

Evaluation of Lower Limb Muscle Synergies in Paediatric Females with and without ACL Injuries

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Master's Thesis

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## List of Acronyms

|           |  |
|-----------|--|
| ACL       | Anterior Cruciate Ligament   |
| ACLc      | Anterior Cruciate Ligament Contralateral Limb                        |
| ACLd      | Anterior Cruciate Ligament Deficient Limb                            |
| BF        | Biceps Femoris   |
| BMI       | Body Mass Index  |
| CI        | Co-Activation Index  |
| CNMF      | Concatenated Non-Negative Matrix Factorization                       |
| CNS       | Central Nervous System   |
| CoM       | Center of Mass   |
| DVJ       | Drop Vertical Jump   |
| EMG       | Electromyography   |
| GMed      | Gluteus Medius   |
| GRF       | Ground Reaction Force  |
| iEMG      | Integrated Electromyography  |
| LG        | Lateral Gastrocnemius  |
| MAO       | Moment Arm Orientation   |
| MG        | Medial Gastrocnemius   |
| MVIC      | Maximum Voluntary Isometric Contraction                              |
| NMF       | Non-Negative Matrix Factorization                                    |
| Pedi-FABS | Pediatric Functional Activity Brief Scale                            |
| Pedi-IKDC | Pediatric International Knee Documentation Committee Subjective Form |
| RF        | Rectus Femoris   |
| ST        | Semitendinosus   |
| VL        | Vastus Lateralis   |
| VM        | Vastus Medialis  |

## General Abstract

**Purpose:** Young adolescent females are at the highest risk of sustaining an ACL injury, which may alter their movement and muscle activation patterns yet there is a lack sex- and age-specific guidelines for ACL injury management. The purpose of this study was to (1) evaluate the effects of limb dominance in a healthy uninjured population to serve as a baseline for the ACL-deficient cohort and (2) provide evidence of the neuromuscular patterns and biomechanical loading of uninjured and ACL-deficient knee joints in a female paediatric population.

**Methods:** Eighteen active female adolescents with ACL rupture (ACLd) and 21 uninjured female adolescent controls matched for limb dominance (CON) participated in this study. Participants completed bilateral squats and drop vertical jumps (DVJ) while lower limb electromyography, kinetics and kinematics data were collected. Muscle synergies were extracted using a concatenated non-negative matrix factorization (CNMF) framework and compared between limbs, (CON dominant vs CON non-dominant and CON vs ACLd) across tasks and between limbs within tasks using intraclass correlation coefficients and statistical parametric mapping.

**Results:** ACLd participants took significantly longer to perform the squat relative to their uninjured peers. No significant differences were found for hip, knee and ankle peak joint flexion angles and moments between populations for the squat. Squat and DVJ muscle synergies were equivalent for dominant and non-dominant uninjured control limbs. ACL injured (ACL deficient and contralateral limbs) exhibited greater variability in DVJ synergy vectors than for the squat task. When comparing across tasks, scaling coefficients were consistently higher for the DVJ for all populations.

**Conclusion:** Differences in lower limb kinematics, muscle activity and muscle activation patterns between dominant and non-dominant limbs indicate that limb symmetry, a clinical tool commonly used to assess rehabilitation and return to play may not provide relevant results. DVJ scaling factors were larger than those of the squat for all groups, likely due to the increased demand of that task. ACLd and CON participants completed squats and DVJ with similar lower limb joint angle patterns and muscle activity. ACL injured groups had fewer consistent vectors across tasks demonstrating greater variability in muscle activation patterns. This increased variability may be due to the ACL injury however, as injured participants were not studied pre-injury it cannot be confirmed.

## Chapter 1. Introduction

Over the past 20 years paediatric (12-18 yrs) anterior cruciate ligament (ACL) injury rates in the United States have been increasing at approximately 2.3% annually (Beck, Lawrence, Nordin, DeFor, & Tompkins, 2017), with similar per capita numbers expected in Canada. The majority of ACL ruptures occur when the lower limb undergoes a sudden deceleration with shallow flexion angles prior to a change in direction (Sakane et al., 1999; Shimokochi & Shultz, 2008; Shin, Chaudhari, & Andriacchi, 2009). This injury occurs 58-71 percent of the time during dynamic movements without any external contact in the form of a tackle or collision, indicating that factors within the individual and their joint are responsible for the ligament's failure (Boden, Feagin, & Garrett, 2000; Waldén, Hägglund, Magnusson, & Ekstrand, 2011).

ACL injury risk factors such as anatomical, biomechanical and neuromuscular aspects evolve as individuals mature and vary between sexes (Hewett et al., 2010). With the rising incidence of paediatric ACL injuries (Shea, Pfeiffer, Wang, Curtin, & Apel, 2004; Werner, Yang, Looney, & Gwathmey, 2016) and reinjuries (Mohtadi, Chan, Barber, & Paolucci, 2016), particularly in females (Leroux et al., 2014; Shea et al., 2004; Shelburne, Torry, & Pandy, 2006), and the lack of sex- and age-specific guidelines for ACL injury management, there is a need to develop sex- and age-specific evidence to inform the clinical decision making process for return-to-play following an ACL injury.

Previous studies suggest that during a static weight-bearing target matching task requiring participants to modulate direction specific loads, females exhibit more generalised stabiliser activity in the lower limb muscles, while males use the muscles as more direction-specific activators and stabilisers (Agel, Rockwood, & Klossner, 2016; Del Bel et al., 2018; Flaxman, Smith, & Benoit, 2013). While this research indicates sex differences in muscle activation patterns, ACL injuries occur primarily during dynamic, not static tasks. Although most rehabilitation programs aim to improve muscle function, there is very little in the literature describing lower-limb neuromuscular control in the paediatric healthy female population, and even less with respect to the ACL injured population. As such, a better understanding of

how female lower limb muscle function differs after an ACL rupture is required. The primary objective of this study was therefore to determine how pediatric female lower limb muscle activation patterns differ between individuals with and without ACL ruptures.

## Chapter 2. Literature Review

### 2.1 Functional role of the Anterior Cruciate Ligament

The ACL is one of the four main ligaments within the knee joint that connect the femur to the tibia and allow the knee to dictate effective lower limb function while remaining stable. Knee stability is defined as the ability to remain or quickly return to the initial homeostatic state following a disruption (Riemann & Lephart, 2002). These ligaments maintain the knee joint's six degrees of freedom; three rotations (internal/external, valgus/varus and flexion/extension) and three translations (anterior/posterior, medial/lateral and compression/distraction) (Girgis, Marshall, & Monajem, 1975; Takeda, Xerogeanes, Livesay, Fu, & Woo, 1994). Thus, the ACL's main function is to stabilise the tibia relative to the femur, to prevent anterior tibial translation, knee varus and valgus movements, and tibial rotation (Girgis et al., 1975; Micheli, Metzl, Di Canzio, & Zurakowski, 1999; Takeda et al., 1994). The ACL also protects the menisci from shear forces during athletic manoeuvres like landing, pivoting and decelerating that put additional push and pull forces on the tibia (Goldblatt & Richmond, 2003). The ACL is also composed of mechanoreceptors that relay somatosensory information generated by changes in ligament tension and length to the central nervous system (CNS) (Johansson, Sjölander, & Sojka, 1991; Schultz, Miller, Kerr, & Micheli, 1984; Schutte, Dabezies, Zimny, & Happel, 1987). This feedback is used to maintain knee joint stability by regulating the contributions of the musculature surrounding the joint (Johansson et al., 1991; Krogsgaard, Fischer-Rasmussen, & Dyhre-Poulsen, 2011).

### 2.2 Muscular contributions to knee joint stability

Dynamic knee joint stability, is achieved collaboratively through active and passive components. While passive components such as ligaments aid in maintaining stability, only the active components, muscle groups, can be regulated to maintain knee joint stability. Muscles crossing the knee joint are

typically categorised as either flexors (such as semitendinosus, biceps femoris) or extensors (such as vastus lateralis, vastus medialis and rectus femoris). When activated, these muscles generate tension across the knee joint which can both load and unload soft tissues and stabilise the knee (Markolf, Graff-Radford, & Amstutz, 1978; Olmstead, Wevers, Bryant, & Gouw, 1986; Pope, Johnson, Brown, & Tighe, 1979). However, when the knee joint is compromised, such as a ligament injury, the function of the surrounding musculature may be altered (Del Bel et al., 2018; Johansson et al., 1991). Therefore, to improve treatment for individuals with ACL injuries, it is important to develop a better understanding of individual muscle contributions to joint stability before and after an ACL injury.

Studies have aimed to understand the individual contributions of the muscles surrounding the knee and their impacts on knee ligament function. Using a reliable quasi-static weight-bearing protocol (Smith, Flaxman, Speirs, & Benoit, 2012), our research group has identified three functional muscle roles general joint stabiliser, moment actuator and specific joint stabiliser of the lower limb based on muscle activations relative to their moment arm orientations (MAO) during various loading conditions, in an uninjured adult population (Flaxman, Alkjær, Simonsen, Krogsgaard, & Benoit, 2017). General joint stabilisers exhibited symmetrical activation patterns while moment actuators exhibited relatively high specificity and an asymmetrical activation pattern about its reported MAO. Lastly, specific joint stabilisers exhibited relatively high specificity and an asymmetrical activation pattern opposite of its reported MAO (Flaxman et al., 2017). Using a regression model, the quadriceps were classified as contributors to knee extensor moments (Flaxman, Alkjær, Simonsen, et al., 2017) agreeing with previous studies (Fleming et al., 2001), suggesting that they compress the knee joint thus providing stability. Flaxman (2017) also classified the vastii as general joint stabilisers with weak prediction accuracy for internal joint moments, implying that they are activated under multiple loading directions. Contrarily, the rectus femoris was classified as a moment actuator for knee extensions reiterating the quadriceps contributions to knee extension. Flaxman suggests that in load bearing conditions, the vastii muscles contract to increase compressive forces acting to brace the knee and protect the ACL. The protective role of the hamstrings as an antagonist to quadriceps activation preventing anterior tibial translation has also

been reported (Giove, Miller, Kent, Sanford, & Garrick, 1983; Solomonow et al., 1987) yet the significance of this protection is debated (Kvist & Gillquist, 2001; Simonsen et al., 2000). Hamstring activation is also essential to transverse plane stability under torsional loads (Flaxman, Alkjær, Simonsen, et al., 2017) as extension of the knee using isolated quadriceps force results in internal tibial rotation (Victor, Labey, Wong, Innocenti, & Bellemans, 2009).

Flaxman et al. (2017) also demonstrated significant associations between the lateral and medial gastrocnemius with respect to internal and external knee rotation moments. Conversely, conflicting reports regarding the gastrocnemius role in increasing ACL strain (Fleming et al., 2001) make it unclear whether the gastrocnemius muscle plays a protective role for the ACL. In brief, a muscles contributions cannot be determined solely on its anatomical orientation but rather by also considering its role in maintaining joint stability. As such, a muscle's contributions may vary according to external loads in order to maintain stability in the frontal and transverse planes (Flaxman, Alkjær, Simonsen, et al., 2017; Zajac & Gordon, 1989).

### **2.3 Anatomical changes and long term complications following an ACL rupture**

When an ACL is ruptured, the structural integrity of the knee joint is affected. The tibia's anterior translation with respect to the femur, as well as the valgus/varus deformities and knee joint hyperextension, are less constrained. In addition to providing mechanical support to the physical integrity of the joint, the ACL also uses stress and strain mechanoreceptors to provide proprioception and motor sensory feedback (Krogsgaard et al., 2011). Thus, when the ACL is ruptured, individuals experience structural stability losses and compromised neuromuscular function (Krogsgaard et al., 2011). This neuromuscular function may be permanently lost or severely diminished (Krogsgaard et al., 2011), leading individuals with ACL ruptures to adopt compensation strategies.

Decreased structural stability and compromised neuromuscular control may lead to damaging long term effects, including reduced stability leading to the shifting of joint contact regions causing wear to thinner regions of articular cartilage (Andriacchi & Dyrby, 2005). This degradation of articular surfaces has the potential of resulting in osteoarthritis (OA) (Lohmander, Englund, Dahl, & Roos, 2007), a

condition associated with pain, stiffness, and difficulty or loss of movement. Age, genetics, history of injury, cartilage loading, muscle weakness and instability (Lohmander et al., 2007), predispose an individual to developing OA (Lohmander et al., 2007). As such, active individuals who have suffered an ACL injury are over 50% more likely to develop OA than their non-injured counterparts (Lohmander et al., 2007).

#### **2.4 Altered Muscle Activation Patterns following an ACL rupture**

Individuals with ACL injuries often exhibit varied movement patterns and muscle activation strategies compared to healthy counterparts (Alkjær, Simonsen, Jørgensen, & Dyhre-Poulsen, 2003; Rudolph, Axe, Buchanan, Scholz, & Snyder-Mackler, 2001; Sinkjaer & Arendt-Nielsen, 1991; Williams, Barrance, Snyder-Mackler, Axe, & Buchanan, 2003). These variations may result from the injury however the cause-and-effect nature of this relationship cannot be confirmed. The same quasi-static weight bearing protocol previously mentioned revealed that participants with a ruptured ACL showed altered muscle roles compared to their healthy counterparts (Del Bel et al., 2018). The ACL-deficient (ACLd) participants exhibited more generalised stabiliser activity indicated by their symmetrical activation patterns, regardless of the direction of the load (Del Bel et al., 2018). This activity is also characterised by a failure to adapt their neuromuscular control strategies to a variety of externally applied loads, in their lower limbs compared to the uninjured participants (Del Bel et al., 2018). These findings illustrate how different populations use the same muscles for different functions to accomplish the same task. Although the direct cause of these discrepancies was not determined, it could be a result of underlining knee flexor or extensor strength deficits (Del Bel et al., 2018 Chmielewski, Stackhouse, Axe, & Snyder-Mackler, 2004; Palmieri-Smith, Thomas, & Wojtys, 2008; Rudolph et al., 2001).

Reduced quadriceps strength, often attributed to incomplete voluntary activation in the injured limb, is the most common symptom following an ACL rupture (Chmielewski, Stackhouse, Axe, & Snyder-Mackler, 2004; Palmieri-Smith, Thomas, & Wojtys, 2008; Rudolph et al., 2001) and may require changes in hamstring and gastrocnemii activity to maintain knee joint stability. However, while these adaptations may play compensatory roles, they may also reduce rotational stability and potentially

contribute to an increased risk of re-injury (Flaxman, Alkjær, Shourijeh, Krogsgaard, & Benoit, 2017). This reduction in quadriceps strength is also correlated with poor functional scores for dynamic tasks and activities of daily living (Reinke et al., 2011; Rudolph et al., 2001), thus potentially contributing to the debilitating nature of the injury and prompting individuals to adopt compensation strategies. These strategies include earlier onset of muscle activation (Sinkjaer & Arendt-Nielsen, 1991); increased quadriceps activity (Rudolph et al., 2001); and increased co-activation of the quadriceps and hamstring (Aalbersberg, Kingma, & Dieën, 2009; Alkjær, Simonsen, Magnusson, Dyhre-Poulsen, & Aagaard, 2012; Chmielewski, Hurd, Rudolph, Axe, & Snyder-Mackler, 2005; Kvist & Gillquist, 2001; Rudolph et al., 2001; Sinkjaer & Arendt-Nielsen, 1991). Co-activation may therefore be a compensation strategy post ACL rupture used to promote joint stability by increasing the compressive force and minimising internal joint translations and rotations. However, it remains unclear if these adaptations help to protect the knee, are simply results of reduced knee function or a combination of the two (Alkjær et al., 2012; Chmielewski et al., 2005; Kvist & Gillquist, 2001; Rudolph et al., 2001; Shanbehzadeh, Bandpei, & Ehsani, 2015; Sinkjaer & Arendt-Nielsen, 1991; Slemenda et al., 1997; Williams et al., 2003)

## **2.5 Increased Injury Risk for Females**

While there are biomechanical and neuromuscular differences between males and females, research and rehabilitation programs are commonly based on evidence gathered using adult male participants (McLean, Huang, & van den Bogert, 2008). The anatomy of the adult female lower limb and knee joint differ from males via an increased quadriceps angle (Q-angle) (Haycock & Gillette, 1976), defined as the angle between the force vector of the quadriceps muscle group and the patellar tendon (Brattström, 1964), narrower intercondylar notch (LaPrade & Burnett, 1994; Tillman et al., 2002), and 20-35% smaller articulation surfaces making valgus loading more dangerous (McLean et al., 2008).

Neuromuscular sex differences have also been identified during the vertical stop-jump, a task where participants take a two to three step running approach, land on two feet and immediately perform a two-footed takeoff for maximum height. During the first landing, where participants are absorbing the momentum from the running approach, females demonstrate decreased hip and knee flexion, increased

quadriceps activation and decreased hamstring activation relative to males, thereby potentially increasing the shear force on the ACL (Chappell, Creighton, Giuliani, Yu, & Garrett, 2007; LaBella, Hennrikus, & Hewett, 2014). In the same static weight bearing task previously described (Flaxman et al., 2017), females maintained joint stability by symmetrically recruiting muscles in the test leg, regardless of the direction of the applied loads relative to males whose muscle recruitment is more load specific (Del Bel et al., 2018). These differences are consistent in both healthy and ACLd populations and indicate that adult males and females use their muscles in the lower extremity differently (Del Bel et al., 2018). It remains unclear if these different activation strategies compensate for anatomical differences between males and females or also place females at a higher risk of injury (McLean et al., 2008).

The biomechanical and functional differences between sexes make it clear that adult males and females should be considered independently. Furthermore, preadolescent (under the age of 12) females experience similar rates of ACL injuries as their male counterparts (Shea et al., 2004) and exhibit similar muscle activity (Del Bel et al., 2018), indicating changes associated with puberty may be responsible for the discrepancies in injury rates.

Females between the ages of 13 and 17 years possess the highest injury incidence of any sex-age strata (Herzog et al., 2018), indicating puberty induced physiological changes, which occur around the age of 12 years (Coleman & Coleman, 2002), could make them susceptible to ACL injuries. During puberty, both sexes experience rapid growth of the tibia and femur (Tanner & Davies, 1985), translating into greater torques on the knee (Tanner & Davies, 1985). The increase in body weight and height throughout puberty generates greater joint force and raises the location of the center-of-mass complicating balance (Hewett, Myer, & Ford, 2006; LaBella et al., 2014). Females also experience widening of the hips, resulting in a larger Q-angle accompanied by a higher pelvic width-femur length ratio (Shelburne et al., 2005). These higher ratios predict increased knee valgus during dynamic loading (Shelburne et al., 2005), a primary component of one of the proposed non-contact ACL injury mechanisms (Kiapour et al., 2016).

## **2.6 Objective measures of neuromuscular control**

Objective measures of assessing knee joint function are essential when studying knee joint functionality following an ACL injury. Clinical measures often include symmetry indices between injured and uninjured limbs (Adams, Logerstedt, Hunter-Giordano, Axe, & Snyder-Mackler, 2012; Paulos, Noyes, Grood, & Butler, 1991a). However, this method is limited by several assumptions: i) all individuals use their two limbs equally, ii) individuals' limbs were symmetric prior to their injury, and iii) pre-exhaustion test results are an accurate representation of fatigued state results (Augustsson, Thomeé, & Karlsson, 2004). Therefore, visual examinations of clinical tasks may not provide accurate assessments of patient performance in real-world settings (Adams, Logerstedt, Hunter-Giordano, Axe, & Snyder-Mackler, 2012; Paulos, Noyes, Grood, & Butler, 1991).

### **2.6.1 Muscle Synergy Analysis**

Electromyography (EMG) is a commonly used tool to record and analyse the activation of skeletal muscles. Traditional EMG analyses have been used to interpret information regarding muscle force and muscle activation (Luca, 1997). However, these types of analyses often investigate discrete values, such as peak activation over the course of a dynamic task without considering the values for the entire movement. Muscle synergy analysis provides an alternate form of EMG analysis that addresses the contributions of various muscles over the entire movement pattern (Ting, 2004). Muscle synergies are underlying patterns of co-active muscles used in different combinations to execute all movements (Chvatal, Torres-Oviedo, Safavynia, & Ting, 2011; Neptune, Clark, & Kautz, 2009; Ting, 2004; Torres-Oviedo, Macpherson, & Ting, 2006). They are suggested to represent basic neural mechanisms that are common across different dynamic conditions, allowing us to respond to our ever changing environments. Traditional measures of muscle activity such as co-activation indices are limited as they assume certain groups of muscles are antagonistic to each other. This assumption may not be appropriate as it does not address how multiple muscles can work collectively to oppose direction specific loads. Alternatively, muscle synergy analysis looks to identify patterns of muscle activation, while taking in to account that each muscle can be simultaneously activated by multiple synergies (Tresch, Saltiel, & Bizzi, 1999).

This analysis is an objective method of measuring and comparing knee joint function during dynamic tasks within and between different populations without making contralateral limb comparisons (Chvatal et al., 2011; Luca, 1997; Neptune et al., 2009; Shelburne et al., 2005; Torres-Oviedo et al., 2006).

Synergy analysis has traditionally been done through non-negative matrix factorization (NMF) (Chvatal et al., 2011; Moghadam, Aminian, Asghari, & Parnianpour, 2013; Neptune et al., 2009; Ting, 2004; Torres-Oviedo et al., 2006). This technique uses linear decomposition to output the optimised basis and synergy vectors and the corresponding weights vectors representing the coefficients. This method has successfully accounted for over 80% of the total variability in EMG signals during direction dependant perturbation in cats (Torres-Oviedo et al., 2006) and to compare patients who have undergone a knee replacement (Ardestani, Malloy, Nam, Rosenberg, & Wimmer, 2017) yet, the limitations of NMF may render it physiologically irrelevant. For example, if an NMF solver algorithm is run over a single set of data multiple times it will continuously output different results. This drawback stems from the algorithm's ability to find either the coefficients or the synergy vectors, but not both simultaneously, and its necessity of always using one of the iterations as a reference (Lee & Seung, 2000).

An alternative to overcome these limitations is to use a concatenated non-negative matrix factorization (CNMF), where the input data are vertically concatenated in the matrix (Shourijeh, Flaxman, & Benoit, 2016). This approach fixes the synergies across all participants but allows coefficient weight to fluctuate, accounting for variability between participants (Flaxman, Shourijeh, Alkjær, Krogsgaard, & Benoit, 2017). CNMF is highly reliable ( $>0.99$ ;  $0.99-1.0$ ) compared to NMF ( $0.26$ ; range  $0.26-0.98$ ) when used to analyse the synergies present during the weight bearing, force matching task. Similar muscle synergies used for two legged squats and forward lunges with and without induced muscle pain have also been identified (Flaxman et al., 2017). These findings support the repeatability and robustness of the CNMF, suggesting this method could be used to explore and compare muscle synergies used by young females with and without ACL injuries.

## **2.7 Bilateral Movement use in Rehabilitation and Clinical Decision Making**

Bilateral squats and drop vertical jumps (DVJ) are used in clinical decisions making, rehabilitation programs and training programs aimed at preventing ACL injuries (Bizzini et al., 2013; Impellizzeri et al., 2013). Multi joint movements simulate everyday activities, require individuals to activate multiple muscle groups, avoid putting excessive strain on the recently operated joint, and are performed in addition to joint isolating exercises (Wright et al., 2015). ACL rehabilitation guidelines established by the Multicenter Orthopaedic Outcomes Network (MOON) group outline a five phase progression beginning with preoperative benchmark recommendations, progressing through rehabilitation exercise suggestions and key stages, and finishing with the final phase of return-to-sport. Early stages include quarter-depth squats which gradually progress to full squats in later stages (Wright et al., 2015). In contrast, DVJs, are a more demanding tasks and are not introduced until the fourth phase (Wright et al., 2015). This exercise progression from squats to jumps in rehabilitation programs highlights the varied difficulty of the two tasks.

### **2.7.1 Bilateral Squats**

The bilateral squat is an example of a multi-joint movement primarily governed by the quadriceps that has become an essential part of most conservative and postoperative ACL rehabilitation programs (Bynum, Barrack, & Alexander, 1995; Salem, Salinas, & Harding, 2003; Wright et al., 2015). Squats simulate everyday activities, require individuals to activate multiple muscle groups and avoid excess strain on the injured or recently operated joint (Wright et al., 2015).

Squat movements are separated in two phases: descent and ascent. Prior to the descent, the individual starts in a standing position with their hip and knee joints fully extended. Preparation for the descending phase occurs by decreasing hamstring activity (Cheron, Bengoetxea, Pozzo, Bourgeois, & Drayc, 1997; Hase, Sako, Ushiba, & Chino, 2004) and increasing the gastrocnemius activity (Dionisio, Almeida, Duarte, & Hirata, 2008). The movement is initiated by flexing the trunk, then the knees and lastly the ankles (Hase et al., 2004) to lower the center of mass (CoM). As the individual decelerates to reach the target position, the quadriceps activation increases (Cheron et al., 1997; Dionisio et al., 2008;

Hase et al., 2004) with the vastus medialis and laterals activating 40-50% more than the rectus femoris (Escamilla, 2001; Isear, Erickson, & Worrell, 1997). Trunk flexion angle may influence the rectus femoris contributions with more erect trunk positions lengthening the rectus femoris and increasing its activation (Escamilla 2001).

The second phase, the ascent, is initiated by the activation of the quadriceps to extend the knee and raise the individual's CoM. Greater activations are once again seen in the vastii muscles (Isear et al., 1997). The biarticular gastrocnemii also contribute to the ascent by acting about the ankle to increase plantarflexion (Escamilla, 2001). Previous studies have reported greater hamstring activation during the ascent and speculate this occurred to increase hip extension (Escamilla et al., 1998; Isear et al., 1997). However, due to the bi-articular nature of the hamstrings, their length remains relatively constant during a squat: they lengthen at the hip and shorten at the knee during the descent and vice versa for the ascent (Jönhagen, Halvorsen, & Benoit, 2009).

### **2.7.2 Drop Vertical Jumps (DVJ)**

Drop vertical jumps (DVJ) follow a similar movement pattern to squats with an additional landing and jump making them a plyometric exercise (i.e exercise with rapid stretching and contracting of muscles (as by jumping and rebounding), ((Merriam-Webster, 2019)) have been validated as an accurate movement screening tool (Padua et al., 2009). Instead of being initiated by flexing from the proximal to distal joints as seen in the squat (Hase et al., 2004), DVJ are initiated by stepping off a raised platform. Individuals then land from the drop on both feet and immediately perform a maximum vertical jump (Hewett et al., 2005). Aside from the initial stepping forward off the platform all movements occur without any forward displacements. The added difficulty of the plyometric component of the task requires effective landing strategies to stabilise the lower limb joints. However, these landing strategies may place the knee in vulnerable positions. Individuals landing with greater vertical ground reaction force and knee abduction angles, decreased flexion angle, and increased external knee abduction moments are at an increased risk of injuring their ACL (Hewett et al., 2005; Mizner, Kawaguchi, & Chmielewski, 2008). Weaker quadriceps and hamstrings musculature relative to bodyweight in females compared to males

may be responsible for the increased landing knee stiffness observed through shallow flexion angles in females (Lephart, Ferris, Riemann, Myers, & Fu, 2002).

The bilateral nature of this movement, similar to the bilateral squat, provides individuals with the opportunity to employ compensation strategies. In complex manoeuvres involving a jump, such as countermovement jumps, individuals may not equally distribute their weight between their limbs when landing (Romanick et al., 2018). Additional weight may be placed on the contralateral limb as a means of protecting the affected limb (Romanick et al., 2018). It is therefore important that rehabilitation, training and screening programs focus on identifying between limb neuromuscular and biomechanical imbalances more thoroughly than simply visually examining between limb symmetry (Hewett et al., 2005).

Drop jump landings have been validated as an accurate movement screening tool to identify individuals with an elevated ACL injury risk (Padua et al., 2009), and can retrospectively predict ACL injury (Hewett et al., 2005). Additionally, landing tasks are incorporated in later stages of rehabilitation, requiring higher levels of joint stability (Wright et al., 2015). Thus, similarly to the bilateral squat, DVJs are a clinically relevant task for healthy and ACLd populations.

## Chapter 3. Purpose and Hypotheses

### 3.1 Study Rationale

With the rising incidence of ACL injuries in the paediatric population (Shea et al., 2004; Werner et al., 2016), the increased prevalence of injuries in female athletes (Friel & Chu, 2013; Leroux et al., 2014; Shea et al., 2004), and the lack of sex- and age-specific guidelines for ACL injury management, there is a growing need to provide evidence-based clinical decision making guidelines. ACL injury risk factors including genetic, hormonal, anatomical, biomechanical, neuromuscular and physiological aspects, transition as individuals mature. As such, studying the biomechanics of an uninjured paediatric population will provide valuable insight as to how these factors may predispose maturing individuals to ACL injuries and may explain the rising rates of ACL injuries (Shea et al., 2004; Werner et al., 2016).

### 3.2 Research objectives and hypothesis

The overall purpose of the proposed thesis was to provide evidence of the neuromuscular patterns and biomechanical loading of uninjured and ACL-deficient knee joints in a female paediatric population. Since the neuromuscular control of this population has yet to be fully characterised, we first evaluated the effects of limb dominance in a healthy uninjured population to serve as a baseline for the ACL-deficient cohort. We then compared the performance of the injured population and determine what neuromuscular compensation strategies this population uses to accomplish the same tasks as their uninjured peers.

Since to our knowledge, the effects of limb dominance on neuromuscular control have not been investigated in this population, our first objective was (O1) *to determine if muscle synergies in uninjured paediatric females during bilateral squats and drop vertical jumps differ between dominant and non-dominant limbs and across tasks.*

Due to the bilateral nature of the tasks, we hypothesised that similar muscle synergies would be used in the dominant and non-dominant limbs of healthy paediatric females. Secondly, we hypothesised that the decent phase of the DVJ would require greater contributions of the hamstrings and quadriceps

muscles to account for the increased difficulty and effort required to stabilise the knee and hip joints relative to the squat (Hewett 2005).

Our second objective was to determine how ACL status contributes to muscle activation patterns for the same tasks, (O2) *do muscle synergies used to accomplish bilateral squats and drop vertical jumps differ between paediatric females with and without ACL injuries.*

We hypothesised that muscle synergies would differ between uninjured controls and ACLd participants, reflecting the lack of proprioception from the ACL (Krogsgaard et al., 2011). Secondly, we hypothesised that the injured population would experience quadriceps strength deficits commonly experienced following an ACL injury (Palmieri-Smith et al., 2008) and, to compensate would adopt different muscle synergies or movement patterns. Note that should O1 identify an effect for limb dominance, we would then account for this effect when analysing the results of O2.

## Chapter 4. Methodology

### 4.1 Study Design

The data in this study was collected by the Clinical Biomechanics Research Unit at the University of Ottawa under the supervision of Dr. Daniel Benoit, as part of a collaboration with the Children's Hospital of Eastern Ontario (CHEO). This project was part of a larger research project aiming to better understand how sex and age influence the neuromuscular patterns and biomechanical loading of healthy and injured knee joints in paediatric populations. Participants completed a series of hop, functional and endurance during the expanded protocol, however this thesis only evaluated the data from the DVJ and squats (See Appendix 2 for full protocol). The data in this thesis includes healthy controls and ACLd female participants, all between the ages of 10 and 18 years. A cross-sectional experimental study design was implemented to evaluate the differences in muscle synergies during dynamic tasks between healthy and ACLd females.

### 4.2 Participants

An *a priori* power analysis in G\*Power (3.1.9.2, Dusseldorf, Germany), based on previous data evaluating female vastus medialis muscle activation and GRF production between uninjured and ACLd females performing dynamic tasks (Del Bel et al., 2018; DeMont, Lephart, Giraldo, Swanik, & Fu, 1999), revealed that to achieve a power of 0.8, with an input effect size of 0.8 at  $\alpha = 0.05$ , a total sample size of 42, with 21 per group, is required. Therefore, 21 uninjured and 21 ACLd female participants were recruited for this study. All 21 uninjured control participants were included in Study 1, examining the effects of limb dominance and task. However, due to the variability in age and development stages between the injured and control groups, a subset of 15 participants from each group were included in Study 2, investigating the effects of and ACL injury. While selecting a smaller sample underpowers Study 2, this was done to ensure the research questions were being addressed while also mitigating the confounding variables of age and developmental stage. ACLd participants were recruited through CHEO, where they were referred to the research program by their orthopaedic surgeon. Uninjured participants

were recruited from the Ottawa/Gatineau region and, in an attempt to match activity levels to those of the athletic injured population, must have been actively participating in an organised sport. Controls were matched with ACLd participants to minimize the confounding variables of age, limb dominance, BMI, Tanner stages (puberty stage) and physical activity prior to injury. Exclusion criteria for controls included a history of previous traumatic lower extremity injury, pain in the lower extremity before testing and any other musculoskeletal impairment that might bias the results of this study. Throughout testing, participants were instructed to inform the researchers if they were not comfortable completing any of the tasks, and in turn were reassured that if they chose to abstain, their choice would not have an impact on their treatment or testing.

### **4.3 Experimental Protocol**

#### **4.3.1 Informed consent and questionnaires**

Prior to data collection, all participants completed a consent form approved by the University of Ottawa Research Ethics Board (H09-17-10) and by the Children's Hospital of Eastern Ontario Ethics Board (17/74X). Participants also completed the following questionnaires: i) an assessment of physical activity (Pedi-FABS) (Fabricant et al., 2013), ii) a subjective assessment of knee joint function (Pedi-IKDC) (Kocher et al., 2011) and iii) a self-assessment form regarding puberty stages (Tanner Stage) (Taylor et al., 2001). All participants had the option of completing the forms with a guardian present and in either French or English.

#### **4.3.2 Participant and equipment preparation**

Participants were then asked to change into a provided set of tight fitted spandex shirt and shorts. Anthropometric measurements including weight (kg), height, pelvic, knee and ankle width, leg and shank length and thigh and shank circumference (cm) were recorded. Surface electromyography (EMG) electrode placements in line with the SENIAM guidelines (Hermens, Freriks, Disselhorst-Klug, & Rau, 2000) were identified for the following muscles: gluteus medius (GMed), vastus lateralis (VL), vastus medialis (VM), rectus femoris (RF), biceps femoris (BF), semitendinosus (ST), lateral gastrocnemius (LG) and medial gastrocnemius (MG) of both limbs (Benoit, Lamontagne, Cerulli, & Liti, 2003). Identified

sensor locations were shaven and cleaned with an alcohol swab to reduce skin impedance and minimise signal noise (Cram & Rommen, 1989). Correct electrode placement was confirmed by having participants individually activate all studied muscles while visually inspecting their output signals. This allowed confirmation that placed sensors were accurately measuring the desired muscle activity. Sensors were then secured with first aid tape (3M Transpore Medical Tape, Uline, Wisconsin, USA) and pro-wrap.

Full body kinematics were recorded by placing 84 retroreflective markers (14 mm diameter) on anatomical landmarks according to a hybrid cluster marker set (Appendix 3). Marker trajectories were recorded at 200 Hz using a 10 camera infrared motion analysis system (8 Vero and 2 Vantage cameras; Vicon, Oxford, UK) and its supporting software (Nexus, v2.8, Vicon, Oxford, UK). Nexus simultaneously collected EMG and force plate data at 2000 Hz. Participants were given five minutes to warm up on a cycle ergometer (Monark 828, Vansbro, Sweden) with minimal resistance.

### **4.3.3 Maximum Voluntary Isometric Contractions**

Maximum voluntary isometric contractions (MVICs) were used to identify the maximum activation of each of the muscles studied. MVICs were performed using a Biodex isokinetic dynamometer (System 4, Pro, Biodex Medical Systems, New York, USA) with the participant in the following positions, each chosen to maximise the targeted muscle group's activity; i) seated with the hip joint at 90 degrees and the knee joint flexed at 60 degrees (knee extension; vastus laterals, vastus medialis, rectus femoris and knee flexion: biceps femoris, semitendinosus), ii) seated with the hip at 90 degrees, knee at 0 degrees of flexion and the ankle held at -10 degrees (plantar flexion: medial and lateral gastrocnemii) and iii) standing with their hip at 190 degrees, knee with 0 degrees of flexion (hip abduction: gluteus medius) (Worrell et al., 2001). Participants received standardised vocal encouragement instructing them to maintain their maximal force for 5 seconds. Each MVIC was first performed on the uninjured/non-dominant limb and repeated three times for both limbs with at least one minute rest between trials.

### **4.3.4 Dynamic task protocol**

Participants performed a static trial with one foot on each of the force plates while standing with their arms abducted to shoulder height and elbows flexed to 90 degrees, for 10 seconds. The dynamic

protocol was divided in two, with the first half requiring participants to complete a series of hops typically used to gauge return to play eligibility (Adams et al., 2012; Noyes, Barber, & Mangine, 1991) the second half of the protocol included tasks typically used in rehabilitation and injury prevention programs such as squats, lunges and DVJ (Bizzini et al., 2013; Impellizzeri et al., 2013; Wright et al., 2015). As with the MVICs, all tasks were first performed on the uninjured or dominant limb such that the participant was more comfortable and prepared to perform them on their injured/non-dominant limb.

Participants performed a series of two legged squats, and drop jumps (squat Figure 1A and DVJ Figure 1B).



**Figure 1: Squat (A) and DVJ (B) tasks**

Squats were performed by standing with one foot on each force plate and their hands on their head. Beginning from an upright position, they were instructed to squat down to a comfortable position and return to their upright starting position (Trulsson, Miller, Hansson, Gummesson, & Garwicz, 2015). Minimal instructions were given to ensure participants performed the movement as naturally as possible

and elicited potential differences in timing and squat depth. A platform set to the height of the participant's tibial plateau was used for the drop jumps to standardise take-off height. Participants began on the platform, stepped off on to the force plates landing with one foot on each plate, squatted down and immediately performed a vertical jump aiming to get as high as possible. All movements, aside from the initial step off the platform, occurred without any forward movement (Hewett et al., 2005). Participants were given two practice trials for each task and completed as many trials as necessary for five successful trials. For both tasks, trials were considered unsuccessful if either of the participant's feet were not entirely on the force plate, if for the squats their hands did not remain on their head, or if they did not properly return to the upright position. Researchers intermittently asked the participant if they are experiencing any pain or discomfort.

## **4.4 Data Processing**

### **4.4.1 Filtering and Data Reduction**

EMG data was processed using custom made Matlab (R2018a, Mathworks Inc, Natick, USA) scripts. Raw EMG data from all muscles was high-pass filtered (second order, dual pass Butterworth filter, cut-off frequency of 20 Hz), cleaned to remove drop outs, full wave rectified, low-pass filtered (second order, dual pass Butterworth filter, cut-off frequency of 6 Hz) and linear enveloped. Maximum EMG amplitudes for each muscle were determined through the MVIC trials and used to normalise the experimental EMG data by dividing experimental muscle activity by that muscle's maximal muscle activity recorded throughout the MVICs (Winter, 2009)

Marker trajectories were reconstructed, labelled and filtered using a 2<sup>nd</sup> order dual pass Butterworth filter in Nexus (v2.8 Vicon, Oxford, UK). A residual analysis of the differences between filtered and unfiltered marker trajectories was used to determine the optimal cut-off frequency (Winter, 2009). Raw ground reaction force (GRF) data was also filtered in the same manner as the kinematic data (Bisseling & Hof, 2006; Kristianslund, Krosshaug, & Bogert, 2012). Lower limb hip, knee and ankle joint angles and moments were computed through using inverse kinematics and dynamics in Nexus and exported to Matlab. The integrated EMG (iEMG) was calculated in Matlab as the area under the

activation curve for each muscle. Squats trials were time normalized from maximal to maximal pelvis origin height with minimal pelvis height occurring at 50% of squat cycle. DVJ trials were time normalized to time spent on both force plates with minimal pelvis height occurring at 50% of the cycle. Matlab (R2018a, Mathworks Inc, Natick, USA) scripts extracted lower limb kinetics and kinematics and calculated integrated EMG (iEMG) for each muscle as the area under the activation curve.

#### 4.5 Data Analysis

The first study compared lower limb joint angles, moments and muscle synergies for the dominant and non-dominant control limbs for both the squat and DVJ tasks. The second study compared lower limb joint angles, moments and muscle synergies between the injured and uninjured groups for both tasks. Muscles synergies were extracted for uninjured control dominant and non-dominant limbs and ACL injured participants' deficient and contralateral limbs.. Synergy vectors and coefficients were extracted for each group using a concatenated non-negative matrix factorization (CNMF) framework (Flaxman, Shourijeh, et al., 2017; Shourijeh et al., 2016) to assess the effects of limb dominance (dominant vs non-dominant limbs) and ACL state (injured vs control) for each task. CNMF fixes the synergy vectors across all participants within a group but allows coefficient weights to fluctuate and account for the inter-participant variability making it a more reliable and robust method than traditional non-negative matrix factorization (NMF) (Flaxman, Shourijeh, et al., 2017; Shourijeh et al., 2016). The framework was applied to each subpopulation for each task.

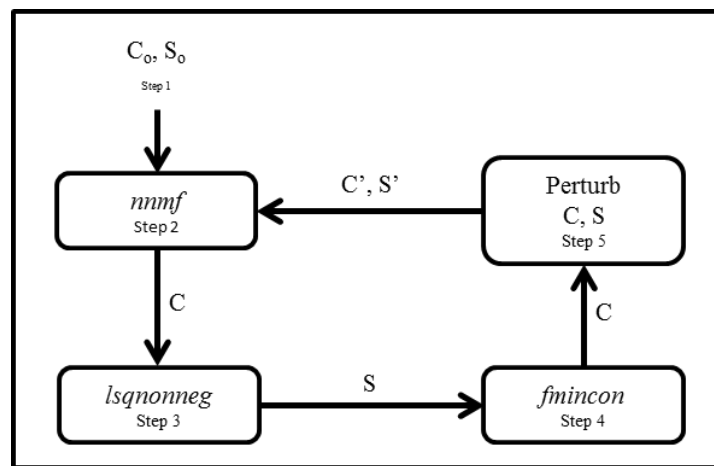
To conduct the CNMF analysis, each participant's processed EMG data was placed in an  $n \times m$  matrix where  $n$  is the number of time normalised frames for that task and  $m$  is the number of muscles studied ( $m=8$ ). The data from each participant within a sub group was concatenated to create the input matrix ( $A$ ),  $N \times m$  where  $N$  is the number of normalised frames times the number of participants in each sub group. The framework then used the concatenated input  $A$  to determine optimised matrices  $C$  and  $S$  representing the coefficients and synergies respectively.

$$A = CS \quad (1)$$

The synergy coefficients represent the relative contributions of the muscle synergy to the overall muscle activity pattern while the muscle synergy vectors represent the relative weighting of each of the muscles within each synergy (Hug, Turpin, Couturier, & Dorel, 2011).

#### 4.5.1 Factorization framework

The *CNMF* solver produced two output matrices through a series of optimization steps (Figure 2, Shourijeh et al., 2016) where the first matrix  $S$  consisted of time invariant synergy vectors each specifying the relative contributions of each muscle to that activation pattern.



**Figure 2: Factorization framework schematic illustrating the 5 steps used to optimise the coefficient (C) and synergy (S) matrices (Shourijeh et al., 2016)**

Each synergy vector  $S$  had a corresponding time-variant coefficient matrix  $C$ , represented the relative scaling factor of that synergy vector for each participant throughout the task. Preliminary analysis revealed that fixing the synergy analysis to three synergies per population per task accounted for at least 80 % of the variance and insured balanced comparisons were being made. As such, three synergies were extracted for each task for each population and synergy vectors were reordered for each comparison based on visual inspection (Flaxman et al., 2017).

#### 4.6 Statistical Analysis

The first study investigated the effects of limb dominance on lower limb movement and muscle activation patterns in young adolescent females while performing bilateral squats and drop vertical jumps.

The second study investigated the effects of ACL injury state including the ACL deficient and contralateral limbs and a control group. The control group was matched to the injured population for limb dominance, age and Tanner stage. The second study followed the same analysis as the first study, looking at lower limb movement and muscle activation patterns through the use of kinetic, kinematic and muscle synergy analyses.

### **Study 1:**

#### **The effects of limb dominance on limb kinetics, kinematics and muscle activity in youth females during squats and drop vertical jumps**

##### *Kinetics, Kinematics and iEMG*

The assumption of normality for continuous group means for joint angles, moments and muscle activations were evaluated using statistical parametric mapping (SPM). Normally distributed continuous data were compared between dominant (DOM) and non-dominant (ND) limbs, using an SPM independent *t*-test whereas a statistical non-parametric mapping (SnPM) independent *t*-test was used for data that rejected the assumption of normality. Statistical significance for all continuous joint angles and moments was defined as  $p < 0.025$  following a Bonferroni correction for multiple comparisons (two comparisons).

The assumption of normality for the discrete variables (integrated EMG (iEMG), peak joint angles and moments) was evaluated through a Shapiro-Wilk test. For normally distributed data differences were compared using an independent *t*-test whereas a Man-Whitney U test was used for data that rejected the assumption of normality. Statistical significance for all discrete tests was defined as  $p < 0.05$  prior to a Benjamini-Hochberg correction for multiple comparisons with a false discovery rate (FDR) of 0.05 (Benjamini & Hochberg, 1995). Discrete statistical analyses were conducted in excel (2016, Microsoft, Washington, USA).

## ***Synergy Analysis***

### **Effects of Limb Dominance**

Limb dominance was compared within tasks (comparisons: DOM Squat vs ND Squat and DOM DVJ and ND DVJ). Synergy vectors were compared between tasks using intraclass correlation coefficients ( $ICC_{(1,k)}$ ) (Flaxman et al., 2017; McGraw & Wong, 1996), where vectors were considered equivalent ( $ICC \geq 0.80$ ), similar ( $0.60 \leq ICC < 0.80$ ) or uncorrelated ( $ICC < 0.60$ ). Time varying activation coefficient matrices  $C$  we compared using statistical parametric mapping (SPM) independent t-tests (Pataky, 2010). Statistical significance for activation coefficient comparisons for limb dominance was defined as  $p < 0.05$ .

Cross-reconstruction of each task using the synergy vectors of the opposing task was used to compare the uniqueness of each task and limb's synergies (Flaxman et al., 2017; Gizzi, Muceli, Petzke, & Falla, 2015). Cross reconstruction was assessed by reconstructing the input matrix of one limb with the vectors of the other (i.e. input matrix from DOM Squat was reconstructed using the synergy vectors from ND Squat). Variance accounted for (VAF) in the cross-reconstruction provides a measure of accuracy for the reconstruction. For example, to cross reconstruct the non-dominant limb squat data matrix, the three synergy vectors extracted for the dominant limb squat matrix would be used. These dominant limb vectors would be inputted to the *CNMF* algorithm, along with the original non-dominant limb squat data matrix. The algorithm is forced to use the synergy vectors of the dominant limb squat data and adjusts the non-dominant limb coefficients to try and optimise VAF. As such, if muscle activation patterns were consistent between limbs, the algorithm would not have any difficulty adjusting the coefficients and account for more of the variance. Similarly, if synergy vectors are consistent across tasks, we would expect them to be able to cross-reconstruct each other's input matrices and account for similar variance as their own original synergy vectors (Flaxman et al., 2017).

### **Effects of Tasks**

Task similarity was compared within limbs (comparisons: DOM Squat vs DOM DVJ, and ND Squat vs ND DVJ). Synergy vectors and coefficients were compared using the same methods and criteria

as those used for the comparisons investigating limb dominance. Similarly, the uniqueness of each tasks' synergies was compared through cross-reconstructions.

## **Study 2:**

### **The effects of ACL injury on lower limb biomechanics and muscle synergies in youth females during squats and drop vertical jumps**

#### ***Kinetics, Kinematics and iEMG***

Group means for continuous and peak joint angles, moments and iEMG were evaluated using the same methods as in Study 1. Statistical analyses compared between limbs, evaluating the effects of the ACL injury state (ACL deficient (ACLd), ACL contralateral (ACLc) and an uninjured control group (CON)). and between tasks, comparing the similarities across the squat, and DVJ.

#### ***Synergy Analysis***

##### **Effects of ACL injury State**

Results from Study 1 indicated minor differences between dominant and non-dominant limbs in uninjured young females. To account for this, a group of controls matched for limb dominance to the injured population was used (CON) for comparisons looking at the effect of ACL injury (comparisons: CON vs ACLd, CON vs ACLc, ACLd vs ACLc). Synergy vectors and coefficients were compared using the same methods and criteria as those used for Study 1. Statistical significance for coefficient comparisons was defined as  $p < 0.0167$  following a Bonferroni correction for multiple comparisons (3 comparisons: ACLd vs ACLc, CON vs ACLd and CON vs. ACLc).

##### **Effects of Tasks**

Task similarity in the injured population was compared within limbs (comparisons: CON Squat vs CON DVJ, ACLd Squat vs ACLd DVJ and ACLc Squat vs ACLc DVJ) using the same methods and criteria as those used for Study 1.

## Chapter 5. Manuscript 1

### **The effects of limb dominance on lower limb kinetics, kinematics and muscle synergies in youth females during squats and drop vertical jumps**

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## Abstract

**Background:** The effect of limb dominance on neuromuscular control has not been investigated in young females during clinically relevant functional tasks. Therefore, prior to investigating neuromuscular control in injured individuals, the potential effects of limb dominance must be established. Thus, the purpose of this study was to describe the lower limb joint angles, moments and muscle synergies in young athletic adolescent females while performing bilateral squats and drop vertical jumps (DVJ).

**Methods:** Twenty-one female adolescent completed bilateral squats and DVJs while lower limb electromyography (EMG), kinetics, and kinematics data were collected. Integrated EMG (iEMG), hip, knee and ankle sagittal plane joint angles and moments were compared between dominant (DOM) and non-dominant (ND) limbs. Muscle synergies were extracted using a concatenated non-negative matrix factorisation framework and compared between limbs (DOM vs ND) and across tasks (squat and DVJ) using intraclass correlation coefficients and statistical parametric mapping.

**Results:** No significant differences were found for hip, knee, and ankle peak joint flexion angles and moments between limbs. Squat movement patterns are consistent between DOM and ND limbs of uninjured adolescent females. DVJ sagittal plan hip angles and frontal plane knee moments were significantly greater in the non-dominant limb (29.4-71.6% and 0.8-1.7% of cycle respectively). The dominant limb biceps femoris had greater iEMG (squat ( $p = 0.021$ ) and DVJ ( $p = 0.035$ )) and continuous EMG (squat: 60.5-64.8% and DVJ 64.7-77.4). Whereas the non-dominant limb had greater semitendinosus iEMG during the DVJ ( $p = 0.015$ , Table 2) and greater continuous vastus lateralis activation between 2.9 and 6.9% of the DVJ. The majority of synergy vectors were equivalent for dominant and non-dominant limbs. Bicep femoris iEMG was significantly higher in the DOM limb for both tasks while DVJ ST iEMG was significantly greater in the ND limb. When comparing across tasks, scaling coefficients were consistently higher for the DVJ.

**Conclusion:** Squat movement patterns are consistent between DOM and ND limbs of uninjured adolescent females while differences were observed in the DVJ, perhaps due to the its added difficulty. Differences in BF muscle activation patterns were found in both tasks, indicating the two limbs employ different muscle activation patterns and strategies to accomplish the same task. Synergy vectors were consistent across tasks, reflecting their load dependency while the scaling factors varied between tasks, reflecting their demand dependency. Future work should take into account limb dominance when investigating muscle activation patterns in young females.

## ***1. Introduction***

Dynamic knee joint stability, defined as the knee's ability to remain in or quickly return to its initial homeostatic state following a disruption (Riemann & Lephart, 2002), allows us to participate in our daily activities. Knee joint stability is maintained through the combination of passive components, including ligaments, menisci and bones, and active components comprised of the surrounding muscles. While the passive components aid in restricting joint motion and provide somatosensory feedback, the muscles are the only components that can be actively regulated.

The central nervous system (CNS) dictates our movements by modulating the activations of our muscles. Although our movements and their complexity are highly variable, a number of studies have shown that muscles' activations to produce a given movement consist of reproducible patterns, commonly referred to as 'muscle synergies' (Cappellini, Ivanenko, Poppele, & Lacquaniti, 2006; Romanick et al., 2018; Ting & Macpherson, 2005; Torres-Oviedo et al., 2006). A muscle synergy represents an underlying activation pattern(s) of individual or multiple muscles by a single neural control signal (Kipp et al., 2014). Conceptually, muscle synergies consist of a time invariant synergy vector and a time-varying activation coefficient. The synergy vector represents each muscle's relative contribution to that synergy whereas the activation coefficient provides a scaling factor, taking into consideration the amplitude and timing. Therefore, muscle synergy analysis allows a reductionistic approach to examine, identify and compare basic neuromuscular mechanisms across different tasks (Chvatal et al., 2011) and populations (Kipp et al., 2014).

Multi joint movements simulating everyday activities require individuals to activate multiple muscle groups and avoid placing excess strain on a recently operated joint (Wright et al., 2015) allowing these movements to be performed in a safe manner during rehabilitation. Bilateral squats and drop vertical jumps (DVJ) are included in training protocols shown to decrease unfavourable biomechanical measures related to ACL injury in adult male athletes (Bizzini et al., 2013; Impellizzeri et al., 2013), ideally reducing the injury risk. Similarly, these tasks have been incorporated into rehabilitation programs for lower limb injuries (Wright et al., 2015) and clinical decision making protocols. Clinical measures often

include symmetry indices between injured and uninjured limbs (Adams et al., 2012; Paulos, Noyes, Grood, & Butler, 1991b). Simple visual symmetry tests comparing contralateral limbs require minimal resources making them an appealing clinical measure. However, this method is limited by several assumptions: i) all individuals use their two limbs equally, ii) individuals' limbs were symmetric prior to their injury and iii) pre-exhaustion tests are an accurate representation of fatigued state (Augustsson et al., 2004). Therefore, visual examinations of clinical tasks may not provide accurate assessments of patient performance (Adams et al., 2012; Paulos et al., 1991b). In order to use these tasks in clinical decision-making and rehabilitation programs, a better understanding of a 'normal' performance is required. By investigating the performance of an uninjured population, reference criteria can be established and used when making clinical decision for an injured individual.

In females, the majority of non-contact ACL injuries occur in their non-dominant limb (Brophy, Silvers, Gonzales, & Mandelbaum, 2010). Differences in muscle activations between non-dominant limbs have been identified in single leg tasks where the limbs have different roles (i.e. support leg vs. kicking leg) (Del Bel, Fairfax, Jones, Steele, & Landry, 2017; Ford, Myer, Schmitt, Uhl, & Hewett, 2011). However, the effects of limb dominance on neuromuscular control have not been investigated in a population of paediatric females completing a bilateral task where both limbs act as support legs. Potential effects of limb dominance must therefore be taken into account when establishing reference criteria for uninjured female youth.

As these tasks are frequently visually examined for limb symmetry (Adams et al., 2012; Paulos et al., 1991b), the first objective of this study was to determine if lower limb joint angles and moments differed between dominant and non-dominant limbs in young females performing squats and DVJs. These findings will support or challenge the relevance of limb symmetry as a measure in injured populations. Our second objective was to determine if muscle synergies in young females during bilateral squats and drop vertical jumps differ between dominant and non-dominant limbs and across tasks. Due to the bilateral nature of the tasks, we hypothesised that similar muscle synergies would be used in the dominant and non-dominant limbs of healthy paediatric females. Secondly, we hypothesised that the decent phase

of the DVJ would require greater contributions of the hamstrings and quadriceps muscles to account for the increased difficulty and effort required to stabilise the knee and hip joints relative to the squat (Hewett et al., 2005).

## **2. Methods**

### *2.1. Participants*

This study received approval from the University of Ottawa Research Ethics Board (H09-17-10); all participants provided informed written consent. Twenty-one athletic females (age: 13.0 +/- 1.69 yrs; BMI: 18.8 +/- 2.3 kg/m<sup>2</sup>; Tanner Stage: 3.1 +/- 0.94) were recruited from local organised sport associations. Participants were excluded if they had any history of lower limb injuries and pain in either limb on the day of testing. Sample size provided an effect size and power of 0.8 with  $\alpha = 0.05$ .

### *2.2. Set up*

Anthropometric data (pelvis, knee, and ankle width, height, and weight, leg length and thigh and shank circumference) were recorded followed by the placement of bipolar EMG surface electrodes (Trigno-16, Delsys Inc., Boston, USA) on the bellies of the gluteus medius (GMed), semitendinosus (ST), biceps femoris (BF), rectus femoris (RF), vastus medialis (VM) and lateralis (VL) and medial (MG) and lateral gastrocnemii (LG) of each limb according to SENIAM guidelines (Hermens et al., 2000). Knee flexion and extension, plantar flexion and hip abduction maximum voluntary isometric contractions (MVICs) were recorded using an isokinetic dynamometer (Systems 4 Pro, Biodex Medical Systems, New York, USA).

Marker trajectories of 84 retroreflective markers placed on anatomical landmarks were sampled at 200 Hz using a 10-camera infrared motion analysis system (8 Vero, 2 Vantage; Vicon, Oxford, UK). The supporting software (Nexus v2.7, Vicon, Oxford, UK) simultaneously recorded marker trajectories and ground reaction forces (GRF) from two force plates sampled at 2000 Hz (FP4060-08, Bertec Corporation, Columbus, OH, USA ).

### *2.3. Protocol*

Participants were instructed to perform the squats by standing with their feet at a comfortable width, hands on their head while squatting as low as possible and returning to their original position at a self-selected pace (Trulsson et al., 2015). DVJ were performed by stepping off a platform set to the height of the participant's tibial plateau, landing with one foot on each plate, squatting down and immediately performing a maximum height vertical jump (Shelburne et al., 2005). Participants successfully completed five trials of each task. For both tasks, trials were considered successful if the participant kept their balance, their feet were entirely on the force plate and if they properly returned to the upright position. For the squat, participants were also required to keep their hands on their head. Researchers intermittently asked the participant if they were experiencing any pain or discomfort.

#### *2.4. Data processing*

Marker trajectories and GRFs were filtered using a 4<sup>th</sup> order zero-lag low pass Butterworth filter at 6 Hz (Bisseling & Hof, 2006; Kristianslund et al., 2012). Hip, knee and ankle angles and moments in the frontal and sagittal planes were calculated using a modified cluster University of Ottawa Motion Analysis Model (Mantovani & Lamontagne, 2017). EMG waveforms were high-pass filtered at 20 Hz with a 2<sup>nd</sup> order dual-pass Butterworth filter, full-wave rectified, filtered with a 2<sup>nd</sup> order dual-pass low-pass Butterworth filter at 6 Hz and normalised to maximal EMG amplitude, identified using a 10 ms moving average of the MVIC trials.

Squat trials were time normalised using the pelvis origin, such that the cycle began with the participant upright, 50 % of the cycle occurred at maximum squat depth and the cycle finished when the participant returned to their upright position. Similarly, DVJ trials were time normalised to time spent on the force plate with the cycle starting when the participant landed; 50% of the cycle occurring at maximal pelvis depth and the cycle finishing at takeoff for the vertical jump. As such, for both tasks the first half of the cycle (1- 50%) corresponded to the descent, and the second half (51-100 %) to the ascent. Custom Matlab (R2018a, Mathworks Inc, Natick, USA) scripts extracted lower limb kinetics and kinematics and calculated integrated EMG (iEMG) as a measure of overall muscle activity (Pincivero, Aldworth, Dickerson, Petry, & Shultz, 2000) for each muscle.

## 2.5. Statistical analysis

The assumption of normality for continuous group means for joint angles, moments and muscle activations were evaluated using statistical parametric mapping (SPM). Normally distributed data were compared between groups (DOM, ND) using SPM independent  $t$ -tests, whereas a statistical non-parametric mapping (SnPM) independent  $t$ -test was used for data that rejected the assumption of normality. Statistical significance for continuous muscle activations required  $p < 0.005$ . Following a Bonferroni correction for multiple comparisons, statistical significance for continuous group means for joint angles and moments was defined as  $p < 0.025$ .

The assumption of normality for the discrete variables (iEMG, peak joint angles and moments) was evaluated through Shapiro-Wilk tests. For normally distributed data, differences were compared using independent  $t$ -tests whereas Mann-Whitney U tests were used for data that rejected the assumption of normality. Statistical significance for all tests was defined as  $p < 0.05$ . Discrete statistical analyses were conducted in Excel (2016, Microsoft, Washington, USA). A Benjamini-Hochberg correction for multiple comparisons was performed for all discrete results (peak joint angles and moments and iEMG) with a false discovery rate (FDR) of 0.05 (Benjamini & Hochberg, 1995).

## 2.6. Muscle synergy analysis

Muscles synergies were extracted for the dominant (DOM) and non-dominant (ND) limbs to assess the effects of limb dominance for each task using a concatenated non-negative matrix factorisation (CNMF) framework (Flaxman, Shourijeh, Alkjær, Krogsgaard, & Benoit, 2017; Shourijeh, Flaxman, & Benoit, 2016). A matrix with each participant's data formed an  $n \times m$  matrix where  $n$  is the number of time normalised frames (160 for squats, 100 for DV) and  $m$  is the number of muscles in one limb ( $m = 8$ ). A concatenated  $N \times m$  input matrix  $A$  was created for each of the subgroups for each of the tasks where  $N$  is equal to  $n$  times the number of participants in that group (DOM and ND;  $n = 18$ ).

The CNMF solver produced two output matrices through a series of optimisation steps (Shourijeh et al., 2016) where the first matrix  $S$  consisted of time invariant synergy vectors each specifying the

relative contributions of each muscle to that activation pattern. Each synergy vector  $S$  had a corresponding time-variant coefficient matrix  $C$ , and represented the relative scaling factor of that synergy vector for each participant throughout the task. Preliminary analyses revealed that fixing the synergy analysis to three synergies per population per task accounted for at least 80 % of the variance and insured balanced comparisons were being made. As such, three synergies were extracted for each task for each population and synergy vectors were reordered for each comparison based on visual inspection (Flaxman et al., 2017).

### *2.6.1. Synergy similarity across tasks:*

Task similarity was compared within limbs (comparisons; DOM Squat vs DOM DVJ and ND Squat vs ND DVJ). Synergy vectors were compared between tasks using intraclass correlation coefficients ( $ICC_{(1,k)}$ ) (Flaxman et al., 2017; McGraw & Wong, 1996), where vectors were considered statistically equivalent ( $ICC \geq 0.80$ ), statistically similar ( $0.60 \leq ICC < 0.80$ ) or uncorrelated ( $ICC < 0.60$ ). Time varying activation coefficient matrices  $C$  we compared using statistical parametric mapping (SPM) independent t-tests in Matlab (R2018a, Mathworks Inc, Natick, USA). Statistical significance required  $p < 0.05$ .

Cross-reconstruction of each task using the synergy vectors of the opposing task were used to compare the uniqueness of the respective task's synergies (Flaxman et al., 2017; Gizzi, Muceli, Petzke, & Falla, 2015). Similar synergy vectors across tasks would be expected to cross-reconstruct each other's input matrices and account for similar variance as that task's own synergy vectors (Flaxman et al., 2017). Cross-reconstruction was assessed by reconstructing the input matrix of one task with the vectors of the other (i.e. input matrix from DOM Squat was reconstructed using the synergy vectors from DOM DVJ). Variance accounted for (VAF) in the cross-reconstruction provided a measure of accuracy for the reconstruction.

### *2.6.2. Effects of limb dominance*

Limb dominance was compared within tasks (comparisons: DOM Squat vs ND Squat and DOM DVJ and ND DVJ). Synergy vectors and coefficients were compared using the same methods and criteria as those used for the comparisons across tasks. Similarly, the uniqueness of each limb's synergies was compared through cross-reconstructions.

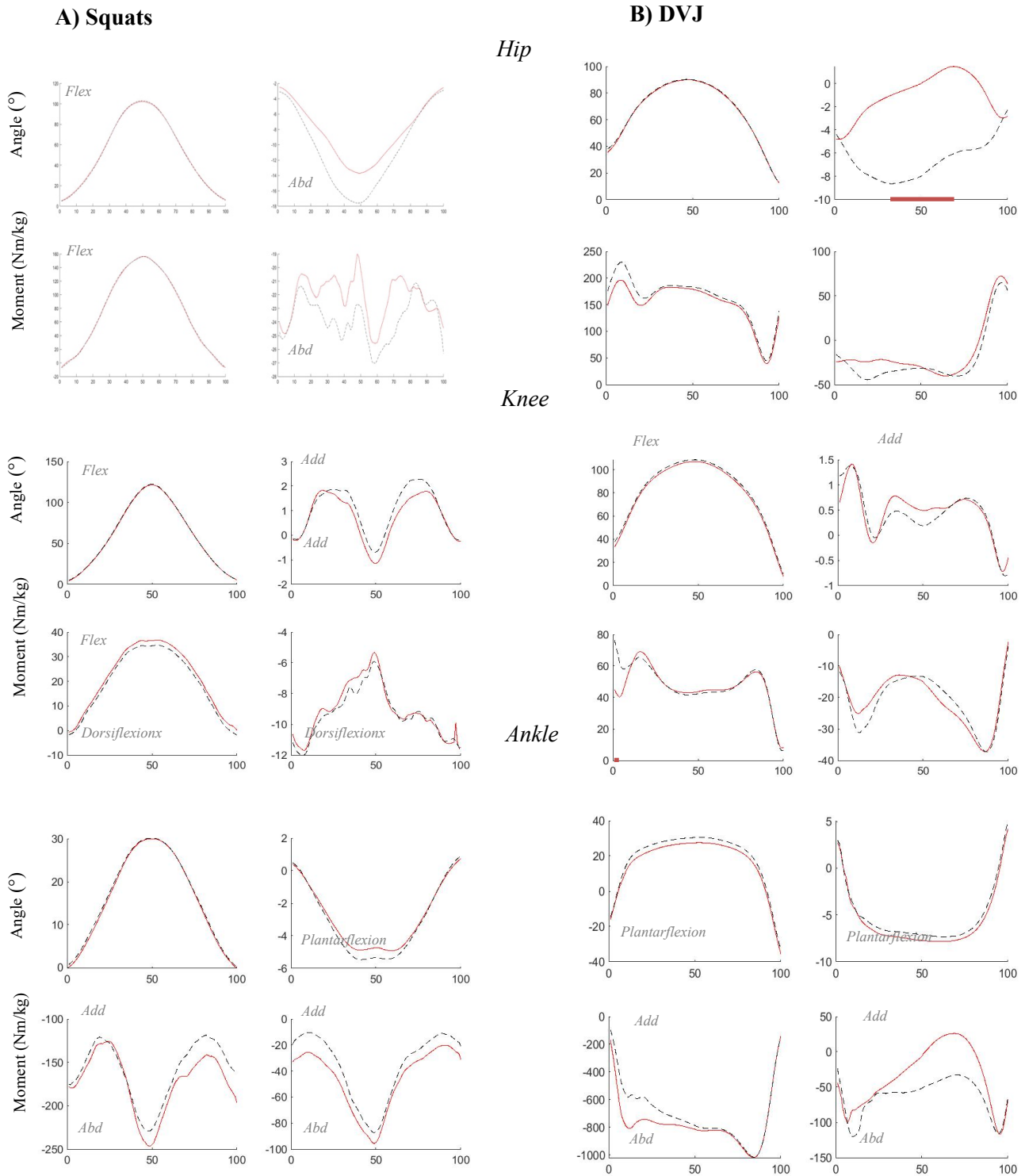
## **3. Results**

### 3.1: Kinematics, kinetics and iEMG:

There were no differences in peak joint angles or moments between dominant and non-dominant limbs during either of the tasks (Table 1). No differences in continuous hip, knee and ankle angles and moments in the sagittal plan were found in the squat (Figure 1). DVJ sagittal plan hip angles and frontal plane knee moments were significantly greater in the non-dominant limb (29.4-71.6% and 0.8-1.7% respectively).

**Table 1:** Descriptive and statistical test results for total time (s), peak flexion/extension angles (°) and moments (Nm/kg) for the hip, knee and ankle during the squatting and drop vertical jump (DVJ) tasks for dominant (DOM) and non-dominant (ND) limbs. No kinematic/kinetic comparisons were statistically significant following a Benjamini-Hochberg correction.

| Variables           | Mean (SD)         |                     | Normality |         | Equal Variances | Statistical Test | Statistical Significance |
|---------------------|-------------------|---------------------|-----------|---------|-----------------|------------------|--------------------------|
|                     | DOM               | ND                  | DOM       | ND      |                 |                  |                          |
| <b><i>Squat</i></b> |                   |                     |           |         |                 |                  |                          |
| Hip Angle           | 102.36<br>(12.24) | 103.12<br>(11.14)   | 0.024     | < 0.005 | 0.67            | Mann-Whitney U   | 0.86                     |
| Hip Moment          | 159.20<br>(10.66) | 159.32<br>(9.41)    | 0.50      | 1.00    | 0.58            | <i>t</i> -test   | 0.97                     |
| Knee Angle          | 121.42<br>(17.68) | 122.22<br>(17.25)   | 0.56      | 0.20    | 0.91            | <i>t</i> -test   | 0.88                     |
| Knee Moment         | 40.05<br>(5.47)   | 38.17<br>(4.62)     | 0.59      | 0.55    | 0.30            | <i>t</i> -test   | 0.24                     |
| Ankle Angle         | 30.58<br>(6.59)   | 30.83<br>(7.25)     | 0.55      | 0.68    | 0.64            | <i>t</i> -test   | 0.91                     |
| Ankle Moment        | -3.38<br>(40.18)  | 4.53<br>(53.74)     | 0.35      | 0.99    | 0.13            | <i>t</i> -test   | 0.59                     |
| <b><i>DVJ</i></b>   |                   |                     |           |         |                 |                  |                          |
| Hip Angle           | 89.79<br>(14.55)  | 90.18<br>(11.40)    | 0.96      | 1.00    | 0.28            | <i>t</i> -test   | 0.92                     |
| Hip Moment          | 195.47<br>(24.24) | 203.83<br>(26.37)   | 0.071     | 0.012   | 0.64            | Mann-Whitney U   | 0.33                     |
| Knee Angle          | 106.89<br>(14.84) | 108.49<br>(13.80)   | 0.30      | 0.45    | 0.69            | <i>t</i> -test   | 0.72                     |
| Knee Moment         | 77.57<br>(8.31)   | 78.92<br>(16.02)    | 0.35      | < 0.005 | 0.25            | Mann-Whitney U   | 0.76                     |
| Ankle Angle         | 29.57<br>(8.87)   | 32.10<br>(5.96)     | < 0.005   | 0.93    | 0.76            | Mann-Whitney U   | 0.50                     |
| Ankle Moment        | -61.08<br>(69.28) | -70.15<br>(44.5682) | < 0.005   | 0.79    | 0.82            | Mann-Whitney U   | 0.92                     |



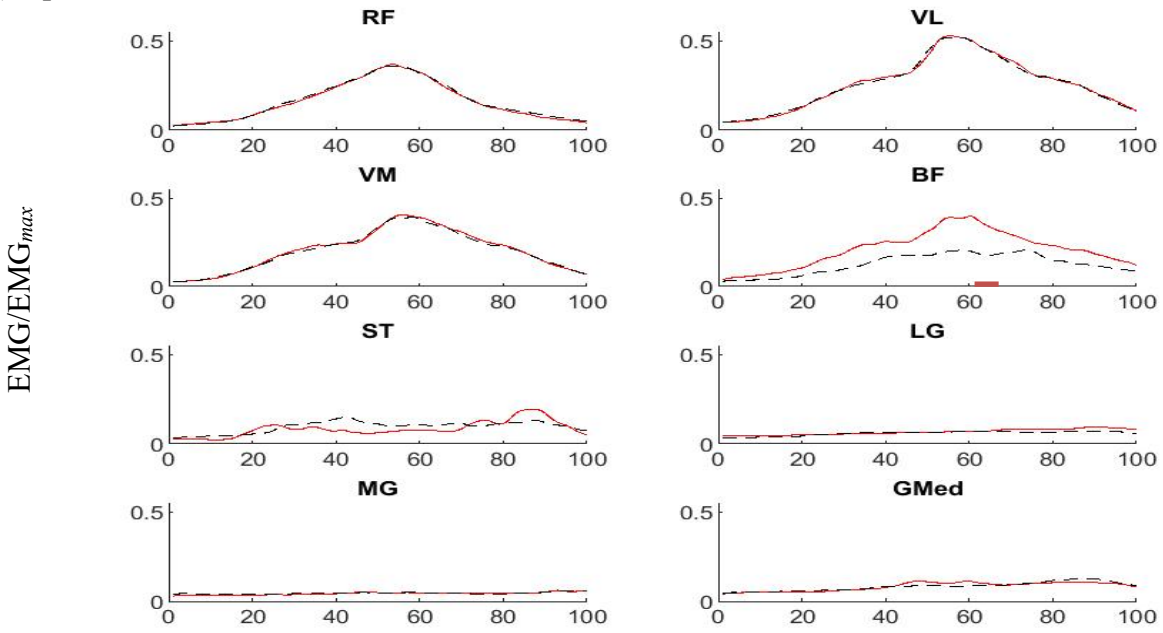
**Figure 1:** Group mean hip, knee and ankle joint angles and moments in the sagittal and frontal plans for dominant (red, solid line) and non-dominant (black, dashed line) limbs during squatting (A) and DVJ (B) tasks. Squats trials are time normalised from maximal to maximal pelvis origin height with minimal pelvis height occurring at 50% of squat cycle. DVJ trials are time normalised to time spent on both force plates with minimal pelvis height occurring at 50% of the cycle. Significant differences following a Bonferroni correction ( $p < 0.025$ ) are identified by the red bar on the x-axis.

The dominant limb BF had greater iEMG (squat ( $p = 0.021$ , Cohen's  $d = 0.76$ ) and DVJ ( $p = 0.035$ , Cohen's  $d = 0.37$ ) and continuous EMG (squat: 60.5-64.8% and DVJ 64.7-77.4, Figure 2). Whereas the non-dominant limb had greater ST iEMG during the DVJ ( $p = 0.015$ , Cohen's  $d = 0.76$ , Table 2) and greater continuous VL activation between 2.9 and 6.9% of the DVJ.

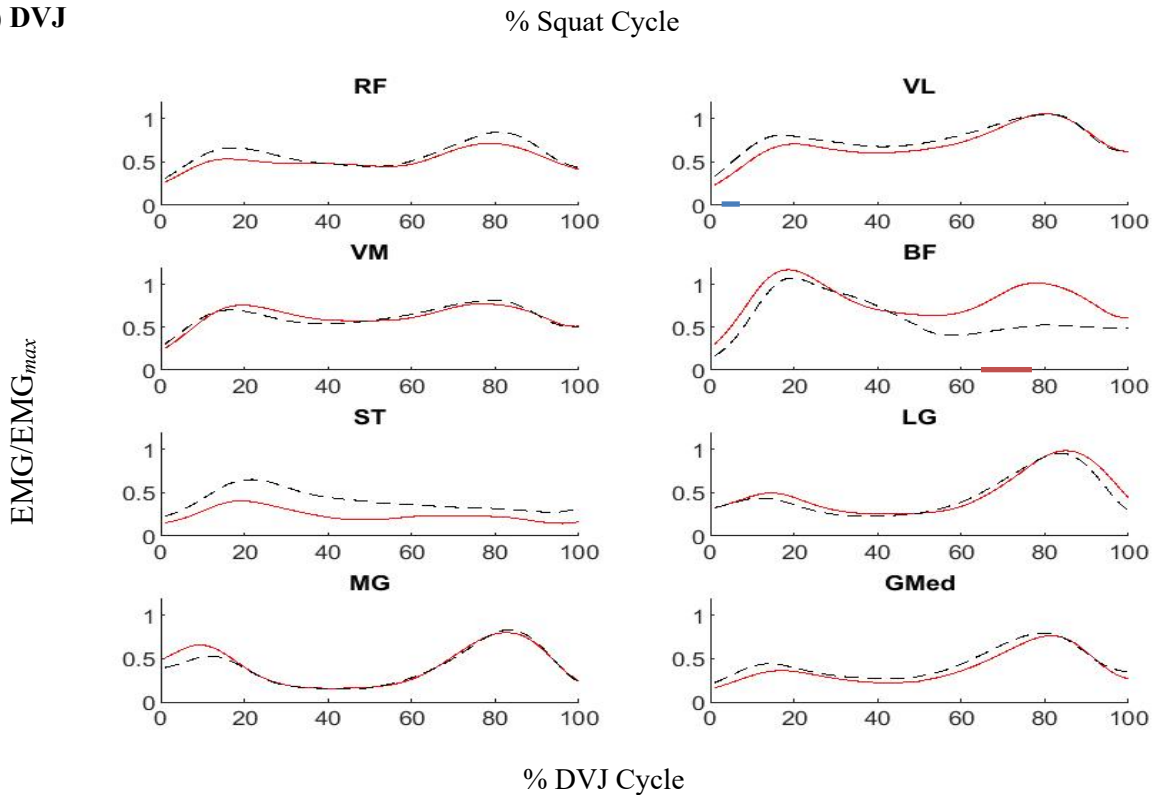
**Table 2:** Descriptive and statistical test results for integrated EMG (iEMG) for the squat and drop vertical jump (DVJ) tasks for dominant (DOM) and non-dominant (ND) limbs. Statistically significance differences following a Benjamini-Hochberg correction are denoted by an asterisk (\*).

| Muscles      | Mean (SD)        |                   | Normality |         | Equal Variances | Statistical Test | Statistical Significance |
|--------------|------------------|-------------------|-----------|---------|-----------------|------------------|--------------------------|
|              | DOM              | ND                | DOM       | ND      |                 |                  |                          |
| <i>Squat</i> |                  |                   |           |         |                 |                  |                          |
| <b>RF</b>    | 26.04<br>(11.30) | 26.55<br>(12.00)  | 0.056     | 0.042   | 0.80            | Mann-Whitney U   | 0.80                     |
| <b>VL</b>    | 42.53<br>(11.32) | 42.46<br>(9.68)   | 0.87      | 0.16    | 0.44            | <i>t</i> -test   | 0.98                     |
| <b>VM</b>    | 33.24<br>(11.62) | 32.69<br>(11.17)  | 0.35      | < 0.005 | 0.39            | Mann-Whitney U   | 0.96                     |
| <b>BF</b>    | 34.25<br>(19.80) | 20.55<br>(16.16)  | 0.70      | < 0.005 | 0.36            | Mann-Whitney U   | 0.021*                   |
| <b>ST</b>    | 13.77<br>(22.01) | 15.46<br>(12.58)  | < 0.005   | < 0.005 | 0.76            | Mann-Whitney U   | 0.14                     |
| <b>LG</b>    | 10.75<br>(4.07)  | 9.55<br>(3.76)    | 0.78      | 0.24    | 0.53            | <i>t</i> -test   | 0.33                     |
| <b>MG</b>    | 7.00<br>(2.97)   | 7.18<br>(2.41)    | 0.064     | 0.94    | 0.50            | <i>t</i> -test   | 0.83                     |
| <b>GMed</b>  | 13.36<br>(5.05)  | 13.29<br>(4.48)   | 0.06      | 0.36    | 0.54            | <i>t</i> -test   | 0.96                     |
| <i>DVJ</i>   |                  |                   |           |         |                 |                  |                          |
| <b>RF</b>    | 52.86<br>(15.80) | 59.23<br>(16.82)  | 0.41      | 0.73    | 0.97            | <i>t</i> -test   | 0.21                     |
| <b>VL</b>    | 72.44<br>(20.40) | 78.94<br>(14.34)  | 0.82      | 0.88    | 0.18            | <i>t</i> -test   | 0.24                     |
| <b>VM</b>    | 64.30<br>(18.55) | 63.92<br>(16.29)  | 0.56      | 0.82    | 0.49            | <i>t</i> -test   | 0.94                     |
| <b>BF</b>    | 83.02<br>(44.42) | 62.54<br>(65.04)  | 0.33      | < 0.005 | 0.74            | Mann-Whitney U   | 0.035*                   |
| <b>ST</b>    | 24.59<br>(17.04) | 41.19<br>(25.95)  | 0.0072    | 0.017   | 0.096           | Mann-Whitney U   | 0.015*                   |
| <b>LG</b>    | 49.99<br>(13.29) | 47.17<br>(16.03)  | 0.56      | 0.80    | 0.25            | <i>t</i> -test   | 0.54                     |
| <b>MG</b>    | 42.70<br>(20.78) | 40.07<br>(16.190) | < 0.005   | 0.028   | 0.45            | Mann-Whitney U   | 0.98                     |
| <b>GMed</b>  | 39.57<br>(14.42) | 45.07<br>(19.67)  | 0.15      | 0.067   | 0.068           | <i>t</i> -test   | 0.31                     |

**A) Squats**



**B) DVJ**



**Figure 2:** Group mean EMG activation patterns for dominant (red, solid line) and non-dominant (black, dashed line) limbs during squatting (A) and DVJ (B) tasks. Squats trials are time normalised from maximal to maximal pelvis origin height with minimal pelvis height occurring at 50% of squat cycle. DVJ trials are time normalised to time spent on both force plates with minimal pelvis height occurring at 50% of the cycle. Significant differences are identified by the red bar on the x-axis

### *3.1. Synergy Analysis - Effect of Limb Dominance*

#### ***Bilateral Squat***

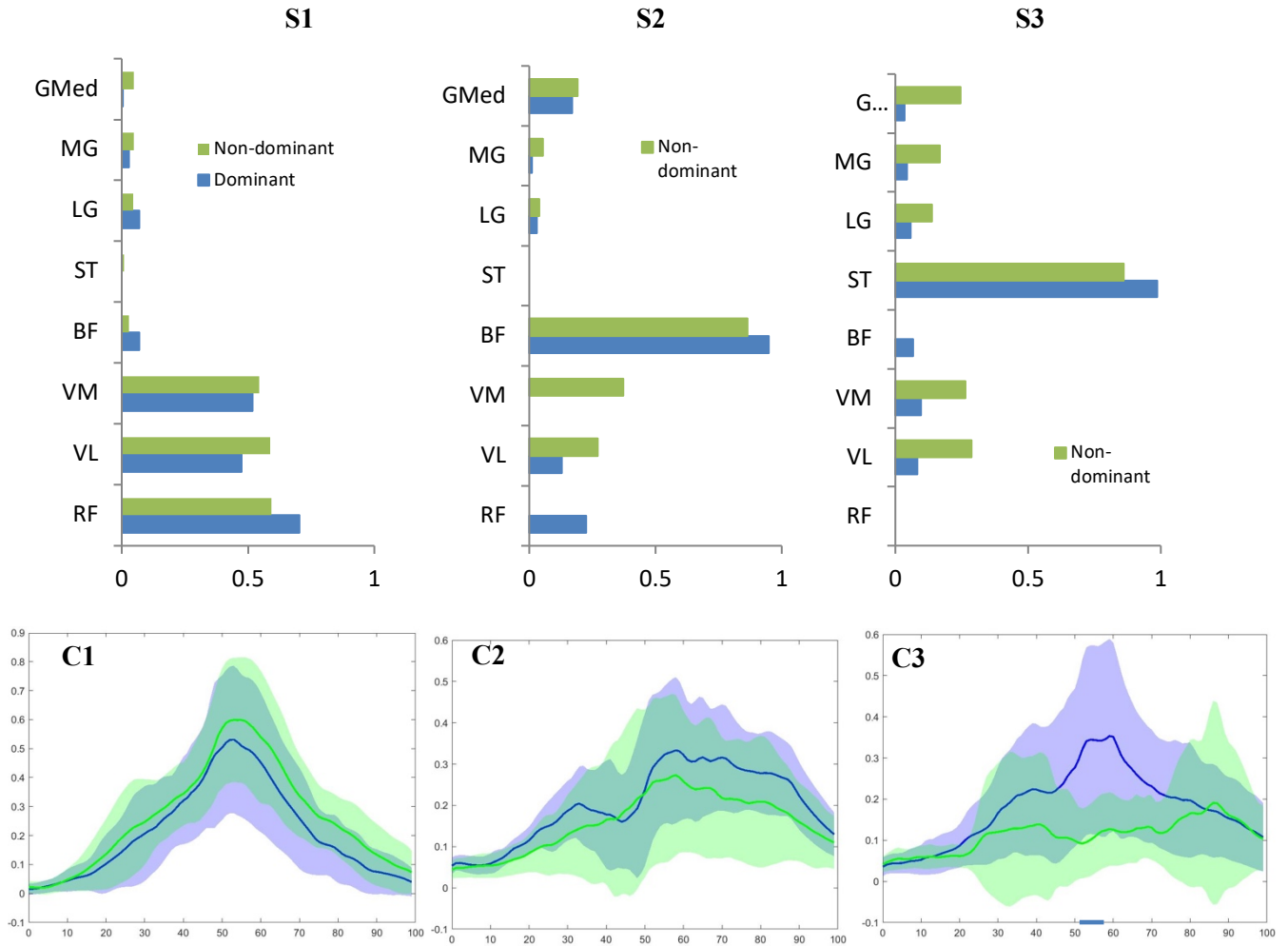
The three muscle synergy vectors used to reconstruct both the squat DOM and ND limb data were equivalent (ICCs > 0.80) with no differences in activation coefficients (Table 3). The first and second synergy vectors, primarily dominated by the quadriceps and BF reached peak activation at around the 50% mark of the squat cycle (Figure 3). While the third synergy vectors were equivalent, their scaling factors were different between the two limbs. Both vectors from the third pair of synergies had the largest contributions from the ST yet the dominant limb had greater activations in the first and last quarters of the task, whereas activation in the non-dominant limb was more evenly distributed throughout the movement.

#### ***Drop Vertical Jumps***

The first two synergies for the DVJ had equivalent vectors between the DOM and ND limbs of the uninjured population (Table 3). Similar to the squat, the first vector was primarily governed by the quadriceps, with higher activations in the first and last 25% of the movement cycle. The second set of vectors were governed by the BF and reached peak activations in the first and last 25% of the movement cycle. The third synergy had higher contributions in the medial and lateral gastrocnemii in the DOM limb with activations peaking in the first and last quarters of the task. In contrast the ND limb had a more even distribution of muscle contributions with activations peaking at around the 25% mark and slowly decreasing for the remainder of the task. However, cross-reconstruction of the squat dominant limb data using the non-dominant limb vectors accounted for greater variance than the reconstruction of the ND limb using the dominant limb synergies, whereas the opposite was seen for the DVJ.

**Table 3:** Summary of synergy analyses for between limb (DOM and ND ( $n= 21$ )) and within task (squat and DVJ) comparisons. Variance accounted for (VAF) each comparison, VAF following cross reconstruction and the amount of synergy vectors deemed equivalent, similar and poorly correlated for each comparison. SMP independent t-test identified significant differences in coefficients among equivalent synergy vectors. Statistical significance following required ( $p < 0.05$ ). Complete results of synergy analysis located in Appendix 1.

| Comparison                | VAF (%)               | xReconstruction VAF (%) | Synergy Vectors      |                   |                             | Synergy coefficients (Statistically significant differences in coefficients of equivalent synergy vectors)                                       |
|---------------------------|-----------------------|-------------------------|----------------------|-------------------|-----------------------------|--|
|                           |                       |                         | Equivalent Synergies | Similar Synergies | Poorly Correlated synergies |  |
| <b>Squat</b><br>DOM vs ND | DOM: 93.8<br>ND: 87.3 | DOM: 96.9<br>ND: 78.6   | 3                    | 0                 | 0                           | Yes,<br>-Synergy 3 (synergy vector primarily reflecting hamstring contributions): higher activations between 52 and 53% in the DOM limb.         |
| <b>DVJ</b><br>DOM vs ND   | DOM: 89.6<br>ND: 96.6 | DOM: 63.9<br>ND: 86.4   | 1                    | 1                 | 1                           | Yes,<br>- Synergy 1 (synergy vector primarily reflecting quadriceps contributions) : higher activations between 1-6 and 78-91 % in the DOM limb. |



**Figure 3:** Squat muscle synergies and respective weighting coefficients for DOM and ND limbs. Squat cycles were time normalised to 100% using height of pelvis origin. Significant differences were found between the DOM and ND coefficients (C3). For coefficient plots (C1, 2, 3) DOM coefficients are in blue and ND coefficients in green.

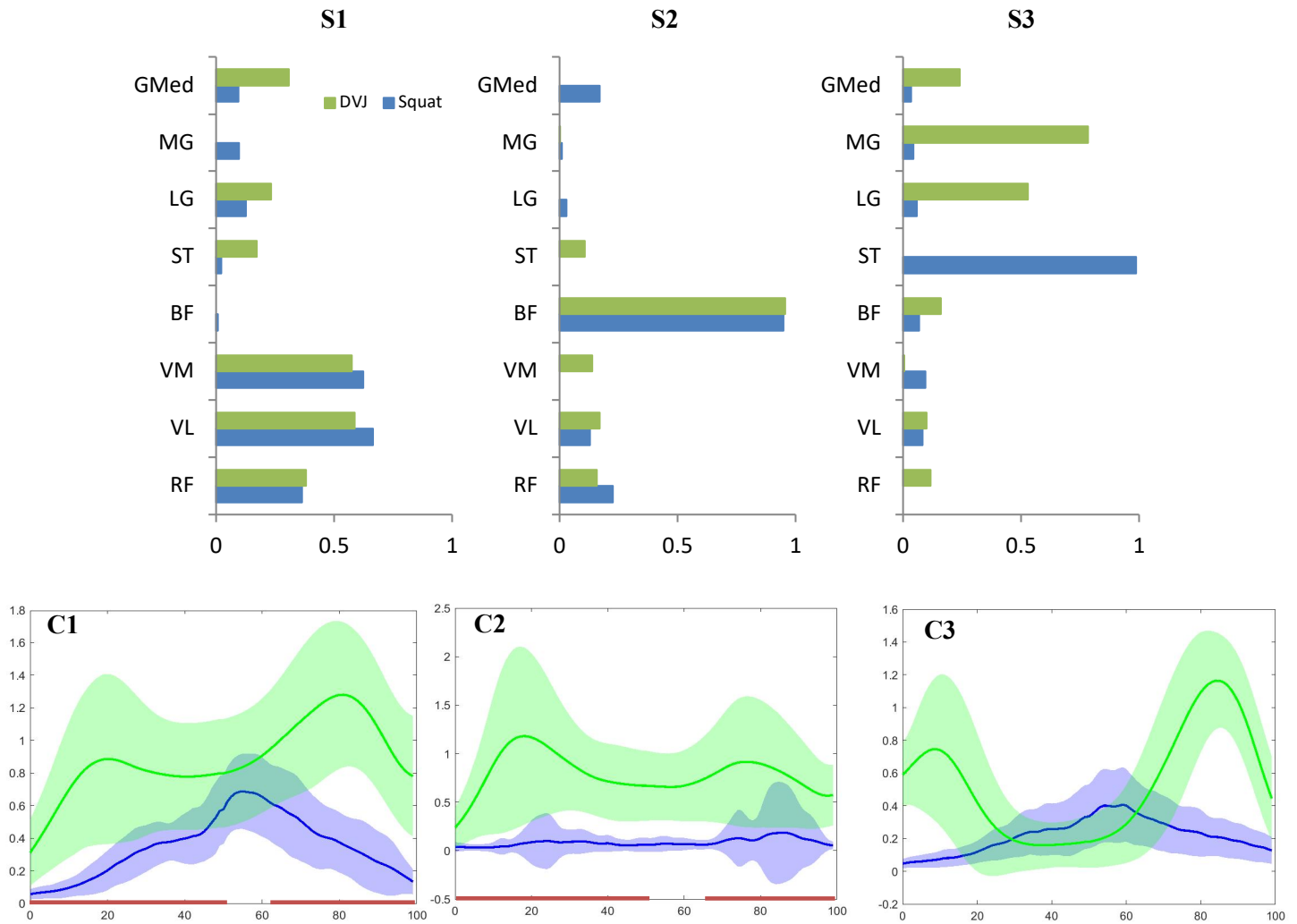
### 3.2. Synergy Analysis - Effect of Task

The majority of synergy vectors were consistent across tasks between limbs (Table 4). Each limb had a synergy vector governed by each of the hamstring muscles (BF and ST) and a third vector governed by the quadriceps muscle group (Figure 4). Differences were found between the scaling coefficients for all groups, due to the higher scaling factors for the DVJ and the timing of peak activations. Squat scaling factors were relatively consistent throughout the task compared to the DVJ yet, when they peaked, the

peak corresponded with peak knee flexion. Alternatively, greater variability was observed in the DVJ scaling factors with peaks either occurring at 25 or 75% but never at 50% of the task.

**Table 4:** Summary of synergy analyses for between task (squat and DVJ) and within limb (DOM and ND ( $n = 21$ )) comparisons. Variance accounted for (VAF) each comparison, VAF following cross reconstruction and the amount of synergy vectors deemed equivalent, similar and poorly correlated for each comparison. SMP independent t-test identified significant differences in coefficients among equivalent synergy vectors. Statistical significance following required ( $p < 0.05$ ). Complete results of synergy analysis located in Appendix 1.

| Comparison                 | VAF (%)                  | xReconstruction VAF (%)  | Synergy Vectors      |                   |                             | Synergy coefficients (Statistically significant differences in coefficients of equivalent synergy vectors)   |
|----------------------------|--------------------------|--------------------------|----------------------|-------------------|-----------------------------|--|
|                            |                          |                          | Equivalent Synergies | Similar Synergies | Poorly Correlated synergies |  |
| <b>DOM</b><br>Squat vs DVJ | Squat: 93.8<br>DVJ: 89.6 | Squat: 53.9<br>DVJ: 63.8 | 2                    | 0                 | 1                           | Yes,<br>- Quadriceps dominated synergy (Syn 1): higher activations between 1-48 and 56-100 % in the DVJ.<br>- BF dominated synergy (Syn 2): higher activations between 1-53 and 63-100 % in the DVJ. |
| <b>ND</b><br>Squat vs DVJ  | Squat: 87.3<br>DVJ: 96.6 | Squat: 71.0<br>DVJ: 88.0 | 1                    | 2                 | 0                           | Yes,<br>- BF governed synergy (Syn 2): higher activations between 1-78 % in the DVJ.   |



**Figure 4:** Squat and DVJ muscle synergies and SPM analysis on respective weighting coefficients for DOM limbs. DVJ were time normalized to 100% of time spent on force plate, squats cycles were time normalised to 100% using height of pelvis origin. Significant differences in coefficients (C) tested with SPM (t) statistic for independent measures and indicated by red line at the bottom of figures C1 and C2. For coefficient plots (C1, 2, 3) squat coefficients are in blue and DVJ coefficients in green.

#### **4. Discussion**

The purpose of this study was to compare movement patterns and muscle synergies in young athletic uninjured adolescent females performing bilateral squats and DVJs. Our results in part support our hypothesis as no differences were found in the kinetics and kinematics between the two limbs for the squat and minimal differences were found for the DVJ. However, we did observe greater hamstrings and quadriceps contributions during the DVJ relative to the squat.

##### *4.1. Effects of limb dominance*

The first task investigated was the bilateral squat, a movement primarily considered a quadriceps-dominant activity (Bynum et al., 1995; Salem et al., 2003), which is consistent with our findings where the quadriceps were eccentrically contracted during the descent phase to control the rate of descent of the body's center of mass (CoM), followed by concentric activation to raise the CoM. Descent phase muscle activations were equally distributed within the quadriceps muscle group, contradicting previous work that suggested greater activations in the vastus medialis and lateralis relative to the rectus femoris (Escamilla, 2001; Isear et al., 1997). This discrepancy could be due to the age and developmental stage of the population studied. Previous work comparing two-footed landings in children and adults demonstrated greater vastus medialis in children relative to adults (Russell, Croce, Swartz, & Decoster, 2007). As the participants in this study were going through puberty (Tanner Stage: 3.1 +/- 0.94), their muscle activation patterns may also be undergoing developmental changes, explaining the discrepancies when comparing their performance to an adult population. Hamstring activations increased as knee flexion increased, contributing to greater hip extension during the ascent portion of the movement (Escamilla, 2001; Isear et al., 1997). DOM and ND groups exhibited similar biomechanics, as no significant differences were found in joint angles and moments, supporting our first hypothesis. However, dominant limb BF activity was significantly greater in both tasks with a medium effect size in the squat and small effect size in the DVJ. Conversely, ST activity was greater in the non-dominant limb than the dominant limb with a medium effect size. While this difference only reached statistical significance in the DVJ, it was consistent across

tasks indicating the dominant limb may rely more on the BF and the non-dominant limb on the ST to complete movements.

The second objective of this study was to determine if lower limb muscles synergies differed between dominant and non-dominant limbs of young female adolescents. We hypothesised that due to the bilateral nature of the tasks, the relatively low demand and our participants having no history of lower limb injury, there would not be any differences between limbs. Our hypothesis was partly confirmed as all three squat synergy vectors were statistically equivalent between limbs. However, only one of the three pairs of vectors were equivalent for the DVJ, indicating different muscle patterns were used by the dominant and non-dominant limbs for this more dynamic and demanding task. The DVJ requires the absorption and generation of momentum for the two jumps whereas the squat is a more controlled task with no initial momentum to compensate for. This increased complexity may explain the difference in number of equivalent vectors within the tasks.

#### *4.2. Similarities across tasks*

While our analyses revealed few significant differences in lower limb kinematics and kinetics for both tasks, previous work has shown higher peak knee joint extension moments in the dominant limb (Edwards, Steele, Cook, Purdam, & McGhee, 2012) during landing, as well as between-limb differences in muscle activity magnitudes and timing during unanticipated side-cuts (Del Bel, Fairfax, Jones, Steele, & Landry, 2017). The varied demands of the two tasks may account for the different correlations between vectors: the squat's vectors were all correlated yet only two of the DVJ vectors were correlated. While both tasks required descending and ascending the body's center of mass, the movement is much more controlled in the squat relative to the higher velocities and momentum present in the DVJ. The decent phase of both tasks require lowering the CoM, however in addition to lowering the CoM in the DVJ, one also needs to control and absorb the potential energy from stepping off the platform.

Both limbs (DOM and ND) had consistent synergy vectors across tasks, indicating that similar patterns of muscle activity were used to accomplish the squat and DVJ. Muscle synergy vectors have been reported as being load direction dependent (Chvatal et al., 2011; Shelburne et al., 2006; Shourijeh et

al., 2016) supporting our observations of correlated vectors across similar movement tasks with consistent loading directions. This finding is also in line with that of previous work investigating various pairs of similar tasks (Cappellini et al., 2006; Flaxman et al., 2017).

#### *4.3. Limitations*

Minimal instructions were given for both tasks. While this ensured participants performed the tasks as naturally as possible, peak CoM descent was not standardised. Furthermore, both tasks studied were predominantly sagittal plane tasks, with limited demands in the frontal plane, therefore limiting the need to stabilise against transverse or rotational loads. Athletic young females were recruited to participate in this study, which may explain why the sample's average BMI was within the normal range (Government of Canada, 2004). This may limit the generalizability of the results to other populations with differing BMIs.

As the majority of ACL ruptures occur when the lower limb undergoes a sudden deceleration with shallow flexion angles prior to a change in direction (Sakane et al., 1999; Shimokochi & Shultz, 2008; Shin et al., 2009), further research should investigate muscle activity, joint kinetics and kinematics in young adolescent females during more challenging tasks such as a cutting maneuver.

#### *5. Conclusion*

This study demonstrated that squat muscle synergy performance is consistent between the dominant and non-dominant limbs of uninjured adolescent females. Both limbs had consistent synergy vectors across tasks, indicating that similar patterns of muscle activity were used to accomplish the squat and DVJ, supporting the load direction dependency of synergy vectors (Chvatal et al., 2011; Shelburne et al., 2006; Shourijeh et al., 2016). Synergy coefficients were larger for the DVJ than the squat, reflecting their demand dependency.

DVJ performance differed slightly between dominant and non-dominant sagittal plan hip angles and frontal plane knee moments, while muscle activity differed slightly between the two limbs with greater dominant limb BF activity briefly occurring in both tasks. These finding indicate dominant and

non-dominant limbs may employ different muscle activation patterns and strategies to accomplish the same task (Del Bel et al., 2017). Observed differences between similar movements with varied demands may be further amplified in tasks with varied difficulty such as cutting maneuvers that require stabilising against multi-directional loads. Differences between dominant and non-dominant limbs in uninjured youth indicate that limb symmetry, a clinical tool commonly used to assess rehabilitation and return to play (Adams et al., 2012; Paulos et al., 1991a), may not provide relevant results.

As such, future work should take into account limb dominance when investigating movement and muscle activation patterns in young females and include more challenging tasks.

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## Chapter 6. Manuscript 2

### **The effects of ACL injury on lower limb biomechanics and muscle synergies in youth females during squats and drop vertical jumps**

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## Abstract

**Background:** Young adolescent females are at the highest risk of sustaining an anterior cruciate ligament (ACL) injury, which may lead to modified movement and muscle activation patterns. The purpose of this study was to investigate both hip, knee and ankle sagittal plane joint angles and moments, and muscle activation patterns during clinically relevant tasks in young females with and without an ACL deficiency.

**Methods:** Fifteen female adolescents with ACL ruptures (ACLd) were matched for limb dominance with 15 uninjured female adolescent controls (CON). Participants completed bilateral squats and drop vertical jumps (DVJ) while lower limb electromyography, kinetics, kinematics data were collected. Muscle synergies were extracted using a concatenated non-negative matrix factorisation framework and compared within limbs, across tasks and between limbs within tasks using intraclass correlation coefficients and statistical parametric mapping.

**Results:** Peak hip flexion moment was greater in CON group for both tasks, although only statistically significant for the squat. Continuous hip flexor moments were larger in the control group for both tasks however, significant intervals were less than 5% of the movement cycles. The ACL deficient and contralateral limbs of ACL deficient participants exhibited greater variability in DVJ synergy vectors than in the squat task. When comparing across tasks, scaling coefficients were consistently higher for the DVJ for both populations.

**Conclusion:** Individuals with an ACL rupture exhibit greater variability in muscle activation patterns in both their limbs than their uninjured peers. Synergy vectors were consistent across tasks, indicating that they are load dependent, while the synergy scaling factors varied between tasks, reflecting their demand dependency. Synergy vectors between injured limbs correlated higher with each other than with controls indicating that potential changes following a unilateral injury may be present in both limbs.

## ***1. Introduction***

The anterior cruciate ligament (ACL) is one of the most frequently injured knee ligaments (Hewett, Di Stasi, & Myer, 2013) with injury rates varying by sport, sex and age (Prodromos, Han, Rogowski, Joyce, & Shi, 2007). Young adolescent females are at the highest risk of sustaining this injury, likely due to the developmental changes they are experiencing with puberty (Kiapour et al., 2016). When the ACL is ruptured, the knee experiences compromised structural stability and neuromuscular function (Krogsgaard et al., 2011). Individuals with ACL injuries often exhibit varied movement patterns and muscle activation strategies relative to their uninjured counterparts (Alkjær et al., 2003; Rudolph et al., 2001; Sinkjaer & Arendt-Nielsen, 1991; Williams et al., 2003). However, these adaptations have rarely been evaluated in the pediatric population.

Bilateral squats and drop vertical jumps (DVJ) included in training protocols are shown to decrease unfavourable biomechanical characteristics related to ACL injury in adult male athletes (Bizzini et al., 2013; Impellizzeri et al., 2013). Such tasks have been incorporated into rehabilitation programs for lower limb injuries (Wright et al., 2015). These multi joint movements simulate everyday activities, require individuals to activate multiple muscle groups and avoid excess strain on the recently operated joint (Wright et al., 2015) allowing movements to be performed in a safe manner during rehabilitation. An example of a framework for ACL rehabilitation guidelines was established by the *Multicenter Orthopaedic Outcomes Network* (MOON) group. The MOON group guidelines, developed for adult males and females, outline a five-phase progression beginning with preoperative benchmark recommendations, progressing through rehabilitation exercise suggestions and key stages, and finishing with the final phase of return-to-sport (Wright et al., 2015). Early stages include quarter-depth, progressing to full squats, mimicking sitting down and standing up from a chair (Wright et al., 2015). In contrast, DVJs are a more demanding and dynamic task that is not introduced until the fourth phase (Wright et al., 2015). This exercise progression from squats to jumps highlights the increased difficulty of remaining stable during plyometric tasks. The DVJ is used to identify athletes at risk of severe knee injuries by identifying athletes who land with valgus knee motion; from this, they are encouraged to

perform neuromuscular training emphasising ‘hip-knee-toe line’ positioning before participating in sporting activities (Hewett et al., 2005). While these tasks have been heavily studied (Aglietti, Ponteggia, & Giron, 2001; Alkjær, Henriksen, & Simonsen, 2011; Cereatti et al., 2011; Cheron, Bengoetxea, Pozzo, Bourgeois, & Drayc, 1997; Flaxman et al., 2017; Hase, Sako, Ushiba, & Chino, 2004; Isear, Erickson, & Worrell, 1997; MacLean, Taunton, Clement, Regan, & Stanish, 1999) and observed compensation strategies such as decreased squat depth, and hip and knee ranges of motion in ACLd populations identified (Button, Roos, & van Deursen, 2014; Trulsson et al., 2015) to our knowledge, no studies have investigated how squats and DVJs are performed by adolescent females with an ACL deficiency and how this performance may differ from that of uninjured adolescent females. ACL injuries and potential compensations strategies may alter individual muscle activity yet, they may also modify overall muscle activation patterns. As such, to compressively compare populations with and without an ACL deficiency, individual muscle activations and overall muscle activations must be compared. Muscle synergy analysis provides an alternate form of analysis that addresses the contributions of various muscles over an entire movement pattern (Ting, 2004). Muscle synergy analysis looks to identify patterns of muscle activation, while taking in to account that each muscle can be simultaneously activated by multiple synergies (Tresch et al., 1999).

Conceptually, muscle synergies consist of a time invariant synergy vector and a time-varying activation coefficient. The synergy vector represents each muscle’s relative contribution to that synergy whereas the activation coefficient provides a scaling factor, taking into consideration the amplitude and timing. Therefore, muscle synergy analysis allows a reductionistic approach to examine, identify and compare basic neuromuscular mechanisms across different tasks (Chvatal et al., 2011) and populations (Kipp et al., 2014).

As these tasks are frequent visually examined for limb symmetry (Adams et al., 2012; Paulos et al., 1991b), the objective of this study was to determining if lower limb movement patterns and muscle synergies used to accomplish squats and DVJ differ between young females with and without an ACL injury. It was hypothesised that ACL deficient (ACLd) individuals would employ an avoidance strategy

by reducing their hip and knee ranges of motion and joint moments (Button et al., 2014) to minimize their squat depth. We also expected synergy vectors to differ between ACLd and uninjured groups due to previous work identifying neuromuscular differences in adults with and without ACL injuries (Alkjaer, Simonsen, Jørgensen, & Dyhre-Poulsen, 2003; Rudolph et al., 2001; Sinkjaer & Arendt-Nielsen, 1991; Williams, Barrance, Snyder-Mackler, et al., 2003).

## **2. Methods**

### *2.1. Participants*

This study received approval from the University of Ottawa Research Ethics Board (H09-17-10) and from CHEO's Ethics Board (17/74X); all participants provided informed written consent. Fifteen females with a confirmed ACL rupture/deficiency (ACLd, age: 15.0 +/- 1.73 yrs; BMI: 22.3 +/- 2.39 kg/m<sup>2</sup>; Tanner Stage 4.1 +/- 0.96; time since injury: 7.9 +/- 12.3 months) were recruited through CHEO and 15 uninjured females (CON, age: 13.6 +/- 1.55 yrs; BMI: 18.9 +/- 2.14 kg/m<sup>2</sup>; Tanner Stage 3.5 +/- 0.74) were recruited from local organised sport associations. CON participants were excluded if they had any history of lower limb injuries and pain in either limb on the day of testing.

### *2.2. Participant preparation*

Anthropometric data (pelvis, knee, and ankle width, height, and weight, leg length and thigh and shank circumference) were recorded followed by the placement of bipolar EMG surface electrodes (Trigno-16, Delsys Inc., Boston, USA) on the bellies of the gluteus medius (GMed), semitendinosus (ST), biceps femoris (BF), rectus femoris (RF), vastus medialis (VM) and lateralis (VL) and medial (MG) and lateral gastrocnemii (LG) of each limb according to SENIAM guidelines (Hermens et al., 2000). Knee flexion and extension, plantar flexion and hip abduction maximum voluntary isometric contractions (MVICs) were recorded using an isokinetic dynamometer (Systems 4 Pro, Biodex Medical Systems, New York, USA).

Marker trajectories of 84 retroreflective markers placed on anatomical landmarks were sampled at 200 Hz using a 10-camera infrared motion analysis system (8 Vero, 2 Vantage; Vicon, Oxford, UK). The

supporting software (Nexus v2.7, Vicon, Oxford, UK) simultaneously recorded marker trajectories and ground reaction forces (GRF) from two force plates sampled at 2000 Hz (FP4060-08, Bertec Corporation, Columbus, OH, USA).

### *2.3. Protocol*

Participants were instructed to perform the squats by standing with their feet at a comfortable width, hands on their head while squatting as low as possible and returning to their original position at a self-selected pace (Trulsson et al., 2015). DVJ were performed by stepping off a platform set to the height of the participant's tibial plateau, landing with one foot on each plate, squatting down and immediately performing a maximum height vertical jump (Shelburne et al., 2005). Participants successfully completed five trials of each task. For both tasks, trials were considered successful if the participant kept their balance, their feet were entirely on the force plate and if they properly returned to the upright position. For the squat, participants were also required to keep their hands on their head

### *2.4. Data processing*

Marker trajectories and GRFs were filtered using a 4<sup>th</sup> order zero-lag low pass Butterworth filter at 6 Hz (Bisseling & Hof, 2006; Kristianslund et al., 2012). Hip, knee and ankle angles and moments in the frontal and sagittal planes were calculated using a modified cluster University of Ottawa Motion Analysis Model (Mantovani & Lamontagne, 2017). EMG waveforms were high-pass filtered at 20 Hz with a 2<sup>nd</sup> order dual-pass Butterworth filter, full-wave rectified, filtered with a 2<sup>nd</sup> order dual-pass low-pass Butterworth filter at 6 Hz and normalised to maximal EMG amplitude, identified using a 10 ms moving average of the MVIC trials.

Squats trials were time normalised using the pelvis origin, such that the cycle began with the participant upright, 50 % of the cycle occurred at maximum squat depth and the cycle finished when the participant returned to their upright position. Similarly, DVJ trials were time normalised to time spent on the force plate with the cycle starting when the participant landed; 50% of the cycle occurring at maximal pelvis depth and the cycle finishing at takeoff for the vertical jump. As such, for both tasks the first half of

the cycle (1- 50%) corresponded to the descent, and the second half (51-100 %) to the ascent parts of the cycles. Custom Matlab (R2018a, Mathworks Inc, Natick, USA) scripts extracted lower limb kinetics and kinematics and calculated integrated EMG (iEMG) as a measure of overall muscle activity (Pincivero et al., 2000) for each muscle as the area under the activation curve.

## 2.5. Statistical analysis

Preliminary results indicated minor differences between dominant and non-dominant limbs in the uninjured controls (Study 1). To account for this, a group of controls matched for limb dominance to the injured population (CON) was used for comparisons looking at the effect of ACL injury (comparisons: CON vs ACLd, CON vs ACLc, ACLd vs ACLc).

### 2.5.1. Kinetics, Kinematics and iEMG analysis

The assumption of normality for continuous group means for joint angles, moments and muscle activations were evaluated using statistical parametric mapping (SPM). Normally distributed data were compared between groups (ACLd, CON) using SPM independent *t*-tests, whereas a statistical non-parametric mapping (SnPM) independent *t*-test was used for data that rejected the assumption of normality. Statistical significance for continuous muscle activations was defined as  $p < 0.05$ . Following a Bonferroni correction for multiple comparisons, statistical significance for continuous group means for joint angles and moments was defined as  $p < 0.025$ .

The assumption of normality for the discrete variables (iEMG, peak joint angles and moments) was evaluated through Shapiro-Wilk tests. For normally distributed data, differences were compared using independent *t*-tests whereas Mann-Whitney U tests were used for data that rejected the assumption of normality. Statistical significance for all tests was defined as  $p < 0.05$ . A Benjamini-Hochberg correction for multiple comparisons was performed for all discrete results (peak joint angles and moments and iEMG) with a false discovery rate (FDR) of 0.05 (Benjamini & Hochberg, 1995). Discrete statistical analyses were conducted in Excel (2016, Microsoft, Washington, USA).

### 2.5.2. Muscle synergy analysis

Muscles synergies were extracted for each group (ACLd, ACLc and CON), to assess the effects of ACL status (injured vs control) for each task using a concatenated non-negative matrix factorisation (CNMF) framework (Flaxman, Shourijeh, Alkjær, Krogsgaard, & Benoit, 2017; Shourijeh, Flaxman, & Benoit, 2016). A matrix with each participant's data formed an  $n \times m$  matrix where  $n$  is the number of time normalised frames (160 for squats and 100 for DVJ) and  $m$  is the number of muscles in one limb ( $m = 8$ ). Three ACLd participants were excluded from the synergy analysis due to incomplete data sets. A concatenated  $N \times m$  input matrix  $A$  was created for each of the subgroups for each of the tasks where  $N$  is equal to  $n$  times the number of participants in that group (ACLd, ACLc and CON;  $n = 15$ ).

The CNMF solver produced two output matrices through a series of optimisation steps (Shourijeh et al., 2016) where the first matrix  $S$  consisted of time invariant synergy vectors each specifying the relative contributions of each muscle to that activation pattern. Each synergy vector  $S$  had a corresponding time-variant coefficient matrix  $C$ , and represented the relative scaling factor of that synergy vector for each participant throughout the task. Preliminary analyses revealed that fixing the synergy analysis to three synergies per population per task accounted for at least 80 % of the variance and insured balanced comparisons were being made. As such, three synergies were extracted for each task for each population and synergy vectors were reordered for each comparison based on visual inspection (Flaxman et al., 2017). Statistical analyses compared between limbs (comparisons; CON vs ACLd, CON vs ACLc, ACLd vs ACLc) and between tasks (comparison; squat vs DVJ).

#### 2.5.2.1. Synergy similarity across tasks:

Task similarity was compared within limbs (comparisons; CON Squat vs CON DVJ, ACLd Squat vs ACLd DVJ and ACLc Squat vs ACLc DVJ). Synergy vectors were compared between tasks using intraclass correlation coefficients ( $ICC_{(1,k)}$ ) (Flaxman et al., 2017; McGraw & Wong, 1996), where vectors were considered statistically equivalent ( $ICC \geq 0.80$ ), statistically similar ( $0.60 \leq ICC < 0.80$ ) or uncorrelated ( $ICC < 0.60$ ). Time varying activation coefficient matrices  $C$  we compared using statistical

parametric mapping (SPM) independent t-tests in Matlab (R2018a, Mathworks Inc, Natick, USA). Statistically significance following a Bonferroni correction required  $p < 0.0167$ .

Cross-reconstruction of each task using the synergy vectors of the opposing task were used to compare the uniqueness of the respective task's synergies (Flaxman et al., 2017; Gizzi, Muceli, Petzke, & Falla, 2015). Similar synergy vectors across tasks would be expected to cross-reconstruct each other's input matrices and account for similar variance as that task's own synergy vectors (Flaxman et al., 2017). Cross reconstruction was assessed by reconstructing the input matrix of one limb with the vectors of the other (i.e. input matrix from ACLd Squat was reconstructed using the synergy vectors from ACLd DVJ). Variance accounted for (VAF) in the cross-reconstruction provided a measure of accuracy for the reconstruction.

#### 2.5.2.2. *Effects of limb*

Synergy vectors and coefficients were compared using the same methods and criteria as those used for the comparisons across tasks. Similarly, the uniqueness of each limb's synergies was compared through cross-reconstructions.

### 3. *Results*

Control participants completed the squat significantly faster ( $p = 0.023$ , Cohen's  $d = 0.88$ ) and with greater continuous and peak hip extension moments (50.0-54.9%, Figure 1;  $p = 0.0019$ , Cohen's  $d = 1.25$ , Table 1) than the participants with an ACL deficiency. DVJ Frontal plan hip moments were greater in the control group (19.6-21.6 and 32.3-39.2 %) while sagittal plan moments were greater in the ACLd group (99-100%, Figure 1). There were no differences in peak hip, knee and ankle joint angles and moments between groups during the DVJ (Table 1). ACLd GMed iEMG ( $p = 0.0014$ , Cohen's  $d = 1.23$ , Table 2) and continuous activation (6.8-22.8, 27.2-29.0 and 54.9-71.1 %, Figure 2) were greater than CON during the squat. MG iEMG ( $p = 0.0028$ , Cohen's  $d = 1.20$ ,) and continuous activation (84.6-86.2%) during the squat. Continuous MG activations were also greater in the DVJ (82.3-99.0%). No differences in iEMG were observed between groups during the DVJ (Table 2, Figure 2).

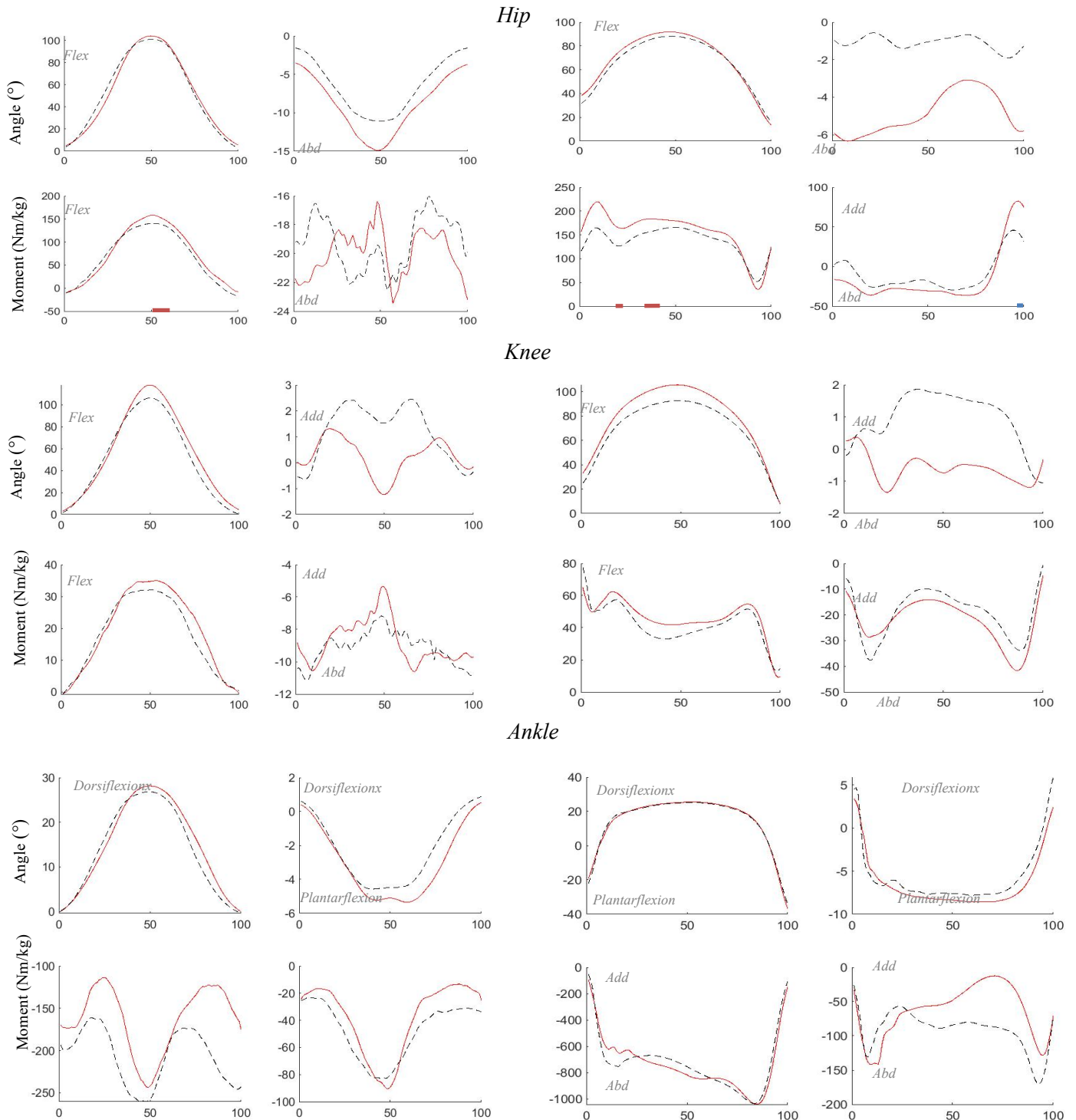
**Table 1:** Descriptive and statistical test results for total time (s), peak flexion/extension angles (°) and moments (Nm/kg) for the hip, knee and ankle during the squatting and drop vertical jump (DVJ) tasks for control (CON) and ACL deficient (ACLd) groups. Significant differences following a Benjamini-Hochberg correction denoted by an asterisk (\*).

| Variables           | Mean (SD)         |                   | Normality |        | Equal Variances | Statistical Test | Statistical Significance |
|---------------------|-------------------|-------------------|-----------|--------|-----------------|------------------|--------------------------|
|                     | CON               | ACLd              | CON       | ACLd   |                 |                  |                          |
| <b><i>Squat</i></b> |                   |                   |           |        |                 |                  |                          |
| Hip Angle           | 104.23<br>(8.91)  | 101.21<br>(13.99) | 0.74      | < 0.05 | 0.55            | Mann-Whitney U   | 0.56                     |
| Hip Moment          | 160.62<br>(9.49)  | 143.86<br>(16.39) | 0.42      | 0.84   | 0.086           | <i>t</i> -test   | 0.0019*                  |
| Knee Angle          | 118.22<br>(17.17) | 106.32<br>(16.15) | 0.68      | 0.55   | 0.68            | <i>t</i> -test   | 0.061                    |
| Knee Moment         | 38.23<br>(5.69)   | 34.83<br>(8.29)   | 0.62      | 0.31   | 0.11            | <i>t</i> -test   | 0.20                     |
| Ankle Angle         | 28.57<br>(5.75)   | 27.24<br>(6.81)   | 0.98      | 0.089  | 0.31            | <i>t</i> -test   | 0.57                     |
| Ankle Moment        | 15.58<br>(11.48)  | -30.05<br>(74.42) | 0.48      | 0.42   | 0.00010         | Mann-Whitney U   | 0.12                     |
| Total Time          | 2.57<br>(0.73)    | 3.18<br>(0.64)    | 0.27      | 0.36   | 0.33            | <i>t</i> -test   | 0.023*                   |
| <b><i>DVJ</i></b>   |                   |                   |           |        |                 |                  |                          |
| Hip Angle           | 91.86<br>(13.68)  | 88.27<br>(21.58)  | 0.16      | 0.029  | 0.24            | Mann-Whitney U   | 0.71                     |
| Hip Moment          | 200.31<br>(28.31) | 180.14<br>(29.00) | 0.11      | 0.46   | 0.99            | <i>t</i> -test   | 0.057                    |
| Knee Angle          | 105.72<br>(16.34) | 92.71<br>(20.45)  | 0.26      | 0.31   | 0.62            | <i>t</i> -test   | 0.064                    |
| Knee Moment         | 76.19<br>(9.54)   | 70.50<br>(13.19)  | 0.29      | 0.078  | 0.71            | <i>t</i> -test   | 0.19                     |
| Ankle Angle         | 27.53<br>(9.64)   | 26.82<br>(5.43)   | < 0.05    | 0.89   | 0.54            | Mann-Whitney U   | 0.32                     |
| Ankle Moment        | -76.04<br>(43.09) | -64.47<br>(35.56) | 0.35      | 0.44   | 0.46            | <i>t</i> -test   | 0.43                     |
| Total Time          | 0.63<br>(0.14)    | 0.62<br>(0.11)    | 0.080     | 0.74   | 0.19            | <i>t</i> -test   | 0.95                     |

A) Squats Master's Thesis

B) DVJ

L Kemp

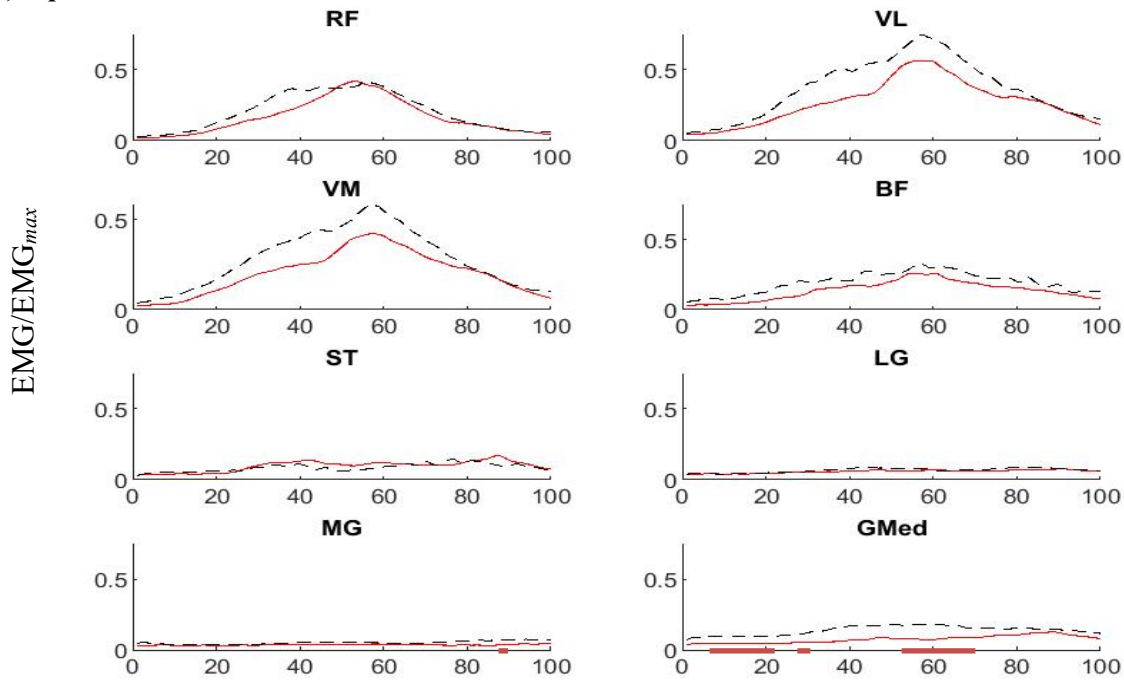


**Figure 1:** Group mean hip, knee and ankle joint angles and moments in the sagittal and frontal planes for uninjured control (red, solid line) and ACL deficient (black, dashed line) groups during squatting (A) and DVJ (B) tasks. Squats trials are time normalised from maximal to maximal pelvis origin height with minimal pelvis height occurring at 50% of squat cycle. DVJ trials are time normalised to time spent on both force plates with minimal pelvis height occurring at 50% of the cycle. Significant differences following a Bonferroni correction ( $p < 0.025$ ) and noted by the red bar on the x-axis.

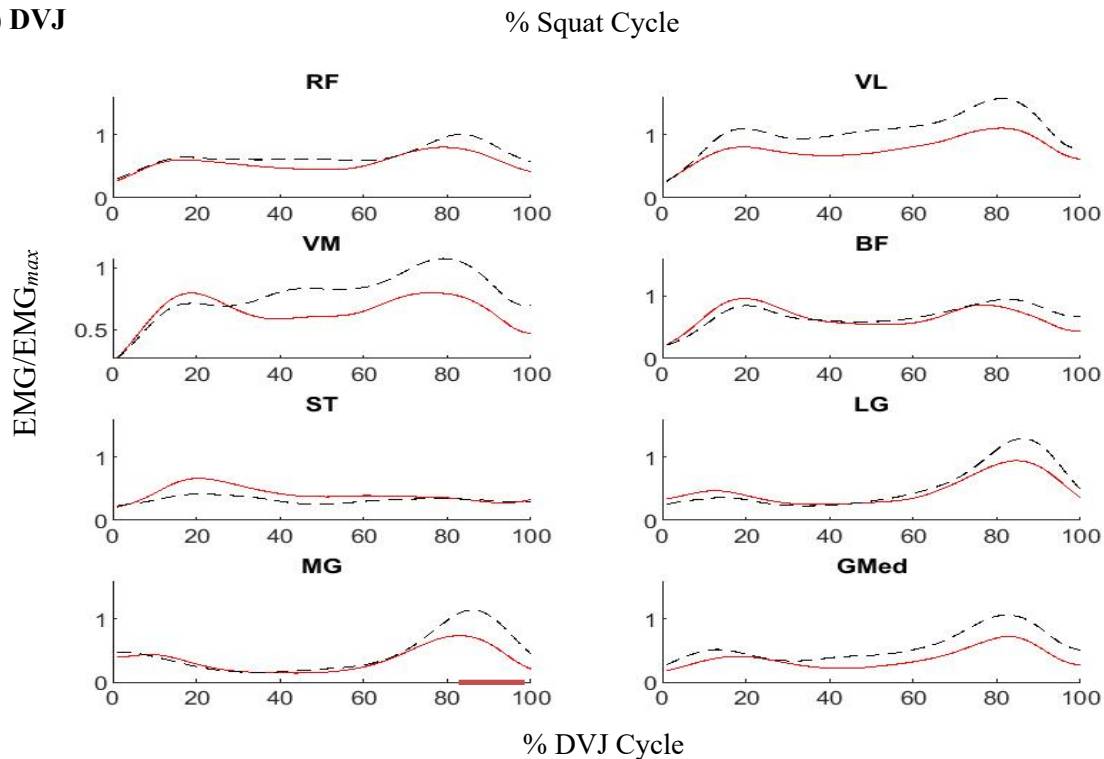
**Table 2:** Descriptive and statistical test results for integrated EMG (iEMG) for the squat and drop vertical jump (DVJ) tasks for control (CON) and ACL deficient (ACLd) groups. Statistically significant differences following a Benjamini-Hochberg correction are denoted by an asterisk (\*).

| Muscles      | Mean (SD)        |                   | Normality |         | Equal Variances | Statistical Test | Statistical Significance |
|--------------|------------------|-------------------|-----------|---------|-----------------|------------------|--------------------------|
|              | CON              | ACLd              | CON       | ACLd    |                 |                  |                          |
| <i>Squat</i> |                  |                   |           |         |                 |                  |                          |
| <b>RF</b>    | 27.36<br>(13.68) | 33.16<br>(17.62)  | 0.032     | 0.36    | 0.20            | Mann-Whitney U   | 0.41                     |
| <b>VL</b>    | 44.21<br>(11.41) | 60.24<br>(28.93)  | 0.31      | 0.67    | 0.0038          | Mann-Whitney U   | 0.12                     |
| <b>VM</b>    | 33.27<br>(13.79) | 45.99<br>(23.20)  | 0.038     | 0.80    | 0.042           | Mann-Whitney U   | 0.12                     |
| <b>BF</b>    | 22.17<br>(17.56) | 31.32<br>(28.59)  | 0.049     | 0.0016  | 0.46            | Mann-Whitney U   | 0.30                     |
| <b>ST</b>    | 15.76<br>(13.72) | 14.05<br>(9.67)   | 0.00072   | 0.0028  | 0.44            | Mann-Whitney U   | 0.93                     |
| <b>LG</b>    | 9.58<br>(4.06)   | 10.97<br>(4.69)   | 0.81      | 0.18    | 0.72            | <i>t</i> -test   | 0.39                     |
| <b>MG</b>    | 5.76<br>(1.62)   | 8.27<br>(2.48)    | 0.32      | 0.95    | 0.12            | <i>t</i> -test   | 0.0028*                  |
| <b>GMed</b>  | 12.17<br>(3.44)  | 22.43<br>(11.25)  | 0.31      | 0.13    | 0.0021          | Mann-Whitney U   | 0.0014*                  |
| <i>DVJ</i>   |                  |                   |           |         |                 |                  |                          |
| <b>RF</b>    | 56.79<br>(16.90) | 70.19<br>(26.94)  | 0.69      | 0.13    | 0.012           | Mann-Whitney U   | 0.15                     |
| <b>VL</b>    | 79.13<br>(14.97) | 105.56<br>(43.57) | 0.49      | 0.11    | <0.0001         | Mann-Whitney U   | 0.14                     |
| <b>VM</b>    | 66.28<br>(16.72) | 84.06<br>(35.32)  | 0.32      | 0.58    | 0.0049          | Mann-Whitney U   | 0.23                     |
| <b>BF</b>    | 67.68<br>(52.68) | 76.24<br>(58.94)  | 0.049     | 0.012   | 0.73            | Mann-Whitney U   | 0.77                     |
| <b>ST</b>    | 41.71<br>(26.33) | 36.04<br>(25.17)  | 0.026     | 0.012   | 0.81            | Mann-Whitney U   | 0.32                     |
| <b>LG</b>    | 47.83<br>(13.07) | 55.21<br>(16.15)  | 0.57      | 0.99    | 0.45            | <i>t</i> -test   | 0.18                     |
| <b>MG</b>    | 35.74<br>(12.49) | 45.91<br>(10.35)  | 0.56      | 0.28    | 0.82            | <i>t</i> -test   | 0.022                    |
| <b>GMed</b>  | 38.45<br>(18.34) | 54.43<br>(35.22)  | 0.0022    | 0.00022 | 0.47            | Mann-Whitney U   | 0.089                    |

**A) Squats**



**B) DVJ**



**Figure 2:** Group mean EMG activation patterns for uninjured control (red, solid line) and ACL deficient (black, dashed line) groups during squatting (A) and DVJ (B) tasks. Squats trials are time normalised from maximal to maximal pelvis origin height with minimal pelvis height occurring at 50% of squat cycle. DVJ trials are time normalised to time spent on both force plates with minimal pelvis height occurring at 50% of the cycle. Significant differences following a Bonferroni correction denoted by a red line on the x-axis.

### 3.2. Synergy Analysis -Effect of ACL Injury State

#### ***Bilateral Squat***

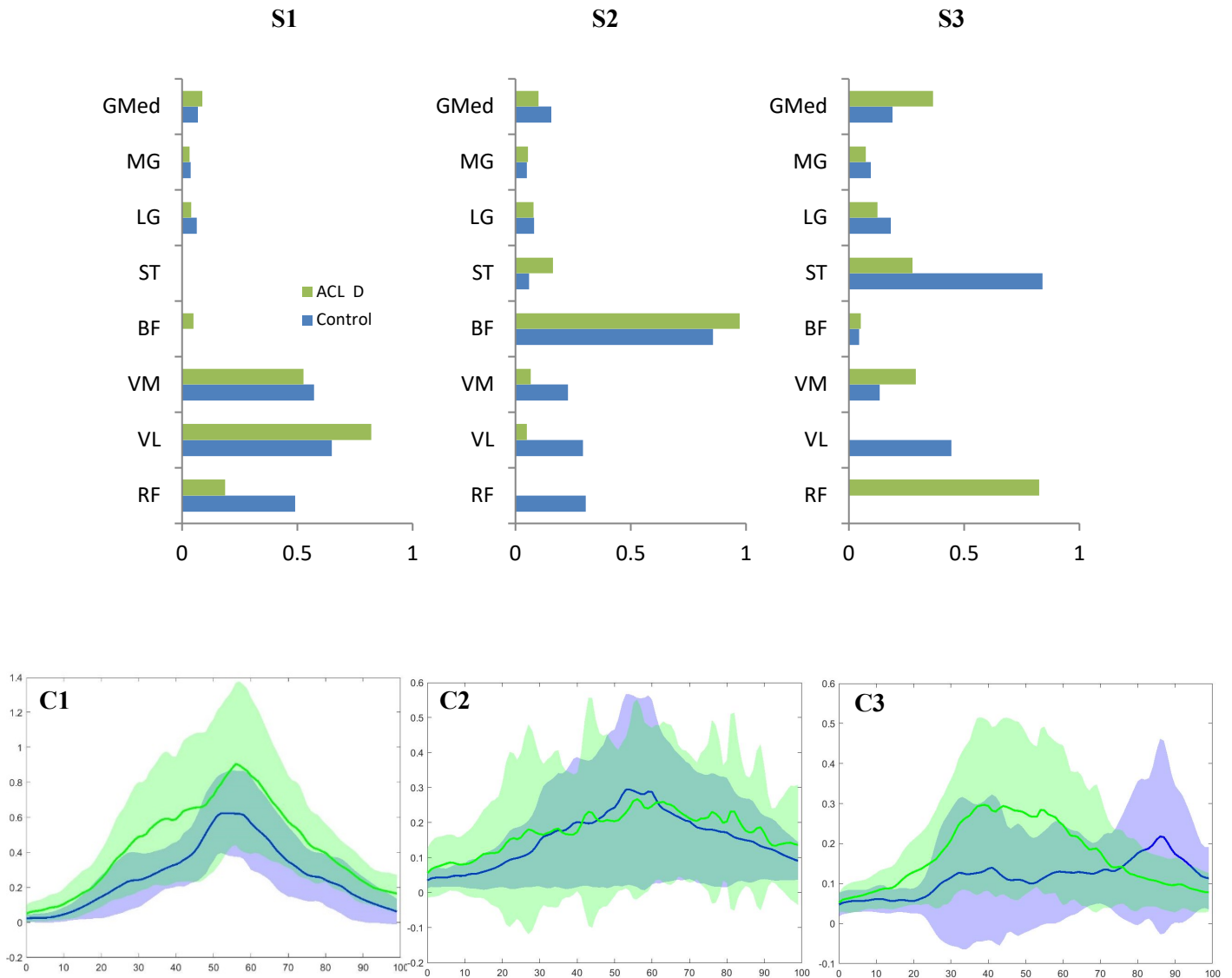
The first pair of synergy vectors were equivalent for each of the three populations (ACLd, ACLc and CON), governed by the quadriceps with scaling coefficients peaking at 50 % of the task cycle (Table 3). The scaling coefficients for the first two synergies of every comparison followed the same scaling pattern of increased activations around peak knee flexion whereas the third set of scaling coefficients for each comparison represented a more constant profile with little variations of scaling activations throughout the task (example of synergy analysis in Figure 3, full synergy analysis results in Appendix 1). No significant differences in scaling coefficients were found. Both the ACLd and ACLc limbs had synergy vectors dominated by the RF (ICC = 0.98), while the CON limb had synergy vectors two and three dominated by the BF and ST respectively.

#### ***DVJ***

There was greater variability in the DVJ synergy vectors and scaling coefficients between populations (ACLd, ACLc and CON) than those of the squat task. While at least one pair of vectors in each comparison was always equivalent, these equivalent vectors were not consistent across populations. Equivalent vectors between CON and ACLd had higher relative contributions of the BF whereas the equivalent vectors between CON and ACLc had higher ST contributions. ACLd and ACLc shared a second set of equivalent vectors, whose primary contributions were from GMed, MG and LG. The scaling of these synergies remained low until the second half of the ascent phase where it quickly peaked before returning to baseline. Although contributions from GMed, MG and LG were also present in CON limbs synergy vectors, they were always accompanied by equal quadriceps contributions. Cross-reconstruction, when possible for the DVJ, did not account for a significant amount of variance (Table 3).

**Table 3:** Summary of synergy analyses for between limb (ACLd, ACLc and CON, ( $n= 15$ )) and within task (squat and DVJ) comparisons. Variance accounted for (VAF) each comparison, VAF following cross reconstruction and the amount of synergy vectors deemed equivalent, similar and poorly correlated for each comparison. SMP independent t-test identified significant differences in coefficients among equivalent synergy vectors. Statistical significance following a Bonferroni correction required ( $p < 0.0167$ ). Complete results of synergy analysis located in Appendix 1.

| Comparison   | VAF (%)                  | xReconstruction VAF (%)  | Synergy Vectors      |                   |                             | Synergy coefficients<br>(Statistically significant differences in coefficients of equivalent synergy vectors)   |
|--------------|--------------------------|--------------------------|----------------------|-------------------|-----------------------------|---|
|              |                          |                          | Equivalent Synergies | Similar Synergies | Poorly Correlated synergies |   |
| <b>Squat</b> |                          |                          |                      |                   |                             |   |
| CON vs ACLd  | CON: 87.9<br>ACLd: 96.7  | CON: 64.5<br>ACLd: 84.6  | 2                    | 0                 | 1                           | No  |
| CON vs ACLc  | CON: 87.9<br>ACLc: 95.9  | CON: 54.3<br>ACLc: 82.9  | 1                    | 0                 | 2                           | No  |
| ACLd vs ACLc | ACLd: 96.7<br>ACLc: 95.9 | ACLd: 70.7<br>ACLc: 80.7 | 2                    | 0                 | 1                           | No  |
|              |                          |                          |                      |                   |                             |   |
| <b>DVJ</b>   |                          |                          |                      |                   |                             |   |
| CON vs ACLd  | CON: 89.6<br>ACLd: 85.5  | CON: -<br>ACLd: -        | 1                    | 1                 | 1                           | No  |
| CON vs ACLc  | CON: 97.8<br>ACLc: 89.6  | CON: -<br>ACLc: -        | 1                    | 1                 | 1                           | No  |
| ACLd vs ACLc | ACLd: 85.5<br>ACLc: 95.3 | ACLd: 24.9<br>ACLc: -    | 2                    | 0                 | 1                           | Yes,<br>- Higher ST activation in ACLc and higher gastrocnemii activation in ACLd (Syn 2): higher activations between 1-4 and 73-99 % in the ACLd limb. |



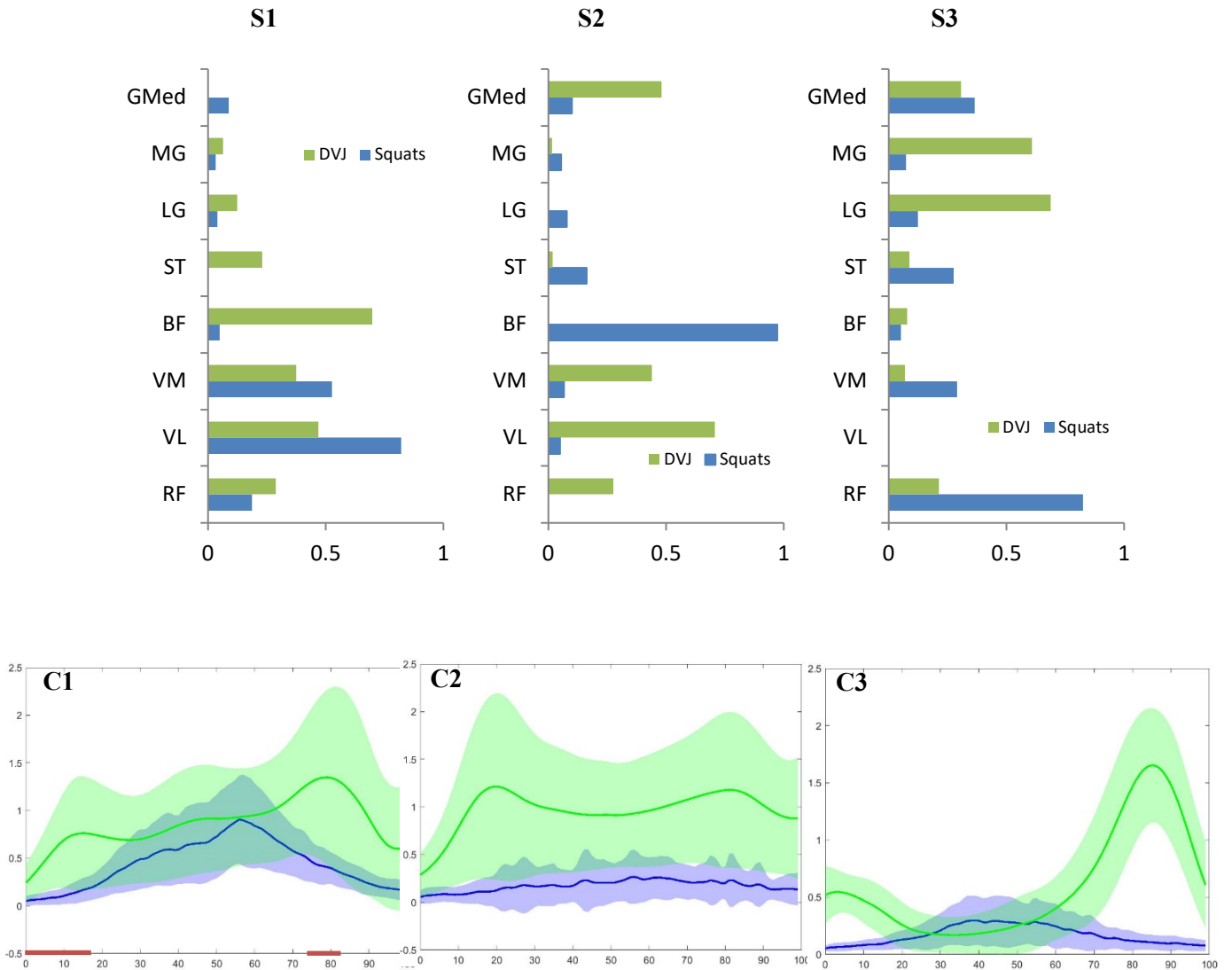
**Figure 3:** Squat muscle synergies and SPM analysis on respective weighting coefficients for ACLd and CON limbs. Squats cycles were time normalised to 100% using height of pelvis origin. No significant differences in coefficients were found (C) with SPM. For coefficient plots (C1, 2, 3) squat coefficients are in blue and DVJ coefficients in green.

### 3.3. Synergy Analysis- Effect of Tasks

The patient population, demonstrated less consistency within limbs (ACLd and ACLc) and between tasks than the control group. While their scaling coefficients followed the same pattern as those of the CON group, with DVJ scaling factors consistently higher than those of the squat, each injured limb (deficient and contralateral) had only one pair of equivalent synergy vectors (Table 4). Equivalent synergy vectors for ACLd and ACLc were dominated by the quadriceps with slightly larger GMed contributions in the DVJ. DVJ synergy vectors partially reconstructed both the ACLd and ACLc squat muscle activation data while the squat synergy vectors failed to reconstruct the DVJ data.

**Table 4:** Summary of synergy analyses for between task (squat and DVJ) and within limb (ACLd, ACLc and CON, ( $n=15$ )) comparisons. Variance accounted for (VAF) each comparison, VAF following cross reconstruction and the amount of synergy vectors deemed equivalent, similar and poorly correlated for each comparison. SPM independent t-test identified significant differences in coefficients among equivalent synergy vectors. Statistical significance required ( $p < 0.0167$ ) following a Bonferroni correction. Complete results of synergy analysis located in Appendix 1.

| Comparison                  | VAF (%)                   | xReconstruction VAF (%)   | Synergy Vectors      |                   |                             | Synergy coefficients<br>(Statistically significant differences in coefficients of equivalent synergy vectors)   |
|-----------------------------|---------------------------|---------------------------|----------------------|-------------------|-----------------------------|---|
|                             |                           |                           | Equivalent Synergies | Similar Synergies | Poorly Correlated synergies |   |
| <b>ACLd</b><br>Squat vs DVJ | Squats: 96.7<br>DVJ: 85.5 | Squats: 39.3<br>DVJ: -    | 1                    | 1                 | 1                           | Yes,<br>- Quadriceps dominated synergy (Syn1): higher activations between 1-18 and 74-83 % in the DVJ.  |
| <b>ACLc</b><br>Squat vs DVJ | Squats: 95.9<br>DVJ: 89.6 | Squats: 86.1<br>DVJ: 1.00 | 1                    | 0                 | 2                           | Yes,<br>- Quadriceps dominated synergy (Syn1): higher activations between 1-51 and 55-100 % of cycle in the DVJ.  |
| <b>CON</b><br>Squat vs DVJ  | Squats: 87.9<br>DVJ: 89.6 | Squats: 76.9<br>DVJ: 78.2 | 2                    | 1                 | 0                           | Yes,<br>-BF dominated synergy (Syn 1): higher activations between 1-37 and 91-100 %.<br>- ST dominated synergy (Syn 2): higher activations between 2-33 and 55-75 % in the DVJ. |



**Figure 4:** Squat and DVJ muscle synergies and SPM analysis on respective weighting coefficients for ACLd limbs. DVJ were time normalized to 100% of time spent on force plate, squats cycles were time normalised to 100% using height of pelvis origin. Significant differences in coefficients (C) tested with SPM (t) statistic for independent measures and indicated by red line at the bottom of figures C1. For coefficient plots (C1, 2, 3) squat coefficients are in blue and DVJ coefficients in green.

#### ***4. Discussion***

This study identified movement patterns and muscle synergies recruited by young adolescent females with and without an ACL rupture during squatting and DVJ tasks. We hypothesised that ACLd individuals may employ a knee flexion avoidance strategy and exhibit different synergy vectors than their uninjured peers. Our results in part support our hypothesis as hip flexor/extensor moments were significantly greater in CON participants than in ACLd limbs. This difference was observed in both tasks but only reached statistical significance in the squat. Synergy vectors were largely consistent within limbs and across tasks, in part supporting our second hypothesis. However, ACL deficient synergy vectors were not consistently different from controls; squat data could be reconstructed between CON and ACLd whereas DVJ data could not, indicating that muscle activation patterns were more consistent between the injured and uninjured participants during the squat than the DVJ. This could be due to the different demands of the two tasks: the DVJ is a more dynamic task that requires the absorption and generation of momentum for the two jumps whereas the squat is a more controlled task with no initial momentum to compensate for.

##### ***4.1. Effect of ACL injury***

While injured participants took significantly longer (large effect size) to complete the squat than their uninjured peers, both groups completed the two tasks with similar movement patterns. Continuous hip flexor moments were larger in the control group for both tasks, however, significant intervals were less than 5% of the movement cycles. Previous work suggests ACLd individuals may employ an avoidance strategy when performing a squat, by reducing both range of motion (ROM) and joint moments for the hip and knee while increasing them at the ankle joint (Button et al., 2014). While we observed greater variability at the ankle joint in our ACLd population relative to the controls, the injured participants did not reduce their ROM and moments at the hip or knee. Our participants were not given a standardised depth while squatting yet the ACLd peak knee flexion angles were consistent with that of the CON group, thus producing similar external (flexion) torque on the knee and potentially requiring similar

quadriceps forces. This lack of avoidance may be due to the paediatric population studied, with their lower centers of mass and body weight relative to adults resulting in less external torque at the knee joint. The iEMG during the squat was also consistent across populations, with the exception of MG and GMed that were significantly higher in the ACLd group with a very large effect size. As expected, squat muscle activations were lower than those of the DVJ, supporting the squat's early use in rehabilitation programs (Wright et al., 2015).

During the DVJ, ACLd participants exhibited slightly greater knee valgus angles than their uninjured peers, however the difference of a few degrees was not statistically significant. Young adolescent females are considered to be at the highest risk for ACL injury (Herzog et al., 2018) therefore we would expect to observe risky landing strategies present in both CON and ACLd groups, contributing to this population's elevated injury risk. Landing positions can be deemed safe or unsafe depending on the relative hip and knee external flexion moment arms. Smaller hip external flexion moment arms and larger knee external flexion moment arms are believed to contribute to unsafe landing positions due to the disproportionately high activation of the quadriceps (Chappell et al., 2007; Ford et al., 2011). However, there were no significant differences in hip external moments and knee extensor moments between our groups, indicating a consistent loading pattern between injured and uninjured participants (Chappell et al., 2007; Ford et al., 2011). The DVJ landing phase was also the preloading phase prior to the maximum height vertical jump. As such, during the landing, participants may have been thinking ahead to the vertical jump, focusing on maximising power generation by flexing their hips, inadvertently placing them in a safer landing position.

The second objective of this study was to determine if young adolescent females with an ACL injury exhibit the same muscle synergies as their uninjured peers. We hypothesised that synergy vectors would differ between groups due to the varied muscle activations and co-contractions of the knee flexor and extensor muscle groups commonly reported in ACLd adults (Alkjaer, Simonsen, Jørgensen, & Dyhre-Poulsen, 2003; Rudolph et al., 2001; Sinkjaer & Arendt-Nielsen, 1991; Williams et al., 2003). Our hypothesis was confirmed as only two pairs of squat vectors and a single DVJ vector pair were

statistically equivalent between injured and uninjured groups. This finding indicates that individuals with an ACL injury use different muscle activation patterns than those without an injury and that the patterns deviate more as the demand of the task increases. However, scaling coefficients of equivalent vectors for both tasks were relatively consistent between ACLd, ACLc and CON groups. This indicates that when patterns with similar contributions of each muscle were used, they were also scaled to similar magnitudes.

The majority of the injured group's squat vectors, found to be dominated by RF contributions, were poorly correlated to the control group's, yet were statistically equivalent between ACLd and ACLc limbs. Similarly, the vector pair, with relatively large and equal contributions from the GMed, MG and LG, was statistically equivalent between injured group's limbs but neither were correlated with the control group's. These observations demonstrate that potential adaptations following an injury occur in both limbs (Ferber, Osternig, Woollacott, Wasielewski, & Lee, 2004). As such, limb symmetry, a clinical tool commonly used to assess rehabilitation and return to play (Adams et al., 2012; Paulos et al., 1991a), may not provide relevant results.

Furthermore, while adult males and females with ACL deficiencies commonly display reduced knee joint range of motion and knee extensor moments (Alkjaer, Simonsen, Magnusson, Dyhre-Poulsen, & Aagaard, 2012; Shelburne et al., 2005), our lower limb squat and DVJ knee joint angles and moments were consistent between ACLd and CON participants. These findings of persistent joint angles and moments alongside different muscle activations further illustrate how the different populations use varying contributions for the same muscles to accomplish the same task (Del Bel et al., 2018). This may also be a difference between adult and youth populations, supporting the need to provide more information on this population and interventions that are developed based on evidence derived from this population.

#### *4.2. Similarities across tasks*

The injured participants used different synergy vectors between the two tasks while the control group exhibited greater task consistency with their synergy vectors. Both ACLd and ACLc used a quadriceps-dominated pattern across tasks yet the relative contributions of each muscle differed in the

other two patterns. Both secondary squat and secondary DVJ patterns for the ACLd limb were dominated by the BF whereas secondary patterns in the ACLc limb were uncorrelated between tasks. ACLd and ACLc participants had notably larger RF contributions in the squat and larger contributions from the medial and lateral gastrocnemii in the DVJ. Reduced quadriceps strength, often a symptom following an ACL injury (Chmielewski et al., 2004; Ford et al., 2011; Palmieri-Smith et al., 2008; Rudolph et al., 2001) may have required ACL injured participants to increase their RF activation to maintain their knee joint stability in the squat. RF activation may have been used to stabilise the hip and knee whereas VL and VM activity would have directly acted about the knee, perhaps explaining the elevated RF contributions in the squat.

The ACL injured participants exhibited larger scaling coefficients indicating greater muscle activations for equivalent, similar and poorly correlated synergy vectors in both limbs compared to controls. Our findings of increased task demand requiring increased scaled muscle activations are consistent with previous work investigating gait where running used similar muscle activation patterns as walking but with larger activations (Cappellini et al., 2006). Therefore, the findings of the control population support the notion that muscle synergy vectors are load dependent whereas the scaling coefficients are demand dependent, with higher demand requiring higher scaling coefficients. The scaling coefficients for the injured population also exhibited demand-dependent activations with larger activations in the DVJ than the squat. However, the injured population's synergy vectors varied within a constant loading direction, lacking the load dependency observed in the control group. This increased variability may be due to the ACL injury however, as injured participants were not studied pre-injury, it cannot be confirmed that the increased in variability results from the injury.

#### *4.3. Limitations*

While all participants were matched for limb dominance, on average the control group was 1.4 years younger ( $p = 0.03$ ) and half a Tanner Stage ( $p = 0.09$ ) behind the ACL injured group. Participants were therefore at similar maturation stages yet the discrepancy in ages may have influenced the results. Regarding protocol, minimal instructions were given for both tasks, ensuring participants performed the

tasks as naturally as possible. This resulted in differences in squat timing between the injured and control groups. Without standardisation, injured participants may have had to adopt different compensation strategies to compete the task within the requirements, but our objective was to represent their natural state. Nevertheless, injured participants were not studied pre-injury therefore we cannot conclude that these differences are a direct result of the injury.

## **5. Conclusion**

Both ACL injured and healthy controls performed the squat and DVJ using similar movement patterns, although the ACL injured participants took more time to complete the squat. Uninjured participants used a more hip-dominant strategy while the ACL injured participants employed relatively greater gastrocnemius activations, further supporting the importance of these muscles following an ACL injury (Benoit et al 2003). This study demonstrated that synergy vectors change, to varying degrees, between populations (with and without an ACL injury) while synergy scaling coefficients of equivalent vectors varied between tasks (squats and DVJ). The consistency of muscle synergy vectors throughout the squat and DVJ suggests that for both groups studied, the difficulty of the plyometric component in the DVJ not only required higher amounts of muscle activity (as observed through higher scaling coefficients), but also slightly different muscle activation profiles. The consistency across tasks supports the theory that synergies are load direction dependent (Chvatal et al., 2011). Future work could investigate muscle activation patterns of injured and uninjured adolescents for tasks ranging in loading directions and demands to test this theory under a wider range of conditions.

Synergy vectors between injured limbs correlated higher with each other than with controls indicating that potential changes following a unilateral injury may be present in both limbs. To gain further insight on this topic, muscle activation in female adolescents would need to be studied before and after an ACL injury.

Taken together, our results indicate that using limb symmetry analysis in the ACLd youth population will not provide information to indicate the status of the patient with respect to their healthy counterpart. Furthermore, as there remains a lack of literature concerning young adolescent

neuromuscular function and biomechanics during functional tasks despite this age group's elevated risk of sustaining ACL injuries, further work should investigate both sexes of this population.

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## Chapter 7. General Discussion

This study identified muscle synergies recruited by young adolescent females in their dominant and non-dominant limbs, and with and without an ACL rupture during squatting and DVJ tasks. The muscle synergy analysis was supported by also investigating lower limb joint angles, moments and iEMG. We first demonstrated that there were no differences in peak lower limb joint angles and moments between the dominant and non-dominant limb for the squat. However, DVJ sagittal plan hip angles were greater in the dominant limb from 29.4-71.6% and non-dominant frontal plane knee moments from 0.8-1.7 % then identified significant differences in iEMG activity in the BF and ST between the uninjured participant's dominant and non-dominant limbs. Lastly, we identified minor differences in muscle synergy vectors between uninjured participants' dominant and non-dominant limbs. These observations informed the next phase of my research, which was to evaluate the youth ACL injured population since we established that, when investigating the effects of ACL injuries compared to uninjured control participants, it was essential to match for limb dominance.

In this second phase, our kinetic and kinematic results did not support our hypothesis since, in general, similar movement patterns and muscle activations (iEMG) were observed across populations within each task. Contrary to research looking at movement and muscle activation patterns in bilateral tasks (Flaxman, 2016), overall few statistically significant differences were found in muscle recruitment through our synergy analysis. For example, the synergy vectors of ACL deficient participants were not consistently different from uninjured participants. Interestingly, the squat data muscle activations could be reconstructed between CON and ACLd, whereas DVJ data could not reconstruct.

### 7.1 Effects of limb dominance

The first aim of this study was to determine if lower limb muscles synergies differed between dominant and non-dominant limbs of young female adolescents. We hypothesise that due to the bilateral nature of both tasks and their similar movement patterns that synergies would be consistent across tasks

(Cappellini et al., 2006) and between dominant and non-dominant limbs (Del Bel et al., 2017). Our hypothesis was partly confirmed as all three squat synergy vectors were statistically equivalent between dominant and non-dominant limbs. However, only one of the three pairs of vectors were equivalent for the DVJ, indicating different muscle patterns were used by the dominant and non-dominant limbs. Few significant differences in lower limb joint angles and moments were found for either task (Study 1) yet previous work has shown higher peak knee joint extension moments in the dominant limb (Edwards et al., 2012). Greater BF iEMG and continuous activations were observed in the dominant limb in both tasks, consistent with previous work (Del Bel et al., 2017). The observation of only the squat's vectors being entirely correlated may be due to the varying demands of the tasks: while both tasks required descending and ascending the body's center of mass, the movement is much more controlled in the squat relative to the higher velocities and momentum present in the DVJ. The differences observed between dominant and non-dominant limbs in uninjured youth indicate that limb symmetry, a clinical tool commonly used to assess rehabilitation and return to play (Adams et al., 2012; Paulos et al., 1991a), may not provide relevant results.

## **7.2 Effect of ACL injury**

The second objective of this study was to determine if young adolescent females with an ACL injury exhibit the same movement patterns and muscle synergies as their uninjured peers. Continuous hip flexor moments were larger in the control group for both tasks however, significant intervals were less than 5% of the movement cycles. Control participants completed the squat significantly faster ( $p = 0.023$ , Cohen's  $d = 0.88$ ) and with greater continuous and peak hip extension moments (50.0-54.9%,  $p = 0.0019$ , Cohen's  $d = 1.25$ ), although both groups reached similar peak knee joint angles ( $p = 0.061$ ). Previous work suggests ACLd individuals may employ an avoidance strategy by reducing ROM and joint moments for the hip and knee while increasing them at the ankle joint (Button, Roos, & van Deursen, 2014). While we observed greater variability at the ankle joint in our ACLd population relative to the control population, the injured participants did not reduce their ROM and moments at the hip or knee. Our

participants were not given a standardized depth to squat down to yet the ACLd peak knee flexion angles were consistent with that of the CON group thus producing similar external (flexion) torque on the knee and potentially requiring similar quadriceps forces. This lack of avoidance may be due to the fact we assessed a paediatric population: their lower centers of mass and body weight relative to adults resulting in less external torque at the knee joint. The iEMG during the squat was also consistent across populations with the exception of MG and GMed that were significantly larger in the ACLd group with a very large effect size. Squat muscle activations were lower than those of the DVJ, supporting the squat's early use in rehabilitation programs (Wright et al., 2015).

The second task, the DVJ is often used to identify athletes at risk of severe knee injuries by identifying athletes who land with valgus knee motion and encouraging them to perform neuromuscular training emphasising 'hip-knee-toe line' positioning before participating in sporting activities (Hewett et al., 2005). ACLd participants exhibited slightly greater knee valgus angles than their uninjured peers, however the difference of a few degrees was not statistically significant. Young adolescent females are considered to be at the highest risk for ACL injury (Herzog et al., 2018), therefore risky landing strategies present in both CON and ACLd groups may in fact be contributing to this population's elevated injury risk. Landing positions can be deemed safe or unsafe depending on the relative hip and knee external flexion moment arms. Smaller hip external flexion moment arms and larger knee external flexion moment arms contribute to unsafe landing positions due to the disproportionately high activation of the quadriceps (Chappell et al., 2007; Ford et al., 2011). However, there were no significant differences in hip external moments and knee extensor moments between our groups, indicating a consistent loading pattern between injured and injured participants. Additionally, both groups appeared to be producing greater hip external moments than their knee extensor moments, consistent with a safe loading pattern (Chappell et al., 2007; Ford et al., 2011). The DVJ landing phase was also the preloading phase prior to the maximum height vertical jump. As such, during the landing, participants may have been thinking ahead to the jump, focusing on maximising power generation by flexing their hip, knee and ankle jumps, inadvertently placing them in a safer landing position. The greater knee joint moments and muscle activations evoked

by simultaneously absorbing the impact of a landing and loading for a subsequent jump requires higher levels of knee joint stability, coinciding with their use in later stages of rehabilitation (Wright et al., 2015). These findings of persistent joint angles and moments yet different muscle activations further illustrate how the different populations use different contributions of the same muscles to accomplish the same task.

We hypothesized that synergy vectors would differ between ACL deficient (ACLd) and uninjured groups based on previous work identifying neuromuscular differences in adults with and without ACL injuries (Alkjaer, Simonsen, Jørgensen, & Dyhre-Poulsen, 2003; Rudolph et al., 2001; Sinkjaer & Arendt-Nielsen, 1991; Williams, Barrance, Snyder-Mackler, et al., 2003). Our hypothesis was confirmed as only two pairs of squat vectors and a single DVJ vector pair were statistically equivalent between injured and uninjured groups. This finding indicates that individuals with an ACL injury use different muscle activation patterns than those without an injury and that the patterns deviate more as the demand of the task increases. However, scaling coefficients of equivalent vectors for both tasks were relatively consistent between injured groups and followed the pattern of control groups.

The majority of the injured group's squat synergy vectors, found to be dominated by RF contributions, were poorly correlated to the control group's, yet there was statistical equivalence between ACLd and ACLc. Similarly, neither of the injured group's DVJ vectors with relatively large and equal contributions from the Gmed, MG and LG were correlated with the control group, yet the vector pair was statistically equivalent between the ACLd and ACLc limbs. These observations are consistent in both the injured population's limbs yet different relative to the uninjured group, demonstrating that potential adaptations following an injury may occur in both limbs (Ferber et al., 2004).

### **7.3 Similarities across tasks**

The first task investigated was the bilateral squat, a movement primarily governed by the quadriceps (Bynum, Barrack, & Alexander, 1995; Salem, Salinas, & Harding, 2003). Our findings were consistent with previous work (Bynum, Barrack, & Alexander, 1995; Salem, Salinas, & Harding, 2003)

showing the quadriceps were contracted during the descent phase to control the rate of descent of the body's center of mass (CoM), followed by concentric activation to raise the CoM. Descent phase muscle activations were relatively equally distributed within the quadriceps muscle group, contradicting work suggesting greater activations in the vastus medialis and lateralis relative to the rectus femoris (Escamilla, 2001; Isear et al., 1997). Hamstring activation increased with increased knee flexion, with activations during the ascent phase potentially contributing to increase hip extension (Escamilla, 2001; Isear et al., 1997).

The DVJ followed similar sagittal plane lower limb joint angle patterns to those of the squat. The initial impact from the landing, qualifying them as a plyometric exercise (Padua et al., 2009) evoked greater knee joint moments than the squat. Continuous muscle activations and discrete iEMG were greater for all muscles during the DVJ. Similar to the squat, eccentric quadriceps contractions were used to control the descent of the center of mass, yet the DVJ also required the knee extensors to aid in dampening the impact of loading at the knee during the landing phase of the jump, observed by higher activations earlier on in the DVJ cycle. Both groups performed the DVJ similarly, with no statistical differences in muscle activations, iEMG or joint angles and moments.

Our synergy analysis supported our kinematic and kinetic observations of consistencies across tasks; all uninjured groups had consistent synergy vectors across tasks, indicating that similar patterns of muscle activity were used to accomplish the squat and DVJ. Muscle synergy vectors have been shown to be load direction dependent (Chvatal et al., 2011; Shelburne et al., 2006; Shourijeh et al., 2016) supporting our observations of correlated vectors across similar movement tasks with a consistent loading direction. This finding is also in line with those of previous work investigating various pairs of similar tasks (Cappellini et al., 2006; Flaxman et al., 2017).

Alternatively, our injured participants exhibited greater variability by using different synergy vectors to accomplish the two tasks. While both injured limbs (ACLd and ACLc) used a quadriceps-dominated pattern across tasks, the relative contributions of each muscle differed in the other two patterns. ACLd and ACLc participants had notably larger RF contributions in the squat and notably larger

contributions from the medial and lateral gastrocnemii in the DVJ relative to the control group. Reduced quadriceps strength, often observed following an ACL injury (Chmielewski et al., 2004; Ford et al., 2011; Palmieri-Smith et al., 2008; Rudolph et al., 2001) may have required ACL injured participants to increase their RF activation to maintain their knee joint stability in the squat. RF activation may have been used to stabilise the hip and knee whereas VL and VM activity would not have directly acted about the knee, perhaps explaining the elevated RF contributions.

Larger scaling coefficients indicating greater muscle activations for equivalent, similar and poorly correlated synergy vectors were observed in all groups, regardless of limb dominance or presence of an ACL injury. Our findings of increased task demand requiring increased scaled muscle activations are consistent with previous work investigating gait where running used similar muscle activation patterns as walking but with larger activations (Cappellini et al., 2006). Therefore, the findings of our control population support the notion that muscle synergy vectors are load dependent whereas scaling coefficients are demand dependent, with higher demands requiring higher scaling coefficients. While our injured population exhibited demand-dependent activations, their synergy vectors varied within a consistent loading direction. This increased variability may be due to the diminished specificity previously observed in ACL deficient populations (Williams, Barrance, Snyder-Mackler, Axe, & Buchanan, 2003; Del Bel et al., 2018) however, as injured participants were not studied pre-injury, it cannot be confirmed that the increased in variability results from the injury or was present prior to the injury.

## **7.4 Limitations**

ACLd participants were matched to uninjured controls for limb dominance yet, on average the control group was 1.4 years younger ( $p = 0.03$ ) and half a Tanner Stage ( $p = 0.09$ ) behind the ACL injured group. Participants were therefore at similar maturation stages yet the discrepancy in ages may have influenced the results. In addition, minimal instructions were given for both tasks with participants completing both tasks at a self-selected pace and to a self-selected depth. While this ensured participants performed the tasks as naturally as possible and elicited the difference squat timing, peak CoM descent

was not standardized. As such, had squat timing and/or squat depth been standardised between participants and groups, perhaps the ACLd group would have had to adapt their movement patterns by modifying their lower limb joint angles, moment and muscle activity.

Additionally, while relatively few differences were found within tasks and between populations the literature suggests that ACL injuries modify dynamic task execution, such as decreased knee flexion moments in the affected limb (Rudolph et al., 2001). This discrepancy may be due to fact that the tasks investigated here required limited movement in the frontal plane. Nevertheless, both the squat and jump are commonly used in clinical assessments (Wright et al., 2015) and as such are findings, in particular the lack of inter-lib differences, are clinically relevant.

## **7.5 General Conclusion**

This study demonstrated that synergy vectors change, to varying degrees, predominantly between populations (limb dominance and ACL injury) while synergy scaling coefficients of equivalent vectors varied between tasks (squats and DVJ). Overall the squat demonstrated higher between limb synergy vector consistency than the DVJ suggesting that, for both groups studied (injured and uninjured), the added absorption and creation of momentum required not only higher amounts of muscle activity, observed through higher scaling coefficients, but also slightly different muscle activation profiles. The observed consistency across tasks supports the loading direction dependency of synergies (Chvatal et al., 2011).

Dominant and non-dominant limbs in uninjured controls exhibited consistent synergy vectors across tasks, indicating that similar patterns of muscle activity were used to accomplish the squat and DVJ. Yet, differences in synergy vectors between the two limbs indicate that limb symmetry, a clinical tool commonly used to assess rehabilitation and return to play (Adams et al., 2012; Paulos et al., 1991a), may not provide relevant results. Synergy vectors between injured limbs (ACLd and ACLc) correlated higher with each other than with controls, indicating that potential changes following a unilateral injury may be present in both limbs.

This thesis demonstrates that, in order to use the squat and DVJ in clinical decision-making and rehabilitation programs, task evaluations should go beyond limb symmetry. Due to the differences in muscle synergies between dominant and non-dominant limbs in uninjured female youth, limb dominance should also be taken into account in ACL injury management. Differences between the ACLc and CON limb muscle synergies indicate that performance of the ACLc limb may not reflect its pre-injury state. Therefore, to gain a more accurate evaluation of an ACLd limb's performance and make informed clinical decisions, ACLd performance should be compared to that of an uninjured control.

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## Appendix 1 – Complete Synergy Analysis Results

### RESULTS – Synergy Analysis

Study 1: Effects of Limb Dominance  
 Study 2: Effects of ACL deficiency and task

**Table 1:** Summary of synergy results for comparisons looking at the effect of limb dominance (within task).

| Comparison            | VAF (%)               | xReconstruction VAF (%) | Synergy Vectors      |                   |                             | Synergy coefficients<br>(Statistically significant differences in coefficients of equivalent synergy vectors)                                    |
|-----------------------|-----------------------|-------------------------|----------------------|-------------------|-----------------------------|--|
|                       |                       |                         | Equivalent Synergies | Similar Synergies | Poorly Correlated synergies |  |
| Squat<br>DOM vs<br>ND | DOM: 93.8<br>ND: 87.3 | DOM: 96.9<br>ND: 78.6   | 3                    | 0                 | 0                           | Yes,<br>-Synergy 3 (synergy vector primarily reflecting hamstring contributions): higher activations between 52 and 53% in the DOM limb.         |
| DVJ<br>DOM vs<br>ND   | DOM: 89.6<br>ND: 96.6 | DOM: 63.9<br>ND: 86.4   | 1                    | 1                 | 1                           | Yes,<br>- Synergy 1 (synergy vector primarily reflecting quadriceps contributions) : higher activations between 1-6 and 78-91 % in the DOM limb. |

**Table 2:** Summary of synergy results for comparisons looking at the effect of ACL injuries (within tasks).

| Comparison              | VAF (%)                  | xReconstruction VAF (%)  | Synergy Vectors      |                   |                             | Synergy coefficients<br>(Statistically significant differences in coefficients of equivalent synergy vectors) |
|-------------------------|--------------------------|--------------------------|----------------------|-------------------|-----------------------------|---|
|                         |                          |                          | Equivalent Synergies | Similar Synergies | Poorly Correlated synergies |   |
| Squat<br>CON vs<br>ACLd | CON: 87.9<br>ACLd: 96.7  | CON: 64.5<br>ACLd: 84.6  | 2                    | 0                 | 1                           | No  |
| CON vs<br>ACLc          | CON: 87.9<br>ACLc: 95.9  | CON: 54.3<br>ACLc: 82.9  | 1                    | 0                 | 2                           | No  |
| ACLd vs<br>ACLc         | ACLd: 96.7<br>ACLc: 95.9 | ACLd: 70.7<br>ACLc: 80.7 | 2                    | 0                 | 1                           | No  |
|                         |                          |                          |                      |                   |                             |   |

|                              |                          |                       |   |   |   |   |
|------------------------------|--------------------------|-----------------------|---|---|---|---|
| <b>DVJ</b><br>CON vs<br>ACLd | CON: 89.6<br>ACLd: 85.5  | CON: -<br>ACLd: -     | 1 | 1 | 1 | No  |
| CON vs<br>ACLc               | CON: 97.8<br>ACLc: 89.6  | CON: -<br>ACLc: -     | 1 | 1 | 1 | No  |
| ACLd vs<br>ACLc              | ACLd: 85.5<br>ACLc: 95.3 | ACLd: 24.9<br>ACLc: - | 2 | 0 | 1 | Yes,<br>- Higher ST activation in<br>ACLc and higher<br>gastrocnemii activation in<br>ACLd (Syn 2): higher<br>activations between 1-4<br>and 73-99 % in the ACLd<br>limb. |

**Table 3:** Summary of synergy results looking at the similarities between tasks (within limb).

| Comparison                     | VAF<br>(%)                | xReconstruction<br>VAF<br>(%) | Synergy Vectors         |                      |                                   | Synergy coefficients<br>(Statistically significant<br>differences in coefficients<br>of equivalent synergy<br>vectors)   |
|--------------------------------|---------------------------|-------------------------------|-------------------------|----------------------|-----------------------------------|--|
|                                |                           |                               | Equivalent<br>Synergies | Similar<br>Synergies | Poorly<br>Correlated<br>synergies |  |
| <b>DOM</b><br>Squat vs<br>DVJ  | Squat: 93.8<br>DVJ: 89.6  | Squat: 53.9<br>DVJ: 63.8      | 2                       | 0                    | 1                                 | Yes,<br>- Quadriceps dominated<br>synergy (Syn 1): higher<br>activations between 1-48<br>and 56-100 % in the DVJ.<br>- BF dominated synergy<br>(Syn 2): higher<br>activations between 1-53<br>and 63-100 % in the DVJ. |
| <b>ND</b><br>Squat vs<br>DVJ   | Squat: 87.3<br>DVJ: 96.6  | Squat: 71.0<br>DVJ: 88.0      | 1                       | 2                    | 0                                 | Yes,<br>- BF governed synergy<br>(Syn 2): higher<br>activations between 1-78<br>% in the DVJ.  |
| <b>ACLd</b><br>Squat vs<br>DVJ | Squats: 96.6<br>DVJ: 96.6 | Squats: 40.3<br>DVJ: -        | 1                       | 1                    | 1                                 | Yes,<br>- Quadriceps dominated<br>synergy (Syn1): higher<br>activations between 1-18<br>and 74-83 % in the DVJ.  |
| <b>ACLc</b><br>Squat vs<br>DVJ | Squats: 95.9<br>DVJ: 85.5 | Squats: 86.2<br>DVJ: -        | 1                       | 0                    | 0                                 | Yes,<br>- Quadriceps dominated<br>synergy (Syn1): higher<br>activations between 1-51<br>and 55-100 % of cycle in<br>the DVJ.   |

|  |                                   |                                   |          |          |          |  |
|--|-----------------------------------|-----------------------------------|----------|----------|----------|--|
| <p><b>CON</b><br/>Squat vs<br/>DVJ</p> | <p>Squats: 89.1<br/>DVJ: 97.8</p> | <p>Squats: 82.2<br/>DVJ: 95.7</p> | <p>2</p> | <p>1</p> | <p>0</p> | <p>Yes,<br/>-BF dominated synergy<br/>(Syn 1): higher<br/>activations between 1-37<br/>and 91-100 %.<br/>- ST dominated synergy<br/>(Syn 2): higher<br/>activations between 2-33<br/>and 55-75 % in the DVJ.</p> |
|--|-----------------------------------|-----------------------------------|----------|----------|----------|--|

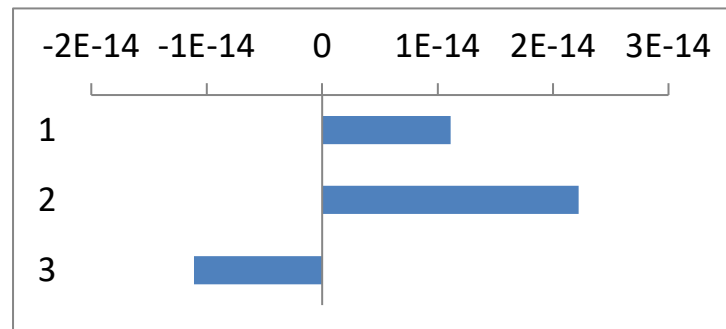
## RESULTS - Study 1: Effects of limb dominance and task SYNERGY ANALYSIS:

### *Effects of limb dominance:*

Three synergies were required to reconstruct the dominant and non-dominant limb squat and DVJ tasks.

### Squats

Squat Synergies 1 (Squat - S1<sub>DOM</sub> and Squat - S1<sub>ND</sub>), S2 (Squat - S2<sub>DOM</sub> and Squat - S2<sub>ND</sub>) and S3 (Squat - S3<sub>DOM</sub> and Squat - S3<sub>ND</sub>) were statistically equivalent (Table 5, Figures 1 and 2). Cross reconstruction of the dominant limb data using the non-dominant limb synergy vectors accounted for 86.9 % of its total variance while reconstructing the non-dominant limb using the dominant limb vectors accounted for 78.6 % of its total variance.



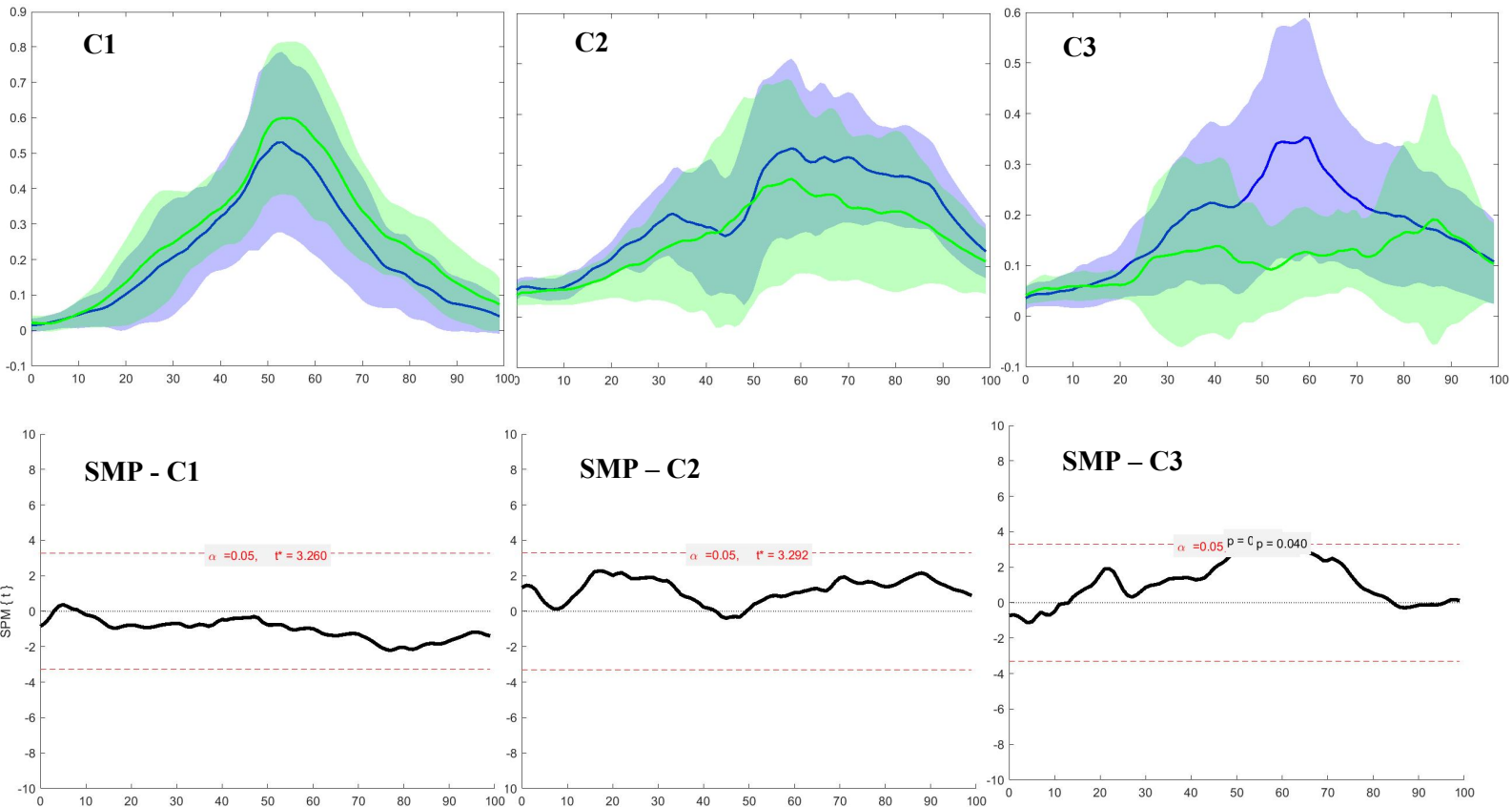
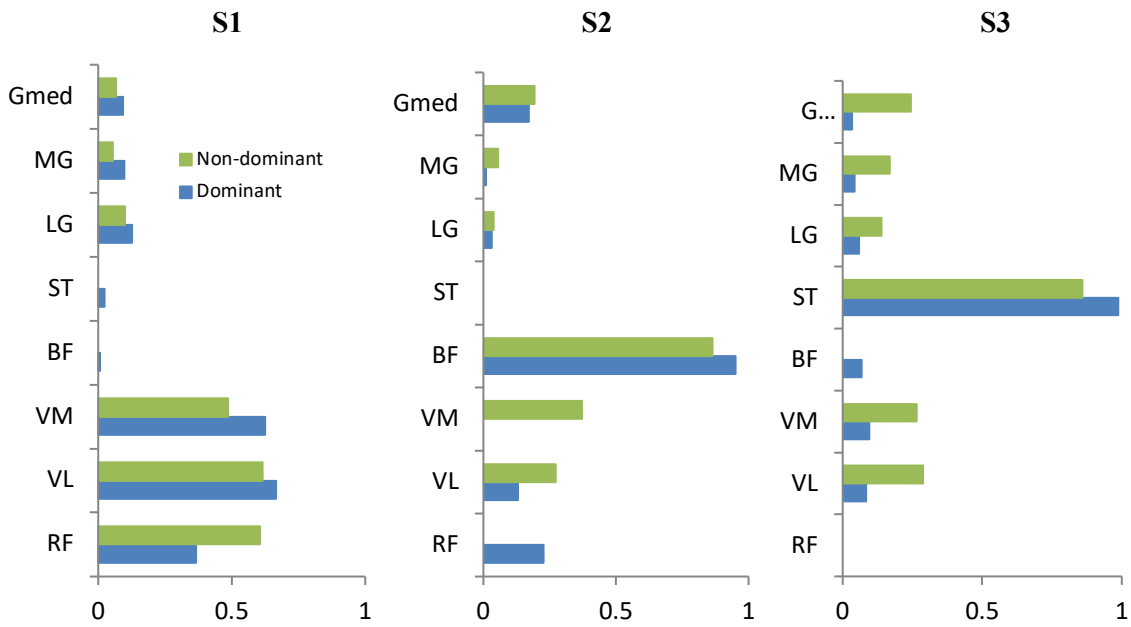
**Figure 1:** Percent relative change (%) in synergy vector norms for the muscle synergies. Dominant limb results used as reference point where positive numbers indicate an overall increase from dominant limb results and negative values represent a decrease relative to the reference.

**Table 4:** Contributions of each synergy.

| Limb / Synergy | Percent of Total Contribution |       |       |
|----------------|-------------------------------|-------|-------|
|                | 1                             | 2     | 3     |
| Dominant       | 0.581                         | 0.278 | 0.141 |
| Non-dominant   | 0.445                         | 0.255 | 0.300 |

**Table 5:** Intraclass correlation coefficients (ICC) comparing synergy vectors for the squat task in the dominant and dominant limb of paediatric females. Statistically *equivalent* defined as ICC > 0.80 and demoted by two asterisks (\*\*) and statistical similarity ICC > 0.60 demoted by an asterisk (\*).

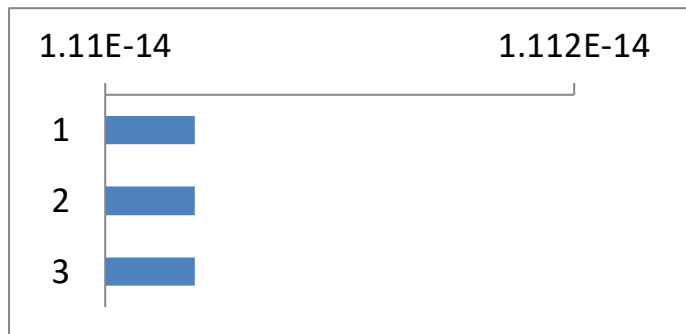
| Synergy #           |   | Dominant Limb |        |        |
|---------------------|---|---------------|--------|--------|
|                     |   | 1             | 2      | 3      |
| Non – Dominant Limb | 1 | 0.96**        | -      | -      |
|                     | 2 | -             | 0.92** | -      |
|                     | 3 | -             | -      | 0.95** |



**Figure 2:** Squat muscle synergies and SPM analysis on respective weighting coefficients for dominant and non-dominant limbs. Squats are time normalized to 100% of squat cycle. Significant differences in coefficients (C) tested with SPM (t) statistic for independent measures.

DVJ

DVJ Synergies 2 (DVJ – S2<sub>Dom</sub> and DVJ – S2<sub>ND</sub>) were statistically equivalent, while synergies 1 (DVJ – S2<sub>DOM</sub> and DVJ – S2<sub>ND</sub>) were similar and S3 (DVJ – S3<sub>DOM</sub> and DVJ - S3<sub>ND</sub>) were uncorrelated (Table 7, Figures 3 and 4). Cross reconstruction of the DVJ dominant limb data using the non-dominant limb vectors accounted for 63.9 % of its total variance while reconstructing the non-dominant limb using the dominant limb vectors accounted for 86.4 % of its total variance.



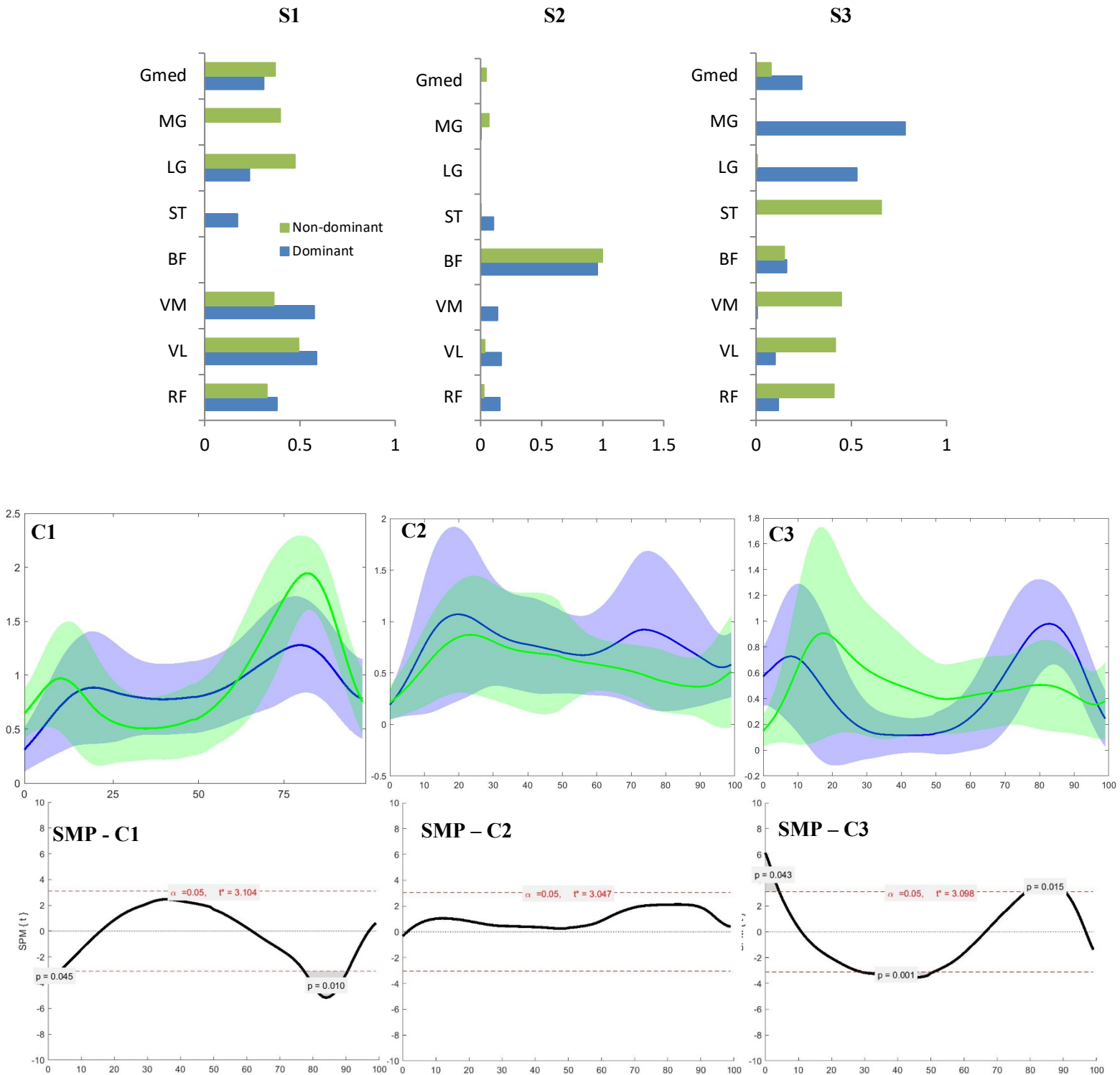
**Figure 3:** Percent relative change (%) in synergy vector norms for the muscle synergies. Dominant limb results used as reference point where positive numbers indicate an overall increase from dominant limb results and negative values represent a decrease relative to the reference.

**Table 6:** Contributions of each synergy.

| Limb / Synergy | Percent of Total Contribution |       |       |
|----------------|-------------------------------|-------|-------|
|                | 1                             | 2     | 3     |
| Dominant       | 0.489                         | 0.228 | 0.283 |
| Non-Dominant   | 0.560                         | 0.116 | 0.324 |

**Table 7:** Intraclass correlation coefficients (ICC) comparing synergy vectors for the squat task in the dominant and dominant ACL deficient limb of paediatric females. Statistically equivalent defined as ICC > 0.80 and demoted by two asterisks (\*\*) and statistical similarity ICC > 0.60 demoted by an asterisk (\*).

|                     | Synergy # | Dominant Limb |        |       |
|---------------------|-----------|---------------|--------|-------|
|                     |           | 1             | 2      | 3     |
| Non – Dominant Limb | 1         | 0.68*         | -      | -     |
|                     | 2         | -             | 0.98** | -     |
|                     | 3         | -             | -      | -8.69 |



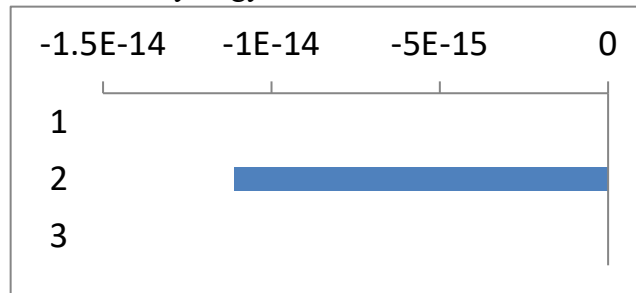
**Figure 4:** DVJ muscle synergies and SPM analysis on respective weighting coefficients for dominant and nondominant limbs. DVJ are time normalized to 100% of time spent on force plate. Significant differences in coefficients (C) tested with SPM (t) statistic for independent measures.

**Synergy similarity across tasks:**

Three synergies were required to reconstruct the dominant and non-dominant limb squat and DVJ tasks.

*Dominant limb*

The first two pairs of dominant limb synergies were statistically equivalent while the third pair were poorly correlated (Table 9, Figures 5 and 6). Cross reconstruction of the squat data using the DVJ synergy vectors accounted for 53.9 % of its total variance while reconstructing the DVJ task using the squat dominant limb synergy vectors accounted for 63.8 % of its total variance.



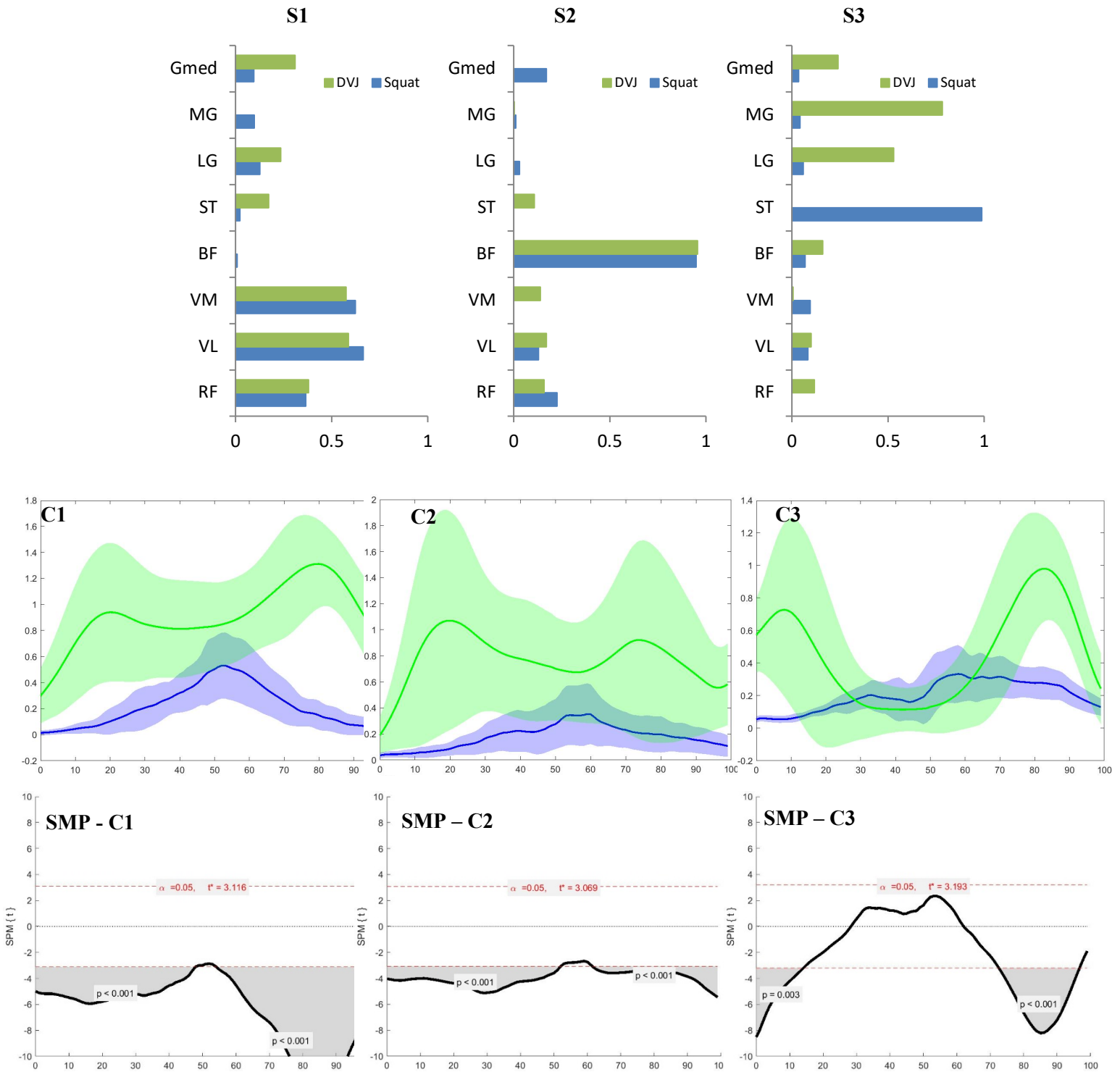
**Figure 5:** Percent relative change (%) in synergy vector norms for the muscle synergies. Dominant limb squat results used as reference point where positive numbers indicate an overall increase from squat results and negative values represent a decrease relative to the reference.

**Table 8:** Contributions of each synergy.

| Task / Synergy | Percent of Total Contribution |       |       |
|----------------|-------------------------------|-------|-------|
|                | 1                             | 2     | 3     |
| Squat          | 0.611                         | 0.250 | 0.139 |
| DVJ            | 0.490                         | 0.228 | 0.283 |

**Table 9:** Intraclass correlation coefficients (ICC) comparing synergy vectors for the squat task in the dominant and dominant ACL deficient limb of paediatric females. Statistically equivalent defined as ICC > 0.80 and demoted by two asterisks (\*\*) and statistical similarity ICC > 0.60 demoted by an asterisk (\*).

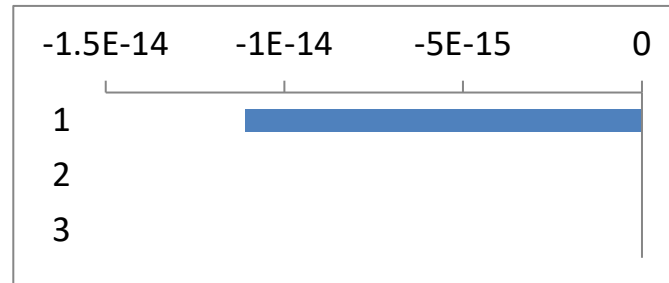
|       | Synergy # | DVJ    |        |       |
|-------|-----------|--------|--------|-------|
|       |           | 1      | 2      | 3     |
| Squat | 1         | 0.94** | -      | -     |
|       | 2         | -      | 0.98** | -     |
|       | 3         | -      | -      | -1.16 |



**Figure 6:** Squat and DVJ muscle synergies and SPM analysis on respective weighting coefficients for the dominant limbs. DVJ are time normalized to 100% of time spent on force plate, squats cycles are time normalized to 100% using height of pelvis origin. Significant differences in coefficients (C) tested with SPM (t) statistic for independent measures. For coefficient plots (C1,2,3) squat coefficients are in blue and DVJ coefficients in green.

Non-dominant limb

The second pair of non-dominant limb synergies was statistically equivalent while the first and third pairs were statistically similar (Table 11, Figures 7 and 8). Cross reconstruction of the squat data using the DVJ synergy vectors accounted for 71.0 % of its total variance while reconstructing the DVJ task using the squat non-dominant limb synergy vectors accounted for 88.0 % of its total variance.



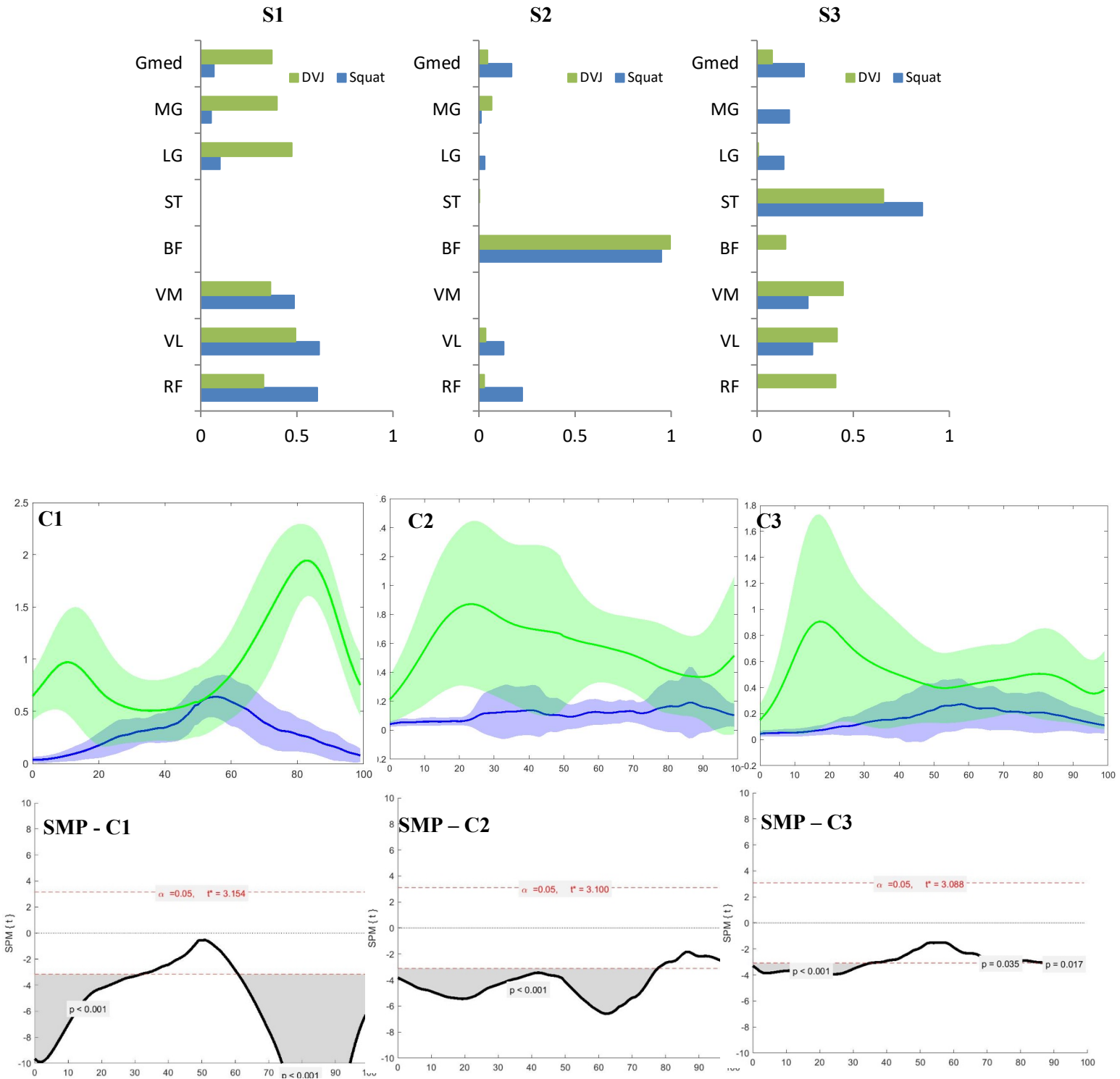
**Figure 7:** Percent relative change (%) in synergy vector norms for the muscle synergies. Non-dominant limb squat results used as reference point where positive numbers indicate an overall increase from squat results and negative values represent a decrease relative to the reference.

**Table 10:** Contributions of each synergy.

| Task / Synergy | Percent of Total Contribution |       |       |
|----------------|-------------------------------|-------|-------|
|                | 1                             | 2     | 3     |
| Squat          | 0.445                         | 0.256 | 0.299 |
| DVJ            | 0.839                         | 0.161 | -     |

**Table 11:** Intraclass correlation coefficients (ICC) comparing synergy vectors for the squat task in the dominant and dominant ACL deficient limb of paediatric females. Statistically equivalent defined as ICC > 0.80 and demoted by two asterisks (\*\*) and statistical similarity ICC > 0.60 demoted by an asterisk (\*).

|       | Synergy # | DVJ   |        |       |
|-------|-----------|-------|--------|-------|
|       |           | 1     | 2      | 3     |
| Squat | 1         | 0.64* | -      | -     |
|       | 2         | -     | 0.93** | -     |
|       | 3         | -     | -      | 0.77* |



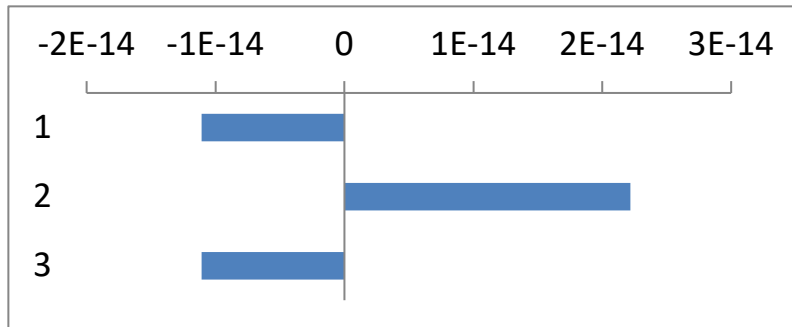
**Figure 8:** Squat and DVJ muscle synergies and SPM analysis on respective weighting coefficients for the non-dominant limbs. DVJ are time normalized to 100% of time spent on force plate, squats cycles are time normalized to 100% using height of pelvis origin. Significant differences in coefficients (C) tested with SPM (t) statistic for independent measures. For coefficient plots (C1,2,3) squat coefficients are in blue and DVJ coefficients in green.

**RESULTS - Study 2: Effects of ACL deficiency and task**

*Squats*

**Control limb vs. ACL deficient limb**

Squat Synergies 1 (Squat - S1<sub>CON</sub> and Squat - S1<sub>ACLd</sub>) and S2 (Squat - S2<sub>CON</sub> and Squat - S2<sub>ACLd</sub>) were statistically equivalent while the third pair of synergies (Squat - S3<sub>CON</sub> and Squat - S3<sub>ACLd</sub>) were poorly correlated (Table 13, Figures 9 and 10). Cross reconstruction of the control data using the ACLd synergy vectors accounted for 50.2 % of its total variance while reconstructing the ACLd limb using the control limb vectors accounted for 84.9 % of its total variance.



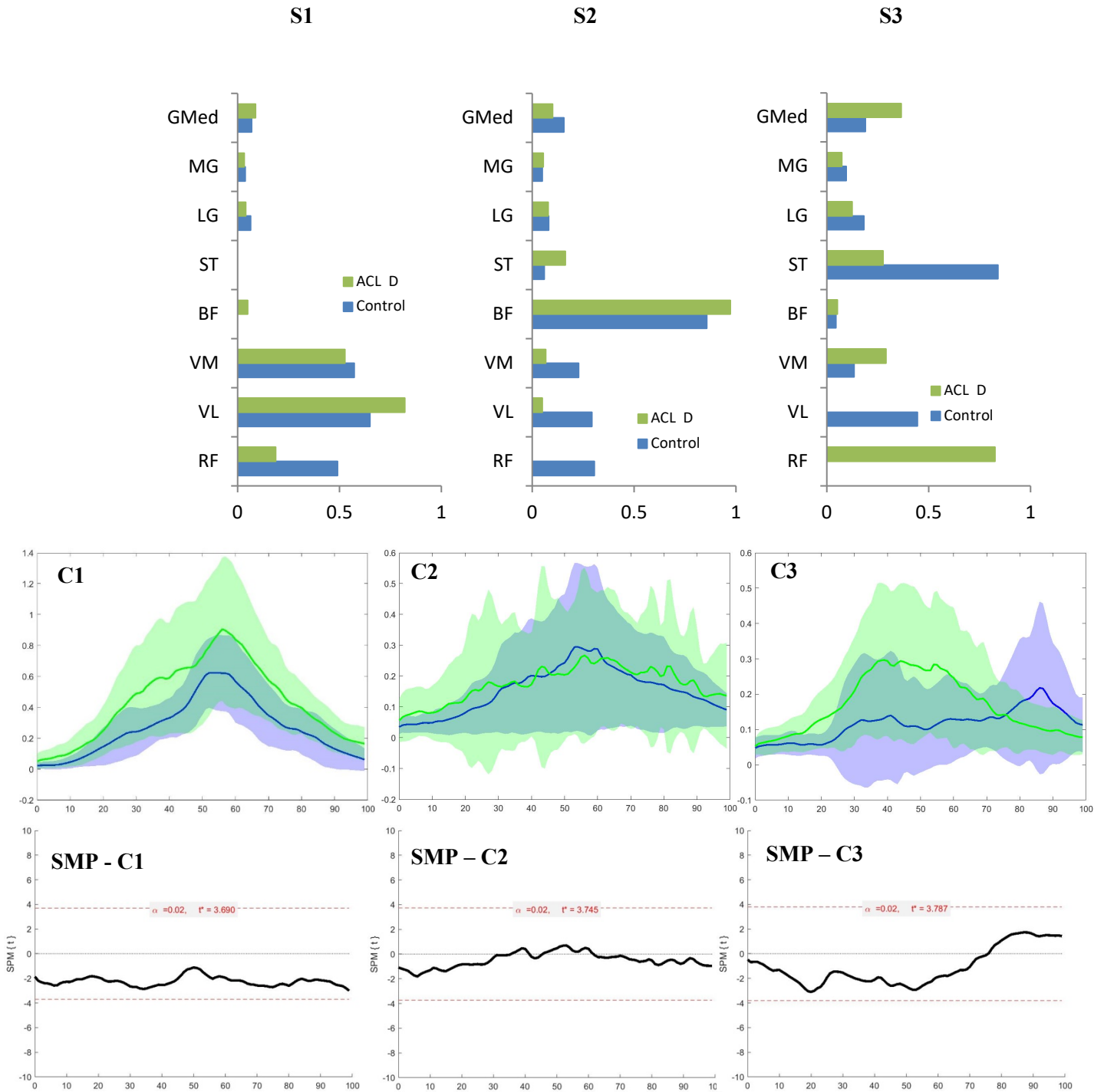
**Figure 9:** Percent relative change (%) in synergy vector norms for the muscle synergies. Control limb results used as reference point where positive numbers indicate an overall increase from the uninjured group results and negative values represent a decrease relative to the reference.

**Table 12:** Contributions of each synergy.

| Limb / Synergy | Percent of Total Contribution |       |       |
|----------------|-------------------------------|-------|-------|
|                | 1                             | 2     | 3     |
| Control        | 0.384                         | 0.321 | 0.294 |
| ACLd           | 0.421                         | 0.235 | 0.344 |

**Table 13:** Intraclass correlation coefficients (ICC) comparing synergy vectors for the squat task in the control ACL deficient limbs of paediatric females. Statistically equivalent defined as ICC > 0.80 and demoted by two asterisks (\*\*) and statistical similarity ICC > 0.60 demoted by an asterisk (\*).

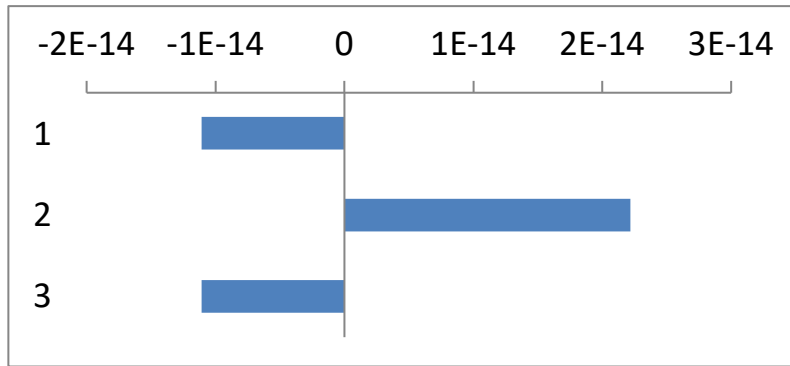
|              |           | ACLd Limb |        |       |
|--------------|-----------|-----------|--------|-------|
|              |           | 1         | 2      | 3     |
| Control Limb | Synergy # |           |        |       |
|              | 1         | 0.94**    | -      | -     |
|              | 2         | -         | 0.92** | -     |
|              | 3         | -         | -      | -0.55 |



**Figure 10:** Squat muscle synergies and SPM analysis on respective weighting coefficients for control and ACLd limbs. Squats are time normalized to 100% of squat cycle. Significant differences in coefficients (C) tested with SPM (t) statistic for independent measures.

**Control limb vs. ACL Contralateral limb**

The first pair of synergy vectors were statistically equivalent while the second and third pairs were poorly correlated (Table 15, Figures 11 and 12). Cross reconstruction of the control data using the ACLc synergy vectors accounted for 54.3 % of its total variance while reconstructing the ACLd limb using the control limb vectors accounted for 82.9 % of its total variance.



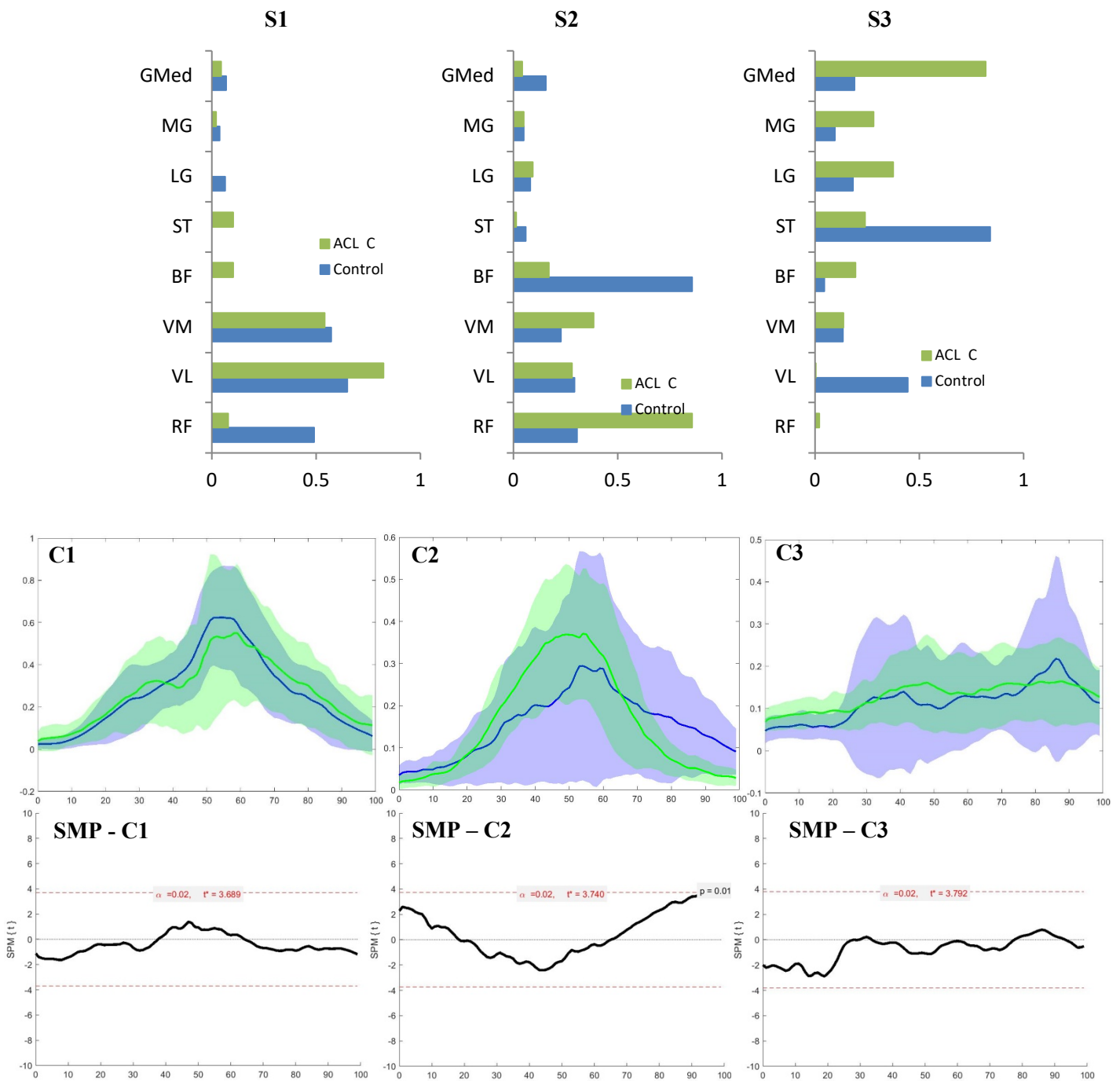
**Figure 11:** Percent relative change (%) in synergy vector norms for the muscle synergies. Control limb results used as reference point where positive numbers indicate an overall increase from the uninjured group results and negative values represent a decrease relative to the reference.

**Table 14:** Contributions of each synergy.

| Limb / Synergy | Percent of Total Contribution |       |       |
|----------------|-------------------------------|-------|-------|
|                | 1                             | 2     | 3     |
| Control        | 0.384                         | 0.322 | 0.294 |
| ACLc           | 0.323                         | 0.279 | 0.398 |

**Table 15:** Intraclass correlation coefficients (ICC) comparing synergy vectors for the squat task in the control and ACL contralateral of paediatric females. Statistically equivalent defined as ICC > 0.80 and demoted by two asterisks (\*\*) and statistical similarity ICC > 0.60 demoted by an asterisk (\*).

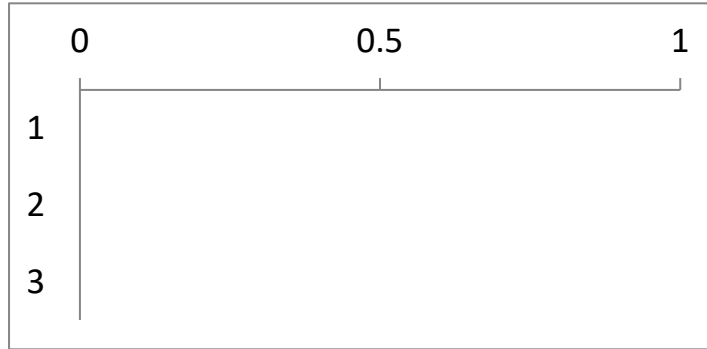
|              |   | ACLc Limb |      |       |
|--------------|---|-----------|------|-------|
|              |   | Synergy # | 1    | 2     |
| Control Limb | 1 | 0.90**    | -    | -     |
|              | 2 | -         | 0.36 | -     |
|              | 3 | -         | -    | -0.08 |



**Figure 12:** Squat muscle synergies and SPM analysis on respective weighting coefficients for control and ACLc limbs. Squats are time normalized to 100% of squat cycle. Significant differences in coefficients (C) tested with SPM (t) statistic for independent measures.

**ACL deficient limb vs. ACL Contralateral limb**

The first and second pairs of synergy vectors were equivalent while the third pair was poorly correlated (Table 17, Figures 13 and 14). Cross reconstruction of the ACLd data using the ACLc synergy vectors accounted for 70.0 % of its total variance while reconstructing the ACLc limb using the ACLd limb vectors accounted for 81.0 % of its total variance. The percent relative change between all pairs of synergy vector norms were 0% (Figure 13).



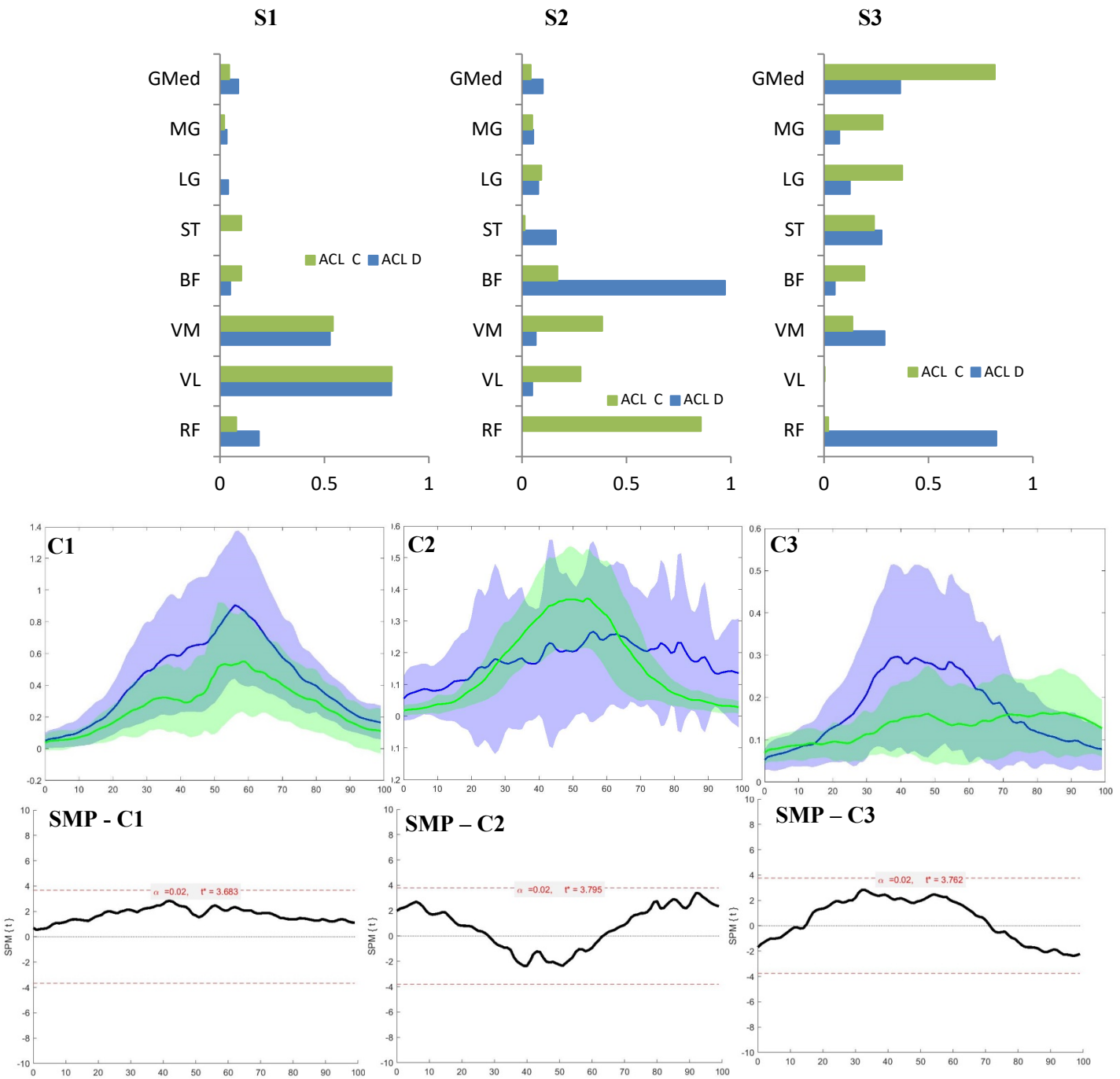
**Figure 13:** Percent relative change (%) in synergy vector norms for the muscle synergies. Control limb results used as reference point where positive numbers indicate an overall increase from the uninjured group results and negative values represent a decrease relative to the reference.

**Table 16:** Contributions of each synergy. Synergies have been renumbered to achieve the least amount of error between the synergy pairs (i.e. Squat - S1<sub>ACLd</sub> and Squat - S1<sub>ACLc</sub>).

| Limb / Synergy | Percent of Total Contribution |       |       |
|----------------|-------------------------------|-------|-------|
|                | 1                             | 2     | 3     |
| ACLd           | 0.421                         | 0.235 | 0.344 |
| ACLc           | 0.323                         | 0.279 | 0.398 |

**Table 17:** Intraclass correlation coefficients (ICC) comparing synergy vectors for the squat task in the ACL deficient and contralateral limbs of paediatric females. Statistically equivalent defined as ICC > 0.80 and demoted by two asterisks (\*\*) and statistical similarity ICC > 0.60 demoted by an asterisk (\*).

|           |           | ACLc Limb |        |       |
|-----------|-----------|-----------|--------|-------|
|           |           | 1         | 2      | 3     |
| ACLd Limb | Synergy # |           |        |       |
|           | 1         | 0.99**    | -      | -     |
|           | 2         | -         | 0.85** | -     |
|           | 3         | -         | -      | -0.07 |

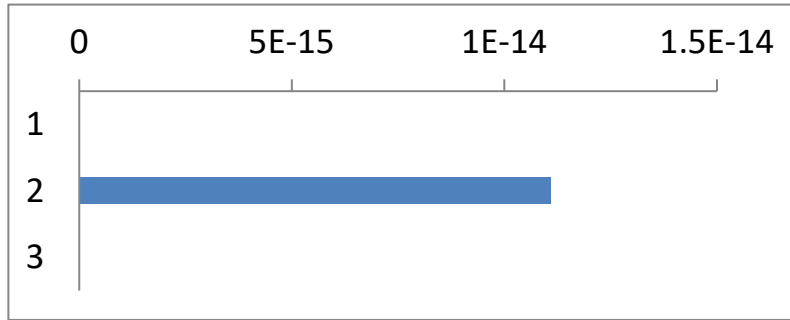


**Figure 14:** Squat muscle synergies and SPM analysis on respective weighting coefficients for ACLd and ACLc limbs. Squats are time normalized to 100% of squat cycle. Significant differences in coefficients (C) tested with SPM (t) statistic for independent measures.

DVJ

**Control limb vs. ACL deficient limb**

DVJ Synergies 1 (DVJ - S1<sub>CON</sub> and DVJ - S1<sub>ACLd</sub>) were statistically equivalent, S2 (SVJ - S2<sub>CON</sub> and SVJ - S2<sub>ACLd</sub>) were statistically similar and the third pair of synergies (DVJ - S3<sub>CON</sub> and DVJ - S3<sub>ACLd</sub>) were poorly correlated (Table 19, Figures 15 and 16). Cross reconstruction of the control data using the ACLd synergy vectors accounted for -38.8 % of its total variance while reconstructing the ACLd limb using the control limb vectors accounted for -39.2 % of its total variance.



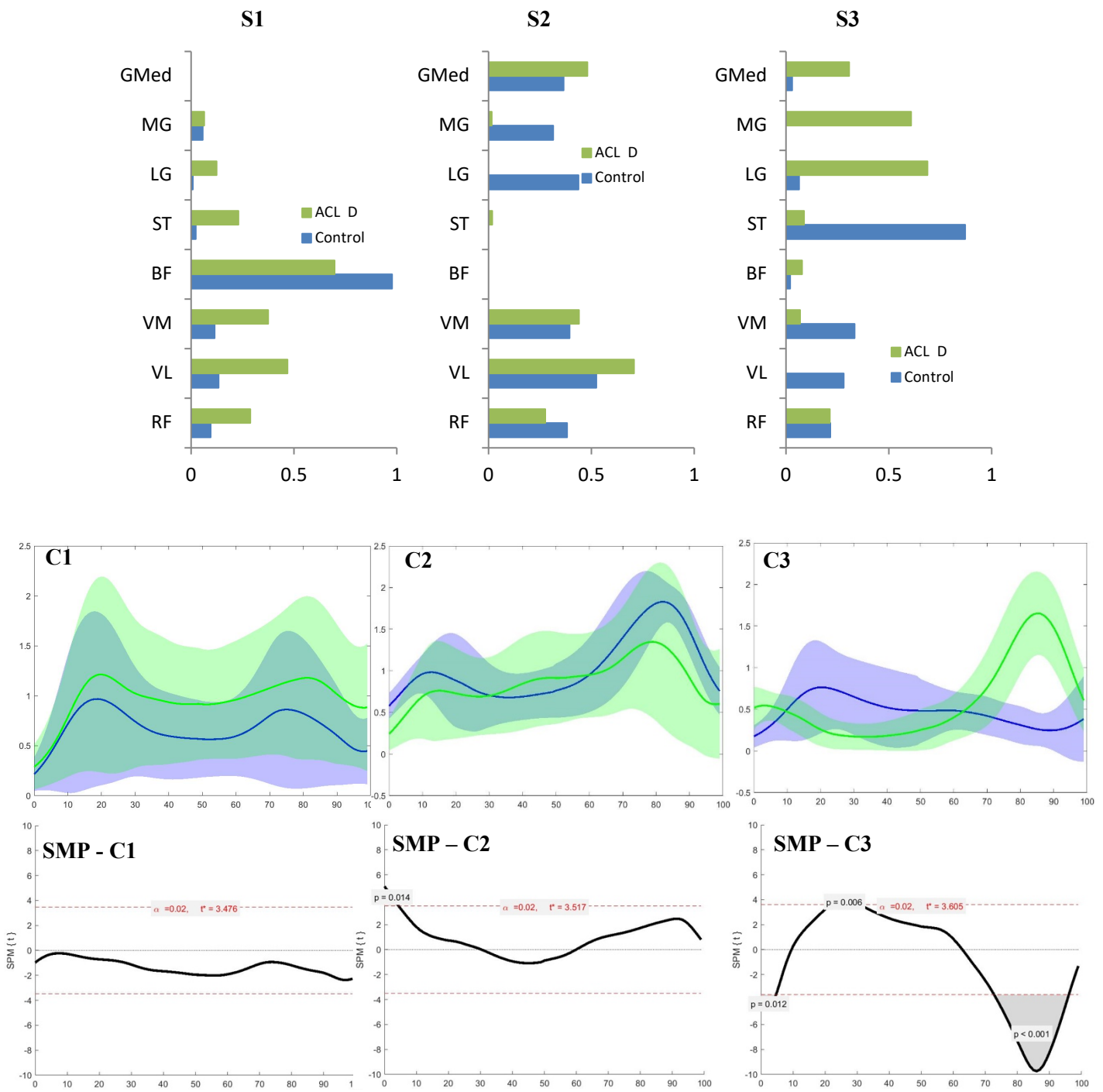
**Figure 15:** Percent relative change (%) in synergy vector norms for the muscle synergies. Control limb results used as reference point where positive numbers indicate an overall increase from the uninjured group results and negative values represent a decrease relative to the reference.

**Table 18:** Contributions of each synergy. Synergies have been renumbered to achieve the least amount of error between the synergy pairs (i.e. DVJ - S1<sub>CON</sub> and DVJ - S1<sub>ACLd</sub>).

| Limb / Synergy | Percent of Total Contribution |       |       |
|----------------|-------------------------------|-------|-------|
|                | 1                             | 2     | 3     |
| Control        | 0.603                         | 0.188 | 0.209 |
| ACLd           | 0.413                         | 0.270 | 0.317 |

**Table 19:** Intraclass correlation coefficients (ICC) comparing synergy vectors for the squat task in the control and ACL deficient limbs of paediatric females. Statistically *equivalent* defined as ICC > 0.80 and demoted by two asterisks (\*\*) and statistical similarity ICC > 0.60 demoted by an asterisk (\*).

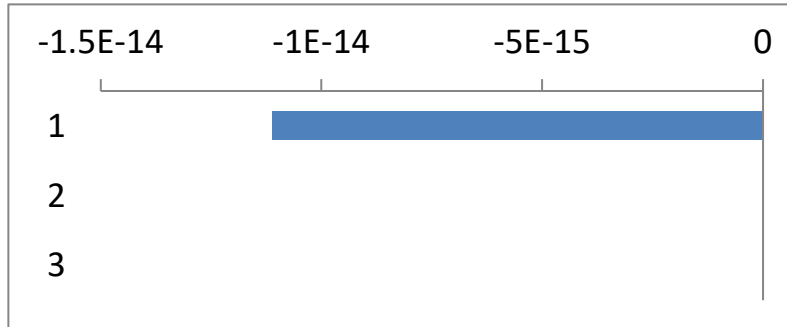
|              |   | ACLd Limb |       |       |
|--------------|---|-----------|-------|-------|
|              |   | Synergy # | 1     | 2     |
| Control Limb | 1 | 0.83**    | -     | -     |
|              | 2 | -         | 0.76* | -     |
|              | 3 | -         | -     | -1.99 |



**Figure 16:** DVJ muscle synergies and SPM analysis on respective weighting coefficients for control and ACLD limbs. DVJ are time normalized to 100% of time spent on force plate. Significant differences in coefficients (C) tested with SPM (t) statistic for independent measures

**Control limb vs. ACL Contralateral limb**

The pair of synergy vectors were statistically equivalent while the second pair was statistically similar and the third pair was poorly correlated (Table 21, Figures 17 and 18). Cross reconstruction of the control data using the ACLc synergy vectors accounted for -20.7 % of its total variance while reconstructing the ACLd limb using the control limb vectors accounted for -25.7 % of its total variance.



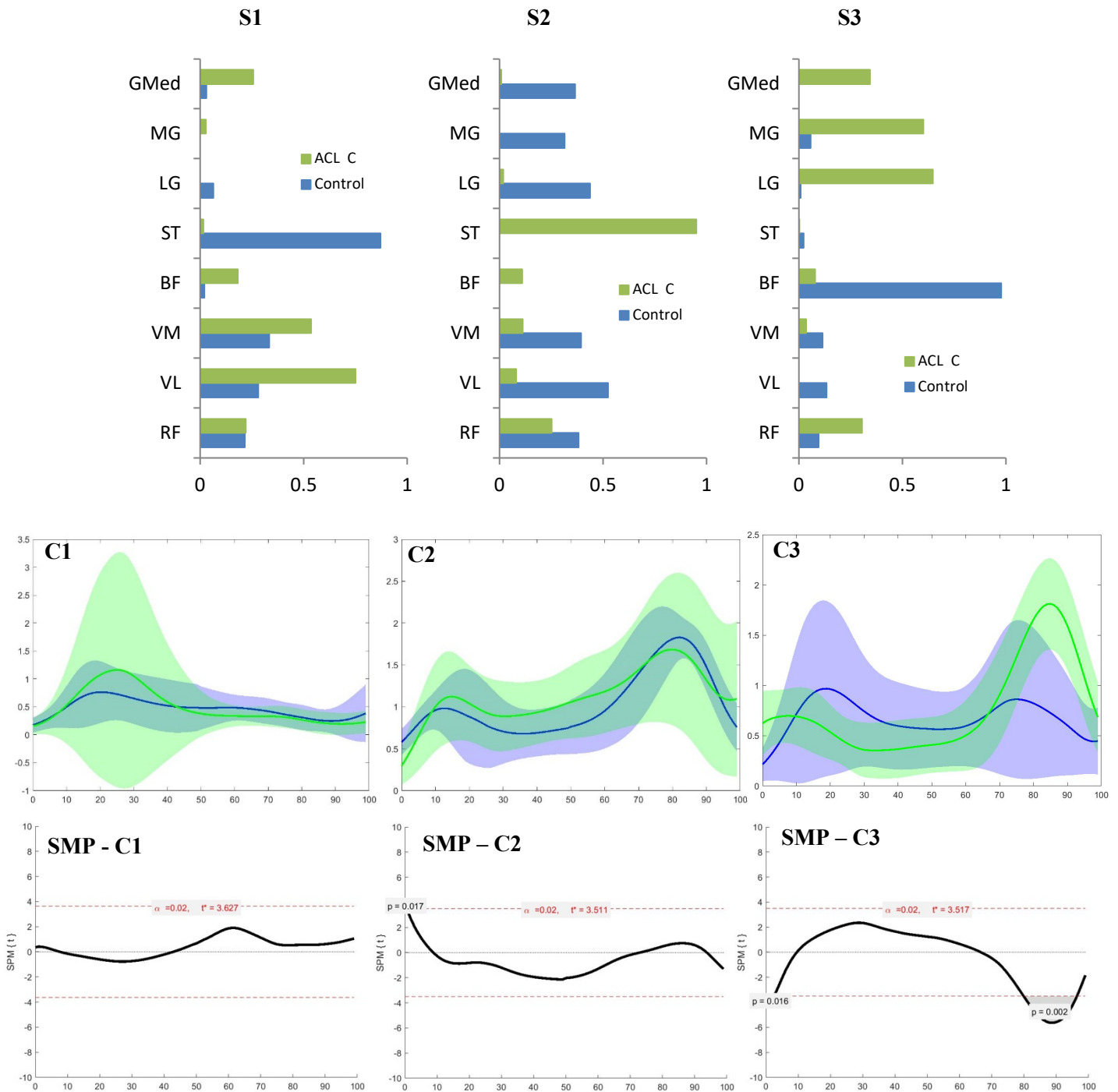
**Figure 17:** Percent relative change (%) in synergy vector norms for the muscle synergies. Control limb results used as reference point where positive numbers indicate an overall increase from the uninjured group results and negative values represent a decrease relative to the reference.

**Table 20:** Contributions of each synergy.

| Limb / Synergy | Percent of Total Contribution |       |       |
|----------------|-------------------------------|-------|-------|
|                | 1                             | 2     | 3     |
| Control        | 0.603                         | 0.188 | 0.209 |
| ACLc           | 0.440                         | 0.177 | 0.384 |

**Table 21:** Intraclass correlation coefficients (ICC) comparing synergy vectors for the squat task in the control and ACL contralateral limbs of paediatric females. Statistically equivalent defined as ICC > 0.80 and demoted by two asterisks (\*\*) and statistical similarity ICC > 0.60 demoted by an asterisks (\*).

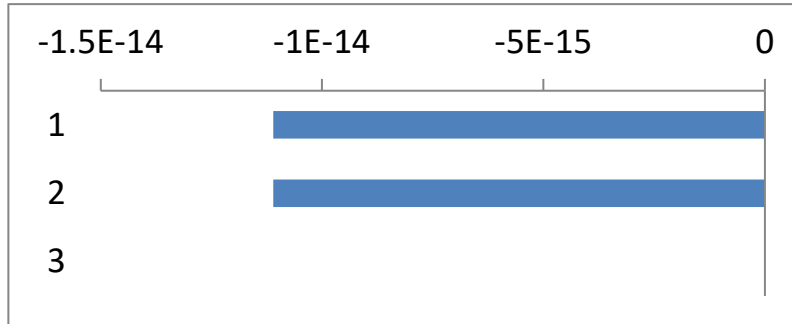
|              |           | ACLc Limb |       |       |
|--------------|-----------|-----------|-------|-------|
|              |           | 1         | 2     | 3     |
| Control Limb | Synergy # |           |       |       |
|              | 1         | 0.96**    | -     | -     |
|              | 2         | -         | 0.66* | -     |
|              | 3         | -         | -     | -0.99 |



**Figure 18:** DVJ muscle synergies and SPM analysis on respective weighting coefficients for control and ACLc limbs. DVJ are time normalized to 100% of time spent on force plate. Significant differences in coefficients (C) tested with SPM (t) statistic for independent measures

**ACL deficient limb vs. ACL Contralateral limb**

The first two pairs of synergy vectors were statistically equivalent while the vectors of the third pair were poorly correlated (Table 23, Figures 19 and 20). Cross reconstruction of the ACLd data using the ACLc synergy vectors accounted for 24.9 % of its total variance while reconstructing the ACLc limb using the ACLd limb vectors accounted for -7.92 % of its total variance.



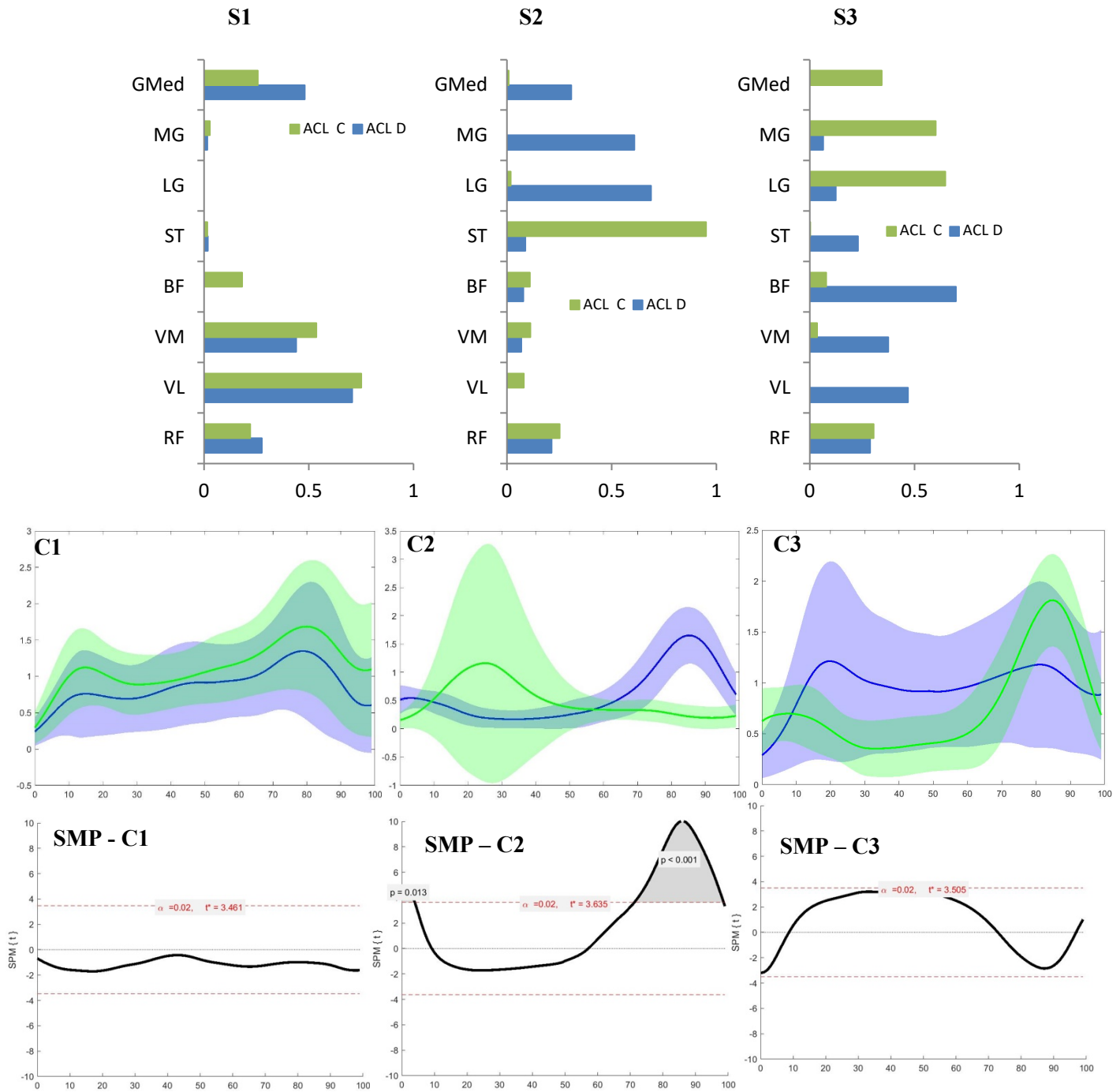
**Figure 19:** Percent relative change (%) in synergy vector norms for the muscle synergies. Control limb results used as reference point where positive numbers indicate an overall increase from the uninjured group results and negative values represent a decrease relative to the reference.

**Table 22:** Contributions of each synergy.

| Limb / Synergy | Percent of Total Contribution |       |       |
|----------------|-------------------------------|-------|-------|
|                | 1                             | 2     | 3     |
| ACLd           | 0.413                         | 0.270 | 0.317 |
| ACLc           | 0.441                         | 0.176 | 0.384 |

**Table 23:** Intraclass correlation coefficients (ICC) comparing synergy vectors for the squat task in the ACL deficient and contralateral limbs of paediatric females. Statistically equivalent defined as ICC > 0.80 and demoted by two asterisks (\*\*) and statistical similarity ICC > 0.60 demoted by an asterisk (\*).

|           |   | ACLcLimb  |        |      |
|-----------|---|-----------|--------|------|
|           |   | Synergy # | 1      | 2    |
| ACLd Limb | 1 | 0.95**    | -      | -    |
|           | 2 | -         | 0.99** | -    |
|           | 3 | -         | -      | 0.07 |

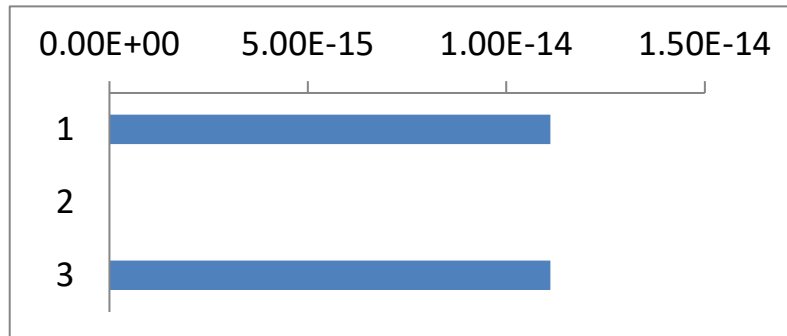


**Figure 20:** DVJ muscle synergies and SPM analysis on respective weighting coefficients for ACLd and ACLc limbs. DVJ are time normalized to 100% of time spent on force plate. Significant differences in coefficients (C) tested with SPM (t) statistic for independent measures.

**Effects of task (within injured limb)**

ACLd limb

The first pair of ACLd limb synergy vectors were statistically equivalent while the second pair was statistically similar and the vectors in the third pair were poorly correlated (Table 25, Figures 21 and 22). Cross reconstruction of the squat data using the DVJ synergy vectors accounted for 39.3 % of its total variance while reconstructing the DVJ task using the squat dominant limb synergy vectors accounted for - 31.9 % of its total variance.



**Figure 21:** Percent relative change (%) in synergy vector norms for the muscle synergies. ACLd limb squat results used as reference point where positive numbers indicate an overall increase from squat results and negative values represent a decrease relative to the reference.

**Table 24:** Contributions of each synergy. Synergies have been renumbered to achieve the least amount of error between the synergy pairs (i.e. Squat -  $S1_{ACLd}$  and DVJ -  $S1_{ACLd}$ ).

| Task / Synergy | Percent of Total Contribution |       |       |
|----------------|-------------------------------|-------|-------|
|                | 1                             | 2     | 3     |
| Squat          | 0.421                         | 0.235 | 0.344 |
| DVJ            | 0.413                         | 0.270 | 0.317 |

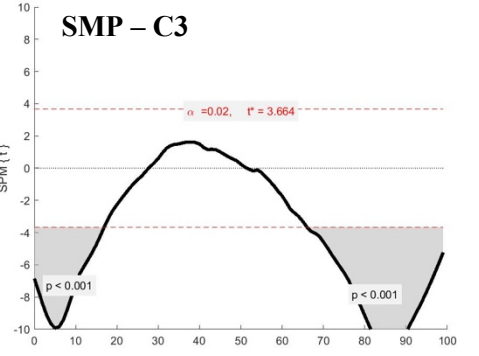
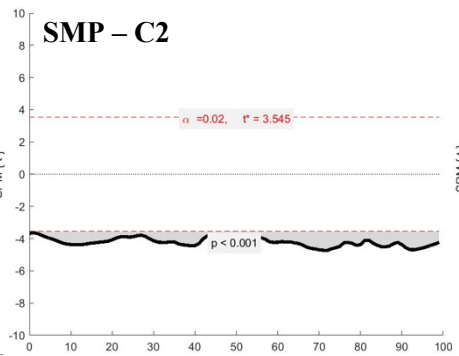
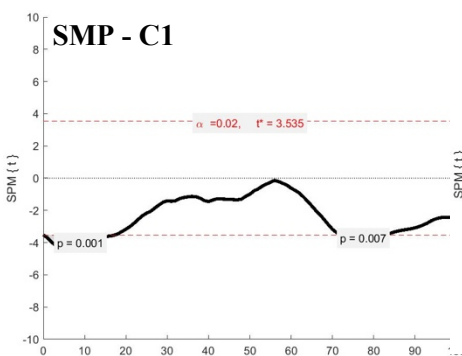
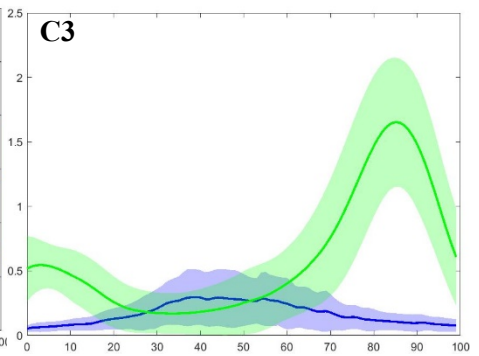
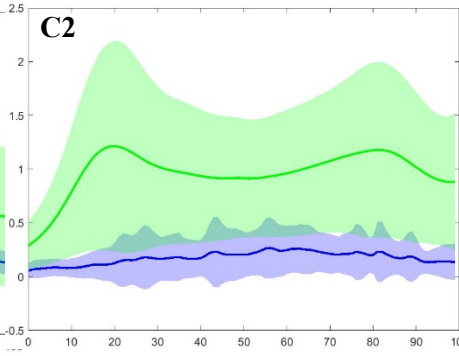
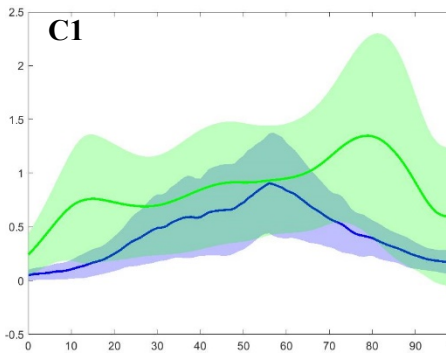
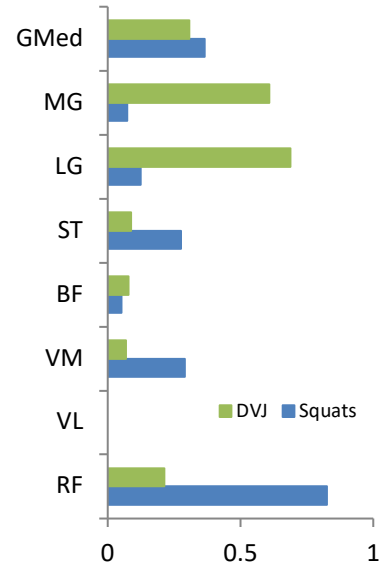
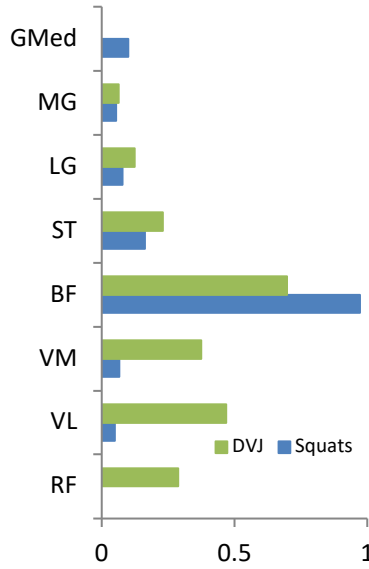
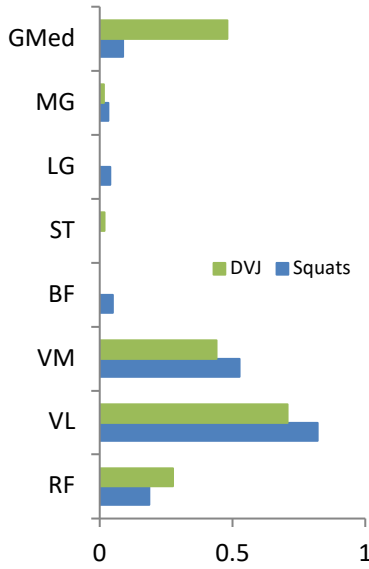
**Table 25:** Intraclass correlation coefficients (ICC) comparing ACL deficient synergy vectors for the squat and DVJ tasks. Statistically equivalent defined as ICC > 0.80 and demoted by two asterisks (\*\*) and statistical similarity ICC > 0.60 demoted by an asterisk (\*).

|       | Synergy # | DVJ    |       |       |
|-------|-----------|--------|-------|-------|
|       |           | 1      | 2     | 3     |
| Squat | 1         | 0.91** | -     | -     |
|       | 2         | -      | 0.79* | -     |
|       | 3         | -      | -     | -0.22 |

S1

S2

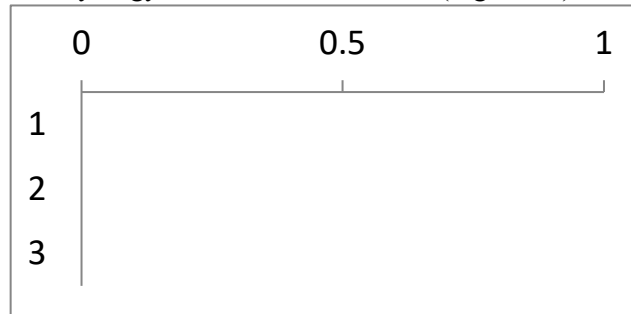
S3



**Figure 22:** Squat and DVJ muscle synergies and SPM analysis on respective weighting coefficients for the ACLd limbs. DVJ are time normalized to 100% of time spent on force plate, squats cycles are time normalized to 100% using height of pelvis origin. Significant differences in coefficients (C) tested with SPM (t) statistic for independent measures. For coefficient plots (C1,2,3) squat coefficients are in blue and DVJ coefficients in green.

ACLc limb

The first pair of ACLd limb synergy vectors were statistically equivalent while the second and third pair were poorly correlated (Table 27, Figures 23 and 24). Cross reconstruction of the squat data using the DVJ synergy vectors accounted for 86.1 % of its total variance while reconstructing the DVJ task using the squat dominant limb synergy vectors accounted for 1.00 % of its total variance. The percent relative change between all pairs of synergy vector norms were 0% (Figure 23).



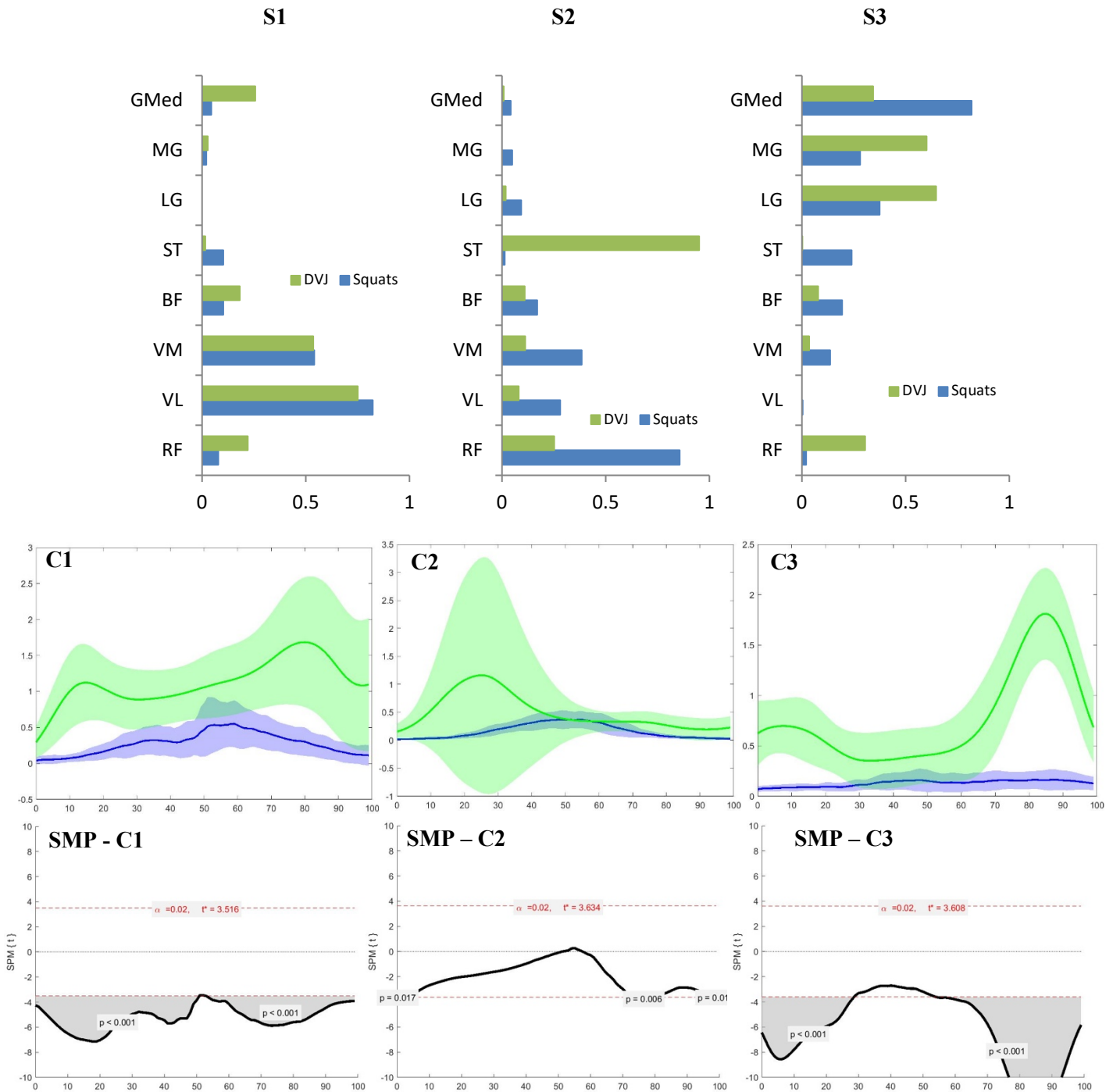
**Figure 23:** Percent relative change (%) in synergy vector norms for the muscle synergies. ACLc limb squat results used as reference point where positive numbers indicate an overall increase from squat results and negative values represent a decrease relative to the reference.

**Table 26:** Contributions of each synergy. Synergies have been renumbered to achieve the least amount of error between the synergy pairs (i.e. Squat -  $S1_{ACLc}$  and DVJ -  $S1_{ACLc}$ ).

| Task / Synergy | Percent of Total Contribution |       |       |
|----------------|-------------------------------|-------|-------|
|                | 1                             | 2     | 3     |
| Squat          | 0.323                         | 0.279 | 0.398 |
| DVJ            | 0.441                         | 0.176 | 0.384 |

**Table 27:** Intraclass correlation coefficients (ICC) comparing ACL contralateral limb synergy vectors for the squat and DVJ tasks. Statistically equivalent defined as  $ICC > 0.80$  and demoted by two asterisks (\*\*) and statistical similarity  $ICC > 0.60$  demoted by an asterisk (\*).

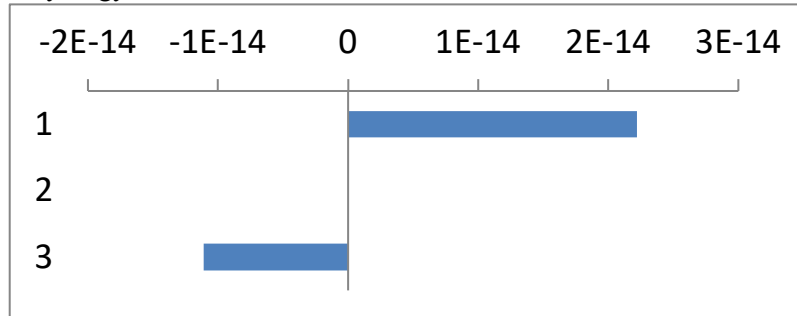
|       | Synergy # | DVJ    |       |      |
|-------|-----------|--------|-------|------|
|       |           | 1      | 2     | 3    |
| Squat | 1         | 0.97** | -     | -    |
|       | 2         | -      | -0.16 | -    |
|       | 3         | -      | -     | 0.58 |



**Figure 24:** Squat and DVJ muscle synergies and SPM analysis on respective weighting coefficients for the ACLc limbs. DVJ are time normalized to 100% of time spent on force plate, squats cycles are time normalized to 100% using height of pelvis origin. Significant differences in coefficients (C) tested with SPM (t) statistic for independent measures. For coefficient plots (C1,2,3) squat coefficients are in blue and DVJ coefficients in green.

Control limb

The first two pairs of synergy vectors were statistically equivalent while the third pair of vectors were statistically similar (Table 29, Figures 25 and 26). Cross reconstruction of the squat data using the DVJ synergy vectors accounted for 76.9 % of its total variance while reconstructing the DVJ task using the squat dominant limb synergy vectors accounted for 78.2 % of its total variance.



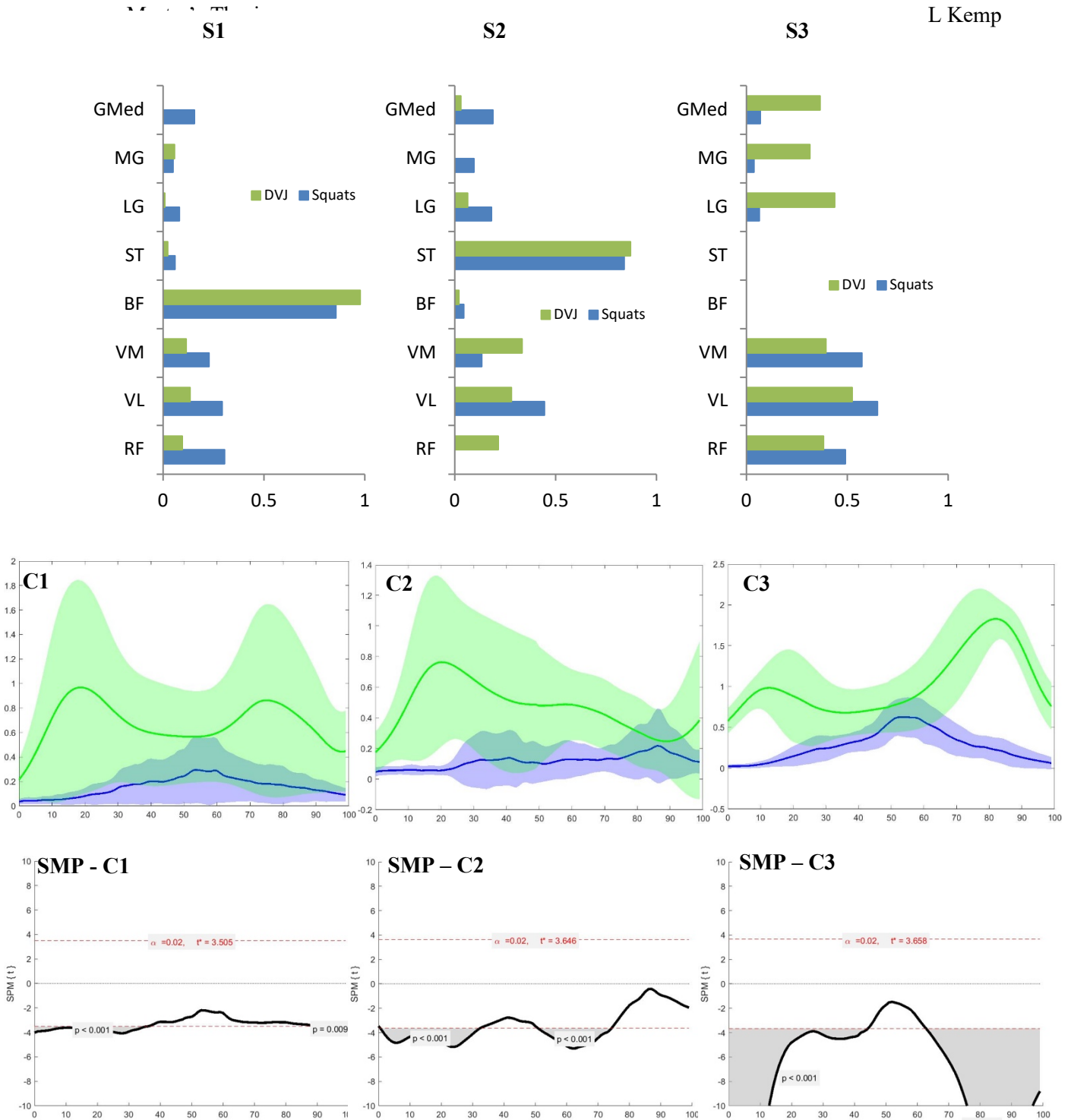
**Figure 25:** Percent relative change (%) in synergy vector norms for the muscle synergies. Control limb squat results used as reference point where positive numbers indicate an overall increase from squat results and negative values represent a decrease relative to the reference.

**Table 28:** Contributions of each synergy. Synergies have been renumbered to achieve the least amount of error between the synergy pairs (i.e. Squat - S1<sub>CON</sub> and DVJ - S1<sub>CON</sub>).

| Task / Synergy | Percent of Total Contribution |       |       |
|----------------|-------------------------------|-------|-------|
|                | 1                             | 2     | 3     |
| Squat          | 0.384                         | 0.321 | 0.294 |
| DVJ            | 0.603                         | 0.188 | 0.210 |

**Table 29:** Intraclass correlation coefficients (ICC) comparing synergy vectors for the squat task in the dominant and dominant ACL deficient limb of paediatric females. Statistically *equivalent* defined as ICC > 0.80 and demoted by two asterisks (\*\*) and statistical similarity ICC > 0.60 demoted by an asterisk (\*).

| Synergy # |   | DVJ    |        |       |
|-----------|---|--------|--------|-------|
|           |   | 1      | 2      | 3     |
| Squat     | 1 | 0.97** | -      | -     |
|           | 2 | -      | 0.92** | -     |
|           | 3 | -      | -      | 0.75* |



**Figure 26:** Squat and DVJ muscle synergies and SPM analysis on respective weighting coefficients for the control limbs. DVJ are time normalized to 100% of time spent on force plate, squats cycles are time normalized to 100% using height of pelvis origin. Significant differences in coefficients (C) tested with SPM (t) statistic for independent measures. For coefficient plots (C1,2,3) squat coefficients are in blue and DVJ coefficients in green.

## Appendix 2 – CBRU/CHEO Collaborative Research Protocol

All participants will read and sign consent forms explaining the procedure, any possible risks, and the purpose of the study. We are recruiting participants between the ages of 8 and 18, therefore some will have full capacity to provide consent on their own. However, some participants may not have full capacity to provide consent on their own behalf (even though there is no age of consent in Ontario) therefore those participants will complete an additional assent form with their guardian. Each participant will then complete a subject assessment of knee function (Pedi-IKDC, American Orthopaedic Society for Sports Medicine), two subjective assessments of activity levels (Hospital for Special Surgery Paediatric Functional Activity Brief Scale (HSS Pedi-FABS), Fabricant et al. 2013; and Tegner Activity Scale, Tegner and Lysholm, 1985) and a pubescent-stage self-assessment form (Tanner Stages, Public Health Agency of Canada) (20min). For patients returning for their 2<sup>nd</sup> and 3<sup>rd</sup> visits to the laboratory, they will be performing an additional questionnaire that focuses on the psychological factors that may influence their return to sport (ACL Return to Sport after Injury (RSI) scale, Webster et al. 2008). Following the completion of these documents, anthropometric measurements will be collected including; pelvis, knee, and ankle width, height, and weight; Q-angle; leg length; and thigh and shank circumference (2min).

Following anthropometric measurements, participants will undergo a full warm-up protocol including cycling on a stationary ergometer (10min). In addition, instructions on how to properly perform each dynamic task will be provided and each participant will be given a chance to practice each task (3min). Wireless, bipolar surface EMG electrodes (16-channel Trigno, Delsys, Boston, USA) will then be placed over the muscle bellies of the rectus femoris, vastus medialis, vastus lateralis, semitendinosus, biceps femoris, medial gastrocnemius, lateral gastrocnemius, and gluteus medius on both limbs (20min). Electrode placements will follow the recommendations by SENIAM (Hermens et al., 2000) and DeLuca (DeLuca, 1997). Maximum voluntary isometric contractions (MVIC) will then be conducted using manual resistance and will include knee flexion and extension, recorded in a seated position with the knee flexed at 45 degrees, and plantarflexion, recorded in a seated position with the knee at 0 degrees flexion and the ankle in a neutral position. Hip flexion, extension and abduction will be recorded in a standing position, with the hip at 0 degree extension. Ten seconds with verbal encouragement and on screen feedback will allow participants to scale perceived force to their maximal effort and hold it for approximately three seconds (20min).

Participants will then have reflective markers (14mm diameter) placed on various landmarks according to a hybrid cluster-marker set (10min). Each participant will then perform each of the following tests in two blocks: clinical/functional tasks and dynamic tasks. Clinical/functional tasks will consist of timed 6m hop tests, triple hop tests for distance, cross-hop tests for distance, single leg anterior and lateral hops for distance, muscular (quadriceps and hamstrings) endurance assessments, and isometric knee flexion and extension strength assessments. Dynamic tasks will consist of side-cuts, two-legged squats, lunges, and drop-vertical jumps. For the purposes of this evaluation, clinically significant differences between the injured/operated and healthy/control limb are generally considered to be greater than a 10% deficit; however this interpretation is at the discretion of the attending clinician and may be test dependent.

### **Clinical/Functional Tasks**

The purpose of these tests is to evaluate the relative difference in functional capacity of the patient's limbs. The tests chosen have been clinically validated in a rehabilitation setting (Adams et al., 2012), however given that the testing is being performed in a laboratory setting; they may not be a true representation of the functional capacity of the individual.

#### **Max Anterior Hops**

Participants will be instructed to hop as far as they can on one foot facing forward.

#### **Max Lateral Hops**

Participants will be instructed to hop as far as they can on one foot to the side (facing perpendicular to the direction of the marked line on the laboratory floor).

#### **Timed 6m Hop**

Participants will be instructed to hop on one foot as fast as they can to cover a distance of six meters.

#### **Triple Hop – Distance**

Participants will be instructed to hop on one foot three times in a row for maximum distance.

**Cross-Hop – Distance**

Participants will be instructed to hop on one foot back and forth across a marked line on the floor, while attempting to cover maximum distance during four hops side to side across the line.

**Muscular Endurance:**

Participants will be on the Biodex Dynamometer and will be instructed to maximally generate knee extension and knee flexion torques sequentially. This will be repeated for a total of 40 repetitions at a set speed of 90 degrees/second to evaluate the muscular endurance of the patient over the 40 repetitions of maximal concentric dynamic contractions on a Biodex Dynamometer. This task will provide an indication of how the patient may perform following repeated exercise as may be the case during practice or game situations. Knee flexion and extension torques will be averaged over the first 5 repetitions (#1) and the last 5 repetitions (#2). The deficit will be determined as a ratio of the (#2)/(#1). A lower deficit score indicate a higher level of fatigue after 40 repetitions.

**Dynamic Hamstring to Quadriceps Strength Ratio:**

The hamstring to quadriceps ratio will be evaluated during the dynamic concentric contractions at 90 degrees/second on a Biodex Dynamometer.

**Isometric Strength:**

Participants will be instructed to maximally generate knee extension and knee flexion torques separately, for 5 seconds (repeated 3 times each) to evaluate the torque generation from the hamstrings and quadriceps during isometric contractions on a Biodex Dynamometer and manual isometric contractions with resistance from the researcher.

**Dynamic Tasks**

The purpose of these tests is, similar to the previous set, to evaluate the relative difference in functional capacity of the patient's limbs during a variety of sport-related movements that are also often used in injury prevention programs (Barengo et al. 2014).

**Side-Cuts:**

Participants will be instructed to run at an approach velocity that is 75% of their maximum sprint velocity, before performing a 45 degree side-cut. They will step and plant the leg of interest on the force plate, and accelerate towards a set of cones that are marked at an angle of 45 degrees from the force plate.

**Two-Legged Squats:**

Participants will be instructed to stand on two force plates that are side-by-side, with one foot on each plate and shoulder-width apart. They will then be instructed to hold their hands on their head while squatting down at a self-selected pace as low as they can comfortably, before returning to the starting position.

**Lunges:**

Participants will be instructed to stand facing a force plate so that when they step forward, their foot of interest is in the center of the force plate while their knee and hip joints are both flexed to 90 degrees. They will be instructed to hold their hands on their head while stepping forward, lunging, and returning to the starting position at a self-selected pace.

**Drop-Vertical Jumps:**

Participants will begin on a platform (adjusted to the height of their tibia) and will drop down onto two force plates, with each foot landing on a separate plate. Immediately after landing, they will be instructed to perform a maximum vertical jump. There will be three additional conditions associated with this task. Similar to the previously described drop-vertical jump task, participants will drop down onto the two force plates, yet will be randomly cued how to complete the second part of the drop-vertical jump. Upon the first landing, the impact on the force plates will trigger a projected image on a screen, which the participant will be facing, instructing them to perform the maximum vertical jump with one of the following types of landings: i) single-leg landings for the left leg, ii) for the right leg and iii) sticking the landing (no maximum vertical jump after first landing).

The 1<sup>st</sup> peak ground reaction force (GRF) will display the mean maximum value from 5 trials of the first impact when the participants lands from the raised platform. For the trials involving two landings, the 2<sup>nd</sup> peak GRF will display the mean maximum value from 5 trials of the second impact after participants performed a maximum jump. We will assess the difference in GRFs between limbs during both impacts to determine whether a functional difference or a shielding effect (protection of injured limb) is taking place.

Participants will complete a minimum of three successful trials for each of the outlined tasks and will have access to water throughout the study (1hr). EMG electrodes and motion capture markers will then be removed from participants (5min).

