/j.1600-
398   0838.2006.00528.x.

399   [21]   Malfait B, Sankey S, Azidin RMFR, Deschamps K, Vanrenterghem J, Robinson MA,
400   et al. How reliable are lower-limb kinematics and kinetics during a drop vertical jump?
401   Med Sci Sports Exerc 2014;46:678–85. doi:10.1249/MSS.0000000000000170.

402   [22]   Malfait B, Dingenen B, Smeets A, Staes F, Pataky T, Robinson M a., et al. Knee and

Hip Joint Kinematics Predict Quadriceps and Hamstrings Neuromuscular Activation Patterns in Drop Jump Landings. PLoS One 2016;11:e0153737. doi:10.1371/journal.pone.0153737.

[23] Dingenen B, Malfait B, Vanrenterghem J, Robinson MA, Verschueren SMP, Staes FF. Can two-dimensional measured peak sagittal plane excursions during drop vertical jumps help identify three-dimensional measured joint moments? Knee 2015;22:73–9. doi:10.1016/j.knee.2014.12.006.

[24] Ford KR, Myer GD, Smith RL, Byrnes RN, Dopirak SE, Hewett TE. Use of an overhead goal alters vertical jump performance and biomechanics. J Strength Cond Res 2005;19:394–9. doi:10.1519/15834.1.

[25] Hermens H, Freriks B, Merletti R, Stegeman D, Blok J, Rau G, et al. SENIAM 8: European recommendations for surface electromyography. 1999.

[26] Fauth ML, Petushek EJ, Feldmann CR, Hsu BE, Garceau LR, Lutsch BN, et al. Reliability of surface electromyography during maximal voluntary isometric contractions, jump landings, and cutting. J Strength Cond Res 2010;24:1131–7.

[27] Bisseling RW, Hof AL. Handling of impact forces in inverse dynamics. J Biomech 2006;39:2438–44. doi:10.1016/j.jbiomech.2005.07.021.

[28] Robinson MA, Vanrenterghem J, Pataky TC. Statistical Parametric Mapping (SPM) for alpha-based statistical analyses of multi-muscle EMG time-series. J Electromyogr Kinesiol 2015;25:14–9. doi:10.1016/j.jelekin.2014.10.018.

[29] Pataky TC, Robinson M a., Vanrenterghem J. Vector field statistical analysis of kinematic and force trajectories. J Biomech 2013;46:2394–401. doi:10.1016/j.jbiomech.2013.07.031.

[30] Friston KJ, Holmes a. P, Worsley KJ, Poline J-P, Frith CD, Frackowiak RSJ. Statistical parametric maps in functional imaging: A general linear approach. Hum Brain Mapp 1995;2:189–210. doi:10.1002/hbm.460020402.

[31] Ford KR, Myer GD, Hewett TE. Valgus knee motion during landing in high school female and male basketball players. Med Sci Sports Exerc 2003;35:1745–50. doi:10.1249/01.MSS.0000089346.85744.D9.

[32] Beynnon BD, Fleming BC. Anterior cruciate ligament strain in-vivo: A review of previous work. J Biomech 1998;31:519–25. doi:10.1016/S0021-9290(98)00044-X.

[33] Hashemi J, Breighner R, Chandrashekar N, Hardy DM, Chaudhari AM, Shultz SJ, et al. Hip extension, knee flexion paradox: A new mechanism for non-contact ACL injury. J Biomech 2011;44:577–85. doi:10.1016/j.jbiomech.2010.11.013.

[34] Beynnon B, Howe J, Pope M, Johnson R, Fleming B. The measurement of anterior cruciate ligament strain in vivo. Int Orhopaedics 1992;16:1–12.

[35] Lloyd DG, Buchanan TS, Besier TF. Neuromuscular biomechanical modeling to understand knee ligament loading. Med Sci Sports Exerc 2005;37:1939–47. doi:10.1249/01.mss.0000176676.49584.ba.

[36] Serpell BG, Scarvell JM, Pickering MR, Ball NB, Newman P, Perriman D, et al. Medial and lateral hamstrings and quadriceps co-activation affects knee joint kinematics and ACL elongation: a pilot study. BMC Musculoskelet Disord 2015;16:348. doi:10.1186/s12891-015-0804-y.

[37] Palmieri-Smith RM, Wojtys EM, Ashton-Miller JA. Association between preparatory muscle activation and peak valgus knee angle. J Electromyogr Kinesiol 2008;18:973–9.

448   doi:10.1016/j.jelekin.2007.03.007.

[38]   Husted RS, Bencke J, Andersen LL, Myklebust G, Kallemose T, Lauridsen HB, et al. A comparison of hamstring muscle activity during different screening tests for non-contact ACL injury. Knee 2016;23:362–6. doi:10.1016/j.knee.2016.02.004.

[39]   Bittencourt NFN, Meeuwisse WH, Mendonça LD, Nettel-Aguirre A, Ocarino JM, Fonseca ST. Complex systems approach for sports injuries: moving from risk factor identification to injury pattern recognition—narrative review and new concept. Br J Sports Med 2016;50:1309–14. doi:10.1136/bjsports-2015-095850.

[40]   Marouane H, Shirazi-Adl A, Adouni M. Knee joint passive stiffness and moment in sagittal and frontal planes markedly increase with compression. Comput Methods Biomech Biomed Engin 2013:37–41. doi:10.1080/10255842.2013.795555.

[41]   Morgan KD, Donnelly CJ, Reinbolt JA. Elevated gastrocnemius forces compensate for decreased hamstrings forces during the weight-acceptance phase of single-leg jump landing: Implications for anterior cruciate ligament injury risk. J Biomech 2014;47:3295–302. doi:10.1016/j.jbiomech.2014.08.016.
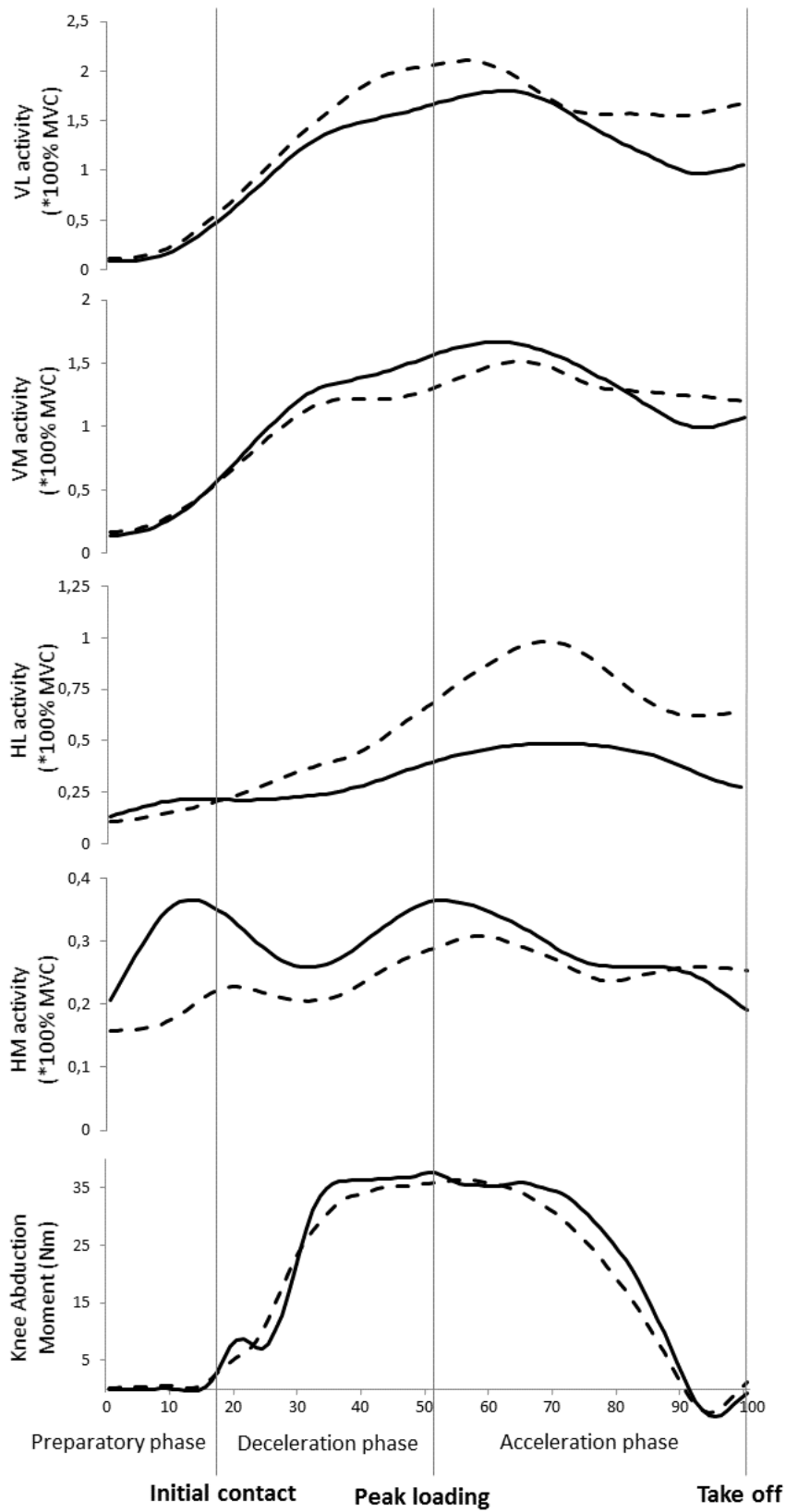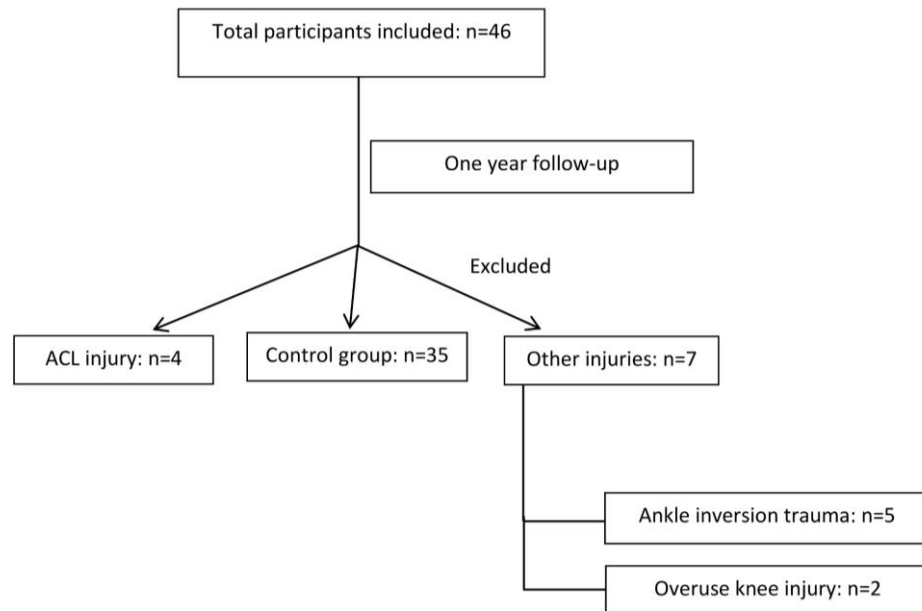
482 **Fig. 1**

483 Mean data of the ACL-injured group (dotted line) versus the control group (bold line) to

484 illustrate the different time periods during DVJ from 100ms before initial contact until take off.
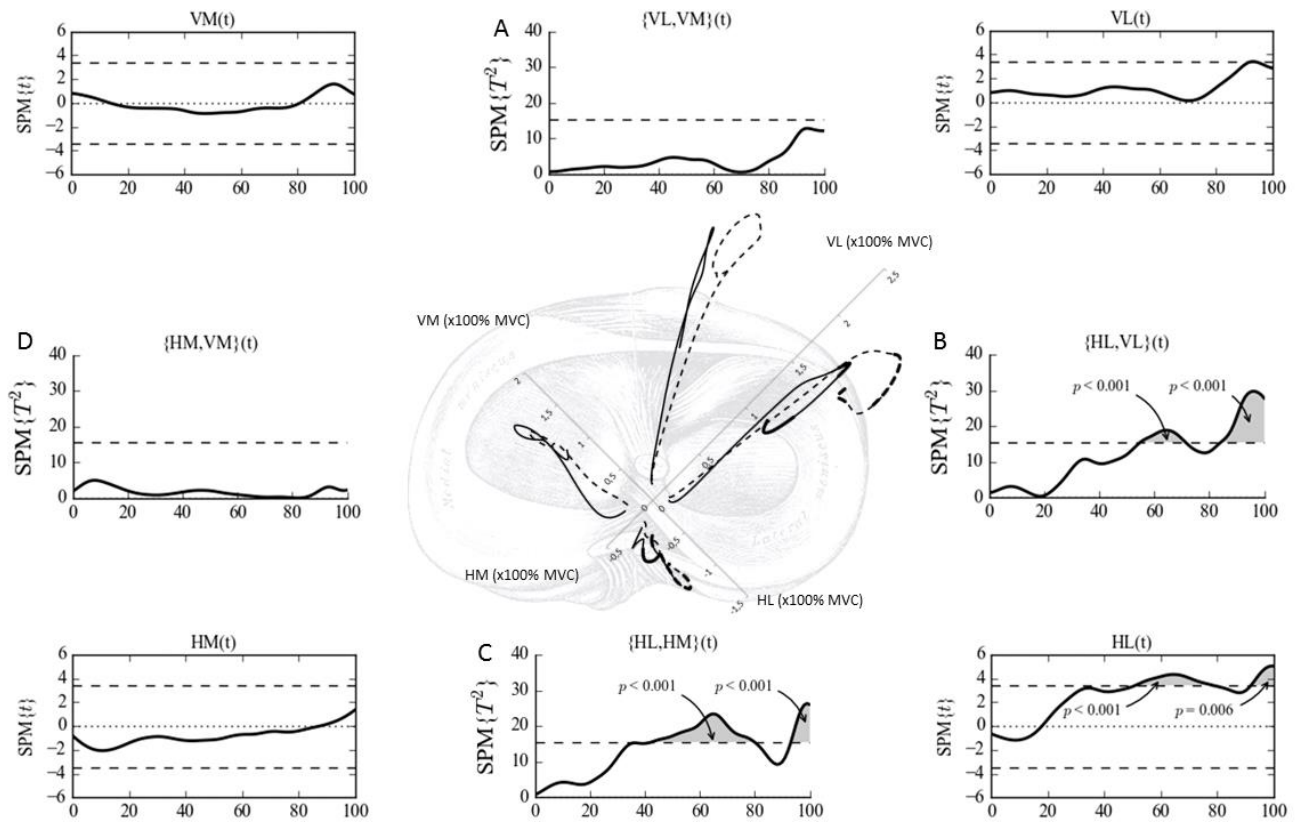
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488 **Fig. 2**

489 Flowchart of the participants

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**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 ($P < 0.05$).
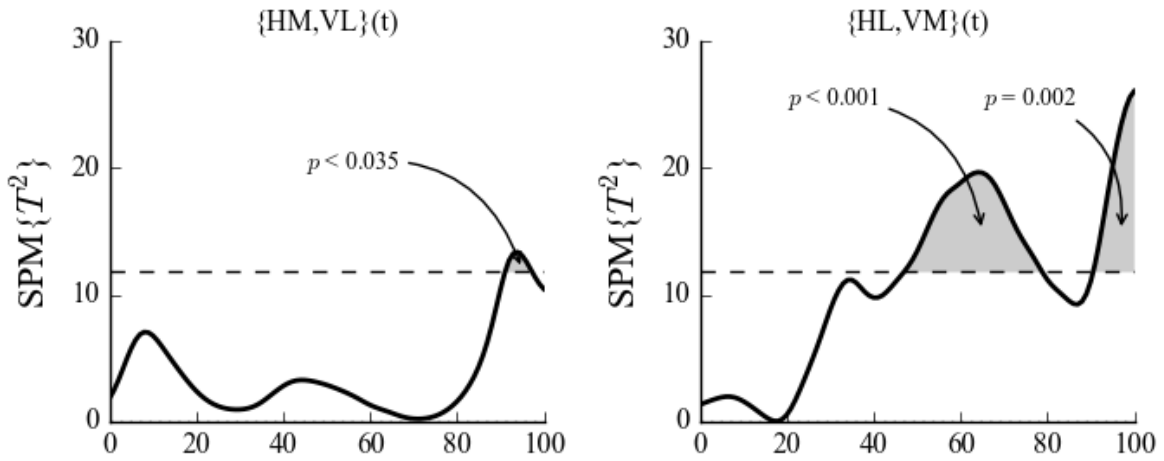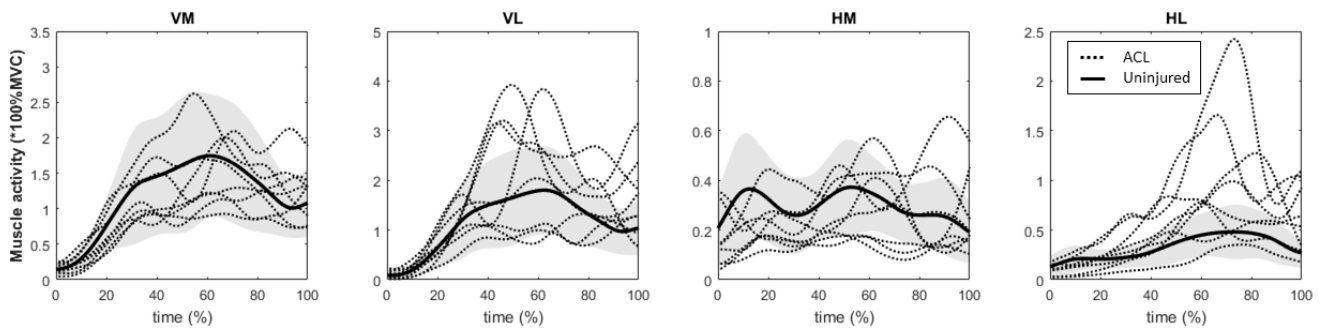
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Fig. 4

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 ($P < 0.05$).
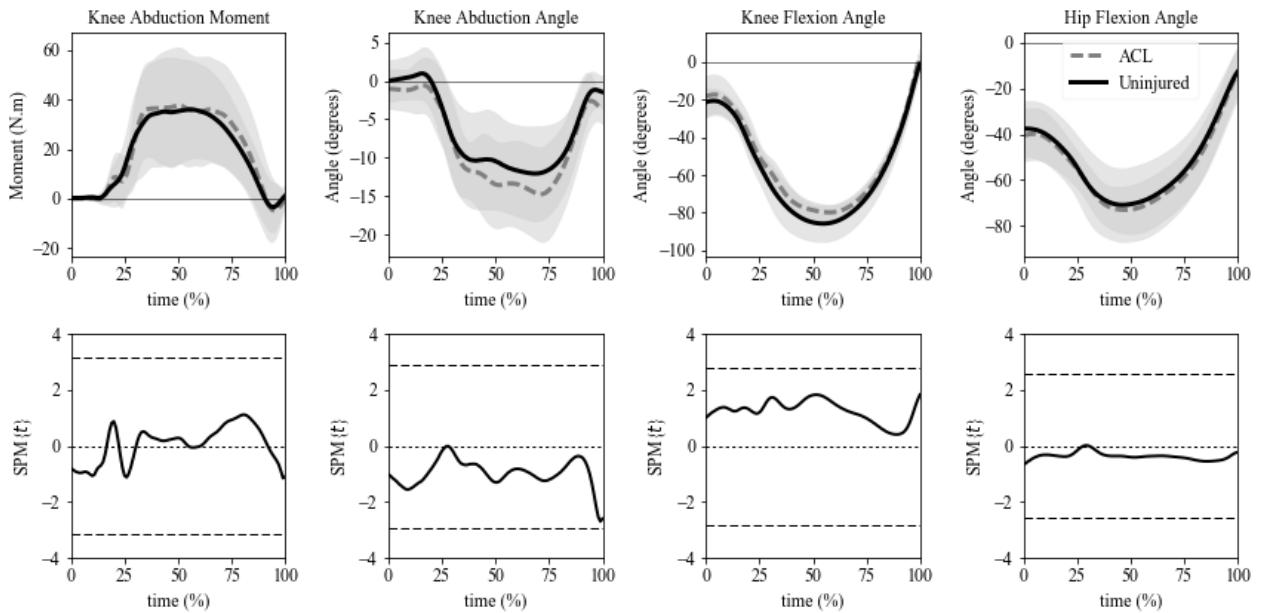
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Fig. 5

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.

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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.

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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.

INTER-SESSION RELIABILITY
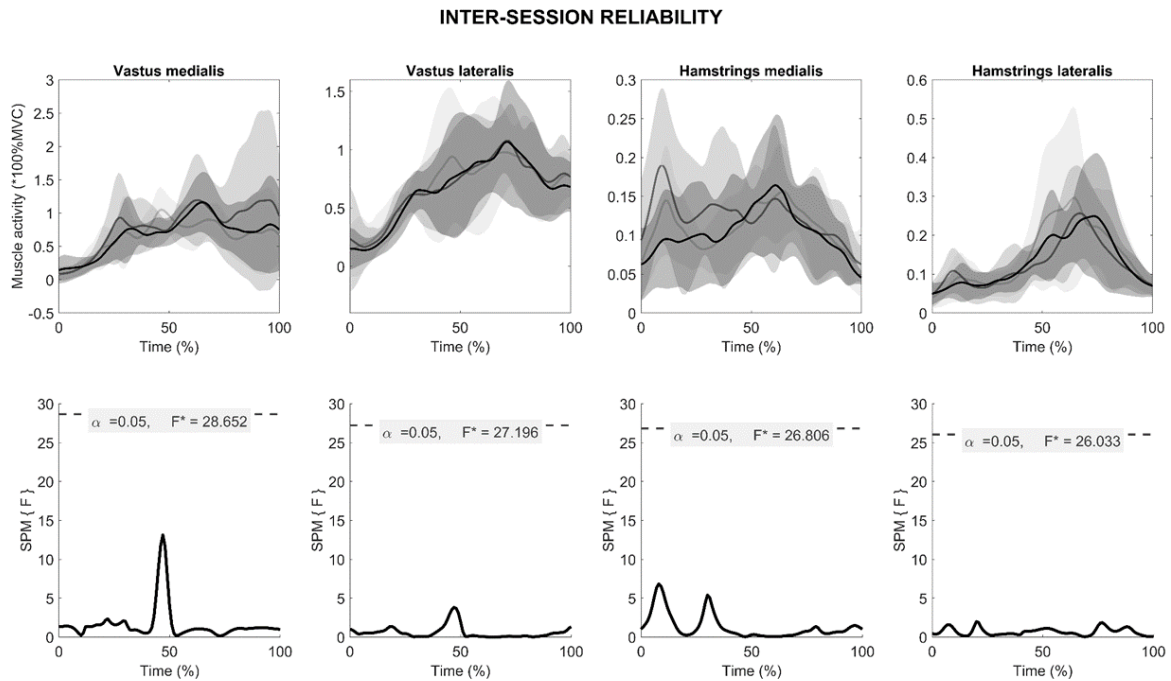
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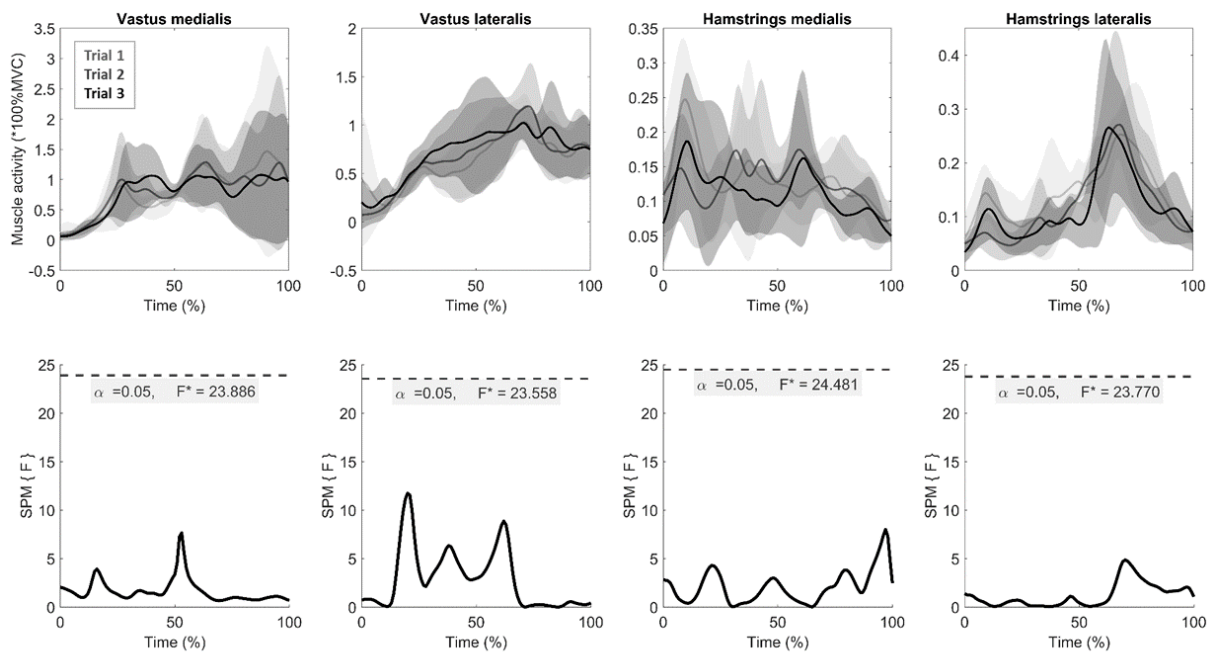555   *Fig. 1.*

556   *The upper figures illustrate the mean data for session 1 (light grey), session 2 (dark grey) and*

557   *session 3 (black) for the respective muscles. Standard deviation clouds are represented by the*

558   *shaded areas. The lower figures illustrate the SPM output. No significant differences were*

559   *found between sessions as the F-curve never exceeds the critical threshold (horizontal red*

560   *dashed line).*

561

562   To implicitly investigate intra-session reliability, we performed another SPM one-way ANOVA

563   with 1 repeated measure (e.g. trial) on the data of session 1. These analyses showed no

564   significant differences in muscle activation between the 3 different trials (see fig. 2). As there

565   was no trial-effect we can conclude that intra-session reliability is high.

INTRA-SESSION RELIABILITY

566

567   *Fig. 2.*

568   *The upper figures illustrate the mean data for trial 1 (light grey), trial 2 (dark grey) and trial 3*

569   *(black) of session 1 for the resprective muscles. Standard deviation clouds are represented by*

570   *the shaded areas. The lower figures illustrate the SPM output. No significant differences were*

571   *found between trials as the F-curve never exceeds the critical threshold (horizontal dashed*

572   *line).*

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

INDIVIDUAL DATA

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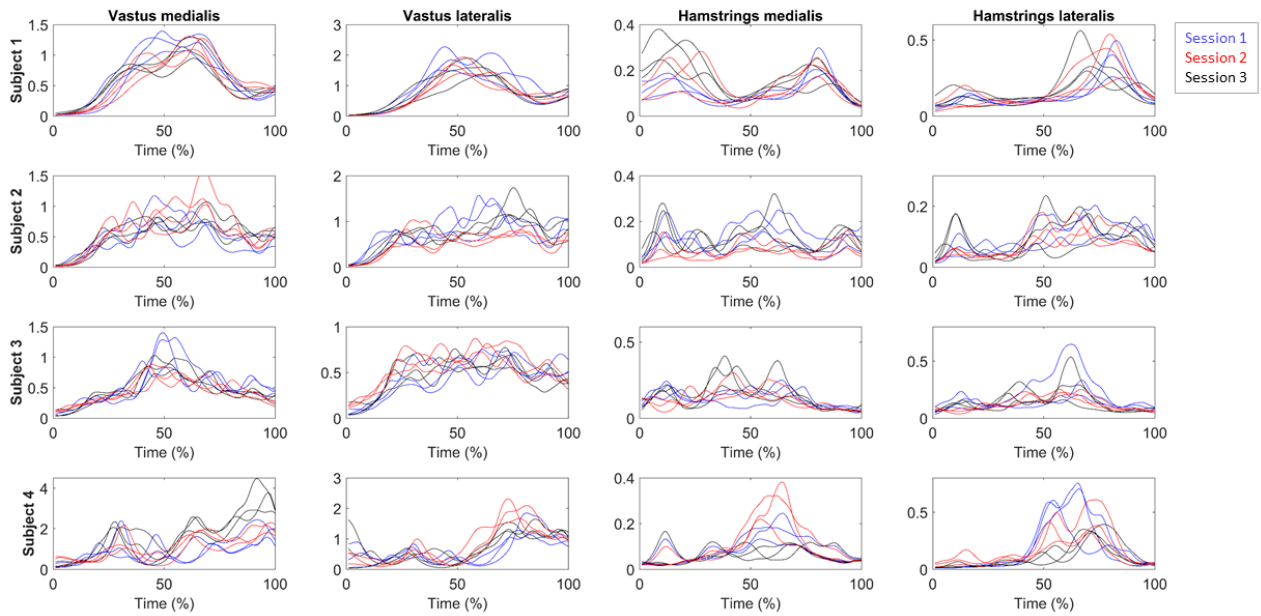
Fig. 3.

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).

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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

590 muscles were very easy to palpate in our athlete population. Furthermore, the rater is a qualified

591 physiotherapist with extensive palpation experience, and used the SENIAM guidelines to

592 standardize his way of electrode placement.

593