ASSESSING THE FUNCTIONAL CAPACITY OF THE KNEE JOINT AMONG BOYS AND GIRLS SUFFERING ANTERIOR CRUCIATE LIGAMENT (ACL) INJURIES

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LIST OF ACRONYMS

ACL	Anterior cruciate ligament
ACLd	Anterior cruciate ligament deficiency
ACLi	Anterior cruciate ligament injury
ACLr	Anterior cruciate ligament reconstruction
ACL-RSI	ACL - Return to Sport after Injury Questionnaire
ADL	Activities of daily life
BF	Biceps femoris
BMI	Body mass index
CHEO	Children's Hospital of Eastern Ontario
CMJ	Countermovement jump
CON	Control participant (uninjured)
EMG	Electromyography
FACL	Females with ACL injury
FCON	Female controls
GM	Gluteus medius
GRF	Ground reaction forces
HMBL	Human Movement Biomechanics Laboratory
IKDC	International Knee Documentation Committee Questionnaire
INJ	Injured participant
KJFE	Knee joint frontal excursion
KJFS	Knee joint flexion stiffness
KJSE	Knee joint sagittal excursion
LG	Lateral gastrocnemius
MACL	Males with ACL injury
MCON	Male controls
MG	Medial gastrocnemius
MVIC	Maximum voluntary isometric contraction
OA	Osteoarthritis
Pedi-IKDC	Pediatric International Knee Documentation Committee Questionnaire
PROM	Patient-reported outcome measure
QOL	Quality of life
RF	Rectus femoris
ROM	Range of motion
RTA	Return-to-activity
SD	Standard deviation
SENIAM	Surface Electromyography for the Non-Invasive Assessment of Muscles
SPM	Statistical parametric mapping
ST	Semitendinosus
VL	Vastus lateralis
VM	Vastus medialis

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PREFACE

While there have been extensive biomechanical analyses performed on adult populations with and without ACL injuries to help identify injury risk factors (i.e., sex and limb-dominance) and inform clinical guidelines, there are little-to-no evidence-based criteria established for adolescent populations. It is therefore not surprising to see the continued upward trend of ACL injuries among adolescents. Addressing the gap in evidence-based clinical guidelines for adolescents suffering from ACL injuries is critical if we are to reduce the risk of primary and secondary ACL injuries in younger cohorts.

The overall goal of this Doctoral thesis was to provide evidence among adolescent cohorts using measures of knee stabilisation that are currently used with adult patients with ACL injuries. The two main objectives of this thesis were to (1) quantify knee stabilisation strategies used by adolescent males and females without an ACL injury and (2) identify the influence of an ACL injury on knee stabilisation strategies in each sex independently. To achieve these goals, four research questions were identified in this thesis: (A) to perform a systematic review of current literature examining sex differences in muscle activation patterns during jump-landing and cutting tasks; (B) to provide both i) a normative data set of uninjured males and females to be used as a benchmark for clinicians and ii) a comparison between the sexes of the uninjured cohort and the injured population; (C) to identify what knee stabilisation strategies were being used by adolescent males and females, during sport-related movement tasks often associated with ACL injuries; (D) to identify how sex and an ACL injury influences knee stabilisation strategies in an adolescent population during dynamic maneuvers.

This thesis is assembled in article format. Chapters 1, 2, 3, and 4 consist of the general introduction, literature review, purpose and hypotheses, and general methodology, respectively. Chapters 5, 6, 7, and 8 each include one independent study whose references are standalone. Chapter 5, Article 1 has been accepted for publication in the journal of *Journal of Electromyography and Kinesiology*. Chapters 6, 7, and 8, (Articles 2, 3, and 4, respectively) are formatted to be submitted for publication. Chapter 9 of this thesis provides a general discussion briefly restating the main findings of the four studies as it relates to the main goals of this thesis and mentions any findings, limitations, and suggestions for future research not mentioned in the discussion section of the articles. Chapter 10 provides a general conclusion of the findings of this thesis and briefly discusses the impact and clinical relevance of the findings.

RESEARCH CONTRIBUTIONS

Chapter 5, Article 1: *Sex differences in muscle activation patterns associated with anterior cruciate ligament injury during landing and cutting tasks: A systematic review. (Published in Journal of Electromyography and Kinesiology 60 (2021): 102583)*

The first study of this thesis was a systematic review. The theoretical conceptions for the purpose and literature search were a combined effort among all co-authors. Michael Del Bel and Dr. Reiko Otsuki performed the search, sequential screening, and scoring for all included articles. The manuscript was developed and written by both Michael Del Bel and Dr. Otuski and edited by all co-authors.

Chapter 6, Article 2: *Functional capacity and limb symmetry among adolescent males and females with and without ACL injuries during a battery of single-limb strength and hopping tasks. (Formatted for submission to Journal of Orthopaedic & Sports Physical Therapy)*

The second study of this thesis explored sex and injury effects during a battery of tests used in assessing return-to-activity readiness. The theoretical conception of these experiments and data collections was a combined effort from all co-authors. For the 141 data collections, either Michael Del Bel, Celine Girard, or Nicholas Romanchuk was leading. Michael Del Bel was responsible for all data/statistical analyses and manuscript development, which was edited by all co-authors.

Chapter 7, Article 3: *Knee joint stabilisation strategies in adolescent males and females during jump-landing and side-cutting. (Formatted for submission to British Journal of Sports Medicine)*

The third study of this thesis explored sex and limb-dominance effects on knee stabilisation strategies during jump-landing and side-cutting tasks. The theoretical conception of these experiments and data collections was a combined effort from all co-authors. During all 36 data collections, either Michael Del Bel, Celine Girard, or Nicholas Romanchuk was leading. Vicon Nexus labelling and electromyography post-processing of the countermovement jumps and side-cutting was completed by Michael Del Bel. Post-processing scripts used for kinematic, kinetic and electromyography outputs were predominately written by Nicholas Romanchuk and Blake Miller, established for the entire research team to use. Modifications of post-processing scripts were completed by Michael Del Bel. Michael Del Bel was responsible for all data/statistical analyses and manuscript development, which was edited by all co-authors.

Chapter 8, Article 4: *Joint stiffness and muscles activation patterns during jump-landing and side-cutting in adolescent males and females with and without ACL injuries. (Formatted for submission to British Journal of Sports Medicine)*

The fourth study of this thesis expanded on study three, by including cohorts of males and females with ACL injuries and comparing them to their uninjured counterparts. The theoretical conception of these experiments and data collections was a combined effort from all co-authors. During all 72 data collections, either Michael Del Bel, Celine Girard, or Nicholas Romanchuk was leading. Vicon Nexus labelling and electromyography post-processing of the countermovement jumps and side-cutting was completed by Michael Del Bel. Post-processing scripts used for kinematic, kinetic and electromyography outputs were predominately written by Nicholas Romanchuk and Blake Miller, established for the entire research team to use. Modifications of post-processing scripts were completed by Michael Del Bel. Michael Del Bel was responsible for all data/statistical analyses and manuscript development, which was edited by all co-authors.

GENERAL ABSTRACT

Despite the well-researched impact of ACL injuries, both acutely and longitudinally, and even though adolescent females have the highest risk of sustaining an ACL injury, there is surprisingly very little biomechanical research conducted on adolescent populations after an ACL rupture has taken place. The increasing rates of ACL injuries and re-injury among adolescents demonstrates a need for improved rehabilitation and return-to-activity (RTA) guidelines that are age/maturity- and sex-appropriate. To accomplish this, the first step is to describe this population alongside a non-injured activity-, maturity- and sex-matched population. Therefore, the overall purpose of this Doctoral thesis was to provide evidence to support the creation of return-to-activity guidelines for adolescent patients with ACL injuries. The findings of this thesis, briefly outlined below, can directly contribute to improving clinical and rehabilitation management of ACL injuries among adolescent males and females.

First, a systematic review was performed to synthesize sex differences in muscle activation patterns in movements associated with ACL injuries in both adult and adolescent populations. Ultimately, 30 articles were included in the review with a wide range of inconsistent findings. In brief, females demonstrated higher pre- and post-landing activation of the quadriceps and lower activation of the hamstrings in 15 of 27 studies. Females also had higher quadriceps-to-hamstring co-contraction ratios during

pre- and post-landing phases compared to their male counterparts in 4 of 9 studies. What's more concerning was the identification of only five research articles that evaluated muscle activations in adolescent or younger cohorts, further emphasising the importance of addressing this gap in literature.

The second article of this thesis explored metrics used to evaluate knee joint function that are often a part of rehabilitation guidelines, such as limb symmetry indices (LSI) during single limb hopping and strength tasks. To date, no evidence exists to support or refute the use of these measures in an adolescent population. Therefore, we sought to investigate LSIs and knee joint function among adolescent males and females with and without ACL-injuries during single-limb assessments. A total of 141 participants were recruited and evaluated during a series of maximum voluntary tasks; isometric knee extension and flexion, and single-limb hopping tasks (anterior-hops, lateral-hops, 6m timed-hops, cross-hops, and triple-hops). In brief, females and males with ACL injuries had lower performance metrics compared to their matched controls, however, the differences were not the same within each sex. Females displayed deficits in all tasks, whereas males with and without ACL injuries were only different in the anterior-hop, triple-hop, and knee extension tasks.

The final two articles of this thesis are complimentary as they both explored knee stabilisation strategies during countermovement jumps (CMJs) and side-cutting tasks. However, Article 3 included only adolescent males and females without ACL injuries to identify the influence of sex and limb-dominance on knee stabilisation strategies, whereas Article 4 included the same uninjured cohorts but compared with matched groups of males and females with ACL injuries. In both studies, surface electromyography and full-body kinematics were captured during CMJs and side-cutting. Knee stabilisation strategies were evaluated using co-activation indices (CIs), full muscle waveforms, knee joint flexion stiffness (KJFS) and knee joint sagittal and frontal excursions (KJSE and KJFE, respectively). In Article 3, sex differences were observed in CIs, with females having higher medial CIs in the non-dominant limb in both CMJs and side-cuts, and higher total CIs in the non-dominant limb during side-cuts. Between-limb differences were present in both sexes. Females had higher total CI in their non-dominant limb in CMJs and higher frontal KJE in their non-dominant limb during the stance phase of side-cuts. Males had higher medial CIs in their dominant limb in CMJs and higher sagittal KJE in their non-dominant limb during the preparatory and landing phases of CMJs. No significant sex or limb differences were identified in individual muscle activations or KJFS. Based on these findings, males and females were treated statistically independent when evaluating the effect of ACL-injury in Article 4. Interestingly, when comparing groups, only one significant effect of injury was observed in knee stability metrics; males with ACL injuries had significantly higher vastus medialis activity during the preparatory phase of the CMJ compared to their uninjured counterparts. There were no other significant differences in either task.

Despite the importance of ACL injury prevention in adolescents, the evidence on sex difference in muscle activation patterns in this population is still scarce. The results of this Doctoral thesis indicate that males and females in adolescent populations have differences in knee stabilisation strategies and overall knee joint function. These findings further support the need for additional research considering risk factors such as, age/maturity-, sex-, and limb-dominance. This research will contribute to the tailoring of rehabilitation programs, thus providing an opportunity to improve the success rate of rehabilitation following ACL-injury among adolescents.

CHAPTER 1: INTRODUCTION

Rationale for Research

Sports and recreational activities play an important role in the lives of children and adolescents (under 18 years of age). In Canada, an estimated 75% of children participate in some form of organized sports (ParticipACTION Report, 2018). Unfortunately, an increase in sport participation translates to an increase in musculoskeletal injuries in this population (Hambidge et al., 2002). In fact, the number of sports-related injuries is estimated to represent up to 68% of all injuries in adolescent populations, with approximately 30-40 injuries occurring per 100 secondary school students (14-18 years old) per year (Billette and Janz, 2011; Damore et al., 2003; Fridman et al., 2013). One of the more common and traumatic injuries in this demographic is an anterior cruciate ligament (ACL) rupture, with rates steadily rising (Beck et al., 2017; Csintalan et al., 2008; Herzog et al., 2017a). This has resulted in ACL-reconstruction (ACLr) surgery being one of the most performed orthopaedic surgeries in North America (Lubowitz and Appleby, 2011).

For patients suffering an initial ACL injury, the experience can be life changing. Some reports indicate that only 65% of patients return to any level of organised physical activity, and only 50% return to pre-injury activity levels in adult populations (Ardern et al., 2011). A portion of this group is surely linked to the high rates of secondary injuries following primary surgical reconstruction, with studies reporting 18-20.6% of these patients re-rupturing their ACL-graft and 11.3-17.7% injuring their contralateral knee, all this despite prescribed rehabilitation and clearance for return-to-activity (RTA; Heath et al., 2019; Paterno et al., 2012; Webster and Feller, 2016). With such outcomes, it is not surprising that many of these patients will also exhibit increased anxiety, and reduced self-confidence, motivation, and overall quality of life (Ardern et al., 2013; Filbay, 2018; Langford et al., 2009; Scott et al., 2018; Tracey, 2003).

Despite the impact of this injury, both acutely and longitudinally, and even though females between the ages of 12 and 18 years have the highest risk of sustaining an ACL injury (Barber-Westin et al., 2006; Shea et al., 2004; Werner et al., 2015), there is surprisingly very little biomechanical research conducted on adolescent populations after an ACL rupture has taken place, defined as ACL-deficiency (ACLd). In fact, substantial knowledge gaps currently limit the tailored management of ACL injuries in these young patients, with a notable disparity in understanding the roles of muscles and the ACL in stabilising the knee joint. In fact, we conducted a systematic review of literature (*Article 1*) to synthesise sex differences in muscle activation patterns in movements associated with ACL injuries in both adult and adolescent populations (Otsuki et al., 2021). We found only five studies that investigated muscle activations in adolescents further highlighting the need to address this gap with new research.

The high rates of injury and re-injury among this cohort clearly demonstrates the need for improved prevention, rehabilitation and RTA guidelines that are evidence-based and specifically tailored for adolescent patients, as these do not currently exist (Romanchuk et al. 2023). The first step in addressing this knowledge gap is to describe this population alongside their non-injured activity-, maturity- and sex-matched population. Given the clinical context, evaluations can include current measures typically used in a clinical setting (i.e. isometric strength, functional symmetry assessments, and subjective questionnaires). Augmenting these with full biomechanical analyses (i.e. neuromuscular control, kinematics, and kinetics) of dynamic movements that are often used in rehabilitation research will help elaborate and explain what potential differences in function are present. In this thesis I will do the above in addition to using biomechanical analysis techniques (i.e. co-activation of lower-limb musculature and metrics of knee joint stability) and psychosocial metrics (i.e. patient reported outcome measures) that are novel to this cohort. Overall, the exploratory findings of this thesis will help determine whether knee joint stability metrics can improve the current rehabilitation model among adolescent males and females. In addition, this doctoral project served as a catalyst for a research program that will track these young patients and uninjured control participants as they progress through puberty to further our understanding of the sex- and developmental-based associations with injuries to the knee joint.

CHAPTER 2: LITERATURE REVIEW

The ACL and Injury Epidemiology

The anterior cruciate ligament (ACL) is one of the four primary ligaments in the knee joint complex and acts as a primary resistor to anterior translation of the tibia with respect to the femur, and a secondary stabiliser in internal rotation, valgus/varus, and hyperextension of the knee joint (Girgis et al., 1975; Takeda et al., 1994). Interestingly, females between the ages of 12 and 18 years old currently have the highest risk of sustaining a non-contact injury to the ACL (Werner et al., 2015). It has been estimated that 1 in 20 females in this age cohort who participate in competitive basketball or soccer will have an ACL injury annually (Prodromos et al., 2007); which is approximately one per competitive sports team. Injuries to the knee joint account for roughly 30% of injuries sustained by females, yet only 16% of injuries in males (Shea et al., 2004). This discrepancy between sexes for these traumatic injuries cannot be ignored with ACL injuries on the rise, with reports of a 2.3% annual increase from 1994-2013 (Beck et al., 2017). Regardless of the treatment strategy/intervention, after an individual has ruptured their ACL, there are increased risks in early onset knee osteoarthritis (OA), dysfunction of the muscles surrounding the knee joint, and a reduced participation in physical activity, which negatively impact these individuals' overall quality of life (Button et al., 2006; Oiestad et al., 2011; Whittaker et al., 2015).

In North America, each complete rupture of the ACL typically leads to a surgical intervention and reconstruction or replacement of the ligament, which costs approximately $6 200, in addition to the costs of post-surgery rehabilitation and loss of time from sports (Nagda et al., 2010). Annual cost estimates have reached $3.9 billion in the United States (Benjaminse et al., 2010; Petushek et al., 2015) and similar costs percapita are expected in Canada. The high incidences of knee injuries occurring beginning at the age of 12 (Shea et al., 2004) are concerning, but what is perhaps even more of a concern is the increased likelihood (50% to 71%) of early onset knee OA within 5 to 20 years after a knee injury (Lohmander et al., 2007, 2004; Oiestad et al., 2011; Roos et al., 1995). Research has already documented these young adults being diagnosed with knee OA in their 20s and 30s (Palmieri-Smith and Thomas, 2009).

The ACL-Deficient Knee Joint

Patients suffering an ACL injury may opt to avoid ACL-reconstruction surgery (ACLr) and choose the conservative approach, where a surgery does not take place and there is greater emphasis on physiotherapy rehabilitation to maximize the function and stability of their ACL-deficient (ACLd) knee (Button et al., 2008; Fitzgerald et al., 2000; Marx et al., 2003). Those who choose the conservative approach will be required to adapt their knee joint stabilisation strategies to maintain function. This has been highlighted in the literature with altered neuromuscular and biomechanical characteristics in the ACLd limb compared to either the contralateral limb or uninjured control (Alkjaer et al., 2012, 2003; Beard et al., 1996; Boerboom et al., 2001; Bohn et al., 2016; Bulgheroni et al., 1997; Ferber et al., 2002; Knoll et al., 2004; Kvist and Gillquist, 2001; Lindström et al., 2010; Rudolph et al., 2001; Rudolph and Snyder-Mackler, 2004; Torry et al., 2000). Interestingly, none of these studies considered sex as an independent variable in their analyses. Sex is often considered a confounding variable in biomechanical analyses of human movement in healthy populations (Bencke and Zebis, 2011; Chappell et al., 2007; Flaxman et al., 2014; Holden et al., 2015; Landry et al., 2009; Landry et al., 2007; Weinhandl et al., 2015), limiting the application of previously reported findings in ACLd populations where sex was not an independent variable.

Within the ACLd population, a distinction has also been made between so called copers and non-copers (Alkjaer et al., 2003; Chmielewski et al., 2001; Rudolph et al., 2001, 2000). Copers are those who can stabilize their ACLd knee joint during high-demand physical activity post-injury, while non-copers cannot (Rudolph et al., 2001). Button and colleagues (2008) recruited 281 patients with ACLd to analyze gait and hop tasks one-year post ACL injury. Patients were then classified on their ability to perform activities pre-injury: 17% were functional copers (individuals who return to their pre-injury work and sport with no limitations in their performance), 45% were adapters (individuals who reduce or changed their work or sport to prevent their knee fully giving way), and 38% were non-copers (individuals who fail to return to their pre-injury sport or work and experience full giving way with work, activities of daily living (ADL) and light non-pivoting sports). These data demonstrate that the injury itself and the functional outcomes and benchmarks of rehabilitation will vary from one patient to the next. Thus, a gap in rehabilitation programs is present, as these programs are not being properly tailored for those who have developed new, adaptive knee stabilisation and control strategies.

Considering the above, it is alarming that very minimal research has considered young patients who have sustained an ACL injury and require surgical/rehabilitative interventions. It is imperative that we establish and understand the mechanisms that affect knee joint function and stability as early as possible, as well as the influence of an ACL injury on these mechanisms. By doing so, rehabilitation interventions could be tailored to adapt for such mechanisms, in particular for young females who have a higher incidence of knee injuries and OA.

Defining and Assessing Knee Joint Stability

Knee stability arises from the integration of muscle forces, compressive loads from weight bearing, articular geometry, and soft tissue passive restraints – such as the ACL (Flaxman et al., 2012; Kaplan, 1957). Of these, muscles are the only active, modifiable regulators of joint loads and any change in neuromuscular control results in a change in knee stability and joint contact forces. The ACL plays an important role in knee joint stability, which is defined here as "the ability to maintain or promptly return to proper positions through an equalisation of forces…" (Riemann and Lephart, 2002). This is achieved in part by providing sensory feedback of the knee joint's motion via stress and strain mechanoreceptors embedded in the ligament's tissues (Georgoulis et al., 2001). When the ACL is ruptured there is a reduced ability to provide sensory feedback, resulting in altered neuromuscular control of the muscles surrounding the knee joint (Krogsgaard et al., 2011). Previous work from our group has demonstrated that an ACL injury influences stabilisation strategies differently in adult males and females (Del Bel et al., 2018a), yet, neuromuscular rehabilitation interventions do not consider sex as a factor in tailoring intervention guidelines, let alone the differences present in adult and adolescent populations. In fact, a recent review by our group found that guidelines for returning adolescents to activity following ACL injury were essentially nonexistent (Romanchuk et al., 2023).

In terms of objective knee stability measures in a clinical context, the Lachman and pivot-shift are common tests that allow the clinician to measure passive stability of the knee joint (Prins, 2006). Unfortunately, these measures of passive anterior laxity are not correlated to subjective functional scores and may significantly over-estimate joint function and stability (Snyder-Mackler et al., 1997). Furthermore, these tests are open-chain, which do not consider the knee's functional state of being weight-bearing during movement, when stability is needed to avoid ACL injury. To account for this inaccuracy, objective measures to reduce the complexity and describe dynamic knee joint stability have been proposed, such as knee joint flexion stiffness (KJFS). Dynamic KJFS is calculated as the change in knee joint flexion moment as a function of change in knee joint flexion angle during a dynamic task (i.e. loading phase of jump-landing), with the slope equaling dynamic stiffness of the knee joint (Davis and DeLuca, 1996; Longpré et al., 2013). This proxy of knee joint stability has yet to be considered in adolescent populations and will serve as an objective measure for evaluating knee stabilisation strategies and if and/or how they are influenced by both sex and injury.

Knee Joint Function and the Influence of ACL Injury

The overall function of the knee joint is directly influenced by its health-status. When being examined by a clinician, the following variables of interest will be assessed and used in the decision-making process: range of motion, joint laxity, effusion, concomitant injuries, extensor and flexor strength symmetries (Adams et al., 2012; Fältström et al., 2016; Gudas et al., 2018; Tay and Tan, 2018; Wright et al., 2015). While many of these variables (i.e. extensor and flexor strength symmetries) play a role in determining the functional status of the knee joint, they do not provide full insight into a patient's stabilisation strategy. The complete understanding of a patient's knee joint stabilisation strategy cannot be undervalued and is crucial when determining the necessary treatment/intervention path to reach the patient's rehabilitation goals.

Extensive research has been conducted on adults to improve the management of ACL injuries. These studies have found the following characteristics to be associated with a high-risk for injury or re-injury: small knee flexion range of motion, high hip abduction, low knee extensor activations, or high quadriceps-to-hamstring activation ratios during a variety of dynamic and isometric tasks (Aalbersberg et al., 2009; Blackburn et al., 2009; Carson and Ford, 2011; Davies et al., 2017; Hewett et al., 2005, 2015; Huston and Wojtys, 1996; Jacobs et al., 2007; Landry et al., 2007; Larsen et al., 2015; Mirkov et al., 2017; Myer et al., 2005, 2015; Petterson et al., 2007; Prieske et al., 2014; Rambaud et al., 2017; Vairo et al., 2008; Weinhold et al., 2007; Wojtys et al., 2002). Given that muscles are the only modifiable contributors to knee joint control, there has been a focus on their management in rehabilitation research (Ithurburn et al., 2019, 2015; Khaiyat and Norris, 2018; Myer et al., 2006; Nawasreh et al., 2019; Sharifmoradi et al., 2019; Zebis et al., 2019, 2016; Zult et al., 2018). After an ACL injury, increased co-activation of the quadriceps and hamstrings is considered a hallmark characteristic in stabilising the knee joint among adults with ACL injuries (Alkjaer et al., 2002; Chmielewski et al., 2005; Myer et al., 2015; Rudolph et al., 2001; Williams et al., 2003).

The co-activation index (CI) of the knee joint musculature is a measurement used to describe the muscle activation strategies about the knee joint based on the integrated electromyography signal (EMGi) (Equation 1; Rudolph et al., 2001).

$$CI = (\text{lower } EMG_i / \text{ higher } EMG_i) \bullet (\text{lower } EMG_i + \text{higher } EMG_i) \quad (1)$$

The CI is defined as the ratio between the antagonist's and agonist's activation during each phase of a task multiplied by the summed activity from both muscles (Lewek et al., 2005; Rudolph et al., 2001). Low co-activation indices represent lower activation of both muscles or low-level activation of one muscle and high activation of the other muscle in the pair. Larger co-activation values denote higher activation of both agonist and antagonist muscles, representing a more general activation pattern. However, increasing co-activation to improve joint stiffness should not be the rehabilitation goal after an ACL injury since a prolonged increase in co-activation about the joint alters knee joint loads and is associated with the onset and progression of knee OA in adults (Griffin and Guilak, 2005; Hodges et al., 2016). Failure to appropriately adapt one's neuromuscular control strategies may explain why some individuals continue to have knee instability and difficulty returning to their pre-ACL injury activity levels after rehabilitation.

It must be noted that all aforementioned characteristics and epidemiology stem from research exclusively on participants over 18 years of age and adults. Despite the extensive work with adult populations, a consensus of what neuromuscular differences exist between the sexes nor the influence of an ACL injury have yet to be identified (Del Bel et al., 2018a; Otsuki et al., 2021). Also, current research on adults has generally considered only a few muscles within the injured limb, despite the high incidences of injury to the contralateral limb. It is clear there is room for improvement in our understanding of knee joint function among adolescents with and without ACL injuries. Current rehabilitation guidelines for RTA cannot be tailored appropriately, which may be contributing to the high rates of re-injury among adolescents (Webster and Hewett, 2019).

For my doctoral research, I will determine whether the above-noted differences (i.e. muscle activation strategies and lower-limb biomechanics) exist in uninjured and injured adolescents. This is an important first step in understanding why there is an increased injury rate in females after the onset of puberty.

Sex Differences in Knee Joint Biomechanics

The focus of sex comparisons stems from the consistently reported sex-bias in the incidences of ACL injuries (Agel et al., 2016; Arendt and Dick, 1995; Hewett et al., 2006a). Several factors associated with this sex-bias have been discussed in the literature such as; neuromuscular, biomechanical, anatomical, and hormonal composition. The general consensus in the literature for approximately the last 20 years is that the disparity in ACL incidences between males and females arises from a combination of these factors (Arendt et al., 1999; Simon et al., 2015), however few studies have been fully driven by said consensus.

When sex is considered, differences between males and females become apparent with females being in a high-risk position for injury more often than their male counterparts (Beaulieu et al., 2009; Ireland, 1999). This high-risk position, often seen during side-cuts and jump-landings, entails a coupling of medial and internal rotational loading, which is when the ACL is most likely to rupture (Andrews et al., 1977). Sex differences in neuromuscular and biomechanical strategies have been analyzed in a wide range of populations performing various maneuvers where medial and internal rotational loads are observed. Maneuvers such as a side-cut (Beaulieu et al., 2009; Bencke and Zebis, 2011; Hanson et al., 2008; Iguchi et al., 2013; Landry et al., 2009; Sigward and Powers, 2006), and a countermovement jump (Chappell et al., 2007; Cowling and Steele, 2001; Fagenbaum and Darling, 2003; Hewett et al., 2015; Holden et al., 2015; Quatman et al., 2006; Weinhandl et al., 2015) are frequently used due to non-contact ACL injuries being most likely to occur during similar tasks. Some studies using these tasks showed females are generally quadriceps-dominant (activating their quadriceps at a higher ratio relative to their hamstrings) compared to males, who typically employ a more balanced activation between their quadriceps and hamstrings (Beaulieu et al., 2009; Hanson et al., 2008; Landry et al., 2007). However, the role of the quadriceps in relation to ACL loading has been debated in the literature. It has been suggested that the quadriceps activate to protect the ACL from injury by increasing the compression of the knee joint (Hashemi et al., 2007). Other researchers have suggested that increased quadriceps activity in relation to hamstrings activity may be detrimental to the ACL, as greater anterior translation of the tibia may occur (Hanson et al., 2008; Iguchi et al., 2013; McLean et al., 2004).

The following neuromuscular characteristics have been identified in adult females as risk factors associated with ACL injury when compared to their male counterparts: lower quadriceps and hamstring strength (Blackburn et al., 2009), lower active hamstring stiffness (Staron et al., 2000), more time to generate peak hamstring torque (Blackburn et al., 2009; Huston and Wojtys, 1996), lower muscular contribution to joint stiffness (Wojtys et al., 2002), and lower knee joint proprioception (Rozzi et al., 1999). Additionally, co-activation ratios being analyzed have demonstrated higher quadriceps-hamstrings co-activation ratios (Myer et al., 2015), a mediolateral imbalance in thigh muscle activations (Landry et al., 2007) and altered activation timings of the quadriceps and gastrocnemii (Bigham, 2015; Petterson et al., 2007; Smale et al., 2015). These neuromuscular differences consequently result in altered knee joint motion with females demonstrating the following during sport maneuvers: lower flexion angles (Prieske et al., 2014), higher knee valgus (Hewett et al., 2005; Jacobs et al., 2007), higher abduction moments (Chappell et al., 2007; Ford et al., 2003; Gehring et al., 2009), and lower internal rotation angles (Beaulieu et al., 2008; Benjaminse et al., 2010; McLean et al., 2004; Pollard et al., 2004). A combination of these factors puts the lower extremity in a higher-risk position for knee injury and may contribute to the higher incidences of knee injuries seen in females.

As a result of this research, it has been routinely suggested that sex directly influences the neuromuscular and biomechanical outputs of an individual, influencing the risk of sustaining an ACL injury. Nevertheless, the specific mechanisms of an ACL injury and how sex is a major contributing factor are still not well understood in all age cohorts

(Benjaminse et al., 2010; Sugimoto et al., 2015) and therefore, sex will be one of the main independent variables considered in this Doctoral thesis.

Influence of Puberty on Adolescent Knee Joint Control

Understanding the role that puberty plays in the biomechanics of an adolescent population is crucial when evaluating this cohort. During puberty there are numerous anatomical and hormonal changes that occur and directly influence biomechanical outputs. Most notably, rapid growth takes place in the body such as bone length, weight, and height; all of which contribute to increasing the neuromuscular demand of movement patterns (LaBella et al., 2014) and increased torques at the knee joint (Hewett et al., 2004; Jensen and Nassas, 1988). Males have more testosterone during puberty, facilitating the necessary increase in muscular power and strength to accommodate for the rapid bone-growth taking place and arguably leading to an improved neuromuscular control of the knee joint compared to females during puberty (LaBella et al., 2014). These changes in anatomical features, in addition to pelvic growth/dimorphism differing between males and females (Coleman, 1969), consequently alter the alignment of the lower extremity differently in males and females. Moreover, the quadriceps angle (Q-angle; (Tillman et al., 2005) is greater in females and has been commonly linked to an increased strain on the knee joint and consequentially, the ACL (Gray et al., 1985; Haycock and Gillette, 1976; Zelisko et al., 1982).

With the onset of puberty, there is a greater influx of estrogen in females compared to males (Sperling, 2008). Interestingly, estrogen has been found to directly regulate the ligamentous structure and function by altering the synthesis of collagen types I and III, and elastin (Kim et al., 2002; Sanada et al., 1978), which are integral to the tissue's structure (Ross and Pawlina, 2010). In addition to collagen synthesis, estrogen has been linked to influencing three other traits of ligamentous tissue: stiffness, failure loading, and healing (Leblanc et al., 2017). In this context, stiffness is a term used to describe the amount of force needed to cause deformation of the ligament, while failure loading refers the force necessary to rupture a ligament (Baumgart, 2000; Hansen et al., 2009; Maitland et al., 1995). There are estrogen receptors on the ACL (Liu et al., 1996) and, as explored in animal models (Slauterbeck et al., 1999; Wild et al., 2012), increased estrogen exposure leads to a reduction in knee stiffness and increased risk of ligament failure (Liu et al., 1996; Rozzi et al., 1999; Wojtys et al., 2002).

Puberty is clearly an important factor in the development of the knee joint and injury risk factors. To properly track patients/participants in their pubescent development, a self-classification of Tanner Stages can be performed (Marshall and Tanner, 1970, 1969). These forms have been deemed reliable and valid for self-assessment, with a rate of 95% accuracy compared to a trained physician's assessment (Rasmussen et al., 2015; Taylor et al., 2001). Given that each individual progresses through puberty differently, knowing the puberty stage of tested participants could provide insight on the influence of pubertal stage on knee stabilisation strategies before and after an ACL injury in children ages 10-18 years old.

Subjective Measurements of Knee Joint Function

In addition to physical trauma, how a patient handles their new physical condition may have as great, if not greater impact on their recovery than the injury itself (Brewer et al., 2010; Tracey, 2003). Collegiate patients with various injuries who do not have adequate support, resilience, coping strategies, and/or emotional outlets have suboptimal outcomes of rehabilitation, such as emotional instability, decline in sport-confidence, inferior performance and, ultimately, re-injury (Clement et al., 2013; Podlog et al., 2013).

Data related to the experience of adolescent patients recovering from an ACL injury could be critical to the development of optimal interventions and tailoring the decision-making process of clinicians, patients, and parents, yet it is sorely lacking.

Current methodologies for assessing subjective knee joint function (patient reported outcome measures; PROMs) do not comprehensively assess the multifaceted cognitive, emotional, and capacities of patients (Webster et al., 2008). PROMs have traditionally been viewed as being invalid in determining knee joint function, but have nevertheless become more popular due to their ease of both administering and incorporating a patient's perspective (Bent et al., 2009). There has been little research in determining the relationship between subjective outcome measures and objective functional tasks, let alone within an adolescent population (Failla et al., 2015; Hildebrandt et al., 2015; Hopper et al., 2002; Reid et al., 2007; Smale et al., 2019a). Many instruments and rating scales have been developed in an attempt to measure outcomes from a patient's perspective when recovering from an ACL injury, however only a few methods have been evaluated for reliability, validity, and responsiveness (Wang et al., 2010), including the International Knee Documentation Committee (IKDC) score form (Irrgang et al., 2001) and the ACL-Return to Sport after Injury (ACL-RSI) scale (Webster et al., 2008). The ACL-RSI scale is a noteworthy tool as it is one of the first assessments designed to incorporate three types of psychological components believed to be associated with returning to activity after an injury: i) emotions, ii) confidence in performance, and iii) risk appraisal (Podlog and Eklund, 2007; Webster et al., 2008).

Although the ACL-RSI is more progressive in considering a patient's subjective influence on their knee joint function, it has yet to be tailored for an adolescent population. The majority of PROMs used in research today were first created for adult populations, but some have since been adapted for younger cohorts. The IKDC was adapted and validated for children with knee disorders (pedi-IKDC), as the original version posed difficulty in comprehension for the younger population (Kocher et al., 2011). The pedi-IKDC is a psychosocial assessment tool tailored for adolescents that captures aspects of the patient's perspective on how difficult it is to perform a range of physical tasks and the pain and/or swelling associated with their knee joint injury. Since its conception in 2011, the pedi-IKDC has been cited by 28 studies, of which, only four studies (Burland et al., 2018; Sabatino et al., 2019; Wall et al., 2017; Willson et al., 2018) used the tool in their analyses, clearly demonstrating the lack of not only its use in the literature, but also of the overall number of publications considering psychosocial assessments in adolescents.

While a great deal of clinical research on ACL injuries among adults includes PROMs, a significant gap still exists regarding our understanding of the experience of a patient who has suffered and is recovering from an ACL injury, no matter the age cohort. The current research overlooks important details that may influence perceived knee joint health, pain, competence, and ultimately, mental readiness and capacity for RTA. An ACL injury reduces the functional capacity of the knee joint and the potential for confounding psychosocial implications has yet to be fully understood, particularly in adolescent populations. Recent research has shown cohorts of patients with ACL injuries to have the same functional capacity before and after their reconstructive surgery, while self-reporting increased perceived function of their knee joint (Failla et al., 2015; Smale et al., 2019a). These findings alone indicate a clear disconnect in objective and subjective measurements in knee joint function during ACL injury rehabilitation.

With these considerations, it is crucial that patient-specific assessments are tailored for the target population in question to accurately measure rehabilitation progression

(Poppel et al., 2010). In order to accomplish this goal, a necessary first step is to determine how currently accepted PROMs relate to an adolescent patient's objective knee joint function, while considering the variables of both an ACL injury and sex. Once this is established, it is then possible to identify if/what information is missing from the current subjective evaluation of patients rehabilitating from an ACL injury.

CHAPTER 3: PURPOSE and HYPOTHESES

Purpose

While there are clinical guidelines to help clinicians judge if an adult patient is ready for RTA following ACL injury (Adams et al., 2012; Cristiani et al., 2019; Noyes et al., 1991) no evidence-based criteria currently exist for adolescents. The competency of the orthopaedic surgeon is crucial for a successful ACL-reconstruction surgery, so too is the implementation of appropriate and effective pre- and post-operative rehabilitation guidelines. The present gap in age/maturity- and sex-based injury prevention and rehabilitation guidelines is likely a major contributor to the current rise of what is already a common injury/re-injury among adolescents. Therefore, these guidelines must be tailored for a younger population, and we must determine whether sex is a factor needed to be considered at this age. As a starting point, we first need to identify which knee stabilisation strategies are used by uninjured adolescent males and females. Once there is a better understanding of the typical biomechanical profile of adolescent males and females without injuries, we can then investigate how an ACL injury alters these movement strategies. These findings can then directly inform guidelines for the development and implementation, helping to reduce the currently high rates of injury and re-injury – particularly in adolescent females.

The overall aim of this Doctoral thesis was to create evidence to support the revision of the measures of knee stabilisation that are currently used when working with adolescent patients with an ACL injury. Through the identification of differences in objective and subjective measures related to knee joint function in males and females with and without ACL injuries, I set out to identify the key biomechanical and clinical variables, and how they differ among these cohorts. The findings of this thesis will contribute to the literature surrounding prevention, clinical and rehabilitation management of ACL injuries among adolescent males and females.

Research Questions and Hypotheses

1) ***(RQ1) What is the current knowledge of sex differences in muscle activation patterns during tasks associated with high-risk of ACL injury?***

The role of muscles in knee stabilisation cannot be undervalued yet has been rarely considered in the context of ACL injuries, let alone in an adolescent population. Lower limb muscles are the only active regulators of knee joint loads that are used to prepare for and adapt to loading. Furthermore, evidence suggests that under weight-bearing conditions, the stabilising role of lower limb muscles is different between males and females (Bigham et al., 2018, Del Bel et al., 2018, Flaxman et al., 2014). Moreover, with the increasing rates of ACL injuries in adolescent populations it is critical to investigate the effect of sex on muscle activation strategies in adolescent populations with and without injuries. As an important first step, the purpose of this investigation was to perform a systematic search and review of current literature examining sex differences in muscle activation patterns during jump-landing and cutting tasks. It was hypothesised that there would be limited literature considering sex differences among an adolescent population. Additionally, it was hypothesised that sex differences would be identified warranting the revisiting of current rehabilitative guidelines that are not tailored by the patient's sex.

2) ***(RQ2) What are the baseline measures and sex differences of non-injured adolescent clinical tests commonly used to assess return-to-activity following ACL injury?***

As previously discussed, high re-injury rates indicate that RTA guidelines are not optimal for younger cohorts of patients with ACL injuries. Therefore, we sought to address this gap in the literature by providing both i) a normative data set of uninjured males and females to be used as a benchmark for clinicians and ii) a comparison between the sexes of the uninjured cohort and the injured population. It was hypothesised that there would be significant differences between sexes in the uninjured population in single-limb performance during hop and isometric strength tasks (Del Bel et al., 2018c), to support the use of sex-specific guidelines in assessing an adolescent patient. Furthermore, it was hypothesised that significant differences would be present between the injured and uninjured populations in the single-limb hop tasks, their isometric strength performances and PROMs (Cristiani et al., 2019). Specifically, females with ACL injuries were expected to have the smallest isometric flexion and extension strength values and would perform more poorly than their male counterparts on the single-limb hop tasks. Despite these differences, it was also hypothesised that the limb symmetry indices (LSI) of injured to healthy limb would not identify significant differences in the injured cohorts (Wellsandt et al., 2017b).

3) ***(RQ3) Are knee stabilisation strategies influenced by sex and limb-dominance during side-cuts and countermovement jumps among healthy adolescent athletes?***

The purpose of this investigation was to identify what knee stabilisation strategies were being used by uninjured adolescent males and females, ages 12-18 years old, during sport-related movement tasks, which are often associated with ACL injuries. There is currently limited research evaluating neuromuscular control of this age cohort (Landry et al., 2007; Landry et al., 2007; Landry et al., 2009; Medina et al., 2008), and even less considering limb-dominance. Therefore, it was hypothesised females will demonstrate unfavourable knee stabilisation strategies such as higher co-activation indices (CIs) between the quadriceps and the hamstrings, lower KJFS, KJSE, and higher KJFE compared to males, as this is after the onset of the sex-bias in ACL injuries, at the age of 12 years (Barber-Westin et al., 2006; Shea et al., 2004; Werner et al., 2015). Furthermore, limb-dominance differences were expected within each cohort of males and females, with higher CIs and lower KJFS in the non-dominant limb compared to the dominant limb during both tasks.

4) ***(RQ4) Do adolescent males and females with ACL injuries use different knee stabilisation strategies during side-cuts and countermovement jumps compared to matched uninjured controls?***

The purpose of this investigation was to identify how sex and an ACL injury influences knee stabilisation strategies in an adolescent population during dynamic maneuvers. Given the influential role of sex on knee stabilisation strategies after an ACL injury in adult populations (Del Bel et al., 2018a), it was hypothesised that adolescent males and females with ACL injuries would demonstrate different knee joint stabilisation strategies compared to their uninjured counterparts. More specifically, it was expected that female patients with ACL injuries would have higher muscle CIs between the quadriceps and hamstrings during the stance and preparatory phases (Chappell et al., 2007; Hanson et al., 2008; Landry et al., 2009; Padua et al., 2005), lower KJFS and KJSE, and higher KJFE during the stance phase of both side-cuts and CMJs (Miranda et al., 2013; Oberländer et al., 2014; Shultz and Schmitz, 2009; Smale et al., 2019a; Stasi et al., 2015).

CHAPTER 4: GENERAL METHODOLOGY

Study Design

This Doctoral Thesis was a part of a larger longitudinal research program being conducted in collaboration between the Children's Hospital of Eastern Ontario (CHEO) and the Clinical Biomechanics Research Unit (CBRU) led by Drs. Sasha Carsen and Daniel Benoit, respectively. At the time of completion of this thesis, the recruitment, enrollment, and data collections of participants was continuing at the Human Movement Biomechanics Laboratory (HMBL), Ottawa, ON. Recruited patients with ACL injuries were enrolled to participate in data collections at three-time points: 1) after sustaining an injury (ACL-deficient; ACLd) and prior to surgery to repair the injury, 2) after ACL-reconstruction surgery (ACLr) but with approval from clinician to return-to-activity (RTA), and 3) two years post-ACLr. Healthy controls were recruited to match the patient population demographics and followed a similar time-based schedule for data collections. Each visit consisted of the same protocol and sequence of tasks on both limbs, which included the following: collection of patient-reported outcome measures (PROMs), isometric and isokinetic strength tests, single-limb hop tasks, bilateral squats, countermovement jumps (CMJs), lunges, side-cutting, and drop-vertical jump landings (see Appendix A for the complete protocol). This thesis focused on the first visit to the laboratory (timepoint 1 above) and the following tasks for all recruited participants: all PROMs (Appendix C), isometric strength, single-limb hop tasks, CMJs, and side-cutting. Ethical approval was given and maintained by both research ethics boards at CHEO (17/74X) and the University of Ottawa (H09-17-10, Appendix B).

To comprehensively address RQ1, a systematic review was performed with PRISMA guidelines being used during reporting and writing of the review. The study designs for RQ2, RQ3, and RQ4 were consistent, using a cross-sectional design, where data from males and females with and without ACL injuries was used from their first visit to the HMBL.

Participants

Adolescent patients between the ages of 10 and 18 years old who had an ACL rupture (ACLd), confirmed via clinical assessment and/or MRI and subsequent arthroscopy, were recruited for this study by Orthopaedic Consultants from CHEO, Ottawa, Canada. Patients who were seeking an ACL-reconstruction revision were not included in this dissertation. Eligible patients did not participate in the study until they were given approval by their physician before their study visit. These approvals occurred when the physician was confident in the patient's ability to fully participate in the study and perform all dynamic tasks required in the protocol. Recruitment of uninjured control participants took place via convenience and snow-ball sampling of regional youth sports associations and club teams in Ottawa. These participants were matched accordingly with recruited patients with ACL injuries for height, weight, age, developmental (Tanner) stage, activity/competition levels, limb-dominance, and sport; although this matching is currently ongoing. The dominant leg of each participant was distinguished by which leg was used to kick a soccer ball a maximum distance (van Melick et al., 2017).

Experimental Protocol

Consent and Questionnaires

Once at the laboratory, all participants were required to read and sign a consent form explaining the protocol, any possible risks, and the purpose of the study, with the help of a parent/guardian if needed. Each participant was given the opportunity to discuss the study or ask any questions with a member of the research team before providing their consent. In addition, a set of self-assessments and surveys were completed in their preferred language (English or French: the Pedi-IKDC (Kocher et al., 2011), the HSS Pedi-Fabs (Del Bel et al., 2019; Fabricant et al., 2014), Tegner activity scale (Tegner and Lysholm, 1985), and the Tanner Stages Classification form (Taylor et al., 2001). Participants were then provided with tight-fitted spandex shorts and a long-sleeved shirt. The following anthropometric measures were then recorded: pelvis, knee, and ankle width, height, and weight; leg and tibial length; and thigh and shank circumference.

Participant Preparation & Equipment

After anthropometric measurements, participants were provided with a pair of running shoes (KBS7FW3343; MS7F505027, Joe Fresh, Canada) followed by a warm-up protocol consisting of cycling on a stationary ergometer for five minutes (Monark 828E, Vansbro, Sweden) and an opportunity to stretch. Wireless, bipolar surface EMG electrodes connected to a 16-channel EMG system (Trigno Wireless System, Delsys, USA) were placed over the muscle bellies of interest following the recommendations by SENIAM (Hermens et al., 2000) and DeLuca (DeLuca, 1997), with minor adjustments being made to account for varying body dimensions. The EMG electrodes contained two sets of differential EMG inputs; with detection surfaces 5mm long, 1mm wide and located 10mm apart. The EMG data were sampled at 2000Hz, band-pass filtered at 20-450Hz with a 12dB/octave filter roll off and a common mode rejection ratio of > 80dB. Prior to placement, areas of the skin were shaved and wiped with alcohol. The muscles of interest on both limbs were: the rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), semitendinosus (ST), biceps femoris (BF), medial gastrocnemius (MG), lateral gastrocnemius (LG), and gluteus medius (GM).

Participants then had 84 reflective markers (14mm diameter) placed on pre-determined landmarks according to a hybrid cluster-marker set adapted from Mantovani and Lamontagne, 2016 (Figure 4.1). During all movement tasks, ground reaction forces (GRFs) were sampled at 1000Hz, amplified with an internal gain of 1000 and recorded through two force platforms (FP4060-08, Bertec Corp., USA), while marker trajectories were sampled at 200Hz using a 10-camera infrared motion analysis system (8 Vero and 2 Vantage; Vicon, Nexus, UK) and synchronously recorded using the support software (Nexus v2.7, Vicon, UK).

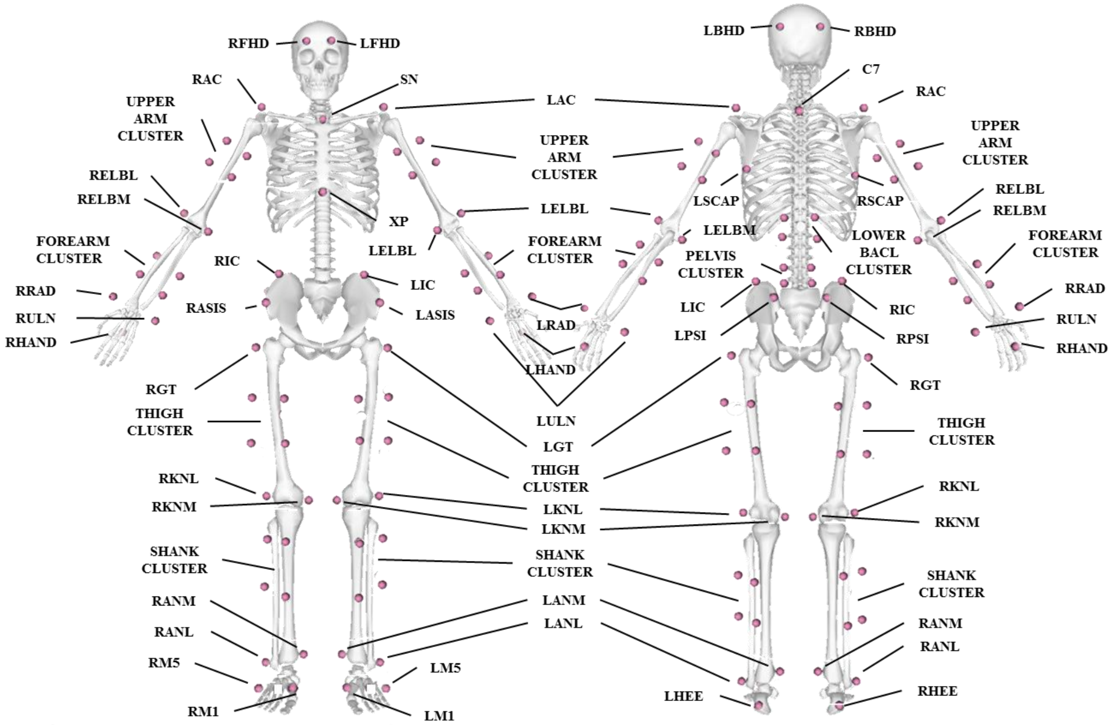

Figure 4.1: Hybrid cluster-marker set adapted from Mantovani and Lamontagne (2016).

Maximum Voluntary Isometric Contractions

Maximum voluntary isometric contractions (MVICs) were performed using an isokinetic dynamometer (System 4 Pro, Biodex Medical Systems, USA) and included i) knee flexion and ii) extension, recorded in a seated position with the knee flexed at 60^{o}, and iii) plantarflexion, recorded in a seated position with the knee at 0^{o} flexion and the ankle in a neutral position; iv) hip abduction was recorded in a standing position, with the hip at 10° abduction and 0° extension (Worrell et al., 2001). Participants were instructed to begin gradually contracting until achieving their maximal contraction and to hold for five seconds. This was repeated three times with at least one minute of rest between each trial. EMG and isometric torque were synchronously collected using Nexus, sampled at 2000Hz (v2.7, Vicon, UK). Torque data were filtered using a 4th order zero-lag low-pass Butterworth filter at 15Hz. Torque values were then normalised for body mass and assigned to be positive to simplify their interpretation during data analysis. Verbal encouragement and on-screen biofeedback were provided to encourage participants to achieve their maximum effort.

Single-Limb Hop Tasks

Participants were instructed to first perform a set of single-limb hop tasks (Figure 4.2) that are often used in evaluating the relative difference in functional capacity of the patient's limbs in a clinical setting (Abrams et al., 2014; Adams et al., 2012; Cristiani et al., 2019; Gokeler et al., 2017a; Logerstedt et al., 2012; Myer et al., 2006; Noyes et al., 1991; van Grinsven et al., 2010; Wellsandt et al., 2017b). These tasks include: Max Anterior Hops (participants were instructed to hop as far as they could on one foot facing forward); Timed-6m Hops (participants were instructed to hop on one foot as fast as they could to cover a distance of six meters); Triple Hops (participants were instructed to hop on one foot three times in a row for maximum distance); and Cross-Hops (participants were instructed to hop on one foot back-and-forth three times across two marked lines separated by 15cm on the laboratory floor, while attempting to cover maximum distance). Additionally, we added a Max Lateral Hop task, where participants were instructed to hop as far as they could on one foot laterally, facing perpendicular to the direction of the marked line on the laboratory floor. The lateral hop is not currently a part of the single-limb battery of hop tests; however, we deemed it appropriate to be added as an assessment given that ACL injuries often occur in the frontal plane (Hewett et al., 2010; Kiapour et al., 2015; Quatman et al., 2014).

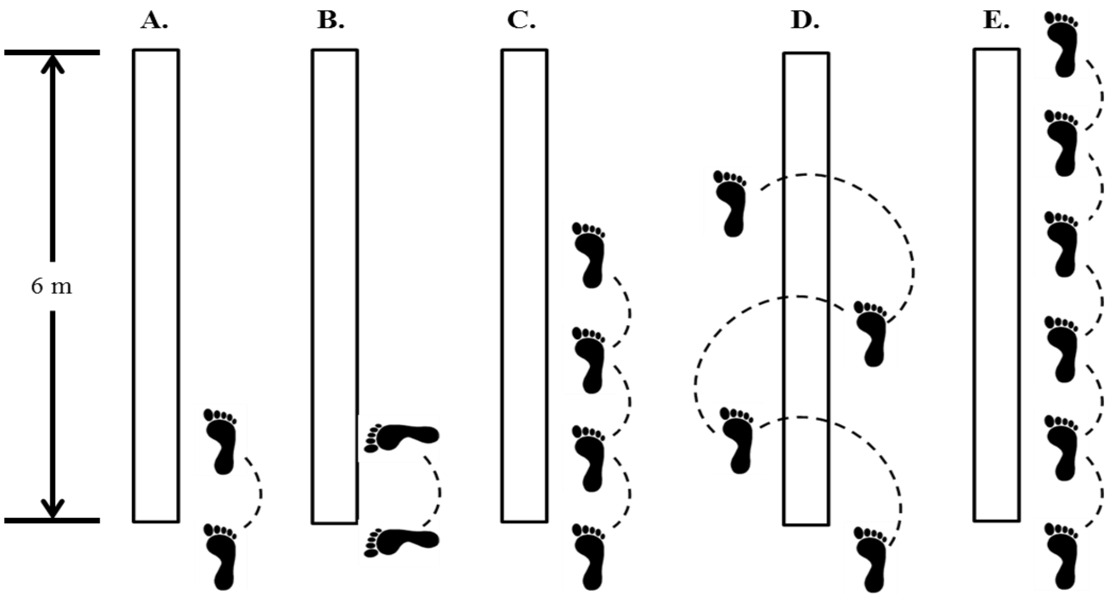

Figure 4.2: Single-limb hop tests on the right foot: A) Max Anterior Hop, B) Max Lateral Hop, C) Triple Hop, D) Cross-Hop, and E) Timed-6m Hop.

Participants were provided detailed instruction and a demonstration prior to completing two practice trials. Participants performed a minimum of two successful trials (consisting of using proper technique and holding the landing for a minimum of two seconds) for both limbs. To account for variability in arm swing, participants were instructed to keep their hands on their hips throughout each trial. A minimum of one-minute rest was provided between trials to prevent fatigue. All hop tests were performed at a self-selected pace. For this dissertation, only the spatiotemporal variables (distances and times) were included as a part of evaluating current RTA standards in pediatrics.

Countermovement Jumps and Side-Cuts

Similar to the set of single-limb hop tasks, tasks such as countermovement jumps (CMJs) and side-cuts are often used in evaluating the relative between-limb difference in functional capacity of a participant, particularly in injury prevention and rehabilitation programs (Barengo et al., 2014; Ireland, 1999; Wright et al., 2015). Participants were instructed to perform five successful trials of both CMJs and side-cuts (on both limbs). CMJs consisted of participants standing with feet shoulder-width apart on separate force platforms, with their hands on their head to account for arm motion variability, and were instructed to squat down in order to help them jump as high as possible (Hildebrandt et al., 2015). A CMJ trial was deemed successful if the participant kept their hands on their head, landed with both feet entirely on their respective force platforms and held the landing without losing their balance (Figure 4.3).

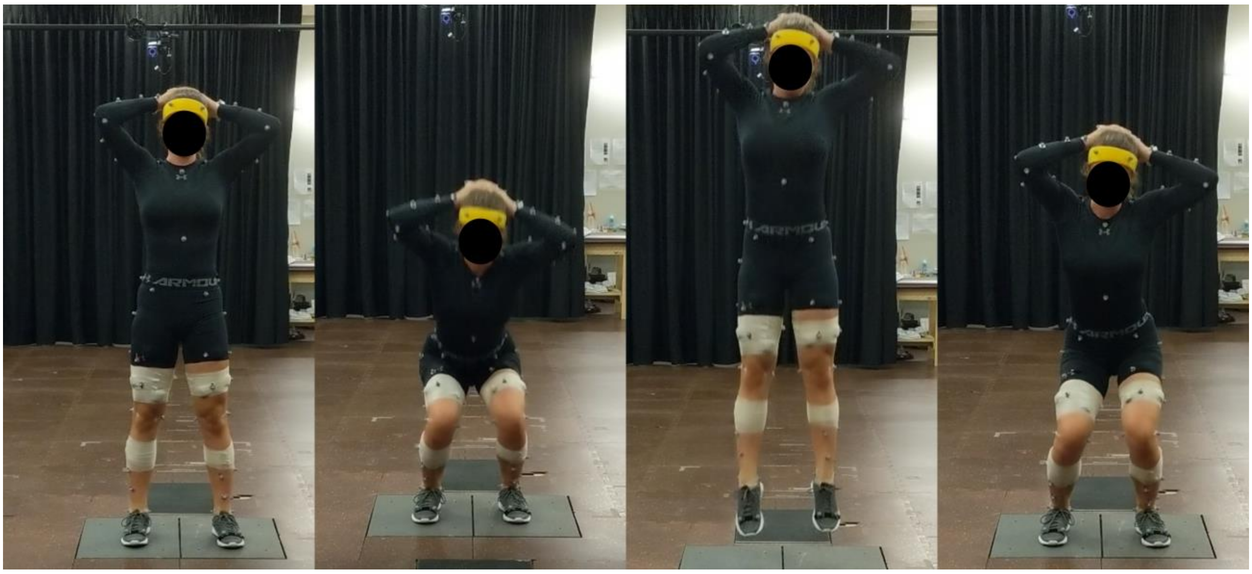

Figure 4.3. Sequence of events for a countermovement jump in motion laboratory. From left to right: starting position, preparatory phase, flight phase, and landing phase.

Side-cuts were performed with participants starting at one end of the laboratory and running linearly as fast as possible while maintaining control to plant and cut with the foot of interest on the force platform, cutting at an angle of 45^{o} (Beaulieu et al., 2009a;

Del Bel et al., 2017a) (Figure 4.4). A side-cut trial was deemed successful if the participant planted and cut with the appropriate foot, planted their entire foot on the force platform, and accelerated out of the foot-plant at a 45° angle (pylons in the lab provided a ±10° target area).

Figure 4.4. Sequence of events for a 45° side-cut maneuver in motion laboratory. From left to right: preparatory phase, stance phase, and push-off in new direction.

Data Reduction

Marker trajectories and GRFs were filtered with a 2nd order zero-lag dual low-pass Butterworth filter with matching cut-off frequencies of 15Hz (Bisseling and Hof, 2006; Kristianslund et al., 2012) using custom pipelines in Vicon Nexus (v2.10, Vicon, UK). A Vicon Nexus model adapted from (Mantovani and Lamontagne, 2016) was used for scaling and to compute inverse kinematics (relative to a standing reference trial) and inverse dynamics for the hip, knee, and ankle sagittal and frontal plane angles. Raw EMG signals were exported as .c3d files into Matlab (R2018a, Natick, USA) to be high-pass filtered at 20Hz with a 2nd order dual-pass Butterworth filter, full-wave rectified, and low-pass filtered at 6Hz with a 2nd order dual-pass Butterworth filter. A 10ms moving average algorithm was used to identify maximum EMG amplitude for each muscle during the

MVICs; these maxima were used to amplitude-normalise EMG data in the CMJs and side-cuts.

All data were time-normalised in accordance with the task being analysed. The CMJs trials were parsed into three phases: take-off (initiation of upward acceleration from the lowest point of the squat (maximum knee flexion)), flight (the duration of the task spent jumping with no contact on the force platforms (GRF < 10)), and landing (the total duration after the flight phase between foot and the force platform until standing with maximum knee extension). The side-cuts trials were parsed into two phases: the preparatory phase (100ms prior to initial contact with the force platform (GRF$>$ 10N)) (Bencke et al., 2000; Besier et al., 2003; Cavanagh and Komi, 1979; DeMont et al., 1999; DeMont and Lephart, 2004; Landry et al., 2009; Zazulak et al., 2005) and the stance phase (the total duration between foot and the force platform). The time normalised waveforms were averaged over the trials for each participant.

A large number of continuous kinematic and kinetic variables were collected (hip, knee, and ankle sagittal and frontal plane angles and internal joint moments) and aggregated to provide the following dependent variables as proxies of knee stabilisation: knee joint flexion stiffness (KJFS) (Davis and DeLuca, 1996) and knee joint angle excursions, in both the sagittal (KJSE) and the frontal (KJFE) planes (Smale et al., 2019a). The KJFS was calculated as the change in knee joint flexion moment as a function of change in knee joint flexion angle during a dynamic task, with the slope equaling dynamic stiffness of the knee joint. The values of KJFS at initial impact (GRF $>$ 10N) was reported for both CMJs and side-cuts. Neuromuscular data from all eight muscles was evaluated throughout the various phases of the two dynamic tasks; however, primary outcomes were based on muscle co-activation (CI) to explain gross stabilisation strategies of the joint.

The CIs were calculated (Chapter 2, Equation 1) for both legs separately between the lateral thigh muscles (VL and ST), the medial thigh muscles (VM and BF), and the summed quadriceps (VL and VM) and hamstrings (ST and BF) muscles.

Statistical analysis

All variables were assessed for normality to determine whether parametric or non-parametric statistical tests are more appropriate to be used (Field, 2013). All statistical analyses were performed using Matlab (2018a, Mathworks, Natick, USA) or SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA) with a significance level set at $p = 0.05$. The alpha level was adjusted using a Bonferroni Correction in each study to account for multiple variables. For discrete variables, independent t-tests (or the non-parametric equivalent, Mann-Whitney U-tests) were used to evaluate group differences between limb symmetry indices (LSIs), spatiotemporal variables from single-limb hop tests, anthropometrics, questionnaires, and co-activation indices (CIs).

Statistical parametric mapping (SPM) is a technique that compares the entire time-dependent waveforms between data sets (i.e. the whole flight phase of a CMJ or stance phase of a side-cut), as opposed to other conventional techniques that only evaluate discrete values between data set (i.e. magnitudes at heel strike event) (Pataky et al., 2013). Independent t-tests in SPM were applied to determine if differences existed between knee status (uninjured and ACLd) for all continuous dependent variables.

CHAPTER 5: ARTICLE 1

Sex differences in muscle activation patterns associated with anterior cruciate ligament injury during landing and cutting tasks: A systematic review.

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Abstract

Background: Neuromuscular control is critical for maintaining dynamic joint stability and mitigating the risk of anterior cruciate ligament (ACL) injury. Given the increased risk of ACL injury in females, sex-based differential muscle activation strategies are often associated with this risk. For example, the quadriceps-dominant muscle activation strategy sometimes observed in females has been discussed as a cause of their increased risk of ACL injury. However, there has been no synthesized knowledge on sex differences in muscle activation patterns associated with ACL injuries. Therefore, the purpose of this review was to synthesize sex differences in muscle activation patterns in movements associated with ACL injuries in both adult and adolescent populations.
Methods: A systematic electronic database search was conducted.
Results: Thirty studies were included in the review. Females demonstrated higher pre- and post-landing activation of the quadriceps and lower activation of the hamstrings in 15 of 27 studies. Females also had higher quadriceps-to-hamstring co-contraction ratios during pre- and post-landing phases compared to their male counterparts in 4 of 9 studies.
Conclusion: While some studies supported the quadriceps-dominant activation strategies in females, no consensus can be drawn due to methodological inconsistencies and limitations. Also, despite the importance of ACL injury prevention in children and adolescents, the evidence on sex difference in muscle activation patterns in this population is still scarce.

Introduction

The anterior cruciate ligament (ACL) is the most frequently damaged knee ligament (Brooks et al. , 2005). Despite extensive efforts to prevent ACL injuries over the past few decades (Webster and Hewett, 2018), the incidence of this injury is increasing in the young population (Agel et al. , 2016, Beck et al. , 2017, Weitz et al. , 2020, Werner et al. , 2016), and females have a two to four times greater risk of sustaining ACL injuries than males (Agel et al. , 2005, Arendt and Dick, 1995, Joseph et al. , 2013, Stanley et al. , 2016).

Several studies have reported that multi-planar loading, namely, coupling of knee abduction and internal tibial rotation, as well as anterior tibial translation, are considered mechanisms of ACL injury (Quatman and Hewett, 2009, Shimokochi and Shultz, 2008). Since the vast majority (over 70%) of ACL injuries are classified as non-contact (Arendt and Dick, 1995, Boden et al. , 2000), this implies that the loads causing the tibia to translate and/or rotate to the point of rupturing the ACL are generally a result of a combination of: 1) the foot interacting with the ground, 2) inertia of the body segments and 3) passive (i.e.: ligaments, tendons, menisci, cartilage) and 4) active (muscles) tissues surrounding the joints. Of these, muscle forces regulated from neural and reflex feedback control are the only active regulators of knee joint loads that can both be used to prepare (through pre-tensioning) and adapt to loading conditions which threaten the dynamic stability of the joint (Reeves et al. , 2007) and therefore warrant investigation.

Under controlled experimental conditions, individual or muscle groups can directly affect ACL loading, such as the quadriceps muscles can increase anterior tibial translation (DeMorat et al. , 2004) and subsequently increase strain on the ACL (Withrow et al. , 2006); conversely the hamstrings can act as an antagonist to decrease anterior tibial translation and reduce the ACL strain (Fujiya et al. , 2011, Withrow et al. , 2008). Similarly, under isolated conditions the gastrocnemius contraction can increase ACL strain (Fleming et al. , 2001, Morgan et al. , 2014). However, as noted above ACL injuries do not typically occur in isolated conditions, but rather during non-contact weight-bearing conditions (Arendt and Dick, 1995, Boden et al. , 2000).

During weight-bearing, knee joint muscles display roles beyond that of simple knee extensors and flexor by acting as stabilisers (Flaxman et al. , 2012), with individual muscle activation strategies being associated with maintaining knee joint stability in the frontal and transverse loading planes in addition to those predicated by their moment arm directions (Flaxman et al. , 2017). Furthermore, muscular co-activation of all muscles crossing the knee are required to support it during injury-inducing loading conditions such as externally applied knee abduction and rotation (Flaxman et al. , 2021) and evidence suggests these muscular contributions are altered following ACL injury (Flaxman et al. , 2019).

Given the above, it is important to consider the context of the research that is forming opinion on muscular contributions to joint stability and ACL injury. Also, given the increased injury risk in females, and the evidence that suggests that under weight-bearing conditions biological sex influences the stabilizing role of these muscles (Bigham et al., 2018, Del Bel et al., 2018, Flaxman et al., 2014) it is critical to investigate the effect of sex on neuromuscular control in the ACL injury context. Moreover, with the increasing rates of ACL injuries in adolescent populations (Beck et al. , 2017, Shea et al. , 2004, Werner et al. , 2016), studies that consider this high-risk population must also be explored.

Therefore, the purposes of this systematic review were to identify, evaluate, and synthesize research result which (1) examine sex difference in muscle activation patterns during landing and cutting tasks in adult population; (2) explore muscle activation patterns during landing and cutting in adolescent population. We will then summarize and discuss this evidence in the context of neuromuscular control and ACL injury.

Methods

This systematic review conformed to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Literature Search

A literature search was conducted using the MEDLINE, EMBASE, CINAHL, and SPORTDiscus from January 1980 to January 2019. The following search terms were used: (1) 'electromyography' OR 'neuromuscular control' OR 'muscle activation' OR 'muscle activity' OR 'EMG' AND (2) 'anterior cruciate ligament' OR 'ACL'. Language was limited to English and all subjects were human. In addition, a hand search was performed for the reference lists of the included articles. The titles and abstracts of all studies identified through the literature search were reviewed by the authors (RO and MDB) and the full text of potentially relevant articles were retrieved. The same two independent reviewers assessed the articles based on the criteria identified below.

Selection Criteria

Studies were included if they: (1) evaluated muscle activation patterns (i.e. activation magnitude and timing) in multiple muscles, (2) evaluated landing or cutting tasks, and (3) made comparison between females and males. Since this review aimed to assess muscle activation patterns that were relevant to ACL injuries, studies evaluating multiple muscles were included. Specifically, the muscles crossing the knee joint as well as the hip and shank muscles were included, since the hip (Boden et al. , 2009, Koga et al. , 2018, Leppänen et al. , 2017, Pollard et al. , 2010) and ankle (Koga et al. , 2018, Kristianslund et al. , 2014, Lee and Shin, 2021, Ogasawara et al. , 2020) kinematics during dynamic loading could affect the risk of ACL injury. Also, landing and cutting tasks were selected as ACL injuries commonly occur during these activities (Arendt and Dick, 1995). Abstracts, editorial letters, case reports, reviews, posters, and unpublished data were excluded.

Assessment of Methodological Quality and Risk of Biases

Since the articles included were observational cross-sectional studies, the methodological quality and risk of biases were assessed using the AXIS critical appraisal tool (Downes et al. , 2016). Items 7, 13, and 14 of the AXIS tool regarding the management of non-responders were not deemed relevant for this investigation; therefore, the studies were rated based on the 17-items criteria. Two independent reviewers (RO and MDB) rated all included articles. Inter-rater reliability of the modified AXIS tool was assessed with the kappa statistics. The level of agreement was interpreted according to Cohen's criteria: none (κ = 0–.20), minimal (κ = .21–.39), weak (κ = .40–.59), moderate (κ = .60–.79), strong (κ = .80–.90), and almost perfect (κ >.90) (Cohen, 1960). Any disagreement was resolved with a consensus meeting. If a consensus could not be achieved, a third reviewer (DLB) made the final judgement.

Data Extraction

The following data were extracted: publication details (author and year), participants' characteristics (sample size, age, sex, and sports), tasks performed, phases of landing tested (pre-landing or post-landing), EMG data (muscles tested, electrode location, normalization methods, and EMG variables). An effect size (ES = mean difference/pooled standard deviation) was calculated for each variable to compare the magnitude of sex differences among studies. The magnitude of ES was interpreted according to Cohen's criteria: small ($d = 0.2–0.5$), medium ($d = 0.5–0.8$), and large ($d > 0.8$) (Cohen, 1988).

Results

Search Strategy and Study Characteristics

The initial search strategy identified 2205 articles, with 975 remaining after duplicates were removed. Following title and abstract screening, 53 full-text articles were assessed for eligibility. Based on the selection criteria, a final total of 30 studies were included in this review (Figure 5.1) and their study characteristics are listed in Appendix D: D1.

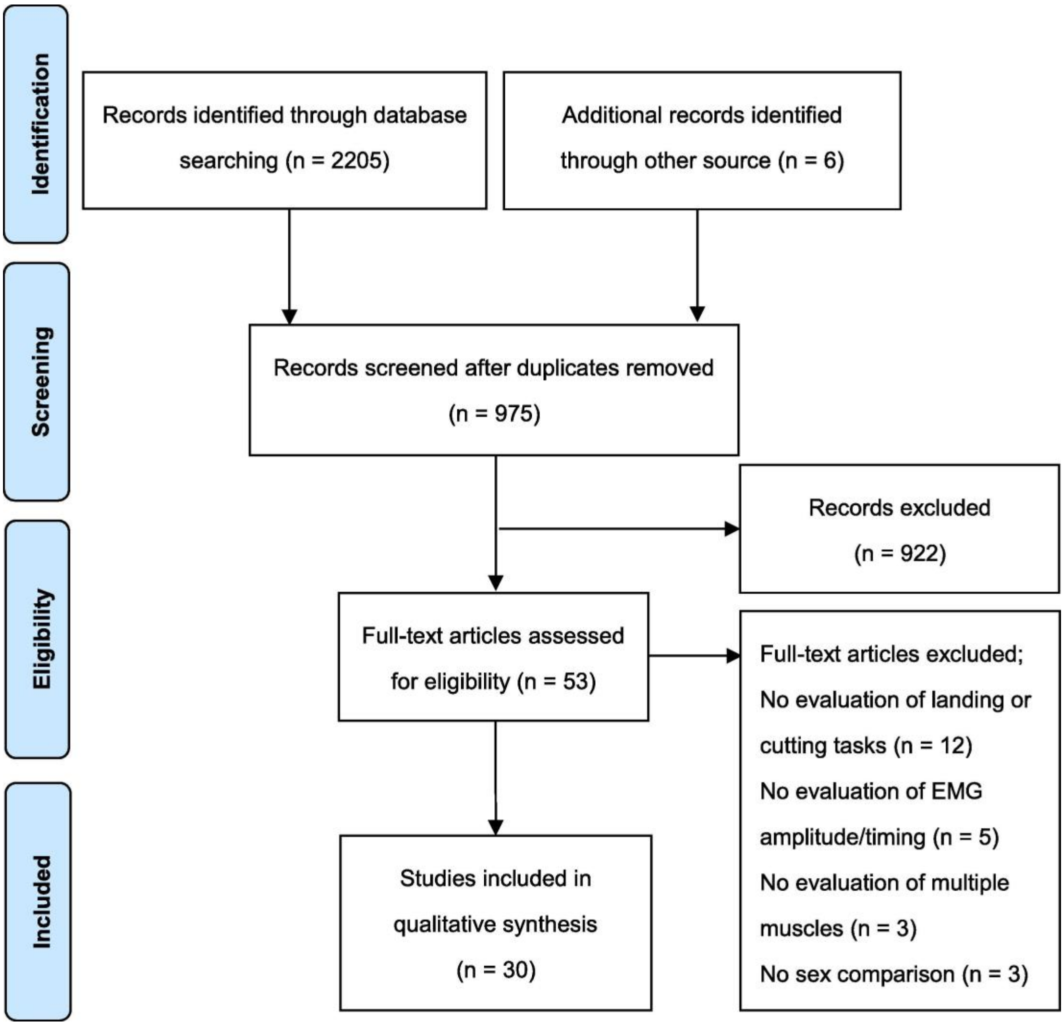

Figure 5.1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of literature search.

Of these 30 articles, 21 studies examined adults (over 17 years of age), 5 studies examined adolescents (aged 13 to 17), 3 studies examined pre-adolescents (aged 8 to 12), and one study examined both adults and pre-adolescents. Nine studies evaluated bilateral landing tasks, 13 studies evaluated unilateral landing tasks, and 11 studies evaluated cutting tasks. Sixteen studies examined pre-landing muscle activity and 25 papers examined post-landing muscle activity.

The muscle activation patterns examined were measured using surface EMG sensors and quantified using 1) amplitudes of muscle activity, 2) onset of muscle activity, and/or 3) indices of muscle co-contraction. Muscles tested included: vastus medialis, vastus lateralis, rectus femoris, medial hamstring (semintendinosus/semimembranosus), lateral hamstring (biceps femoris), medial gastrocnemius, lateral gastrocnemius, soleus, tibialis anterior, gluteus maximus, and gluteus medius. EMG data were normalized to maximum voluntary isometric contractions (MVIC) in 21 papers, maximum amplitude during tested task (% max) in five papers, and average amplitude during static single leg stance in two papers. Two papers did not use any normalization techniques for interpreting their EMG data. The effect sizes were obtained in 17 papers; 13 papers did not report sufficient data to calculate the effect sizes. The characteristics of EMG data collection and analysis and their procedures are described according to the ISEK guidelines (Appendix D: Tables D2, D3).

Methodological Quality

The average methodological quality score using the AXIS tool was 13.8/17, ranging from 9 to 16 (Appendix D: D4). Inter-rater reliability between the two reviewers was moderate to almost perfect (κ = 0.61-1.0). Of the 30 studies evaluated, none adequately reported with sufficient detail the selection process of participants. For items 5 and 6 of the AXIS tool to be satisfied, the authors were required to ensure their sample population is the same composition of the target population and address selection bias (i.e. demographics, exposure to training/coaching, sport specialization, etc.). The majority of studies indicated that convenience sampling was used to recruit their participants; therefore, they were not able to meet said criteria. Moreover, only three studies performed a power calculation to justify their sample sizes used in the recruitment of their sample population.

Sex Differences in Muscle Activation Patterns during Bilateral Landing

Six studies (Chappell et al. , 2007, de Britto et al. , 2014, Ebben et al. , 2010, Padua et al. , 2005, Russell et al. , 2007, Shultz et al. , 2009) evaluated pre-landing muscle activation during bilateral landing tasks. Of these, three studies (Chappell et al. , 2007, Padua et al. , 2005, Shultz et al. , 2009) found that female participants demonstrated significantly higher EMG amplitudes of the quadriceps compared to males (d = 0.70–1.00 (Padua et al. , 2005)), while Ebben et al. (2010) found that females demonstrated delayed activation of vastus lateralis and vastus medialis (d = 1.12 and 1.07, respectively). Three of the five studies reported that females had higher hamstring EMG amplitudes compared to males (Chappell et al. , 2007, de Britto et al. , 2014, Shultz et al. , 2009), with de Britto et al. (2014) specifically identifying that females demonstrated higher pre-landing activity of the medial hamstring. Three of the studies (Ebben et al. , 2010, Padua et al. , 2005, Russell et al. , 2007) evaluated quadriceps-to-hamstrings (Q:H) co-contraction ratios (i.e. calculated by dividing the activation magnitudes of the quadriceps by the hamstrings); only Padua et al. (2005) reported significantly higher Q:H co-contraction ratios in females (d = 0.75–1.02). In addition, Padua et al. (2005) also investigated muscles in the shank, and found that females exhibited higher pre-landing activity of the soleus compared to males during bilateral landings (d = 1.20–1.24).

Five studies (Ebben et al. , 2010, Padua et al. , 2005, Pappas et al. , 2007, Russell et al. , 2007, Shultz et al. , 2009) examined post-landing muscle activation during bilateral landing tasks. Two of these (Padua et al. , 2005, Shultz et al. , 2009) reported higher quadriceps muscle activity in females (d = 0.99–1.01). Ebben et al. (2010) reported lower lateral hamstring activation in females compared to males during a drop jump (d = 0.75), whereas Shultz et al. (2009) reported that both lateral and medial hamstrings activations were lower in females. The other three studies (Padua et al. , 2005, Pappas et al. , 2007, Russell et al. , 2007) reported no significant sex differences in hamstring activity. Ebben et al. (2010), Padua et al. (2005), and Russell et al. (2007) also assessed Q:H co-contraction ratios; only Padua et al. (2005) found higher Q:H ratios in females (d = 0.75–1.04), in addition to finding higher activity of the soleus compared to males (d = 0.68–1.29).

Sex Differences in Muscle Activation Patterns during Unilateral Landing

Five studies (Cowling and Steele, 2001, Fagenbaum and Darling, 2003, Nagano et al. , 2007, Rozzi et al. , 1999, Zazulak et al. , 2005) examined muscle activations during the pre-landing phase of unilateral landing tasks. Two studies (Nagano et al. , 2007, Zazulak et al. , 2005) found significantly higher activity of the rectus femoris in females compared to males (d = 0.96 (Zazulak et al. , 2005)). No sex differences were reported in either the vastus lateralis or vastus medialis. Although there were no sex differences in the amplitude of hamstrings, Cowling and Steele (2001) found that females demonstrated earlier onset of semimembranosus compared to males during single leg landing (d = 1.17). Furthermore, Nagano et al. (2007) evaluated Q:H co-contraction ratios and found that it was significantly higher in females compared to males. No sex differences were observed in the shank or hip muscle groups during unilateral landing tasks (Rozzi et al. , 1999, Zazulak et al. , 2005).

Ten studies (Fagenbaum and Darling, 2003, Garrison et al. , 2005, Hart et al. , 2007, Hughes and Dally, 2015, Nagano et al. , 2007, Palmieri-Smith et al. , 2009, Pappas et al. , 2007, Rozzi et al. , 1999, Urabe et al. , 2005, Zazulak et al. , 2005) examined post-landing muscle activation during unilateral landing tasks. Hughes and Dally (2015) reported higher rectus femoris activation (d = 1.24), and Urabe et al. (2005) found higher vastus medialis activation (d = 1.44), in females compared to. None of the other eight studies (Fagenbaum and Darling, 2003, Garrison et al. , 2005, Hart et al. , 2007, Nagano et al. , 2007, Palmieri-Smith et al. , 2009, Pappas et al. , 2007, Rozzi et al. , 1999, Zazulak et al. , 2005) identified sex differences in quadriceps activations.

Nine of the studies (Fagenbaum and Darling, 2003, Garrison et al. , 2005, Hart et al. , 2007, Hughes and Dally, 2015, Nagano et al. , 2007, Palmieri-Smith et al. , 2009, Pappas et al. , 2007, Rozzi et al. , 1999, Urabe et al. , 2005) examined the activity of the hamstrings. Hughes and Dally (2015) found lower activity in the lateral hamstrings (d = 1.64), whereas Rozzi et al. (1999) found higher activity in the lateral hamstring in females compared to males (d = 1.26). No significant difference in hamstring activation were found in the other seven studies (Fagenbaum and Darling, 2003, Garrison et al. , 2005, Hart et al. , 2007, Nagano et al. , 2007, Palmieri-Smith et al. , 2009, Pappas et al. , 2007, Urabe et al. , 2005). Nagano et al. (2007) evaluated Q:H co-contraction ratios and found that females had higher Q:H ratios compared to males during single-limb drop jumps. Palmieri-Smith et al. (2009) also investigated Q:H co-contraction ratios and provided additional calculations to determine if an imbalance was present between the medial and lateral muscles of the thigh muscle groups: a medial-to-lateral Q:H co-contraction ratio was calculated by dividing the medial Q:H (vastus medialis and medial hamstrings) by the lateral Q:H (vastus lateralis and lateral hamstrings) co-activation index. They found that females had lower medial-to-lateral Q:H co-contraction ratios compared to males (d = 2.48).

Four studies (Garrison et al. , 2005, Hart et al. , 2007, Hughes and Dally, 2015, Zazulak et al. , 2005) examined activation of the hip muscles during the single-limb drop jumps. Zazulak et al. (2005) and Hart et al. (2007) found that females had lower activity of the gluteus maximus (d = 0.91–0.99) and gluteus medius (d = 1.98), respectively. Only two studies (Pappas et al. , 2007, Rozzi et al. , 1999) investigated activation of the gastrocnemius and no sex differences were observed.

Sex Differences in Muscle Activation Patterns during Cutting

Six studies evaluated muscle activation during cutting tasks, using side and cross cutting with or without anticipation. Of these, (Beaulieu et al. , 2009, Bencke and Zebis, 2011, Hanson et al. , 2008) evaluated pre-landing muscle activity. Hanson et al. (2008) found that females had significantly higher vastus lateralis activity during the preparatory phase of the side-step cutting compared to males (d = 0.64–0.67). No sex differences were observed in the rectus femoris or vastus medialis (Beaulieu et al. , 2009, Bencke and Zebis, 2011, Hanson et al. , 2008). Bencke and Zebis (2011) found that females displayed lower semitendinosus (d = 1.00) and biceps femoris (d = 1.29) pre-activities compared to males. Beaulieu et al. (2009) also reported that females demonstrated significantly earlier onset of semitendinosus than males during an unanticipated cutting task (d = 0.75). Both Bencke and Zebis (2011) and Hanson et al. (2008) reported significantly higher Q:H co-contraction ratios during pre-landing phase of cutting (d = 0.61–1.25). Beaulieu et al. (2009) found that females displayed higher activity of the lateral gastrocnemius than males (d = 0.82). In the hip muscles, Hanson et al. (2008) found no sex differences in the activity of the gluteus maximus or gluteus medius.

Six of the studies (Beaulieu et al. , 2009, Ebben et al. , 2010, Hanson et al. , 2008, Hughes and Dally, 2015, Malinzak et al. , 2001, Sigward and Powers, 2006) evaluated post-landing muscle activation during cutting tasks. Of those, five studies (Beaulieu et al. , 2009, Hanson et al. , 2008, Hughes and Dally, 2015, Malinzak et al. , 2001, Sigward and Powers, 2006) found that females had higher activity of the quadriceps compared to males. Beaulieu et al. (2009) and Hughes and Dally (2015) found higher peak rectus femoris activity (d = 0.95, 1.32). Hanson et al. (2008), Malinzak et al. (2001), and Sigward and Powers (2006) reported higher vastus lateralis activation (d = 0.95–1.06 (Hanson et al. , 2008)) in females. Two studies (Ebben et al. , 2010, Malinzak et al. , 2001) reported higher activity of both medial (d = 0.70 (Ebben et al. , 2010)) and lateral (d = 1.00 (Ebben et al. , 2010)) hamstrings in females compared to males during cutting. Ebben et al. (2010) and Hanson et al. (2008) evaluated Q:H co-contraction ratios and Hanson et al. (2008) found significantly higher Q:H ratios in females (d = 0.69–1.09).

Only Beaulieu et al. (2009) reported on shank muscles and found that females had higher activity of the lateral gastrocnemius (peak value: d = 1.02, integral value: d = 1.16) during the post-landing phase, and the tibialis anterior (d = 0.79) at initial contact. Only Hanson et al. (2008) evaluated hip joint muscles and found that females had higher activity of the gluteus medius compared to males during cutting (d = 0.54–0.55).

Sex Differences in Muscle Activation Patterns in Adolescents

Only four studies (Del Bel et al. , 2017, Hamstra-Wright et al. , 2006, Kipp et al. , 2014, Russell et al. , 2007) evaluated muscle activation patterns in pre-adolescents (aged 8 to 12). No sex differences were reported in muscle activity with respect to integrated EMG, Q:H ratios, and onsets/amplitudes (Del Bel et al., 2017, Hamstra-Wright et al. , 2006, Russell et al. , 2007). However, Kipp et al. (2014) reported sex differences using a muscle synergy analysis, with girls having higher activation of the medial hamstrings before landing and at initial contact (d = 0.93), whereas boys had higher activation of the vastus medialis after landing (d = 0.96).

In adolescents (aged 13 to 17), Landry et al. (2007a, 2009) found that females demonstrated higher activation of the lateral and medial gastrocnemius during the pre-landing phase of unanticipated side cutting. During a cross-cutting task, they reported higher activations of the lateral gastrocnemius and rectus femoris, and reduced activation of the medial and lateral hamstring during the post-landing phase among females compared to males (Landry et al. , 2007a). Sell et al. (2006) reported that female adolescents had higher semitendinosus activation during the post-landing phase of bilateral landings (d = 0.45–0.49). Conversely, Medina et al. (2008) did not find sex differences during unilateral jumps in adolescents. Only one study (Russell et al. , 2007) examined developmental differences in muscle activation, reporting that adults had smaller Q:H co-contraction ratios than pre-pubescent children before landing (Russell et al. , 2007).

Discussion

The purposes of this systematic review were to identify, evaluate, and synthesize research result examining sex differences in muscle activation patterns during landing and cutting tasks in adult and adolescent populations. While hundreds of papers have used electromyography in the context of ACL injury research, only thirty studies, including six focused on adolescents, met our study criteria.

Sex Differences in Muscle Activation Patterns in Adults

Eight of the 24 of the reviewed studies (Beaulieu et al. , 2009, Hanson et al. , 2008, Hughes and Dally, 2015, Malinzak et al. , 2001, Padua et al. , 2005, Shultz et al. , 2009, Sigward and Powers, 2006, Urabe et al. , 2005) reported higher post-landing quadriceps activation in females compared to males. On the other hand, post-landing activations of the hamstrings, the synergists for the ACL, were at times lower in female participants (Ebben et al. , 2010, Hughes and Dally, 2015, Malinzak et al. , 2001). This also helps explain why females typically displayed higher Q:H ratios compared to males (Ebben et al. , 2010, Hanson et al. , 2008, Padua et al. , 2005). These findings support the quadriceps-dominant theory (Hewett et al. , 1996) which states that females preferentially rely on the quadriceps to stabilize the knee. This strategy is not necessarily optimal for preventing an ACL injury, as excessive quadriceps contractions could induce anterior tibial translation (DeMorat et al. , 2004), strain the ACL (Withrow et al. , 2006), and ultimately increase the risk of rupture. Furthermore, it has been reported that reduced hamstrings co-contraction with quadriceps can elevate strain (Fujiya et al. , 2011, Withrow et al. , 2008) and/or in-situ forces (Li et al. , 1999) in the ACL.

As for the frontal plane, excessive knee abduction moments are associated with an increased risk of ACL injury (Hewett et al. , 2005). Muscles can nevertheless mitigate risk by generating torque in the frontal plane (Zhang and Wang, 2001). Specifically, contraction of the medial musculature, such as the semitendinosus, medial gastrocnemius, and gracilis produced resistance to knee abduction perturbation, while contraction of the lateral musculature including the biceps femoris, lateral gastrocnemius, and vastus lateralis generated resistance to knee adduction perturbation (Zhang and Wang, 2001). Palmieri-Smith et al. (2009) investigated these contributions through medial-to-lateral

Q:H co-contraction. They reported that females demonstrated a lower ratio of the medial-to-lateral Q:H co-contraction, with lower Q:H co-contraction levels in the medial muscles (VM/MH) compared to the lateral (VL/LH). Landry et al. (2007a) also found a mediolateral activation imbalance in gastrocnemii: female athletes demonstrated greater activity of lateral gastrocnemius compared to medial gastrocnemius. These mediolateral imbalances may contribute to disadvantage facing excessive knee abduction moments, and when coupled with internal rotation loads, may increase the risk of ACL injury (Hewett et al. , 2010, Kiapour et al. , 2015, Quatman et al. , 2014). The importance of medial musculature was further emphasized by a prospective study (Zebis et al. , 2009) which found that female athletes who had reduced pre-activity of the semitendinosus and elevated pre-activity of the vastus lateralis during side-cutting were at increased risk of future ACL injury (Zebis et al. , 2009).

Considering the timing sequences of an ACL injury, pre-landing muscle activation might play a critical role as an injury to ACL is believed to occur approximately 40–50 ms after initial ground contact (Kiapour et al. , 2014, Koga et al. , 2010). In theory, the post-landing reflex response is not fast enough to provide an adaptive response to stabilize the joint within 40–50 ms after foot contact (Dyhre-Poulsen and Krogsgaard, 2000). Thus, the control of the knee joint immediately after landing is determined by the preparatory muscle activity rather than the reflexive muscle activity. The importance of pre-landing preparation is further emphasized, as muscle activation and kinematics began to differ during the flight phase of failed and successful drop vertical jump landings (Romanchuk et al. , 2020b). In this review, higher preparatory quadriceps activation was identified in females during double leg landing (Chappell et al., 2007, Padua et al. , 2005, Shultz et al. , 2009), single leg landing (Nagano et al. , 2007, Zazulak et al., 2005), and cutting

(Hanson et al. , 2008). For the hamstrings, several studies reported that preparatory activation was higher in females compared to males (Chappell et al., 2007, de Britto et al., 2014, Shultz et al., 2009). Also, females demonstrated earlier onset of the medial hamstring compared to males during single leg landing (Cowling and Steele, 2001) and cutting (Beaulieu et al., 2009). These findings suggest that females are attempting to use more preparatory activation strategies to increase knee joint stiffness, as increased preparatory muscle activities are reported to add excitatory input to the motor pool (Fuglevand et al., 1993) and increase the stiffness of the knee joint (Dhaher et al., 2005). While increased stiffness may provide greater joint stability and protection against joint injury (Riemann and Lephart, 2002) during jump landing and cutting where a short amount of time is available to prepare for the landing, increased stiffness may reduce force absorption and potentially cause increased demands on the knee joint (Schmitz et al., 2007). Furthermore, pre-landing Q:H co-contraction ratios were higher in females (Bencke and Zebis, 2011, Hanson et al., 2008, Nagano et al., 2007, Padua et al., 2005), indicating that they demonstrated a quadriceps-dominant pattern during the pre-landing phase. Brown et al. (2014) reported that greater pre-landing activation of the rectus femoris predicted increased peak anterior tibial shear force. Also, elevated quadriceps pre-landing activation could affect the axial compression force, which is another important sagittal plane factor linked to ACL injuries immediately after initial ground contact (Kiapour et al., 2014, Meyer and Haut, 2005), as Wall et al. (2012) reported that the force threshold required to rupture the ACL was lowered when the quadriceps force was added to the axial compression force. These findings suggest that pre-landing quadriceps-dominant activation pattern could be a factor contributing to the sagittal plane mechanism of ACL injury.

Although the quadriceps-dominant activation strategy was observed in females both pre- and post-landing, association and causation are not easily reconciled, and careful consideration must be taken before making a direct connection with ACL injury risk. Post-landing quadriceps activation might not be directly related to the ACL injury risk, since quadriceps forces does not reach the force level shown to cause ACL injuries within 50 ms after ground contact (Domire et al., 2011). It should also be noted that heightened quadriceps activation does not necessarily mean an increase in anterior tibial translation leading to excessive strain on an ACL, in particular if we are comparing across populations. Muscle activation measured by EMG, in particular when normalized within a participant and not to an external force, is not directly related to force (Woods and Bigland-Ritchie, 1983). The observed sex differences in muscle activation patterns can be explained by the sex differences in muscle strength. Females have muscles with smaller cross sectional area (Behan et al., 2018) and greater proportion of type 1 muscle fibers (Staron et al., 2000), leading to lower strength relative to body mass compared to males. As such, females might have to increase their relative muscle activation (i.e. use a higher percentage of their maximal capacity) to produce the force level required to decelerate their body mass during landing. Of the studies included, Shultz et al. (2009) found higher quadriceps activation and lower normalized thigh strength in females, and identified that lower thigh muscle strength was a moderate predictor of greater quadriceps activation amplitudes in females. Also, Nimphius et al. (2019) reported that sex disparity in muscle activation was not evident when males and females were matched for normalized strength. It is therefore reasonable that when performing functional activities such as landing and cutting, females may increase the activations of the quadriceps relative to hamstrings to generate adequate extensor force. Therefore, the quadriceps-dominant activation pattern observed in females could potentially be a result of quadriceps weakness and thus a resultant neuromuscular strategy in females that is required in order to produce the necessary muscle force to absorb impact during landing.

Considering the lower extremity kinetic chain, muscle activation of the proximal hip muscles may play an integral role in controlling the knee joint. The quadriceps-dominant activation strategy could not be discussed without addressing these muscles. However, there were only five studies that investigated the hip joint muscles, and the results of these studies were not consistent. Two studies identified that females had lower post-landing activation of the gluteus maximus and medius (Hart et al., 2007, Zazulak et al., 2005), one study found that females had higher pre-activation of the gluteus medius (Hanson et al., 2008), and the other two studies did not find sex differences (Garrison et al., 2005, Hughes and Dally, 2015). Further investigation is clearly needed to fully elucidate the role of hip muscle activation in relation to ACL injury.

Finally, landing maneuvers require the control of the entire body. As such, the quadriceps-dominant strategy observed in females must be considered in the context of their whole-body landing mechanics. Females appear to land with a more upright or erect posture (Chappell et al. , 2002, Romanchuk et al., 2020a, Schmitz et al., 2007), which requires more quadriceps activation (Blackburn and Padua, 2009). Interestingly, if males perform landing maneuvers in an upright posture, they also demonstrate increased activation of the quadriceps (Blackburn and Padua, 2009). Since trunk and pelvis positioning alters muscle activations in the lower extremity (Allum and Honegger, 1998, Romanchuk et al., 2020b), the quadriceps-dominant strategy that is often discussed in the literature as a direct cause of injury risk might not be a sex specific phenomenon nor a knee joint-specific issue. It is possible that this strategy can be explained by the movement patterns of the whole body and is an adaptive response to the positions generally observed in females during landing. As such, while it may be convenient to directly link activation strategies such as increased quadriceps to hamstring ratios with ACL injury risk, the observed difference in neuromuscular control are multifactorial adaptations associated with whole body movement biomechanics. Therefore, an important consideration moving forward for investigations into the sex differences related to ACL injury risk is the inclusion of the relationship between local knee joint stabilisation strategies and whole-body dynamics.

Sex Differences in Muscle Activation Patterns in Adolescents

The importance of ACL injury prevention in children and adolescents is paramount (Myer et al. , 2013); however, there are very few studies that have investigated muscle activation patterns in this population. Based on these studies, there do not appear to be significant sex differences in muscle activation patterns between pre-pubescent girls and boys (Del Bel et al., 2017, Hamstra-Wright et al., 2006, Russell et al., 2007).

Sex differences in movement biomechanics associated with ACL injury begin to emerge after the onset of puberty (Ford et al., 2010, Hewett et al., 2004, Hewett et al., 2015, Yu et al., 2005). This review identified inconsistent results in adolescents with respect to whether or not sex differences in neuromuscular control appear at this time as well. Three studies found sex differences, but in different muscles (Landry et al., 2007a, b, 2009, Sell et al., 2006), whereas one study did not find sex differences (Medina et al., 2008). Overall, there is a definite need for studies in adolescents. Longitudinal studies are also necessary to evaluate the effects of growth and development on neuromuscular control patterns.

General Observation and Limitations

First of all, this review included only studies written in English and this might have introduced a language bias. In addition, only three studies (Bencke and Zebis, 2011, Russell et al. , 2007, Shultz et al. , 2009) performed sample size calculations to justify their sample sizes, and the results of these three studies were also inconclusive. Bencke and Zebis (2011) found that females had lower pre-activity of semitendinosus and biceps femoris; Shultz et al. (2009) found that females had higher pre- and post-activity of quadriceps and hamstrings; and Russell et al. (2007) found no sex differences. Many of the other reviewed studies had a small number of participants and might have been underpowered to detect a clinically relevant difference between sexes. Conversely, an underpowered study might have reduced the likelihood that a statistically significant result reflected a true difference between sexes (Button et al., 2013). In addition, although we only included studies that evaluated muscle activation patterns during functional tasks associated with ACL injury, such as landing and cutting, great variability within these tasks and the recruited participants were observed. This included varying platform heights for drop-jumps, angles and approach velocities of cutting tasks, skill level of athletes, and/or sport specialization of athletes. This variability might have affected the findings in neuromuscular patterns and the ability to determine the presence of sex differences, while also preventing reliable cross-study comparisons and generalizability. Another important limitation is that not all studies ensured proper electrode location and orientation with respect to the innervation zone of the tested muscle. When electrodes are placed above the innervation zone or close to the tendons, EMG amplitudes become lower than when electrodes are placed between the innervation zone and the tendon (Merletti et al., 2001, Rainoldi et al., 2000). Moreover, the movement of muscle under the electrodes can substantially alter EMG amplitude during dynamic contractions as the innervation zone shifts under the electrode pair (Rainoldi et al., 2000). Thus, the best location to place an electrode is the area between the innervation zone and tendon terminations where EMG amplitudes are less affected by signal generations and terminations and by the displacement of the electrodes. However, none of the included studies identified or addressed the innervation zone, although they reported that the electrodes were placed in the middle of the muscle or over the muscle belly according to the SENIAM guidelines. Recent works overcame such limitations in the SENIAM guidelines and developed an atlas to provide information on the distribution of the innervation zone and anatomical landmarks for superficial 43 muscles (Barbero et al. , 2012). Use of this atlas would improve reliability of EMG measurement and avoid biased conclusions. Another limitation in interpreting the reviewed neuromuscular studies as a collective is that not all studies considered the same normalization technique, which can have a significant impact on the clinical interpretation of EMG results (Benoit et al. , 2003). Furthermore, not all processing stages of the EMG data were consistently described, making it difficult to facilitate inter-study comparisons. While many of the reviewed studies evaluated EMG amplitude, only six evaluated muscle activation timing and reported inconclusive findings. To gain a thorough understanding of this topic area, future investigations should consider activation timing as well as muscle coordination using synergy analysis.

Conclusion

This systematic review identified potential sex differences in neuromuscular patterns during functional tasks associated ACL injuries; however, no consensus from the literature can be drawn. Females demonstrated higher pre- and post-landing activation of the quadriceps and lower activation of the hamstrings in 15 of 27 studies and demonstrated higher Q:H co-contraction ratios during pre- and post-landing phases compared to their male counterparts in 4 of 9 studies. While these findings support the quadriceps-dominance theory, which can result in undesirable strain on the ACL, several studies found no sex differences in neuromuscular patterns. Furthermore, the methodological limitations found in many studies that only reported EMG data does not permit the extrapolation to changes in knee joint loading and stabilisation between the sexes. Consensus in the literature is also limited by the methodological quality reported in the studies, in particular with respect to sample sizes and recruitment, and differing analysis methods. Also, despite the importance of ACL injury prevention in children and adolescents, the evidence on sex and developmental difference in muscle activation patterns was scarce and inconclusive; future studies must address this critical knowledge gap to improve our understanding of neuromuscular patterns associate with ACL injuries in adolescents.

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CHAPTER 6: ARTICLE 2

Functional capacity and limb symmetry among adolescent males and females with and without ACL injuries during a battery of single-limb strength and hopping tasks.

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Abstract

Objective: Increased adolescent participation in sport unfortunately leads to more musculoskeletal injuries, such as a rupture to the anterior cruciate ligament (ACL). Rehabilitation guidelines are currently informed by adult-based research, with measures such as limb symmetry indices (LSI) during single-limb tasks to evaluate knee joint function. To date, no evidence exists to support the use of these rehabilitation measures in an adolescent population. Therefore, the overall purpose of this study was to investigate LSIs and knee joint function among adolescent males and females with and without ACL-injuries during single-limb assessments.

Methods: Seventy-three adolescent patients with confirmed ACL ruptures (51 females) and 68 uninjured controls (38 females) were recruited. Participants performed maximum voluntary isometric contractions for knee extension and flexion, and single-limb hop tasks. Comparisons were determined *a priori* to evaluate sexes independently while evaluating LSIs for injury and limb-dominance effects.

Results: Females with ACL-injuries had lower performance and LSIs in all tasks compared to controls, while a limb-dominance effect was observed among female controls during cross-hops and knee flexion. Males with ACL-injuries had lower performance in anterior-hop, triple-hop, isometric knee extension and flexion, while only the cross-hop elicited a limb-dominance effect among male controls. Differences between male cohorts were identified in anterior-hop, triple-hop, and isometric knee extension LSIs.

Conclusion: Single-limb tasks and LSIs identified functional differences among adolescents with and without ACL injuries but were not consistent between sexes. These findings support the use of a more patient-specific approach for adolescents, tailored with sex as a factor.

Keywords: ACL injury, adolescents, limb symmetry index, sex differences, strength, rehabilitation guidelines

Introduction

Sports and recreational activities play an important role in the lives of children and adolescents (under 18 years of age). Unfortunately, an increase in sport participation translates to an increase in musculoskeletal injuries in this population [1]. In fact, the number of sports-related injuries is estimated to represent up to 68% of all injuries in adolescent populations, with approximately 30-40 injuries occurring per 100 secondary school students (14-18 years old) per year [2–4]. One of the more common and traumatic injuries in this demographic is an anterior cruciate ligament (ACL) rupture, with an estimated 320,000 to 400,000 injuries occurring among adults annually in North America [5]. As a result, ACL-reconstruction surgery is one of the most performed orthopaedic surgeries on our continent [6].

For patients suffering an initial ACL injury, there are high rates of secondary injuries. Studies report that even after surgical intervention and prescribed rehabilitation, 18% of patients re-ruptured their ACL graft and 17.7% of patients injured their contralateral knee [7,8]. These data are further supported by only 65% being able to return to any level of organized physical activity, and only 50% returning to pre-injury activity levels [9]. Despite the impact of this injury, and considering that females between the ages of 12 and 18 years have the highest risk of sustaining an ACL injury [10–12], there is surprisingly very little sex-specific ACL-injury related research conducted with adolescent populations.

Considering this, patient-specific assessments should be tailored for the target population in question to accurately measure rehabilitation progression and prevent further/secondary injury [13]. This is particularly relevant since the time from injury to return to full participation can last up to two years, and adolescents with or without injury will undergo developmental changes in strength and coordination as a result maturation. As a first step, it is imperative to fully describe and compare the functional capacity of adolescent males and females without an injury to those with ACL ruptures (ACL-deficiency) to have additional insight into the injury's effect on knee joint function. The milestones for rehabilitation are most often based on adult datasets and are not tailored to reflect a paediatric patient's age, sex, maturation state, and activity levels [14,15], and are often reported using limb symmetry indices (LSIs) [10,16]. Most studies calculate the limb symmetry index in healthy individuals by dividing the score of the dominant limb by the non-dominant limb's score with an accepted threshold of 0.90 indicating no asymmetry [17–19]. These RTA measurements have yet to be comprehensively measured in uninjured adolescents, and therefore, rehabilitative measures cannot yet be fully tailored for this age cohort. Thus, the overall purpose of this study was to contribute to addressing this gap in adolescent rehabilitation management. The first objective of this study was to provide a comprehensive reference dataset of tasks/measurements often used in RTA assessments for adolescent males and females with and without an ACL injury. The second objective was to quantify the effect of an ACL injury on functional and subjective knee joint performance in adolescent patients. It was hypothesised that an ACL injury would influence the functional capacity of adolescent patients in both limbs, resulting in poorer performance than their uninjured counterparts in all used assessments. It was also hypothesised that, when considering within-group comparisons, there would be between-limb differences present for all groups, with better performance metrics on the self-identified dominant limb among control participants, and the contralateral limb among injured populations.

Methods

Participants

This study was approved by the ethics boards of the Children's Hospital of Eastern Ontario (17/74X) and the University of Ottawa, Canada (H09-17-10). Adolescents (ages 10-18 years) with confirmed ACL rupture via arthroscopy and/or MRI were eligible to be recruited for this study by Orthopaedic Surgeons from CHEO, Ottawa, CA. Patients who required ACL reconstruction revision or had other lower-extremity injuries were not eligible for participation in this study. Eligible patients required approval from their physician, and it was only provided once the physician was confident in the patient's ability to participate fully in the study by performing all tasks. Recruitment of uninjured controls took place via convenience and snow-ball sampling of regional youth sports associations and club teams in Ottawa, CA.

Consent and questionnaires

Upon arrival to the human movement laboratory, participants read and signed a consent form explaining the protocol, any possible risks, and the purpose of the study, with the help of a parent/guardian if necessary. Each participant was given the opportunity to discuss the study or ask any questions with a member of the research team before consenting. In addition, a set of self-assessments and surveys (patient reported outcome measures: PROMs) were completed in their preferred language (English/French): Pedi-IKDC [20], HSS Pedi-Fabs [21,22], Tegner activity scale [23], and Tanner Stages Classification form [24]. The dominant leg of each participant was distinguished by which leg was used to kick a soccer ball a maximum distance [25].

Study procedures

Participants were provided with a set of tight-fitted spandex shorts and long-sleeved shirt, and a standardized set of neutral running shoes (KBS7FW3343; MS7F505027, Joe Fresh, Canada) before completing a warm-up of cycling on a stationary ergometer for five minutes (Monark 828E, Vansbro, Sweden). Maximum voluntary isometric contractions (MVICs) were performed using an isokinetic dynamometer (System 4 Pro, Biodex Medical Systems, USA) for knee flexion and extension: recorded in a seated position with the knee and hip flexed at approximately 60^o and 90^o, respectively. Participants were instructed to begin gradually until achieving their maximal contraction and then to hold for five seconds; this was repeated three times with one minute rest between trials. Isometric torque data were sampled at 2000Hz and collected using Nexus software (v2.7, Vicon, UK). Verbal and on-screen biofeedback provided encouragement for participants to achieve maximal efforts. Torque data were filtered using a 4th-order zero-lag low-pass Butterworth filter at 15Hz, normalized to body mass, and full-wave rectified. Maximum torques were identified using a 10ms moving average algorithm during the MVICs.

Participants then performed single-limb hop tasks, which are used in evaluating the relative difference in functional capacity of a patient's limbs in a clinical setting [26–34]. These unilateral tasks included: anterior hops (hop as far as possible on one foot facing forward); timed-6m hop (hop on one foot as fast as possible to cover a distance of 6 meters); triple hops (hop on one foot three times in a row for maximum distance); and cross-hops (hop on one foot back-and-forth three times across two marked lines separated by 15cm on the laboratory floor, while attempting to cover maximum distance). Additionally, a lateral hop task was added to the battery of tests with participants hopping as far as they could on one foot while facing perpendicular to the direction of the marked line on the laboratory floor. The lateral hop is not currently a part of the cited single-limb battery of hop tests, however, we deemed it appropriate to be added as an assessment given that ACL injuries often occur in the frontal plane [35–37]; its repeatability and reproducibility has been validated [38] (Figure 6.1).

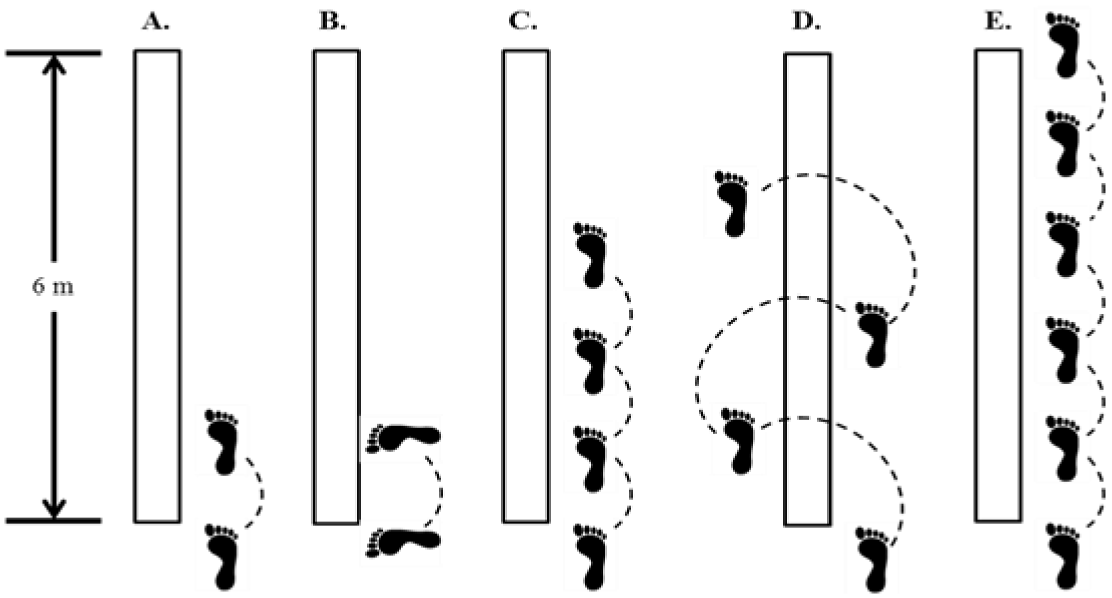

Figure 6.1: Single-limb hop tests on the right foot: A) Max Anterior Hop, B) Max Lateral Hop, C) Triple Hop, D) Cross-Hop, and E) Timed-6m Hop.

Participants were provided detailed instructions and demonstration by a study team member before completing two practice trials on each limb. Participants then performed a minimum of two good trials for both limbs, consisting of using proper technique and holding the landing for a minimum of two seconds before placing the contralateral foot on the floor. To account for variability in arm swing, participants kept their hands on their hips throughout each trial; if their hands came off at any point, the trial was not included. One-minute rest was provided between trials to prevent fatigue and all hop tests were performed at a self-selected pace.

After task completion, each participant was asked three questions pertaining to their perceived performance on their injured limb or their non-dominant limb, for patients with and without injuries, respectively. Five-point Likert scales were used to capture their perceived pain (1: no pain at all, 5: extremely painful) and their perceived difficulty of completing that task (1: not difficult at all, 5: being extremely difficult), and a 20-point Borg scale was used to capture their perceived exerted effort.

Data reduction and statistical analysis

Limb symmetry indices (LSIs) were calculated for each measurement to provide a ratio of symmetrical performance, with a ratio of < 0.90 being deemed clinically significant [9]. LSIs were calculated using the maximum values for each task by dividing the value of the injured limb by the contralateral limb and the value of the non-dominant limb by the dominant limb, for patients with ACL injuries and control participants, respectively [39–42].

All statistical analyses were performed using SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA) with a significance level set at $p = 0.05$ with a Bonferonni correction to account for multiple compared variables. All variables for both males and females were assessed for skewness/kurtosis, normality (Shapiro-Wilks tests), and equality of variance (Levene's test). Independent t-tests (or the non-parametric equivalent, Mann-Whitney U-tests) were used to evaluate group differences (FACL vs. FCON, MACL vs. MCON) on the following variables: demographics and anthropometrics, questionnaires, and LSIs for spatiotemporal variables during single-limb hop tests, and isometric knee flexion and extension. To explore the effect of an ACL injury, comparisons were completed by contrasting the dominant limb of the control cohorts to the ACL-deficient limb of the injured cohort. Paired t-tests were used to evaluate between-limb differences (ACL cohorts: injured vs. contralateral limb; CON cohorts: dominant vs. non-dominant limb) within each study group (FACL, FCON, MACL, and MCON) for spatiotemporal variables and torques.

Results

Males and females with ACL injuries had statistically different ($p < 0.05$) group means compared to their uninjured counterparts for the following demographic variables: age, height, weight, BMI, and Tanner maturation stage (Table 6.1). A post-hoc covariate analysis, was performed to determine the effect of Tanner stages; however, no changes in statistical results were identified. As anticipated, the significant effect of injury was observed in questionnaires related to perceived knee joint function and physical activity levels within each sex, evidenced by smaller group means for the Pedi-IKDC, Tegner Activity Scale, Pedi-FABS, and perceived pain during study tasks compared to their uninjured counterparts (Table 6.1).

Not all participants completed all single-limb hopping tasks due to their comfort level and had the opportunity to skip a task. Female participants with ACL injuries (n = 51) completing each task on both limbs were: 88% for anterior hop, 84% for lateral hop, 82% for 6m-timed hop, 71% for cross hop, and 84% for triple hop. Male participants with ACL injuries (n = 22) completing each task on both limbs were: 91% for anterior hop, 82% for lateral hop, 82% for 6m-timed hop, 68% for cross hop, and 73% for triple hop. All participants with injuries completed MVIC assessments. Every control participant completed all study tasks.

Table 6.1. Group means (standard deviations) for females with ACL injuries (FACL), female controls (FCON), males with ACL injuries (MACL), and male controls (MCON) of participant demographics, subjective questionnaires, and reported pain levels during tasks. Time since injury (T.S.I.) was defined as the time, in months, between the approximate date of injury and the visit to the laboratory. Significant differences ($p < 0.05$) evaluated using independent *t*-tests and Mann-Whitney *U*-tests are denoted with * and §, respectively.

Variable	**FACL**	**FCON**	***p*-value**	**Effect size (*d*)**	**MACL**	**MCON**	***p*-value**	**Effect size (*d*)**
Sample Size	n = 51	n = 38	---	---	n = 22	n = 30	---	---
Age (years)	15.2 (1.36)	13.2 (1.66)	< 0.001*	1.32	15.6 (1.18)	13.3 (1.76)	< 0.001*	1.54
T.S.I. (months)	5.93 (3.44)	---	---	---	6.53 (2.82)	---	---	---
Height (cm)	165.1 (6.08)	161.8 (8.02)	0.033*	0.46	174.9 (8.56)	164.6 (13.5)	0.003*	0.91
Weight (kg)	64.2 (11.3)	50.9 (11.0)	< 0.001*	1.19	76.1 (29.0)	51.8 (12.7)	< 0.001*	1.09
BMI (kg/m^2)	23.5 (3.83)	19.2 (2.82)	< 0.001*	1.28	24.8 (8.89)	18.9 (2.83)	0.001*	0.89
Leg Dominance								
Right	n = 42	n = 32	---	---	n = 20	n = 30	---	---
Left	n = 9	n = 6	---	---	n = 2	n = 5	---	---
Leg Injured								
Right	n = 27	---	---	---	n = 14	N/A	---	---
Left	n = 24	---	---	---	n = 8	N/A	---	---
Dominant	n = 21	---	---	---	n = 14	N/A	---	---
Non-Dominant	n = 30	---	---	---	n = 8	N/A	---	---
Questionnaires								
Tanner Stage (/5)	4.31 (0.74)	3.18 (1.06)	< 0.001§	1.24	4.09 (0.94)	3.13 (1.14)	0.002§	0.92
Pedi-IKDC (/100)	59.38 (9.61)	98.1 (4.09)	< 0.001§	5.24	68.4 (14.7)	99.0 (1.69)	< 0.001§	2.92
Tegner (/10)	3.66 (1.67)	8.34 (1.26)	< 0.001§	3.16	4.43 (2.74)	8.47 (1.25)	< 0.001§	1.90
Pedi-FABS (/24)	6.45 (6.33)	21.7 (5.45)	< 0.001§	2.58	9.67 (8.09)	25.3 (4.47)	< 0.001§	2.39
Pain Level (/5)								
Anterior Hop	1.56 (0.81)	1.03 (0.16)	< 0.001§	0.91	1.31 (0.60)	1.07 (0.25)	0.076	---
Lateral Hop	1.68 (0.91)	1.00 (1.00)	< 0.001§	0.71	1.36 (0.63)	1.03 (0.18)	0.002§	0.71
6m-Timed Hop	1.82 (0.79)	1.03 (0.16)	< 0.001§	1.39	1.50 (0.65)	1.03 (0.18)	< 0.001§	0.99
Cross Hop	1.82 (1.13)	1.00 (1.00)	< 0.001§	0.77	1.45 (0.69)	1.03 (0.18)	0.004§	0.83
Triple Hop	1.92 (0.91)	1.03 (0.16)	< 0.001§	1.36	1.46 (0.66)	1.03 (0.18)	0.002§	0.89
Knee Extension	2.15 (0.83)	1.32 (0.62)	< 0.001§	1.13	2.00 (0.97)	1.43 (0.73)	0.043§	0.66
Knee Flexion	1.85 (0.99)	1.18 (0.51)	< 0.001§	0.85	1.50 (0.82)	1.20 (0.41)	0.265	---

Females with and without ACL injuries

The significant effect of injury was present during each task completed by the two female cohorts. Although the females without ACL injuries were, on average, younger and not as physiologically developed (height, weight, maturity level), they had higher performance metrics (greater distances, less time, and higher force generation) and overall higher levels of symmetry compared to females with ACL injuries (Tables 6.2 and 6.3).

Table 6.2. Means and standard deviations of group means for limb symmetry indices (LSIs) of clinical assessment data for females with ACL injuries (FACL) and without (FCON). Significant differences ($p < 0.05$) evaluated using independent t-tests are denoted with * and effect sizes represented by Cohen's d.

Variable LSI	**FACL**	**FCON**	***p*-value**	***T*-statistic**	**Effect Size (*d*)**
Anterior Hop	0.79 (0.20)	0.99 (.127)	< 0.001*	5.68	1.21
Lateral Hop	0.87 (0.27)	1.00 (0.22)	0.019*	2.40	0.54
6m-Timed Hop	0.89 (0.16)	1.03 (0.10)	< 0.001*	4.79	1.07
Cross Hop	0.87 (0.19)	0.97 (0.16)	0.013*	2.54	0.59
Triple Hop	0.87 (0.14)	0.98 (0.07)	< 0.001*	4.41	0.98
Knee Extension	0.80 (0.16)	0.97 (0.11)	< 0.001*	5.92	1.22
Knee Flexion	0.86 (0.18)	0.93 (0.12)	0.033*	2.17	0.44

Considering the within-group comparisons for females with ACL injuries, as expected, group performance metrics were greater on their contralateral limb compared to their injured limb for each task (Table 6.3). For female controls, two significant leg-dominance differences were observed, with participants jumping further during the single-limb cross hop task ($p = 0.037$) and generating higher isometric knee flexion torque ($p < 0.001$) on their dominant limb compared to their non-dominant limb (Table 6.3).

Table 6.3. Means and standard deviations of group means for each limb of clinical assessment data for females with ACL injuries (FACL) and without (FCON). INJ – limb with ACL injury; CONT – contralateral limb; ND – non-dominant limb; DOM – dominant limb. Significant within-group differences ($p < 0.05$) evaluated using paired-sample *t*-tests are denoted with * and effect sizes represented by Cohen's d.

	FACL					
Variable	**INJ Limb**	**CONT Limb**	***df***	***p*-value**	***T*-statistic**	**Effect Size (*d*)**
Anterior Hop (cm)	70.3 (23.0)	90.3 (21.7)	44	< 0.001*	7.08	1.06
Lateral Hop (cm)	56.1 (17.1)	67.4 (18.4)	42	< 0.001*	4.36	0.67
6m-Timed Hop (s)	3.24 (0.93)	2.78 (0.53)	41	0.001*	3.21	0.49
Cross Hop (cm)	221.2 (52.2)	262.9 (62.1)	35	< 0.001*	4.84	0.81
Triple Hop (cm)	288.5 (55.8)	334.7 (56.7)	43	< 0.001*	5.89	0.89
Knee Extension (Nm)	2.16 (0.62)	2.63 (0.69)	50	< 0.001*	5.58	0.78
Knee Flexion (Nm)	0.96 (0.27)	1.12 (0.28)	50	< 0.001*	5.76	0.81
	FCON					
Variable	**ND Limb**	**DOM Limb**	***df***	***p*-value**	***T*-statistic**	**Effect Size (*d*)**
Anterior Hop (cm)	91.9 (20.1)	93.2 (19.5)	37	0.480	0.71	---
Lateral Hop (cm)	70.4 (16.0)	72.7 (17.8)	37	0.260	1.14	---
6m-Timed Hop (s)	2.68 (0.43)	2.60 (0.38)	37	0.063	1.91	---
Cross Hop (cm)	267.8 (60.5)	279.7 (61.3)	37	0.037*	2.17	0.35
Triple Hop (cm)	344.8 (50.9)	350.2 (44.5)	37	0.175	1.38	---
Knee Extension (Nm)	2.85 (0.43)	2.95 (0.50)	37	0.060	1.94	---
Knee Flexion (Nm)	1.24 (0.23)	1.33 (0.22)	37	< 0.001*	3.95	0.64

Males with and without ACL injuries

For males, similar demographic differences were observed, however; they also had higher performance metrics (greater distances, less time, and higher force generation) and overall higher levels of symmetry compared to males with ACL injuries (Tables 6.4 and 6.5). A significant effect of injury was present during the anterior hop ($p < 0.001$), triple hop ($p = 0.035$) and isometric knee extension ($p = 0.004$), with uninjured controls jumping further and generating higher torque values, respectively (Table 6.4).

Table 6.4. Means and standard deviations of group means for limb symmetry indices (LSIs) of clinical assessment data for males with ACL injuries (MACL) and without (MCON). Significant differences ($p < 0.05$) evaluated using independent t-tests are denoted with * and effect sizes represented by Cohen's d.

Variable LSI	**MACL**	**MCON**	***p*-value**	***T*-statistic**	**Effect Size (*d*)**
Anterior Hop	0.77 (0.23)	0.98 (0.14)	< 0.001*	3.89	1.12
Lateral Hop	0.91 (0.32)	1.00 (0.15)	0.273	1.13	---
6m-Timed Hop	0.94 (0.19)	1.03 (0.11)	0.067	1.59	---
Cross Hop	0.91 (0.17)	0.95 (0.11)	0.269	1.12	---
Triple Hop	0.89 (0.18)	0.98 (0.09)	0.035*	2.18	0.68
Knee Extension	0.84 (0.21)	1.00 (0.17)	0.004*	3.01	0.85
Knee Flexion	0.87 (0.27)	1.00 (0.19)	0.058	1.94	---

Males with ACL injuries had higher performance metrics on their contralateral limb compared to their injured limb on four tasks (Table 6.5), specifically, a significant injury effect was found with poorer performance on their injured limb during the anterior hop ($p < 0.001$), triple hop ($p = 0.028$), and both isometric knee extension ($p < 0.001$), flexion ($p = 0.015$) tasks. For male controls, one significant leg-dominance difference was observed with participants jumping further during the single-limb cross hop task ($p = 0.037$) on their dominant limb compared to their non-dominant limb (Table 6.5).

Table 6.5. Means and standard deviations of group means for each limb of clinical assessment data for males with ACL injuries (MACL) and without (MCON). INJ – limb with ACL injury; CONT – contralateral limb; ND – non-dominant limb; DOM – dominant limb. Significant within-group differences ($p < 0.05$) evaluated using paired-sample *t*-tests are denoted with * and effect sizes represented by Cohen's d.

		MACL				
Variable	**INJ Limb**	**CONT Limb**	***df***	***p*-value**	***T*-statistic**	**Effect Size (*d*)**
Anterior Hop (cm)	81.4 (28.2)	107.4 (30.6)	19	< 0.001*	4.19	0.94
Lateral Hop (cm)	72.9 (27.2)	81.9 (26.2)	17	0.073	1.91	---
6m-Timed Hop (s)	3.00 (0.89)	2.75 (0.99)	17	0.206	1.31	---
Cross Hop (cm)	285.9 (100.8)	314.3 (90.3)	14	0.056	2.08	---
Triple Hop (cm)	351.6 (99.5)	396.9 (94.9)	15	0.028*	2.44	0.61
Knee Extension (Nm)	2.65 (1.08)	3.26 (1.27)	21	< 0.001*	4.99	0.53
Knee Flexion (Nm)	1.15 (0.47)	1.35 (0.51)	21	0.015*	2.64	0.11
		MCON				
Variable	**ND Limb**	**DOM Limb**	***df***	***p*-value**	***T*-statistic**	**Effect Size (*d*)**
Anterior Hop (cm)	103.2 (30.5)	104.8 (24.4)	29	0.558	0.59	---
Lateral Hop (cm)	83.6 (20.7)	83.9 (19.3)	29	0.866	0.17	---
6m-Timed Hop (s)	2.53 (0.45)	2.44 (0.34)	29	0.087	1.77	---
Cross Hop (cm)	327.7 (91.2)	342.1 (80.5)	29	0.037*	2.19	0.40
Triple Hop (cm)	385.7 (88.3)	394.1 (83.9)	29	0.195	1.33	---
Knee Extension (Nm)	3.14 (0.70)	3.19 (0.70)	29	0.566	0.58	---
Knee Flexion (Nm)	1.46 (0.38)	1.49 (0.36)	29	0.572	0.57	---

Discussion

Current rehabilitation milestones for ACL injuries were developed from adult datasets and have not been fully tailored to an adolescent population. Our study sought to evaluate common metrics used in such rehabilitation guidelines among adolescent males and females, with and without ACL injuries. However, it must be noted, that due to Covid-19 restrictions affecting recruitment, there are group differences present for demographic variables. While this does not diminish the descriptions related to the healthy population itself, it does relate to the comparison with the ACL injured. Interestingly, since the healthy cohort is younger and less skeletally mature, our findings of them being the same or better functionally indicates an even greater deficit in the ACL injured cohort. Our results clearly demonstrate the effect of an ACL injury on single-limb performance, as their younger, smaller, and physically less mature counterparts out-performed them on almost every task.

The battery of single-limb hop tests examined in this study offers clinicians, and strength and conditioning experts a reliable method to assess the functional capacity of uninjured, adolescent males and females [44,45]. The reliability of these tasks has also been established by multiple authors [46–48] and additional research has provided insight into the functional capacity in adults with and without injuries, using LSIs [40,44]. However, there is limited evidence for its use in adolescent populations, regardless of injury status. Among our findings, females had higher performance metrics using their dominant limb compared to their non-dominant limb for the cross-hop and isometric knee flexion tasks. A similar observation was observed among male controls, with only one limb-dominance difference being present during the cross-hop task. These findings further support the notion of requiring sex-dependent streams of research and rehabilitation models for patients being evaluated for knee joint function. In addition, it was hypothesised that both males and females with ACL injuries would have significantly lower performance metrics in their injured limb. This was observed for all tasks in females, thus supporting the hypothesis. However, in males the injury-effect was only present during the anterior hop, triple hop, isometric knee flexion, and knee extension tasks, partially supporting this hypothesis.

Injury recovery among adolescents differs from adults, given the complex multifactorial nature of growth during puberty [49]. As such, when evaluating an adolescent patient's recovery from an ACL injury, a clinician's current best practice is to compare their patient's injured limb to their contralateral limb [40,44]. Considering the results of this study, the contralateral limb should not be the baseline measurement of functional status for patient with an ACL injury without a deeper understanding of the contralateral limb's performance compared to their healthy age, sex, and activity-matched cohort. This is more evident among the female participants of this study as the contralateral limb's performance was poorer in every completed task compared to an uninjured, despite them being a younger and less physiologically mature population.

Even though LSIs have become popular when evaluating functional status of a patient's injured knee [16,50,51], this measurement may not adequately represent the functional status of the joint since the contralateral limb's performance in this study was not always up to the standard of uninjured individuals. Wellsandt and colleagues (2017) reported LSIs among patients aged 14-55 years of age before and after ACL-reconstruction surgery and reported that LSIs frequently overestimate knee function, suggesting this is likely due to rehabilitation models focusing on unilateral strengthening and neuromuscular control. Neglecting the contralateral limb before and after ACL-reconstruction has also been proposed to contribute to higher risk of secondary injury after being medically cleared on their affected limb [42]. Given that pre-operatively it is not up to the healthy cohort's standard, it is clear that the contralateral limb requires additional training. Our findings indicate that patient assessments must tailored for the target population in question to accurately measure functional capacity and rehabilitation progression.

Conclusion

Between-group differences in performance were identified using single-limb tasks and calculating LSIs in adolescent males and females with and without ACL injuries. These differences were not consistently present during all tasks for both sexes. Females with ACL injuries had lower performance in all single-limb tasks, while males with ACL injuries only had lower performance during the anterior hop, triple hop, knee extension and flexion, compared to their uninjured counterparts. Considering our findings in both injured and control populations, only using LSIs when evaluating the functional status of an adolescent patient's knee joint is not adequate.

Key Points

- Age-specific rehabilitation guidelines should be enforced for each sex when evaluated between-limb knee joint function.
- The performance of the ACL injured adolescent needs to be contrasted to their age and sex-matched healthy counterpart
- LSIs should not be the sole measure for evaluating functional status of an adolescent patient's knee joint.

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CHAPTER 7: ARTICLE 3

Knee joint stabilisation strategies in adolescent males and females during jump-landing and side-cutting.

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Abstract

Objectives: Non-contact ACL ruptures often occur during multi-planar loading and have routinely been associated with a sex-bias. Tasks such as countermovement jumps (CMJ) and side-cuts require dynamic stabilisation to perform and are often considered in evaluating knee joint function; however, they have yet to be fully explored among adolescent males and females.

Methods: Thirty-six (females, n=20) adolescent participants with no injury history had surface electromyography of eight lower-limb muscles and full-body kinematics captured during CMJs and side-cuts. Knee stability was evaluated using co-activation indices (CIs), full muscle waveforms, knee joint flexion stiffness (KJFS) and knee joint sagittal and frontal excursions (KJSE and KJFE). Between and within statistical comparisons were used to evaluate the effect of sex and limb-dominance on discreet and continuous measures.

Results: Females had higher medial CIs in the non-dominant limb in both CMJs and side-cuts, and higher total CIs in the non-dominant limb during side-cuts compared to males. Females had higher total CI in their non-dominant limb in CMJs and higher KJFE in their non-dominant limb during side-cut stance phase. Males had higher medial CIs in their dominant limb in CMJs and higher KJFE in their non-dominant limb during the preparatory and landing phases of CMJs. No significant sex or limb differences were identified in individual muscle activations or KJFS.

Conclusion: Adolescents require a population-specific approach in evaluating and establishing injury prevention/management guidelines. In conjunction with sex differences, our results support limb-dominance as a relevant factor for consideration during knee stability assessments among adolescent cohorts.

Key Terms: adolescents, knee stability, sex differences, limb-dominance, dynamic loading

Introduction

Approximately 70% of ACL injuries are non-contact in nature [1] and typically occur during movements that entail multi-planar loading, such as jump-landing and side-cutting where medial and internal rotational loading is observed. In addition, researchers have suggested that there may be limb-specific injury risks related to one's dominant or preferred limb, however, the results are inconsistent [2]. Some studies indicate a higher risk is present in the non-dominant limb among females and higher in the dominant limb for males [3,4], whereas other research has found there to be no relationship between limb-dominance and injury risk among either sex [5]. As such, countermovement jumps (CMJs) and side-cuts are often used in evaluating the relative between-limb differences in functional capacity of a participant, particularly in injury prevention and rehabilitation programs [6].

During dynamic loading tasks such as CMJs and side-cuts, the knee's ability to mitigate loading through the joint is imperative to maintaining stability and a healthy joint. Knee joint stability arises from a combination of the articulating geometry, passive soft tissue restraints, compressive loading from bearing weight, and muscle forces – which are the only modifiable contributors to knee joint control [7,8]. Given their role in knee joint stability, there has been a focus on the management of muscle activations and their contributions to motion in ACL injury and rehabilitation research [9]. Despite extensive work being completed with adults as participants, a consensus on the differences in neuromuscular control between sexes and the influence of an ACL injury or limb-dominance have on knee stabilisation strategies have yet to be fully established. Even more concerning is the lack of research related to sex bias, neuromuscular control and ACL injury prevention among adolescents given that this cohort has the highest rates of non-contact ACL injuries [10,11].

Coincidentally, there is no consensus regarding rehabilitation guidelines nor thresholds to be met for return to activity (RTA) in adolescent patients with ACL injury [12], which is likely contributing to the high rates of injury and re-injury among this age cohort [13]. A logical step in developing these guidelines is establishing knee stabilisation strategies used by adolescent males and females without ACL injuries during demanding dynamic tasks and is the main purpose of this study. This knowledge could help tailor guidelines to be age-, limb- and sex-specific for each patient. In addition, this study sought to identify whether the role of limb-dominance influences knee stabilisation strategies among this age group.

Based on the limited literature investigating sex differences in muscle activation strategies in adolescents [14], it was hypothesised that adolescent females would demonstrate unfavourable knee stabilisation strategies such as higher co-activation ratios, knee joint stiffness, and lower joint excursions compared to males, as this is after the onset of the sex-bias in ACL injuries, at the age of 12 years [15]. Furthermore, it was hypothesised that limb-dominance differences would exist among both males and females, manifested as higher co-activation ratios, knee joint stiffness, and smaller joint excursions in their non-dominant limb compared to their dominant limb during the countermovement jump (CMJ) and side-cutting [16].

Methods

Participants

This study was approved by the local research ethics board. Female and male adolescents were recruited via convenience and snow-ball sampling of regional youth sports associations and clubs (see Table 7.1). All participants were required to read and sign a consent form explaining the protocol, any possible risks, and the purpose of the study, with the help of a parent/guardian if needed. Each participant was given the opportunity to discuss the study or ask any questions with a member of the research team before providing their consent. Participants were eligible to participate in this study if they were between the ages of 10 and 18 years old, physically active (participated in exercise/sport at least twice per week), had no history of traumatic injury (i.e. meniscal tear, ligament rupture), had no injury at time of testing (i.e. lower limb sprain) and there was no presence of any other observed physical impairment that may hinder the results of their participation in this study. Limb dominance was self-identified as which leg was used to kick a soccer ball a maximum distance [17].

Questionnaires

A set of self-assessments and surveys were completed in their preferred language (English or French): Pedi-IKDC [18], HSS Pedi-Fabs [19,20], Tegner-activity-scale [21], and Tanner Stages Classification form [22]. Participants were then provided with tight-fitted spandex shorts and a long-sleeved shirt prior to the collection of the following anthropometric measures: pelvis, knee, and ankle width, height, and weight; leg and tibial length; and thigh and shank circumference. Participants were then provided with neutral running shoes in their size (KBS7FW3343; MS7F505027, Joe Fresh, Canada).

Participant preparation and equipment

Wireless, bipolar surface EMG electrodes connected to a 16-channel EMG system (Trigno Wirreless System, Delsys, USA) were placed over the muscle bellies of interest following SENIAM [23] and DeLuca [24] recommendations, with minor adjustments for varying body dimensions. Prior to placement, areas of the skin were shaved and cleaned with alcohol wipes. The muscles of interest on both limbs were: the rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), semitendinosus (ST), biceps femoris (BF), medial gastrocnemius (MG), lateral gastrocnemius (LG), and gluteus medius (GM). EMG data were sampled at 2000Hz, band-pass filtered at 20-450Hz with a 12 dB/octave filter roll off and a common mode rejection ratio of >80dB. After sensor placement, both thigh and calf segments were wrapped with athletic pro-wrap and secured with tape to limit movement artefacts that may arise during dynamic movement. Participants were then asked to complete a warm-up of cycling on a stationary ergometer for five minutes (Monark 828E, Vansbro, Sweden). EMG data for each muscle were visually screened live using Nexus software (v2.7, Vicon, UK) to ensure proper measurement and sensor placement during stationary cycling.

Next, maximum voluntary isometric contractions (MVICs) were performed using an isokinetic dynamometer (System 4 Pro, Biodex Medical Systems, USA) and included i) knee flexion and ii) extension, recorded in a seated position with the knee flexed at 60^o, and iii) plantarflexion, recorded in a seated position with the knee at 0^o flexion and the ankle in a neutral position; iv) hip abduction was recorded in a standing position, with the hip at 10^o abduction and 0^o extension. Participants were instructed to begin gradually contracting until achieving their maximal contraction and to hold for five seconds. This action was repeated three times with at least one minute of rest in between trials. EMG and isometric torque data were synchronously collected using Nexus, sampled at 2000Hz. Torque data were filtered using a 4th-order zero-lag low-pass Butterworth filter at 15Hz. The torque values were then corrected for body mass and assigned to be positive to simplify their interpretation during data analysis. Verbal encouragement and on-screen biofeedback were provided to encourage participants to achieve their maximal effort. Torque data were not considered in the current analysis.

Participants then had 84 reflective markers (14mm diameter) placed on various landmarks according to a hybrid cluster-marker set adapted from Mantovani and Lamontagne [25]. During all movement tasks, ground reaction forces (GRFs) were sampled at 1000Hz, amplified with an internal gain of 1000 and recorded through two force platforms (FP4060-08, Bertec Corp., USA), while marker trajectories were sampled at 200Hz using a 10-camera infrared motion analysis system (8 Vero and 2 Vantage; Vicon, Nexus, UK) and synchronously recorded using Nexus software.

Experimental protocol

Participants performed a standing calibration trial, followed by a series of dynamic trials. For this study, the five successful trials of both CMJs and side-cuts (on each limb) were analysed. CMJs consisted of participants standing with feet shoulder-width apart on separate force platforms, with their hands on their head to account for arm motion variability, and the participants were instructed to squat down to help them jump as high as possible. A CMJ trial was deemed successful if the participant kept their hands on their head, landed with both feet entirely on their respective force platforms and held the landing without losing their balance (Figure 7.1).

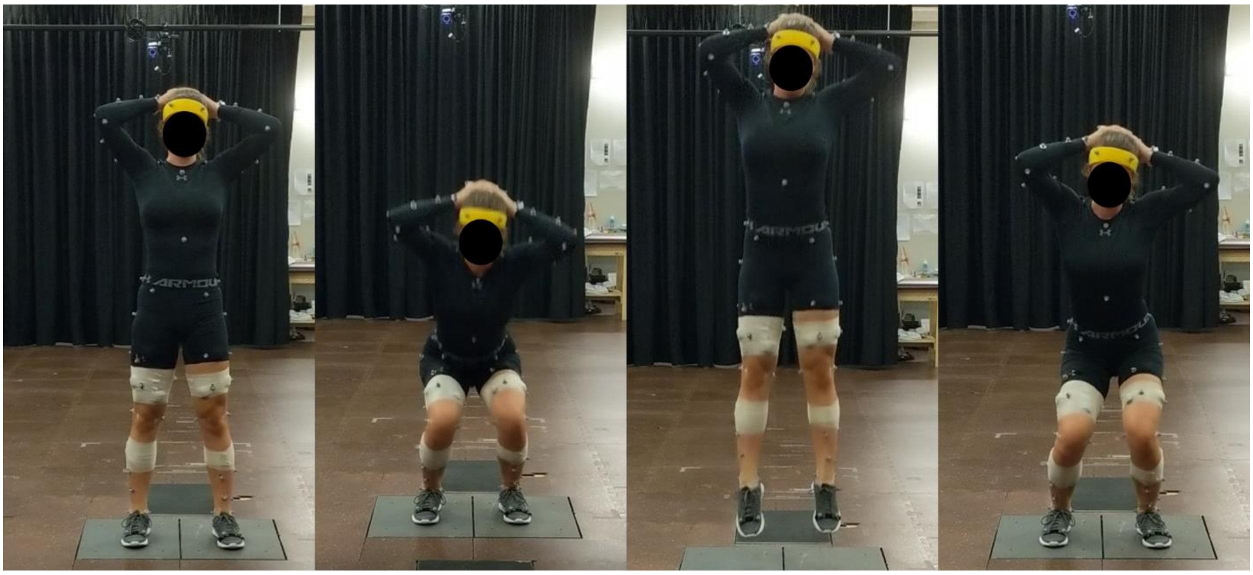


Figure 7.1. Sequence of events for a countermovement jump in motion laboratory. From left to right: starting position, preparatory phase, flight phase, and landing phase.

Side-cuts were performed with participants starting at one end of the laboratory and running linearly as fast as possible while maintaining control to plant and cut with the foot of interest on the force platform to either the left or the right at a 45^o angle. A side-cut trial was deemed successful if the participant planted and cut with the appropriate foot, placed their entire foot on the force platform, and accelerated out of the foot-plant at a 45^o angle (pylons in the lab provided a ± 10-degree range) (Figure 7.2).

Figure 7.2. Sequence of events for a 45-degree side-cut maneuver in motion laboratory. From left to right: preparatory phase, stance phase, and push-off in new direction.

Data processing

Marker trajectories and GRFs were filtered with a 2^{nd}-order zero-lag dual low-pass Butterworth filter with matching cut-off frequencies of 15Hz [26] using custom pipelines in Vicon Nexus (v2.10, Vicon, UK). Modified Vicon Nexus pipelines were used for scaling and computing inverse kinematics (relative to the standing calibration trial) and inverse dynamics for the hip, knee, and ankle sagittal and frontal plane angles and moments. Raw EMG signals were exported into Matlab (R2018a, Natick, USA), high-pass filtered at 20Hz with a 2^{nd}-order dual-pass Butterworth filter, full-wave rectified, and low-pass filtered at 6Hz with a 2^{nd}-order dual-pass Butterworth filter. A 10ms moving average algorithm was used to identify maximum EMG amplitudes for each muscle during MVICs; these maxima were used to amplitude-normalise the EMG data during CMJs and side-cut trials.

All data were time-normalised within the task being analysed. The CMJ trials were parsed into three phases: take-off, flight, and landing. The take-off phase was identified using the initiation of upward acceleration from the lowest point of the squat (maximum knee flexion) until GRF <10N. The flight phase was identified as the duration of the task spent in air with no contact on the force platforms. Lastly, the landing phase was identified by the initial contact with the force platforms (GRF >10N) until the participant returned to a standing position (maximum knee extension). Side-cut trials were parsed into two phases; the first being the preparatory phase, defined as the 100ms prior to initial contact with the force platform (GRF >10N). Next was the stance phase, which was defined as the total time between the participant's foot and the force platform until GRF <10N. The time normalised waveforms within each phase were averaged over five trials for each participant.

Many continuous kinematic and kinetic variables were collected (hip, knee, and ankle sagittal and frontal plane angles and internal joint moments), however, these data were reduced to provide the following dependent variables as proxies of knee function and stabilisation strategies for this study: knee joint flexion stiffness (KJFS) at impact [27] and knee joint angle excursions in both the sagittal (KJSE) and the frontal (KJFE) planes [28]. Full waveform data of all eight muscles in both legs were evaluated throughout all phases of the two dynamic tasks. Primary outcomes for measured muscles were full waveforms and muscle co-activation indices (CI) to explain gross stabilisation strategies of the joint throughout a task and at initial impact, respectively, during CMJs and side-cuts. CIs were calculated for both legs separately between the lateral thigh muscles (VL and ST), the medial thigh muscles (VM and BF), and the summed quadriceps (VL and VM) and hamstrings (ST and BF) muscles using methods by [29]. These CIs were calculated to provide a comprehensive description of the relative muscle activation (ratio) and the magnitude of activation (sum of muscle magnitudes). Low co-activation values represented lower activation of both muscles or low-level activation of one muscle and high activation of the other muscle in the pair. Larger co-activation values denoted higher activation of both agonist and antagonist muscles, representing a more general activation pattern [29].

Statistical analysis

Statistical analyses on discrete variables were performed using SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA), while continuous variables were assessed using statistical parametric mapping (SPM) in Matlab (2018a, Mathworks, Natick, USA) with a significance level set at p=0.05 and a Bonferroni correction to account for multiple comparisons [30]. Independent t-tests (or the non-parametric equivalent, Mann-Whitney

U-tests) were used to compare adolescent males and females on the following variables: demographics, anthropometrics, and questionnaires. To determine if an effect of limb dominance was present in adolescent males and/or females, paired t-tests (or the non-parametric equivalent, Wilcoxon signed-rank tests) were used to evaluate between-limb differences (dominant vs. non-dominant limb) within each sex for all metrics of knee function and stabilisation.

Independent t-tests (or the non-parametric equivalent, Mann-Whitney U-tests) were then used to compare all metrics of knee function and stabilisation between adolescent males and females.

Results

Males were taller and more active (see Pedi-FABS scores) than female participants (Table 7.1). No differences between sexes were found in age, weight, BMI, Tegner Activity Scale, or Tanner maturation stage (Table 7.1).

Table 7.1. Group means (standard deviations) of participant demographics, subjective questionnaires. Significant differences ($p < 0.05$) evaluated using independent *t*-tests and Mann-Whitney U-tests are in bold and denoted with * and §, respectively.

Variable	Females	Males	*p*-value	Effect size (*d*)
Sample Size	n = 20	n = 16	---	---
Age (years)	14.32 (1.67)	14.33 (1.52)	0.48	---
Height (cm)	165.38 (5.91)	173.33 (8.93)	**0.005***	1.05
Weight (kg)	54.97 (9.61)	58.39 (8.41)	0.31	---
BMI (kg/m^2)	20.00 (2.51)	19.45 (2.45)	0.56	---
Leg Dominance				
Right	n = 15	n = 14	---	---
Left	n = 5	n = 2	---	---
Questionnaires				
Tanner Stage (/5)	3.75 (0.77)	3.63 (0.78)	0.25	---
Tegner (/10)	8.40 (1.16)	8.63 (0.99)	0.22	---
HSS Pedi-FABS (/29)	21.45 (4.60)	25.00 (4.40)	**0.014**§	0.80

Knee Joint Stiffness and Excursions

No significant sex differences were observed in either the sagittal or frontal plane stability metrics during CMJ or side-cut trials. However, within-group comparisons revealed an effect of limb-dominance in KJE for each sex. Males had higher sagittal KJE during most of the preparatory and landing phases of CMJs in their non-dominant limb (Figure 7.3), while females had higher frontal KJE during most of the stance phase of side-cuts in their non-dominant limb (Figure 7.4).

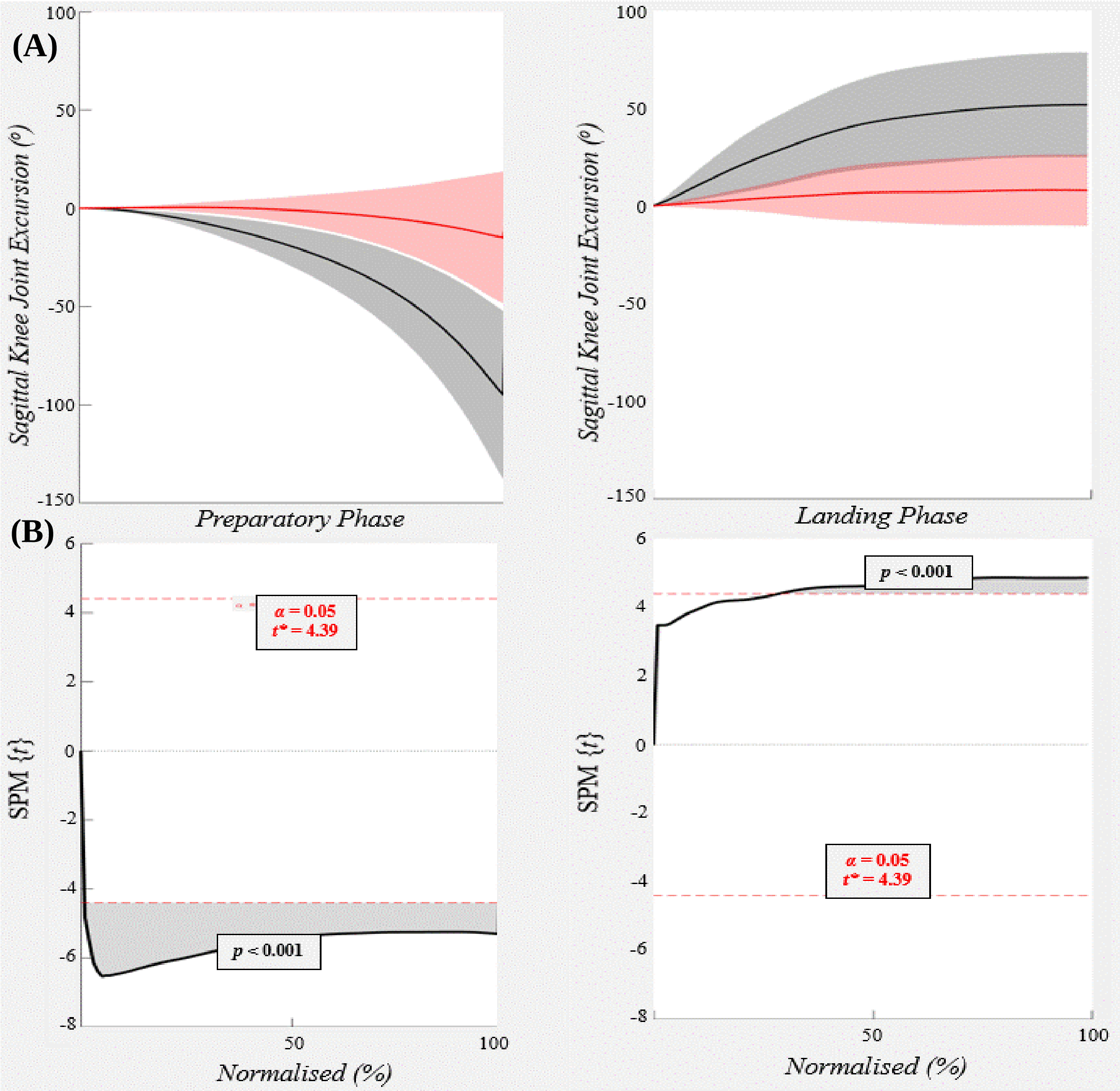


Figure 7.3. (A) Sagittal knee joint excursion of the dominant (black) and non-dominant (red) limbs of adolescent males during the preparatory and landing time-normalised phases of a countermovement jump. **(B)** SPM Mann Whitney-U test statistic SPM{t}. Significant differences ($p < 0.05$) are indicated by the grey regions where SPM{t} passes the critical threshold (red-dashed line).

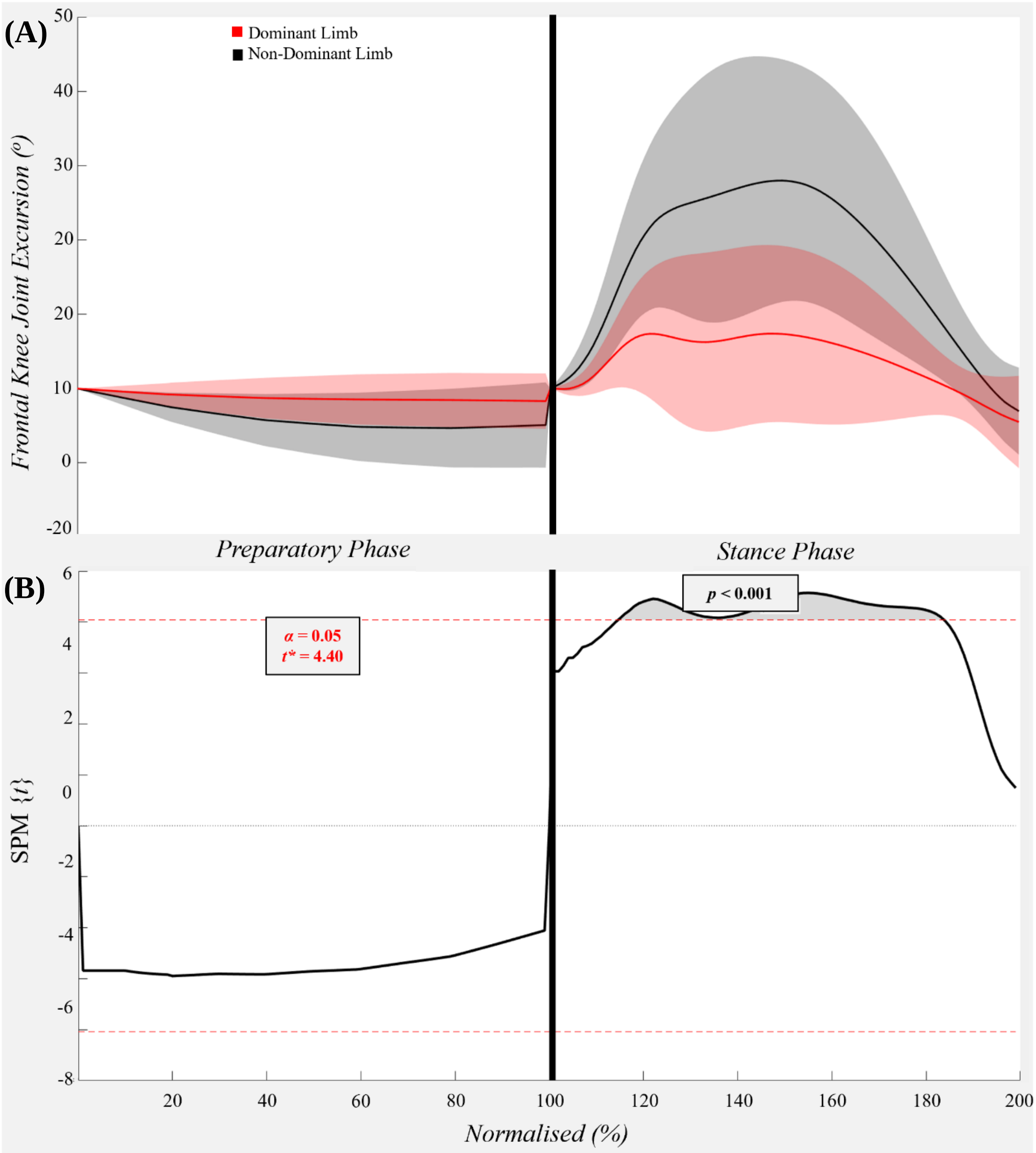
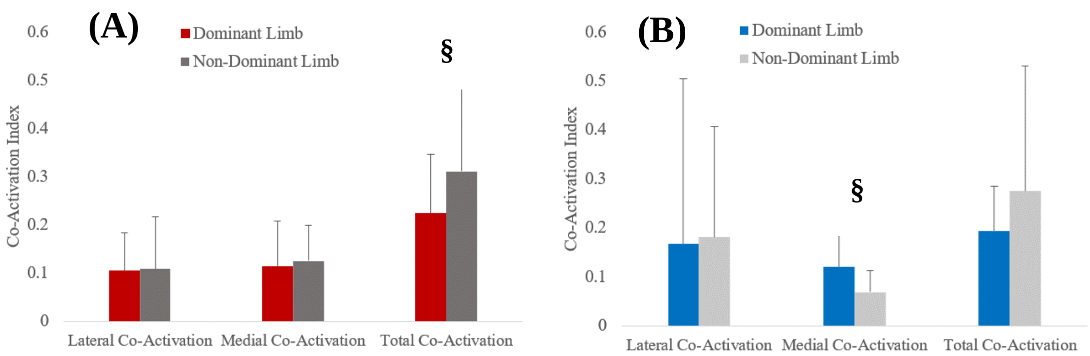


Figure 7.4. (A) Frontal knee joint excursion of the dominant (black) and non-dominant (red) limbs of adolescent females during the two normalised phases of a side-cut. **(B)** SPM Mann Whitney-U test statistic SPM{*t*}. Significant differences ($p < 0.05$) are indicated by the grey regions where SPM{*t*} passes the critical threshold (red-dashed line).

Muscle Activations

During CMJs, a significant effect of limb dominance was seen in muscle co-activations in both females (Figure 7.5A) and males (Figure 7.5B). Females had higher total CIs in their non-dominant limb compared to their dominant limb (p = 0.027, d = 2.24), whereas males had higher medial CIs in their dominant limb compared to their non-dominant limb (p = 0.035, d = 9.28). No limb dominance differences were observed in muscle CIs for females nor males during side-cuts (Figure 7.5C and 5D).



(C)

(D)

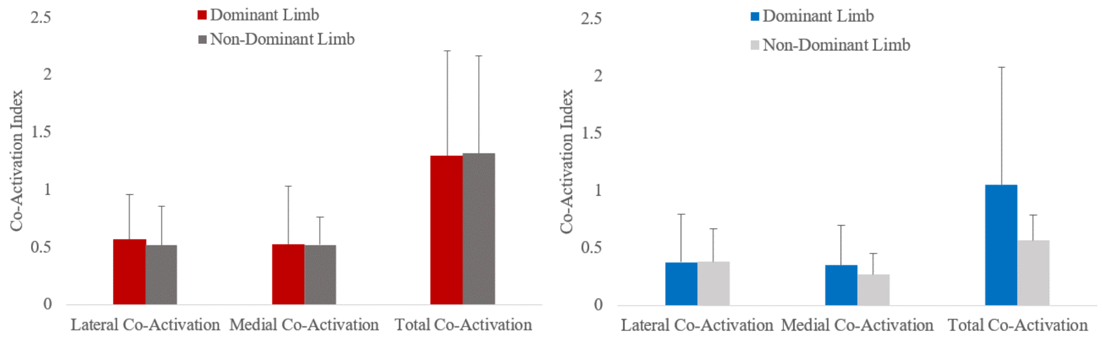


Figure 7.5. Muscle co-activations indices (CI) of dominant and non-dominant limbs at the initiation of the landing phase during a countermovement jump (CMJ) and the stance phase during a side-cut. Female dominant and non-dominant CIs during **(A)** CMJs and **(C)** side-cuts. Male dominant and non-dominant CIs during **(B)** CMJs and **(D)** side-cuts. Significant limb-dominance differences ($p < 0.05$) are denoted with §.

Comparisons between males and females yielded significant differences between muscle CIs during both CMJs and side-cuts (Figures 7.6A and 7.6B). Females had higher medial CIs in the non-dominant limb compared to males during CMJs (p = 0.007, d = 12.7) and side-cuts (p = 0.003, d = 5.02). In addition, females had higher total CIs in the non-dominant limb compared to males during side-cuts (p = 0.002, d = 1.40).

Lateral Medial Total

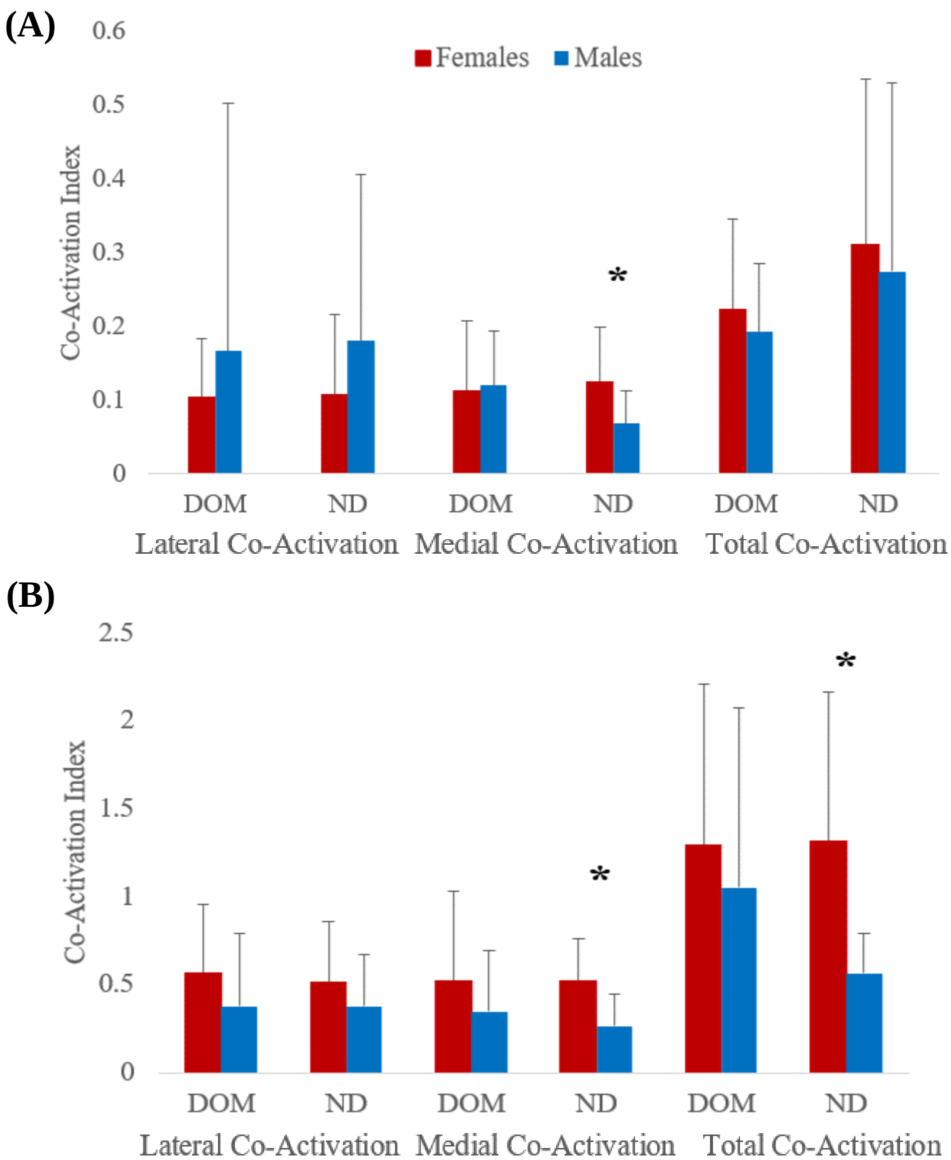


Figure 7.6. Muscle co-activations indices (CIs) of females (red) and males (blue) for their dominant (DOM) and non-dominant (ND) limbs at the initiation of the landing and the stance phases of the **(A)** CMJs and **(B)** side-cuts, respectively. Significant sex differences ($p < 0.05$) are denoted with *.

Upon examining individual muscle waveforms throughout the entirety of both CMJs and side-cuts, no significant differences between limbs nor sexes were observed for any of the eight muscles (Appendix E).

Discussion

Over the last few decades, ACL-related research has attempted to understand the mechanisms and factors that contribute to the injury itself among adults, limiting the interpretations towards adolescent populations. Given the high rates of injury and re-injury among adolescents [31], we investigated the potential effects of limb-dominance and sex on knee stabilisation strategies used by uninjured adolescents. The first hypothesis, being, that adolescent females would demonstrate unfavourable knee stabilisation strategies such as higher CIs, knee joint stiffness, and lower joint excursions compared to males, was partially supported. Females did have higher medial and total CIs in their non-dominant limbs compared to males during both CMJs and side-cuts. Higher CIs have been considered a risk-factor for ACL injuries and is often observed among adults who have suffered ACL injuries [29,32]. The second hypothesis of limb-dominance differences being observed among each sex was accepted, as both males and females had between-limb differences in CIs and KJFE. More specifically, females had higher KJFE during side-cuts in their non-dominant limb, while males had higher KJFE during CMJs in their non-dominant limb. There is currently limited limb-dominance research in muscle activations among adolescents [14] and it is rarely considered in ACL-injury research. Our results confirm that limb-dominance is an important independent variable that should be considered when investigating lower limb biomechanics and muscle activations among adolescents.

The role that sex plays on neuromuscular control and the associated risk of ACL-injuries has been widely researched and speculated upon in the field of biomechanics and will likely continue to be a prominent theme in the field, given the oft-reported sex-bias in injuries [33,34]. The challenge in comparing sexes is that there are several intrinsic/extrinsic factors that are different between the two populations, not to mention within-population variation, such as: anatomical differences (i.e. height/weight, Q-angle, intercondylar notch-width, etc.) [35], hormonal levels [36], physical maturation [37], and gender/social constructs [38]. We accounted for some of the above-mentioned factors by taking sub-cohorts of matched adolescent males and females from our larger dataset. The only significant differences between the two groups used in this study were height and self-reported activity levels through the HSS Pedi-FABS questionnaire, with males being taller and more active than females, while still aligning with the typical range among adolescent athletes [39]. Interestingly, we found two sex differences in knee stabilisation strategies when comparing the non-dominant limbs. Specifically, females displayed higher medial CIs in the non-dominant limb during CMJs and total CIs in the side-cut compared to males. In adults, sex differences are routinely evaluated in dynamic loading tasks, although our recent review found a lack of agreement across studies for neuromuscular control [14]. Furthermore, these sex differences are not always consistent in adolescent populations [40–44]. The presence of high variability among adolescent cohorts may contribute to the varied results within the literature. We found a great deal of variability within the muscle activation patterns and knee stability metrics analyzed despite our efforts to create a controlled/standardized testing environment and protocol. That being said, movement variability is expected in the midst of pubescent development given all the changes occurring in the body [45]. For example, Wren and colleagues (2020) evaluated kinematic variables during drop-jumps, single-leg hops, and heel-touches among 7- to 15-year-olds. Greater variability was observed in most measures (i.e. knee and trunk flexion angles, etc.) in younger athletes (10.5 years) when compared to an older cohort (14.3 years) during drop-jumps and heel-touches. Interestingly, the older cohort had greater variability compared to the younger cohort during single-limb hops, and was suggested as a result of older adolescents attempting to jump further and making it more challenging to maintain stability when landing [45].

Statistically speaking, higher variability will likely lead to less statistically significant findings between two groups; however, variability from an injury-prevention perspective is suggested to be a positive finding. Pollard and colleagues (2005) evaluated sex differences in lower extremity movement variability during unanticipated side-cuts and found higher variability among males in four kinematic couplings: 32% more thigh rotation/leg rotation variability; 40% more thigh abduction-adduction/leg abduction-adduction variability; 46% less knee flexion-extension/knee rotation variability; and 44% less knee flexion-extension/hip rotation variability [46]. They suggest higher coordination variability facilitates the ability to respond to unanticipated changes/perturbations in an individual's environment, and the inability to adapt lower extremity kinematics may be associated with an increased ACL injury risk. Considering this, the amount of variability seen within our dataset of healthy, active adolescent males and females could be an indicator of reduced knee joint injury risk. Future ACL-injury research examining sex differences among adolescents could investigate intra-participant variability during repeated and unanticipated movements to explore the relationship between variability and risk of non-contact ACL injuries.

The influence of limb-dominance on functional performance remains unclear among adult literature, and scarcely explored in adolescent research. A meta-analysis [2] found no statistical effect across a wide range of functional tests in adults. Findings among research that is specific to injury-risk are inconsistent as well. For instance, Negrete and colleagues (2007) reported no significant relationship between limb-dominance and ACL injury among adults, whereas Ruedl and colleagues (2012) reported adult female recreational skiers were more likely to injure their non-dominant limb, while males were more likely to injure their dominant limb. Brophy and colleagues (2010) found a similar relationship among adult soccer players, with females being more likely to injure their non-dominant limb (stance limb), while males were more likely to injure their dominant limb (kicking limb). Limited within-participant research has considered lower-limb biomechanics and neuromuscular control, although, a limb-dominance effect is often found. Researchers have found faster overall velocities during an unanticipated side-cut in adult males [47] , improved task accuracy during a jump-block volleyball task in adult females [16] when using their dominant limb. One study investigating pre-adolescents reported higher muscle activation magnitudes in the VL, both hamstring muscles, and the LG in the dominant limb and earlier peak activations in both gastrocnemii, both hamstrings and the VM of the non-dominant-limb among both sexes during unanticipated side-cuts [48]. Our results identified effects of limb-dominance during knee stabilisation strategies, specifically in CIs among adolescent athletes. This further emphasizes the importance of considering how the results of this study differ from those of studies on adults.

Conclusion

The sex-bias associated with non-contact ACL injuries has led to an abundance of biomechanical and neuromuscular research among adults, with limited consideration for adolescent participants. The results of this study provided insight into the role of sex and limb-dominance in an adolescent population and contributed to understanding knee stabilisation strategies during dynamic loading. Both sex and limb-dominance influence knee stability metrics such as KJFE, medial and total CI among adolescent males and females during CMJs and side-cuts. This data can be referred to when tailoring age- and sex-specific knee stability assessments among adolescents with ACL injuries, and accounting for the effect of limb-dominance.

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CHAPTER 8: ARTICLE 4

Joint stiffness and muscle activation patterns during jump-landing and side-cutting in adolescent males and females with and without anterior cruciate ligament injuries

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Abstract

Background: Adolescent females are the most likely cohort to sustain non-contact anterior cruciate ligament injuries and primarily occur during multi-planar loading tasks. Despite the sex-bias and the injury's impact on quality of life, there is limited research investigating knee stabilisation strategies among healthy and injured adolescent males and females.
Methods: Thirty-six (females, n=20) adolescents with confirmed anterior cruciate ligament ruptures and 36 (females, n=20) matched controls with no history of lower extremity injuries were included. Surface electromyography of eight lower-limb muscles and full-body kinematics were captured during countermovement jumps and side-cuts. Knee stability was evaluated using co-activation indices, full muscle waveforms, knee joint flexion stiffness and sagittal and frontal excursions. Comparisons were determined *a priori* to evaluate each sex independently while evaluating for an injury effect.
Findings: No significant effect of injury was observed in knee stability metrics in either male or female cohorts during countermovement jumps or side-cuts. Additionally, no significant injury effect was observed in females for individual muscles throughout either task. One injury effect was identified in males; males with ACL injuries had higher vastus medialis activity during the preparatory phase of countermovement jumps compared to their controls.
Conclusion: Our results indicate that in adolescents with and without anterior cruciate ligament injuries who can perform dynamic loading tasks, systemic differences in knee stabilization metrics did not manifest between the injured and non-injured groups. This may in part be due to the high level of variability in strategies used to perform the tasks in this maturing population.

Key Terms: anterior cruciate ligament injury, adolescents, knee stability, sex differences, dynamic loading

Introduction

Participation in sport is important in the lives of healthy, active adolescents, and, unfortunately, with sport participation there is an increased likelihood in musculoskeletal injuries [1]. Approximately 30-40 injuries are observed per 100 secondary school students (14-18 years old) per year, with estimates of up to 68% injuries being sustained during sport [2]. One such injury is the rupture of the anterior cruciate ligament (ACL), and is one of the more common and traumatic injuries in this demographic [3]. Patients who suffer an initial ACL injury have high rates of secondary injuries, with studies reporting 18-20.6% of patients re-rupturing their ACL-graft and 11.3-17.7% of patients injuring the contralateral knee [4]. These data are further supported by reports of only 65% of patients returning to any level of physical activity, and only 50% returning to pre-injury activity levels even after surgical intervention, prescribed rehabilitation, and clearance for return-to-activity (RTA) [5].

Despite the impact of this injury, there is surprisingly little biomechanical research conducted on adolescent populations with ACL-deficiency (ACLd). Furthermore, considering that females between the ages of 12 and 18 years have the highest risk of sustaining an ACL injury [3], there is even less biomechanics research focused on sex differences among adolescent cohorts [6]. With that, substantial knowledge gaps limit the tailored management of ACL injuries in these young patients, with a notable disparity in understanding the roles of neuromuscular control and the ACL in stabilising the knee joint. The high rates of injury and re-injury among this cohort clearly demonstrates the need for improved rehabilitation and RTA guidelines that are evidence-based and specifically tailored for adolescent patients.

Therefore, the purpose of this study was to address this gap by investigating in adolescent males and females i) how muscle activation patterns and knee joint stability strategies differ between adolescents with and without ACL injury, and ii) identify how males and females respond differently to such an injury. Knee stabilisation strategies during two movements, side-cuts and countermovement jumps (CMJs), were completed as these two tasks are often used in evaluating the relative between-limb difference in functional capacity of the participant, particularly in injury prevention and rehabilitation programs [7]. Given the influence of an ACL injury on knee stabilisation strategies in adult populations, it was hypothesised that patients with ACL injuries would demonstrate different knee joint stabilisation strategies compared to their uninjured counterparts. More specifically, adolescents with ACL injuries would have higher overall muscle activations, in the form of co-activation indices (CIs) of the thigh musculature and muscle waveforms across the entire task, compared to their uninjured counterparts during movements that challenge both frontal and sagittal knee joint control [8–10]. A secondary hypothesis was that higher knee joint flexion stiffness, lower knee joint excursion in the sagittal and frontal planes during the stance phase of both side-cuts and CMJs would be observed in both sexes with ACL injuries compared to their uninjured counterparts [11–14].

Methods

Participants

This study was approved by the ethics boards of the Children's Hospital of Eastern Ontario (17/74X) and the University of Ottawa, Canada (H09-17-10) and is an analysis of a cohort within a larger research program. Adolescent patients between the ages of 10 and 18 years old who had a confirmed ACL rupture via arthroscopy and/or MRI were eligible to be recruited for this study by Orthopaedic Consultants from CHEO, Ottawa, Canada. Patients seeking an ACL-reconstruction revision (secondary ACL injury) were not eligible for participation in this study. Eligible patients required approval from their physician before scheduling a visit to the laboratory. These approvals occurred when the physician was confident in the patient's ability to participate fully in the study by performing all tasks included in the protocol. Recruitment of uninjured controls took place via convenience and snow-ball sampling of regional youth sports associations and club teams in Ottawa. The dominant leg of each participant was distinguished by which leg was used to kick a soccer ball a maximum distance [15].

Consent and Questionnaires

All participants were required to read and sign a consent form explaining the protocol, any possible risks, and the purpose of the study, with the help of a parent/guardian if needed. Each participant was given the opportunity to discuss the study or ask any questions with a member of the research team before providing consent. In addition, a set of self-assessments and surveys were completed in their preferred language (English or French) including the Pedi-IKDC (to measure subjective knee joint function) [16], the HSS Pedi-Fabs [17,18] and Tegner activity scale (to capture activity levels) [19] , and the Tanner Stages Classification form (to self-identify maturation level) [20]. Participants were then provided with tight-fitted spandex shorts and a long-sleeved shirt prior to the collection of the following anthropometric measures: pelvis, knee, and ankle width, height, and weight; leg and tibial length; and thigh and shank circumference.

Participant preparation and equipment

Participants were provided with neutral running shoes (KBS7FW3343; MS7F505027, Joe Fresh, Canada) followed by a full warm-up including cycling on a stationary ergometer for five minutes (Monark 828E, Vansbro, Sweden) and an opportunity to stretch. Wireless, bipolar surface EMG electrodes connected to a 16-channel EMG system (Trigno Wirreless System, Delsys, USA) were placed over the muscle bellies of interest following the recommendations by SENIAM [21] and DeLuca [22], with minor adjustments being made to account for varying body dimensions. EMG data were sampled at 2000Hz, band-pass filtered at 20-450Hz with a 12 dB/octave filter roll off and a common mode rejection ratio of >80 dB. Prior to placement, areas of the skin were shaved and wiped with alcohol. The muscles of interest on both limbs were: rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), semitendinosus (ST), biceps femoris (BF), medial gastrocnemius (MG), lateral gastrocnemius (LG), and gluteus medius (GM). After EMG sensor placement, both thigh and calf segments of the lower limb were wrapped with pro-wrap and secured with tape to limit movement artefacts within EMG data that may arise during dynamic movement.

Next, maximum voluntary isometric contractions (MVICs) were performed using an isokinetic dynamometer (System 4 Pro, Biodex Medical Systems, USA) and included knee flexion and extension, recorded in a seated position with the knee flexed at 60^o, and plantarflexion, recorded in a seated position with the knee at 0^o flexion and the ankle in a neutral position. Hip abduction was recorded in a standing position, with the hip at 10^o abduction and 0^o extension. Participants were instructed to begin gradually contracting until achieving their maximal contraction and to hold for five seconds, this was repeated three times with at least one minute of rest in between trials. EMG and isometric torque were synchronously collected using Nexus, sampled at 2000Hz (v2.7, Vicon, UK). Torque data were filtered using a 4^{th}-order zero-lag low-pass Butterworth filter at 15Hz. The torque values were then corrected for body mass and assigned to be positive to simplify their interpretation during data analysis. Verbal encouragement and on-screen biofeedback were provided to encourage participants to achieve their maximal effort. Torque data were not considered in the current analysis.

Participants then had 84 reflective markers (14mm diameter) placed on various landmarks according to a hybrid cluster-marker set adapted from Mantovani and Lamontagne [23]. During all movement tasks, ground reaction forces (GRFs) were sampled at 1000Hz, amplified with an internal gain of 1000 and recorded through two force platforms (FP4060-08, Bertec Corp., USA), while marker trajectories were sampled at 200Hz using a 10-camera infrared motion analysis system (8 Vero and 2 Vantage; Vicon, Nexus, UK) and synchronously recorded using the support software (Nexus v2.7, Vicon, UK).

Experimental protocol

Participants were instructed to perform a standing calibration trial, followed by a series of dynamic trials. For this study, the five successful trials of both CMJs and side-cuts (on both limbs) were analysed. CMJs consisted of participants standing with feet shoulder-width apart on separate force platforms, with their hands on their head to account for arm motion variability and they were instructed to squat down to help them jump as high as possible. A CMJ trial was deemed successful if the participant kept their hands on their head, landed with both feet entirely on their respective force platforms and held the landing without losing their balance (Figure 8.1).

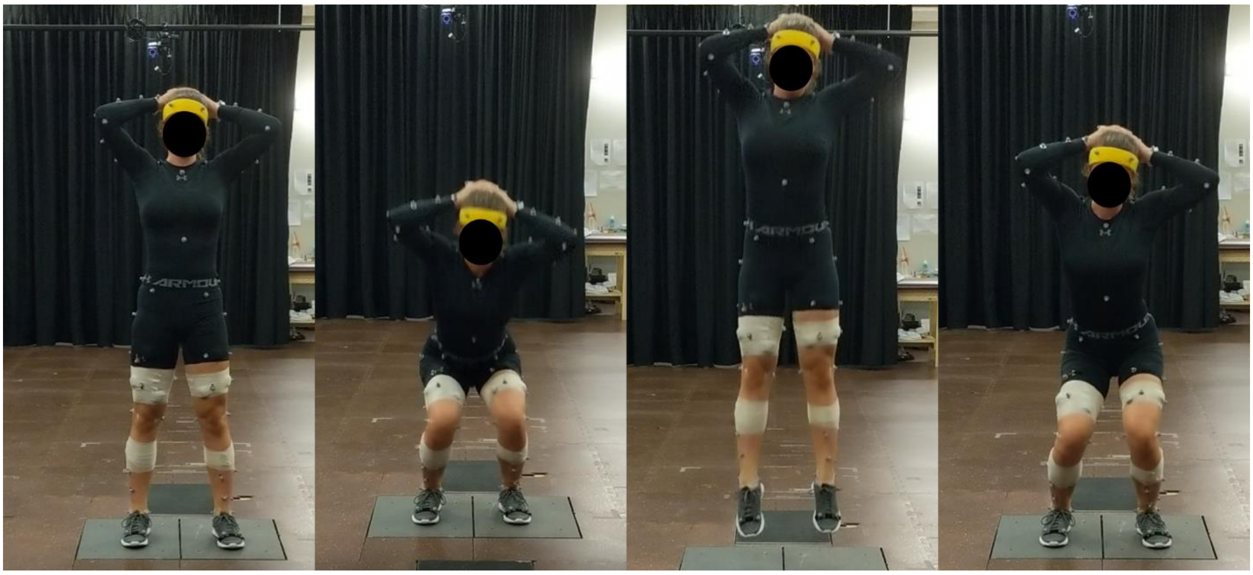

Figure 8.1. Sequence of events for a countermovement jump in motion laboratory. From left to right: starting position, preparatory phase, flight phase, and landing phase.

Side-cuts were performed with participants starting at one end of the laboratory and running linearly as fast as possible while maintaining control to plant and cut with the foot of interest on the force platform to either the left or the right at an angle of 45°. Side-cut trials were deemed successful if the participant planted and cut with the appropriate foot, placed their entire foot on the force platform, and accelerated out of the foot-plant at a 45° angle (pylons in the lab provided a ±10° target range) (Figure 8.2).

Figure 8.2. Sequence of events for a 45-degree side-cut maneuver in motion laboratory. From left to right: preparatory phase, stance phase, and push-off in new direction.

Data processing

Marker trajectories and GRFs were filtered with a 2nd-order zero-lag dual low-pass Butterworth filter with matching cut-off frequencies of 15Hz [24,25] using custom pipelines in Vicon Nexus (v2.10, Vicon, UK). Modified pipelines in Vicon Nexus were used for scaling and computing inverse kinematics (relative to the standing calibration trial) and inverse dynamics for the hip, knee, and ankle sagittal and frontal plane angles and moments. Raw EMG signals were exported into Matlab (R2018a, Natick, USA), high-pass filtered at 20Hz with a 2nd-order dual-pass Butterworth filter, full-wave rectified, and low-pass filtered at 6Hz with a 2nd-order dual-pass Butterworth filter. A 10ms moving-average-algorithm was used to identify maximum EMG amplitude for each muscle during the MVICs; these maximums were used to amplitude-normalise EMG data in CMJs and side-cut tasks.

All data were time-normalised within the task being analysed. The side-cuts trials were parsed into two phases: the preparatory phase (100ms prior to initial contact with the force platform (GRF >10N) and the stance phase (the total duration between foot and the force platform). The CMJs trials were parsed into three phases: take-off (initiation of upward acceleration from the lowest point of the squat (maximum knee flexion)), flight (the duration of the task spent jumping with no contact on the force platforms (GRF < 10)), and landing (the total duration after the flight phase between foot and the force platform). The time normalised waveforms were averaged over five trials for each participant.

Continuous kinematic and kinetic variables (hip, knee, and ankle sagittal and frontal plane angles and internal joint moments) were reduced to provide the following dependent variables as proxies of knee function for this study: knee joint flexion stiffness

(KJFS) [26] at impact and knee joint angle excursions, in both the sagittal (KJSE) and the frontal (KJFE) planes [13]. Electromyography from the injured limb and the matched control limb were evaluated throughout the various phases of the two dynamic tasks. Primary outcomes for measured muscles were full waveforms and muscle co-activation (CI) to explain gross stabilisation strategies of the joint. The CIs were calculated [27] for both legs separately between the lateral thigh muscles (VL and ST), the medial thigh muscles (VM and BF), and the summed quadriceps (VL and VM) and hamstrings (ST and BF) muscles at the beginning of the landing and stance phases for the CMJs and side-cuts, respectively. These CIs were calculated to provide a comprehensive description of the relative muscle activation (ratio) and the magnitude of activation (sum of muscle magnitudes). Low co-activation values represented lower activation of both muscles or low-level activation of one muscle and high activation of the other muscle in the pair. Larger co-activation values denoted higher activation of both agonist and antagonist muscles, representing a more general activation pattern.

Statistical analysis

Given that sex differences related to ACL-injury are routinely reported [6], we deemed it more meaningful to evaluate each male and female cohorts independently in order to focus on the effect of injury within each sex. All statistical analyses were performed using Matlab (2018a, Mathworks, Natick, USA) or SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA) with a significance level set at p=0.05 with a Bonferroni correction to account for multiple comparisons. For discrete variables, Independent t-tests (or the non-parametric equivalent, Mann-Whitney U-tests) were used to evaluate group differences between group demographics, questionnaires, and co-activation indices (CIs).

Statistical parametric mapping (SPM) was used to compare full waveform data sets (i.e. the whole flight phase of a CMJ and stance phase of a side-cut)[28]. Independent t-tests (or the non-parametric equivalent, Mann-Whitney U-tests) in SPM were applied to determine if differences exist between both females and males with and without ACL injuries for all continuous dependent variables.

Results

Of the 141 participants recruited to date for the overall research program, a sub-cohort of the study population was selected based on age, height, weight, BMI, limb-dominance, activity level and ability to complete the CMJ and side-cut tasks. *(Note: to reduce the risk of bias, no other patient information was considered to identify the cohorts)*. From this, 20 females (FACL) and 16 males (MACL) with confirmed ACL ruptures were matched and compared to 20 females (FCON) and 16 males (MCON), who had no present or recent history of injury. Specifically for the side-cut tasks, only 13 full datasets were available for analysis among the MACL group: two participants were unable to complete all trials, while one had incomplete EMG data.

No significant differences were found among females or males with ACL injuries, when compared to their uninjured counterparts when characterising the groups. As anticipated, significant differences were identified for subjective knee joint function and activity level questionnaires (Table 8.1).

Table 8.1. Group means (standard deviations) for females with ACL injuries (FACL), female controls (FCON), males with ACL injuries (MACL), and male controls (MCON) of participant demographics, and subjective questionnaires. Significant differences ($p < 0.05$) evaluated using independent *t*-tests and Mann-Whitney *U*-tests are denoted with * and §, respectively.

Variable	**FACL**	**FCON**	***p*-value**	**Effect size *(d)***	**MACL**	**MCON**	***p*-value**	**Effect size *(d)***
Sample Size	n = 20	n = 20	---	---	n = 16	n = 16	---	---
Age (years)	14.80 (1.29)	14.32 (1.67)	0.19	---	15.25 (1.15)	14.40 (1.14)	0.06	---
T.S.I. (months)	4.45 (2.37)	---	---	---	6.63 (2.78)	---	---	---
Height (cm)	164.5 (6.77)	165.4 (5.91)	0.69	---	174.9 (8.56)	173.3 (8.93)	0.75	---
Weight (kg)	61.04 (9.45)	54.97 (9.61)	0.06	---	66.01 (13.52)	58.39 (8.41)	0.08	---

BMI (kg/m²)	21.93 (2.76)	20.00 (2.51)	0.10	---	21.65 (3.86)	19.45 (2.45)	0.08	---
Leg Dominance								
Right	n = 17	n = 15	---	---	n = 14	n = 14	---	---
Left	n = 3	n = 5	---	---	n = 2	n = 2	---	---
Leg Injured								
Right	n = 11	---	---	---	n = 11	---	---	---
Left	n = 9	---	---	---	n = 5	---	---	---
Dominant	n = 10	---	---	---	n = 11	---	---	---
Non-Dominant	n = 10	---	---	---	n = 5	---	---	---
Questionnaires								
Tanner Stage (/5)	4.05 (0.87)	3.75 (0.77)	0.26	---	4.09 (0.94)	3.63 (0.78)	0.34	---
Pedi-IKDC (/100)	58.75 (8.63)	96.85 (5.09)	< 0.001§	5.66	68.4 (14.7)	99.00 (1.69)	< 0.001§	2.93
Tegner (/10)	3.60 (1.74)	8.40 (1.16)	< 0.001§	3.25	4.43 (2.74)	8.63 (0.99)	< 0.001§	2.04
Pedi-FABS (/24)	6.75 (6.57)	21.45 (4.59)	< 0.001§	2.59	9.67 (8.09)	25.00 (4.40)	< 0.001§	2.35

Muscle Activations

No significant differences were observed in muscle CIs between females with and without ACL injuries nor between males with and without ACL injuries during CMJs (Figure 8.1A and B) or side-cuts (Figure 8.1C and D).

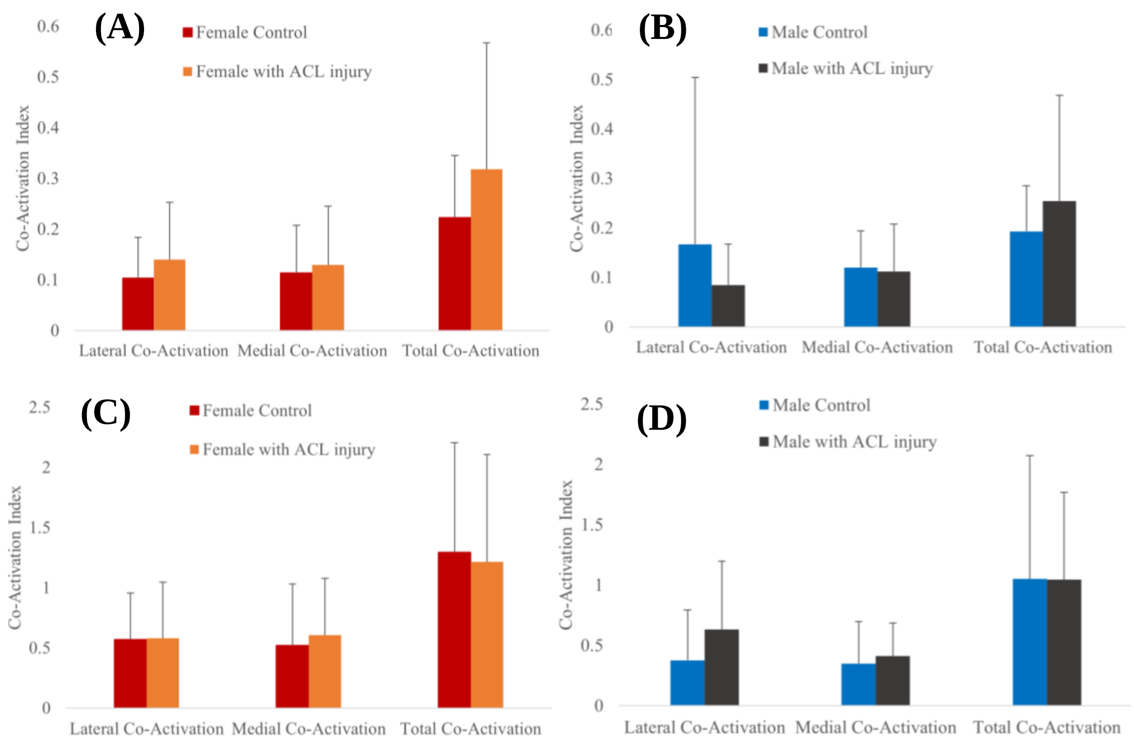

Figure 8.3. Muscle co-activations indices (CI) of adolescent female controls (red), females with ACL injuries (orange), male controls (blue) and males with ACL injuries (black) at the initiation of the landing phase during a countermovement jump (CMJ) and the stance phase during a side-cut. CIs of female with

and without ACL injuries during **(A)** CMJs and **(C)** side-cuts. CIs of male controls and with ACL injuries during **(B)** CMJs and **(D)** side-cuts. No significant injury effects were observed in either task or sex.

When examining individual muscle waveforms, no differences were observed between female cohorts. However, males with ACL injuries had higher activation in their VM during, approximately 25-60%, of the preparatory phase of the CMJ compared to their matched uninjured controls (Figure 8.2). No other significant differences were observed for any of the remaining muscles for either task or sex (Appendix F).

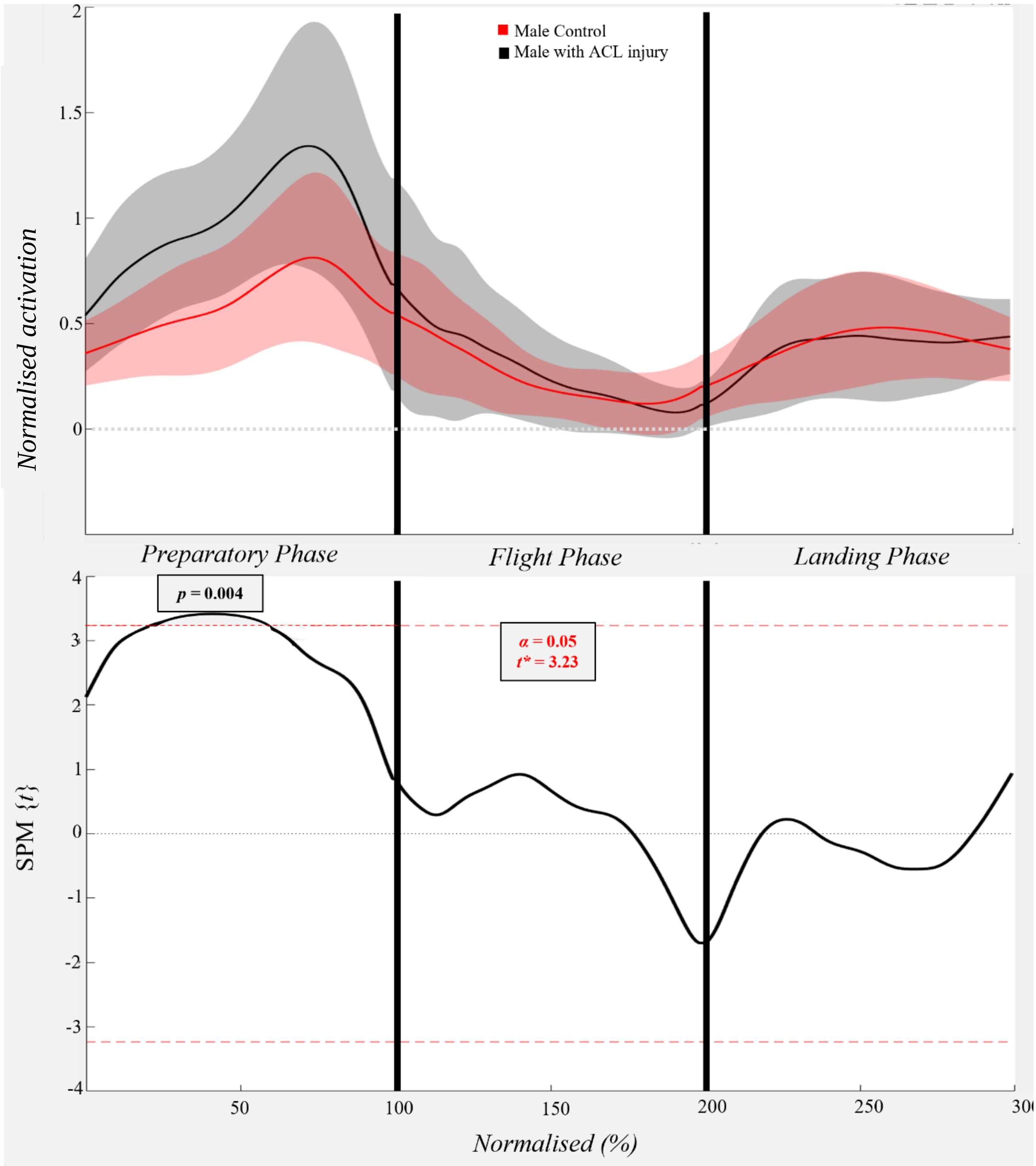

Figure 8.4. SPM Mann Whitney-U test for **(A)** vastus medialis activity of males with ACL injuries (black) and male controls (red) during the three normalised phases of a countermovement jump. **(B)** Significant differences ($p < 0.05$) are indicated by the grey region where SPM{t} passes the critical threshold (red-dashed line).

Knee Joint Stiffness and Excursions

No significant differences were observed in either knee joint stiffness or excursion in the sagittal or frontal planes during CMJ or side-cut trials between controls and their matched ACL-injured cohorts (Appendix F).

Discussion

Despite the increasing incidence rates of ACL injuries reported in literature, specifically among adolescent females, there has been limited biomechanical and neuromuscular analyses performed on this cohort. This study furthers our understanding on the role of the ACL during knee stabilisation strategies and how this differs between males and females. Despite limiting the influence of intrinsic and extrinsic factors (i.e. sex, anatomical, hormonal, physical maturation, limb-dominance, etc.) [29] by taking a sub-cohort of matched adolescent males and females and isolating the influence of an ACL injury within male and female groups, fewer significant differences were observed than expected.

The first hypothesis was partially supported since males with ACL injuries demonstrated differences in muscle activation strategies compared to their uninjured counterparts, however no differences were observed among adolescent females. More specifically, males with ACL injuries had higher vastus medialis (VM) activation amplitudes in their injured limbs during the preparatory phase of the CMJ compared to matched male controls. Furthermore, the secondary hypothesis was rejected as there were no effects of ACL-injury within male or female groups in knee joint flexion stiffness (KJFS) or knee joint excursions in the sagittal (KJSE) or frontal planes (KJFE) during either side-cuts or CMJs.

A recent review conducted by Chamorro-Moriana and colleagues (2022) sought to identify and analyze validated knee functional assessment tools in the literature and found 41 validated tools. Among these 41 tools, only the KOOS-Child [30] and the Pedi-IKDC [16], were tailored for an adolescent population [31]. Although these two validated tools provide subjective insight into the functional capacity of the adolescent patient's knee, they do not include any objective measure of knee joint stability or function. This review highlights the important gap of addressing the means in which knee joint stability is currently considered among an adolescent population. Objective measurements in assessing knee stability are critical when assessing knee joint health and readiness for return-to-activity (RTA) after an ACL injury [32]. Specifically, knee stability should be assessed using weight-bearing conditions and including tasks such as single-limb hopping [27,33] or movements that would challenge translational and rotational stability integrity of the joint, such as jump-landing and side-cuts [34]. We assessed knee stability during CMJs and side-cuts using metrics of joint stiffness and total joint excursions and found no differences in adolescents with and without ACL injuries. Other research using these metrics of knee stability [11,13,35,36], have explored various populations and found inconsistent results: lower knee joint stiffness was found in adults with ACL-deficiency and ACL-reconstruction compared to uninjured controls during a side-cut, while no differences were seen in this population during a hop task [13]. When considering the effect of sex among adults with and without ACL-reconstruction, Miranda and colleagues identified lower knee joint excursions in females during a side-cut compared to males, while, in contrast, did not find an effect of ACL injury [11]. Although the study found an effect of sex and not injury, it is important to note that a small sample size was used with a total cohort of 20 participants with an average age of 26 years: 10 patients at least 5 years post-ACL-reconstruction (6 females) and 10 without ACL injuries (5 females). Even though there are inconsistent results in knee joint stiffness and excursion among cohorts of ACL-injuries, it is nevertheless important that knee stabilisation is being quantified by researchers during dynamic and weight-bearing tasks.

As muscles are the only modifiable contributors to knee joint control and stabilisation, neuromuscular control has been a recent focus in relation to ACL-injury risk and rehabilitation [37–39]. After sustaining an ACL injury, an increase in co-activation indices (CI) in thigh musculature has been considered a distinctive characteristic among adults [40–42]. Increased co-activation leads to increased joint stiffness, which is not necessarily the ideal neuromuscular strategy after an ACL-injury, as increased joint stiffness and compression of the knee would lead to altering joint loads and has been linked to the early onset and progression of knee osteoarthritis (OA) [43]. Although males with ACL injuries had higher activations of the vastus medialis, this finding of increased co-activation among adults, in addition to differences in joint stiffness [13], was not observed in our adolescent population of males and females with ACL injuries. The higher activation of the VM in males with ACL injuries could be a protective mechanism to compress the knee joint, providing frontal plane support, while attempting to generate extensor force to prepare for the flight phase of the CMJ. Given the maturational changes of an adolescent population expressed through anatomical growth of long bones and muscular development, perhaps adolescents are more primed to adapt and are more resilient to a change in the musculoskeletal system as opposed to adults. This may support the presence of the high variability that was seen in our data, specifically in the ST and BF muscles. Although, it is important to also state that variability in movement data from an injury-prevention perspective has been suggested to be a positive finding [44].

Variability in an adolescent population is also to be expected, considering many of these individuals are in the midst of developmental changes in their bodies associated with puberty [45].

The influence of one's sex on neuromuscular control in relation to ACL-injuries has also been a popular research topic in recent years, with differences being linked with the onset of puberty [7]. Before the onset of puberty, no sex differences have been reported in the literature [46–48], however, when evaluating a maturing population, sex differences begin to appear. Landry and colleagues have investigated neuromuscular control among adolescent male and female athletes during sporting maneuvers and reported females to have higher activations of both gastrocnemii prior to the landing phase of an unanticipated side-cut [10,49], and higher activations of the lateral gastrocnemius and rectus femoris and lower activations of the hamstrings during the landing phase of the side-cut [49]. Higher activations of the gastrocnemii and quadriceps with lower activations of the hamstrings, have been presented as high-risk muscle activations strategies and may put the ACL at an increased risk of injury with greater anterior translation of the tibia [50]. This is one of the main reasons why males and females were investigated separately in this study, to isolate the influence of sex and determine whether an ACL injury affects knee joint neuromuscular control differently among the two sexes. Interestingly, the results of the present study only indicated one neuromuscular difference related to ACL injury and was found in males, while none were observed in our female cohorts. Males with ACL-injuries had higher activations amplitudes of the VM during the preparatory phase of the CMJ compared to their matched controls. With no other neuromuscular differences, the higher activation of the VM may be a result of males with

ACL-injuries attempting to increase the compression of the joint and prevent instances of instability.

Conclusion

Although adolescents, more specifically adolescent females, are most at risk of sustaining a non-contact ACL injury, limited research has considered the effect of an ACL rupture on knee stabilisation strategies among adolescents. While controlling for potentially confounding variables such as age, height, weight, BMI, and Tanner stage, only one neuromuscular difference was observed in males, while no differences were identified in female cohorts. Males with ACL injuries had higher vastus medialis activation during the preparatory phase of CMJs compared to their uninjured counterparts. The results of this study demonstrate that an ACL injury may not alter knee stabilisation strategies in adolescent females and males during dynamic tasks. Our findings not only challenge the reported differences related to an ACL injury among adults, but also warrant further investigations on knee stabilisation strategies in adolescents. Future research should consider larger sample sizes and analyze intra-participant variability during repeated and unanticipated movements to explore the relationship between variability and risk of initial and secondary non-contact ACL injuries among adolescent males and females.

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CHAPTER 9: GENERAL DISCUSSION

The overall purpose of this Doctoral thesis was to explore knee stabilisation strategies and knee joint function among adolescent males and females. Although this dissertation accomplished this goal, it must be stated that multiple pivots were required in the overall research design due to the impact of the COVID-19 Global Pandemic. The original outline for this Doctoral thesis aimed to evaluate adolescent males and females with ACL injuries prior to and after their ACL-reconstruction surgery, while comparing them to a cohort of matched controls without injuries. After a year and a half of data collections, the research program was required to halt all operations which lasted almost an entire year. This drastically influenced the total recruitment numbers for initial and secondary data collections and required this dissertation to shift focus. The new direction would focus solely on knee joint function among adolescent patients with ACL injuries prior to their surgery and matched controls, while also exploring the control adolescent population for the effects of limb-dominance and sex. The original intent required adaptation; however, the results of this dissertation and the four included manuscripts successfully contribute to the growing body of literature focusing on improving ACL injury prevention and management, specifically for adolescent males and females.

Female adolescents are the most at-risk group of sustaining non-contact ACL injuries and surprisingly, neuromuscular research among this cohort is scarce, as hypothesised – only five studies were identified in our systematic review and met inclusion criteria (Chapter 5; Otsuki et al., 2021). Among these studies, sex differences were only identified in three of the five studies. It must be noted that the identified sex differences in adolescents were not consistent across studies either, which aligns with the trend in the literature considering adult populations. Twenty-four of the 30 included studies identified sex differences and among those, results were inconsistent. Methodological/analytical variations and limitations may be the major contributor to these inconsistencies among researchers, making it even more challenging to establish a consensus across studies. Identifying consistent and specific sex differences may be difficult, but the identification of any sex difference supports the hypothesis that males and females have different neuromuscular patterns. Regardless of the inconsistencies, sex should always be considered as an independent variable in biomechanical and neuromuscular research, especially when related to ACL injuries. In addition to sex, the changes that occur within the human body during puberty should also be considered as they differ in both males and females (Labella et al., 2014). Currently, the scarcity and inconsistency of research among adolescent males and females prevents the establishment of evidence-based criteria to help in the decision-making process specifically for an adolescent patient's full RTA. Future research with longitudinal research designs is required to fully understand the role of sex and age in the neuromuscular control of the knee joint before we can tailor appropriate injury prevention and management guidelines.

After exploring the current understanding of neuromuscular control among adolescents, the next step of this dissertation was to determine the appropriateness of commonly accepted functional assessments (Adams et al., 2012; Cristiani et al., 2019; Noyes et al., 1991) for knee joint control among an adolescent cohort (Chapter 6). As previously discussed, high re-injury rates clearly demonstrate that RTA guidelines are not optimised for adolescents sustaining ACL injuries. An important first step in tailoring

RTA guidelines is to first establish a benchmark dataset of adolescent males and females without ACL injuries. The battery of single-limb tasks and their LSIs that were assessed in this dissertation were selected as they were commonly used and/or can easily provide clinicians and other health care professionals a method for capturing the functional capacity of the knee joint without complex data acquisition equipment and/or techniques. Considering the anatomical and physiological changes occurring throughout puberty, a clinician's current best option is to compare their patient's injured limb to their contralateral limb and calculate LSIs (Cristiani et al., 2020; Gokeler et al., 2017b). When considering this battery of tests among our adolescent population of males and females with and without ACL injuries, we identified within- and between-group differences among all groups. More specifically, a limb-dominance effect was identified in both uninjured males and females and an effect of injury was observed between injured and uninjured cohorts, and within male and female groups with ACL injuries.

Taking these results into consideration, we caution using the contralateral limb as a baseline measurement for knee joint function related to injury. Research has demonstrated LSIs to frequently overestimate knee joint function (Wellsandt et al., 2017) and suggests that this is likely due to rehabilitation models focusing on unilateral strengthening and neuromuscular control, as neglecting the contralateral limb may increase the risk of secondary injury. Furthermore, the differences identified in our study cohorts were not consistent across sexes. For example, an injury effect was not present for males during the lateral hop, cross-hop, or 6-meter timed hop, whereas females had an injury effect in every single-limb task. This, in and of itself, further supports the notion that males and females should not be assessed using the same RTA guidelines and require sex-specific considerations among adolescents.

Fully establishing sex- and age-matched guidelines for assessing single-limb tasks that can easily be assessed with limited equipment, space, and analyses, is an important step to improving rehabilitation and the management of ACL injuries. However, to optimise RTA guidelines for adolescents, we must also explore knee joint function and stabilisation strategies while performing these dynamic tasks to understand why differences may manifest. Currently, the measurements included in many RTA guidelines, such as hop distances and isometric strength, do not explicitly provide insight on knee joint function. More specifically, these are metrics of overall performance or output and not of the strategy for how the movement was achieved and/or how the knee is being stabilised. The main contributors to knee stabilisation are the muscles of the lower extremity (Kaplan, 1957; Riemann and Lephart, 2002), and the strategy in which they are employed can drastically change the overall performance and/or output for a task, potentially resulting in injury. Furthermore, given that non-contact ACL injuries are most often to occur during tasks that involve multi-planar loading such as CMJs and side-cuts, we sought to evaluate knee stabilisation strategies and muscle activation patterns during these tasks in adolescent males and females.

Knee joint stabilisation strategies are not well understood in an adolescent population and this dissertation is among the few investigations addressing this gap while evaluating for effects of sex, limb-dominance, (Chapter 3), and ACL-injuries (Chapter 4). Metrics of knee stabilisation were quantified in the form of co-activation indices (CIs), knee joint excursions in the sagittal and frontal planes (KJSE and KJFE, respectively) and knee joint flexion stiffness (KJFS). When only considering adolescents without injuries, as hypothesised, sex differences were found with females having higher medial CIs in both tasks and higher total CIs in the non-dominant limb during side-cuts compared to males. Limb-dominance effects were observed in both sexes as well, further emphasizing the importance of including within-participant evaluations. Females demonstrated more unfavorable stabilisation strategies (Ireland, 1999) during both tasks in their non-dominant limb, with higher total CI during the CMJ and higher frontal KJE compared to their dominant limb during the stance phase of side-cuts. Males showed similar differences, with higher frontal KJE in their non-dominant limb during the preparatory and landing phases of the CMJ but had higher medial CIs in their dominant limb during CMJs; no between-limb differences were observed in males during side-cuts. Sex differences in neuromuscular control of the knee joint during dynamic loading have been frequently identified, as elaborated in Chapter 5, further stressing the importance of an individual's sex to be considered in not only research, but also in rehabilitative guidelines. Furthermore, the presence of a limb-dominance effect has been reported by others (Brophy et al., 2010; Del Bel et al., 2017; Mercado-Palomino et al., 2021; Negrete et al., 2007; Rouissi et al., 2016; Ruedl et al., 2012; Weinhandl et al., 2017); however, the research has been inconsistent. Regardless of the inconsistency, likely attributed to varying research designs, these findings and those of this dissertation demonstrate the importance of limb-dominance as an independent variable when researching the lower extremity. The difference between limbs also supports the need for caution when considering only LSIs as a metric for assessing functional capacity in an injured population.

Perhaps the most unexpected findings of this dissertation were identified when exploring the effects of an ACL injury on knee stabilisation strategies among adolescent males and females. The only significant effect of an ACL injury was observed among males, with patients with ACL injuries having higher vastus medialis activity during the preparatory phase of the CMJ compared to their uninjured counterparts. No other significant differences were identified between males with and without ACL injuries, while no significant differences were found for any knee stabilisation metric between females with and without ACL injuries (Chapter 8). An effect of ACL injury has been consistently found in studies analysing knee joint stability and function in adults (Alkjaer et al., 2002; Chmielewski et al., 2005; Myer et al., 2015; Rudolph et al., 2001; Smale et al., 2019b, Williams et al., 2003), but none focused on our age demographic. This dissertation provides new insight into knee stabilisation strategies among adolescents with ACL injuries. Interestingly, the results from this thesis suggest that adolescent males and females do not necessarily adapt their knee stabilisation strategies after an ACL injury in the same capacity as adults, as only one difference was identified. Given the changes that occur within the human body after the onset of puberty, perhaps for those in the midst of skeletal maturation, there is an increase in neuromuscular adaptability and resilience, as observed in our data's high variability. High variability in movement data from an injury-prevention perspective has been suggested to be a positive finding (Pollard et al., 2005; Wren et al., 2020). These researchers suggest that higher variability provides an increased likelihood of having an adaptable neuromuscular control system that could safely respond to unanticipated changes and/or perturbations. The inability to adapt to these scenarios may therefore be linked to having an increased risk of sustaining a non-contact ACL injury and diminishes as individuals mature, as they establish repetitive and consistent neuromuscular activations/pathways. The influence of maturational change on knee stabilisation is one that has been researched to a limited extent, by comparing adults to children (Tumkur Anil Kumar et al., 2021). The consensus remains that it is not yet clear how neuromuscular control adapts to growth during puberty and merits further investigations with longitudinal designs. Nevertheless, the current data further reiterates that current rehabilitative guidelines, which are based on adult databases/studies and do not necessarily translate to an adolescent population. Our findings indicate a needed revision of guidelines for prevention of and management/rehabilitation for ACL injuries among adolescents while also considering for sex and limb-dominance.

The author acknowledges that there were limitations in this dissertation that may limit the interpretation of results and conclusions and were discussed in each of the respective studies. In addition, as previously discussed, the impact of the COVID-19 Global Pandemic cannot be ignored, which ultimately affected the sample size used for each study. Early phases of recruitment were consistent prior to the shut-down of elective surgeries and work/school-from-home mandates. Not only did the pandemic affect recruitment, it also likely affected the lifestyle and activity levels of all eligible participants for the research program, potentially altering motor outputs and ultimately, knee joint control. With the social distancing measures being enforced, physical activity levels decreased by upwards of -90 mins/day (Rossi et al., 2021); however, the impact of this change is yet to be fully documented among adolescent populations from a biomechanical perspective. Furthermore, a high amount of variability was present in some of the datasets and is a limitation in the interpretations of the present study. Specifically, there was high variability in the semitendinosus and biceps femoris muscle data and is likely a result of different EMG sensors being used for data collections. During the first summer of data collections, four Delsys mini-EMG sensors, also a part of the same Delsys Trigno system, were used for collection of the hamstring muscle activation on both limbs. The durability of these sensors was not the same as the larger Trigno sensors and were no longer included in data collections after the first summer. The authors acknowledge that a change in the sensors may have led to a change in data quality, and thus may limit the interpretation of the hamstrings data from this study. With that, these sensors were used in data collections including all cohorts and would not have created a bias between groups.

CHAPTER 10 - GENERAL CONCLUSION

As discussed throughout this thesis, there is a clear gap in research supporting and facilitating the management of ACL injuries while considering sex and/or limb-dominance differences, let alone among adolescent populations. The present gap in age- and sex-based rehabilitation guidelines is likely a major contributor to the current rise of what is already a common injury among adolescents. The overall aim of this Doctoral dissertation was to create evidence to support the necessary revision of adolescent RTA guidelines by providing an assessment of objective and subjective measures related to knee joint function in males and females with and without ACL injuries.

Through a systematic review of the literature, sex differences were identified in neuromuscular patterns during functional tasks associated with ACL injuries. Fifteen of the 27 studies supported the quadriceps-dominance theory among females, who had higher quadriceps-to-hamstring activation ratios during dynamic loading, while the remaining studies found no sex differences. Moreover, despite the importance of ACL injury prevention research and the rise of injury occurrences among adolescent populations, particularly adolescent females, the research is currently quite scarce and inconclusive when considering sex differences in younger cohorts. Furthermore, this review highlighted the current challenge in the attempt to establish a consensus among studies considering muscle activation strategies. The approach used by researchers in their experimental protocols vary greatly from one study to the next, thus limiting the extrapolation of findings and interpretations across studies.

The sex-bias associated with non-contact ACL injuries has led to an abundance of biomechanical and rehabilitative research among adults, with limited consideration for adolescent participants. In addition to the bias among injuries and the common influence of sex when considered in the literature and after identifying sex differences among uninjured males and females, the authors adapted the analysis by focusing on each sex individually with the goal of supporting sex-specific ACL rehabilitation guidelines. The findings of this dissertation directly contribute to the gap in the literature by having focused on each sex and assessing the influence of an ACL injuries in adolescent males and females. Through comparisons of performance during a series of tasks related to return-to-activity (RTA) it was identified that females with ACL injuries had lower performance metrics for all tasks compared to their uninjured counterparts; however, the same could not be said for male cohorts. Males with ACL injuries had lower performance metrics compared to males without injuries in only four of the seven tasks (anterior hop, triple hop, knee extension, and knee flexion). Interestingly, during the assessment of knee stabilisation strategies among these cohorts, we observed only one difference among males with and without ACL injuries: males with ACL injuries had higher vastus medialis activation during the preparatory phase of a CMJ compared to their uninjured counterparts. No other differences were identified in relation to the ACL injury, which challenges the consensus in the literature considering adults, where the ACL-deficient knee is often reported to have poorer biomechanical performance and instability.

These findings encourage the support of sex-specific rehabilitation guidelines as our results indicated that the ACL injury influenced adolescent males and females differently in their capacity to perform tasks often used in evaluating readiness for RTA. Although indirectly, the results of this dissertation suggest that adolescents respond and adapt to an ACL injury differently than adults, further supporting the need for considering age and/or physical maturation when developing RTA guidelines. To fully understand how knee stabilisation strategies change as males and females mature and the effects of an ACL injury, future research should consider a longitudinal design or statistically appropriate representation spanning puberty. Additional efforts are necessary to fully understand and optimally tailor RTA guidelines for adolescent males and females; however, this dissertation demonstrates that there is an opportunity for improving rehabilitation and injury prevention guidelines for non-contact ACL injuries among adolescents.

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APPENDIX A – Research Program Protocol

Participant Arrival (15min)

Complete Consent and Assent From
Complete Questionnaires:
- i) Pedi-FABS
- ii) Pedi-IKDC
- iii) Tanner Stage Self-Assessment

Record Anthropometric Measurements:
- i) Height and Weight
- ii) Pelvis, Knee, and Ankle Widths
- iii) Full leg, Thigh, and Shank Lengths
- iv) Thigh and Shank Circumferences

Participant Preparation (45min)

Complete Warm-Up Protocol
- i) Cycle Ergometer

Instrument with Surface Electromyography Sensors (both limbs):
- i) Rectus Femoris
- ii) Vastus Medialis
- iii) Vastus Lateralis
- iv) Semitendinosus
- v) Biceps Femoris,
- vi) Medial Gastrocnemius
- vii) Lateral Gastrocnemius
- vii) Gluteus Medius

Conduct Maximum Voluntary Isometric Contractions (both limbs):
- i) Knee Extension
- ii) Knee Flexion
- iii) Plantar Flexion
- vi) Hip Abduction
- vii) Hip Extension
- vii) Hip Flexion

Data Collection (1hr)

Instrument with Motion Capture Marker Set
Provide Instructions for each Dynamic Task and Allow Familiarization Period
Complete Clinical/Functional Tasks
- i) Maximum Forward Single-Leg Hops (for distance)
- ii) Maximum Lateral Single-Leg Hops (for distance)
- iii) Timed 6m Single-Leg Hops
- iv) Triple Single-Leg Hop (for distance)
- v) Single-Leg Cross-Hops (for distance)

Complete Dynamic Tasks
- i) Two-legged Squats

ii) Countermovement Jumps
iii) Side-Cuts (quick change in directions)
iv) Drop-Vertical Jumps
v) Lunges

Complete Muscular Endurance Task on Biodex Isokinetic Dynamometer

i) 40 consecutive knee flexion and extension repetitions at a fixed velocity of 90 degrees/second

Participant Debrief (10min)

Remove Surface Electromyography Sensors and Motion Capture Markers

Complete Cool-Down Protocol

i) Cycle Ergometer
ii) Dynamic Stretching

Answer Any Questions

APPENDIX B – Research Ethics Approval

File Number: H09-17-10 **Date (mm/dd/yyyy):** 10/02/2017

Université d'Ottawa University of Ottawa
Bureau d'éthique et d'intégrité de la recherche Office of Research Ethics and Integrity

Ethics Approval Notice

Health Sciences and Science REB

Principal Investigator / Supervisor / Co-investigator(s) / Student(s)

First Name	Last Name	Affiliation	Role
Daniel	Benoit	Health Sciences / Physiotherapy	Principal Investigator
Sasha	Carsen	Medicine / Medicine	Co-investigator
Michael	Del Bel	Health Sciences / Others	Co-investigator
Nicholas	Romanchuk	Health Sciences / Others	Co-investigator

File Number: H09-17-10

Type of Project: Professor

Title: Knee joint function pre- and post-surgery among paediatric patients: a longitudinal perspective

Approval Date (mm/dd/yyyy)	Expiry Date (mm/dd/yyyy)	Approval Type
10/02/2017	05/15/2018	Approval

Special Conditions / Comments:
N/A

1

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APPENDIX C – Patient Reported Outcome Measures (PROMs)

Appendix C1. Pedi-IKDC Subjective Knee Evaluation Form

Pedi-IKDC Subjective Knee Evaluation Form – PATIENT FORM

Section A: General Information

1. Study ID: __ __ __

2. Date distributed: __ __ / __ __ / __ __ __ __
   MM DD YYYY

Section B: Survey

Date you injured your knee: __ __ / __ __ / __ __ __ __
MM DD YYYY

We would like to learn more about your injured knee. Each of the questions asks you a different question about your injured knee. Please answer each question below.

SYMPTOMS

1. If you were asked to do the activities below, what is the most you could do today without making your injured knee **hurt a lot**?

   ❑1 Very hard activities like jumping or turning fast to change direction, like in basketball or soccer
   ❑2 Hard activities like heavy lifting, skiing or tennis
   ❑3 Sort of hard activities like walking fast or jogging
   ❑4 Light activities like walking at a normal speed
   ❑5 I can't do any of the activities listed above because my knee hurts too much now

2. During the past 4 weeks, or since your injury, how much of the time did your injured knee hurt?

Never hurt	0	1	2	3	4	5	6	7	8	9	10	**Hurt all of the time**
	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	

3. How badly does your injured knee hurt today?

Does not hurt at all	0	1	2	3	4	5	6	7	8	9	10	**Hurts so much I can't stand it**
	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	

4. During the past 4 weeks, or since your injury, how **hard has it been to move or bend** your injured knee?

   ❑1 Not at all hard
   ❑2 A little hard
   ❑3 Somewhat hard
   ❑4 Very hard
   ❑5 Extremely hard

5. During the past 4 weeks, or since your injury, how **puffy (or swollen)** was your injured knee?

- ❑1 Not at all puffy
- ❑2 A little puffy
- ❑3 Somewhat puffy
- ❑4 Very puffy
- ❑5 Extremely puffy

6. If you were asked to do the activities below, what is the most you could do today without making your injured knee **puffy (or swollen)**?

- ❑1 Very hard activities like jumping or turning fast to change direction, like in basketball or soccer
- ❑2 Hard activities like heavy lifting, skiing or tennis
- ❑3 Sort of hard activities like walking fast or jogging
- ❑4 Light activities like walking at a normal speed
- ❑5 I can't do any of the activities listed above because my injured knee is puffy even when I rest

	Yes	No
7. During the past 4 weeks, or since your injury, did your injured knee ever **get stuck in place (lock)** so that you could not move it?	❑1	❑2
8. During the past 4 weeks, or since your injury, did your injured knee **ever feel like it was getting stuck (catching)**, but you could still move it?	❑1	❑2

9. If you were asked to do the activities below, what is the most you could do today without your injured knee **feeling like it can't hold you up**?

- ❑1 Very hard activities like jumping or turning fast to change direction, like in basketball or soccer
- ❑2 Hard activities like heavy lifting, skiing or tennis
- ❑3 Sort of hard activities like walking fast or jogging
- ❑4 Light activities like walking at a normal speed
- ❑5 I can't do any of the activities listed above because my injured knee feels like it can't hold me up

SPORTS ACTIVITIES

10. What is the most you can do on your injured knee **most of the time**?

- ❑1 Very hard activities like jumping or turning fast to change direction, like in basketball or soccer
- ❑2 Hard activities like heavy lifting, skiing or tennis
- ❑3 Sort of hard activities like walking fast or jogging
- ❑4 Light activities like walking at a normal speed
- ❑5 I can't do any of the activities listed above most of the time

11. Does your injured knee affect your ability to:

	No, not at all	Yes, a little	Yes, somewhat	Yes, a lot	I can't do this
a. Go up stairs?	1	2	3	4	5
b. Go down stairs?	1	2	3	4	5
c. Kneel on your injured knee?	1	2	3	4	5
d. Squat down like a baseball catcher?	1	2	3	4	5
e. Sit in a chair with your knees bent and feet flat on the floor?	1	2	3	4	5
f. Get up from a chair?	1	2	3	4	5
g. Run?	1	2	3	4	5
h. Jump and land on your injured knee?	1	2	3	4	5
i. Start and stop moving quickly?	1	2	3	4	5

FUNCTION

12. How well did your knee work **before you injured it**?

I could not do anything at all	0 ❑	1 ❑	2 ❑	3 ❑	4 ❑	5 ❑	6 ❑	7 ❑	8 ❑	9 ❑	10 ❑	**I could do anything I wanted to**

13. How well does your knee work **now**?

I am not able to do anything at all	0 ❑	1 ❑	2 ❑	3 ❑	4 ❑	5 ❑	6 ❑	7 ❑	8 ❑	9 ❑	10 ❑	**I am able to do anything I want to do**

14. Who completed the questionnaire? ❑1 Child alone ❑2 Child with help from parent/adult

Appendix C2. Tegner Activity Level Scale

Date: ____________________ Participant: ________________

TEGNER ACTIVITY LEVEL SCALE

Please indicate in the spaces below the HIGHEST level of activity that you participated in BEFORE YOUR INJURY and the highest level you are able to participate in CURRENTLY.

BEFORE INJURY: Level_________ CURRENT: Level_________

Level 10	**Competitive sports- soccer, football, rugby (national elite)**
Level 9	**Competitive sports- soccer, football, rugby (lower divisions), ice hockey, wrestling, gymnastics, basketball**
Level 8	**Competitive sports- racquetball or bandy, squash or badminton, track and field athletics (jumping, etc.), down-hill skiing**
Level 7	**Competitive sports- tennis, running, motorcars speedway, handball** **Recreational sports- soccer, football, rugby, bandy, ice hockey, basketball, squash, racquetball, running**
Level 6	**Recreational sports- tennis and badminton, handball, racquetball, down-hill skiing, jogging at least 5 times per week**
Level 5	**Work- heavy labor (construction, etc.)** **Competitive sports- cycling, cross-country skiing,** **Recreational sports- jogging on uneven ground at least twice weekly**
Level 4	**Work- moderately heavy labor (e.g. truck driving, etc.)**
Level 3	**Work- light labor (nursing, etc.)**
Level 2	**Work- light labor** **Walking on uneven ground possible, but impossible to back pack or hike**
Level 1	**Work- sedentary (secretarial, etc.)**
Level 0	**Sick leave or disability pension because of knee problems**

Y Tegner and J Lysolm. *Rating Systems in the Evaluation of Knee Ligament Injuries*. Clinical Orthopedics and Related Research. Vol. 198: 43-49, 1985.

List of Specific Activities:

	Type	Duration	Sessions/week	Intensity (light/moderate/high)
BEFORE				
NOW				

Version date: 24-MAR-2018

Appendix C3. HSS Pedi-FABS Questionnaire

Date: Participant:

HSS Pedi-FABS Questionnaire

Instructions: Choose one answer for each activity or question. In the grid, please indicate how often you performed each activity in your healthiest and most active condition. IN THE PAST **MONTH**:

	Less than one time per month	One time per month	One time per week	2-3 times per week	More than 4 times per week
Running: running while playing a sport or jogging.					
Cutting: quickly changing directions while running.					
Decelerating: coming to a quick stop while running.					
Pivoting: turning your body with your foot planted (for example: skiing, skating, kicking, throwing, hitting a ball)					
Duration: perform athletic activity for as long as you would like to without stopping.					
Endurance: perform athletic activity for one whole hour without stopping.					

Competition: Do you participate in organized competitive sports or physical activities?

- ☐ No (or gym class only)
- ☐ Yes, but WITHOUT an official or judge (such as club or pickup games)
- ☐ Yes, WITH an official or judge
- ☐ Yes, at a national or professional level

Supervision: Do you participate in supervised (coach, trainer, instructor) sports practice or activities (other than gym class)?

- ☐ No
- ☐ Yes, 1-2 times per week
- ☐ Yes, 3-4 times per week
- ☐ Yes, 5 or more times per week

Version date: 27-JUN-2017

Appendix C4. FC Pedi-FABS Questionnaire

Date: Participant:

FC-Pedi-FABS

<u>**Instructions:**</u> Choisissez <u>une</u> réponse pour chaque activité ou question. Dans le tableau, indiquez combien de fois vous participez à chaque activité quand vous êtes dans votre état le plus actif et en santé.
DANS LE DERNIER <u>**MOIS**</u>:

	Moins qu'une fois par mois	Une fois par mois	Une fois par semaine	2-3 fois par semaine	Plus de 4 fois par semaine
Courir: courir quand vous faites du sport ou du jogging					
Coupe: changer de direction rapidement quand vous courrez					
Décélérer : faire un arrêt rapide quand vous courrez					
Pivoter: tourner votre corps avec votre pied planté au sol (par exemple: skier, patiner, donner un coup de pied, lancer, frapper une balle)					
Durée: faire une activité physique aussi longtemps que vous voulez sans arrêter.					
Endurance: faire une activité physique pour une heure complète sans arrêt					

Compétition: Participez-vous à un sport ou une activité physique de façon compétitive et/ou organisée ?

- ❑ Non (ou seulement pendant vos cours d'éducation physique)
- ❑ Oui, main SANS un arbitre ou un juge (comme des clubs ou des parties improvisées)
- ❑ Oui, AVEC un arbitre ou un juge
- ❑ Oui, au niveau national ou professionnel

Supervision: Participez-vous à un sport ou une activité (autre que les cours d'éducation physique) de façon supervisée (entraîneurs, soigneurs, instructeurs) ?

- ❑ Non (ou seulement pendant vos cours d'éducation physique)
- ❑ Oui, 1-2 fois par semaine
- ❑ Oui, 3-4 fois par semaine
- ❑ Oui, 5 fois ou plus par semaine

Date de version: 27-JUIN-2017

Appendix C5. Self-Classification of Tanner Stage: Pubescent Development

Classification of Tanner Stage: Pubescent Development

Tanner Stages Information

Sexual-maturity ratings have replaced the traditional indicators of growth status such as height, weight and skinfold thickness. Sexual maturity ratings have proven useful in assessing growth and development during adolescence.

Classification of patients may be done as part of a general physical examination and does not require any special procedures.

The scale of development is based on secondary sexual characteristics. The ratings range from stage 1, which represents the pre-pubertal child, to stage 5, which represents the adult.

Boys: Genital Development

- Stage 1: Preadolescent. Testes, scrotum and penis are about the same size and proportion as in early childhood.
- Stage 2: Enlargement of scrotum and testes. Skin of scrotum reddens and changes in texture. Little or no enlargement of penis.
- Stage 3: Enlargement of penis, at first mainly in length. Further growth of testes and scrotum.
- Stage 4: Increased size of penis, with growth in breadth and development of glans. Testes and scrotum larger. Scrotal skin darkened.
- Stage 5: Genitalia are adult in size and shape.

Girls: Breast Development

- Stage 1: Preadolescent. Elevation of papilla only.
- Stage 2: Breast bud stage. Elevation of breast and papilla as small mound. Enlargement of diameter of areola.
- Stage 3: Further enlargement and elevation of the breast and areola, with no separation of their contours.
- Stage 4: Projection of areola and papilla to form a secondary mound above the level of the breast.
- Stage 5: Mature stage. Projection of papilla only, owing to recession of the areola to the general contour of the breast.

Both Sexes: Pubic Hair

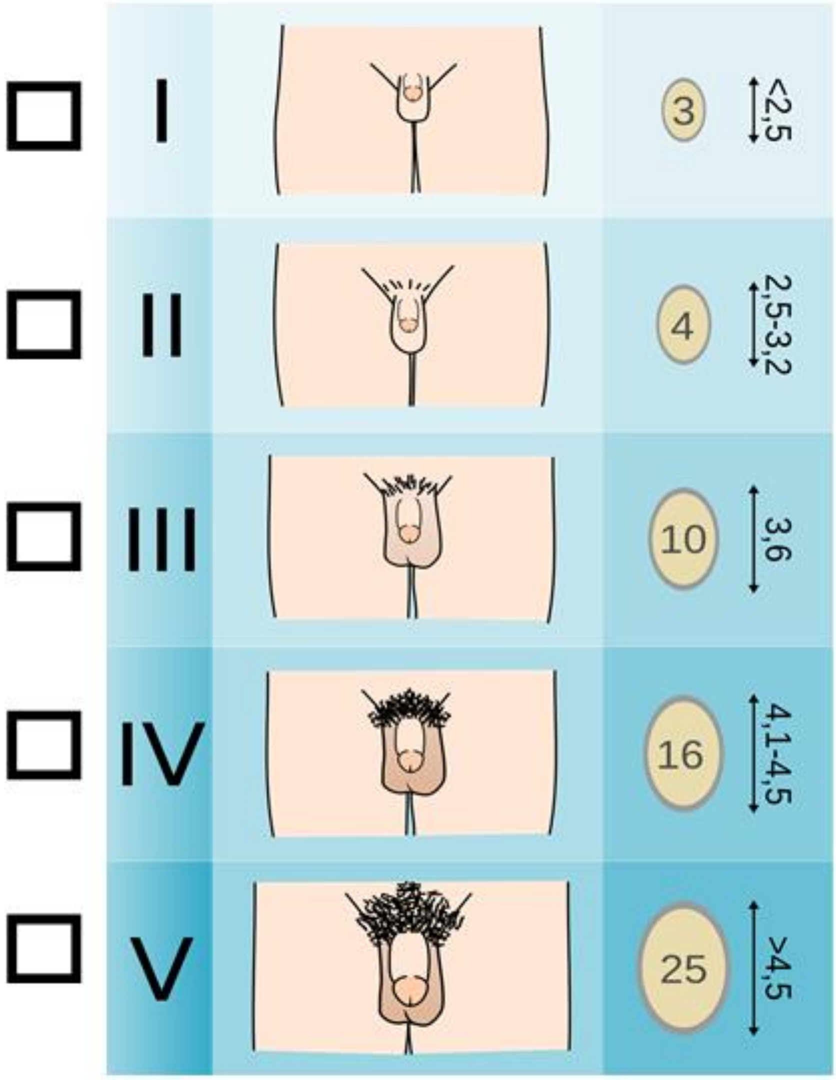
- Stage 1: Preadolescent. Vellus over pubis is not developed further than that over abdominal wall (i.e., no pubic hair).
- Stage 2: Sparse growth of long, slightly pigmented downy hair, straight or slightly curled, chiefly at base of penis and along labia.
- Stage 3: Hair is considerably darker, coarser and more curled. It spreads sparsely over the junction of pubis.
- Stage 4: Hair is adult in type, but area covered is still considerably smaller than in adult. No spread to medial surface of thighs.
- Stage 5: Hair is adult in quantity and type, with distribution of horizontal (or classic "feminine" in females) pattern. Spread to medial surface of thighs but not up linea alba or elsewhere above base of inverse triangle (spread up linea alba occurs late and is rated Stage 6).

Version Date: 17-MAY-2017

Adapted from the Public Health Agency of Canada: Canadian Guidelines on Sexually Transmitted Infections, Section VII, Appendix H. http://www.phac-aspc.gc.ca/std-mts/sti-its/cgsti-ldcits/app-ann-h-eng.php

Classification of Tanner Stage: Pubescent Development

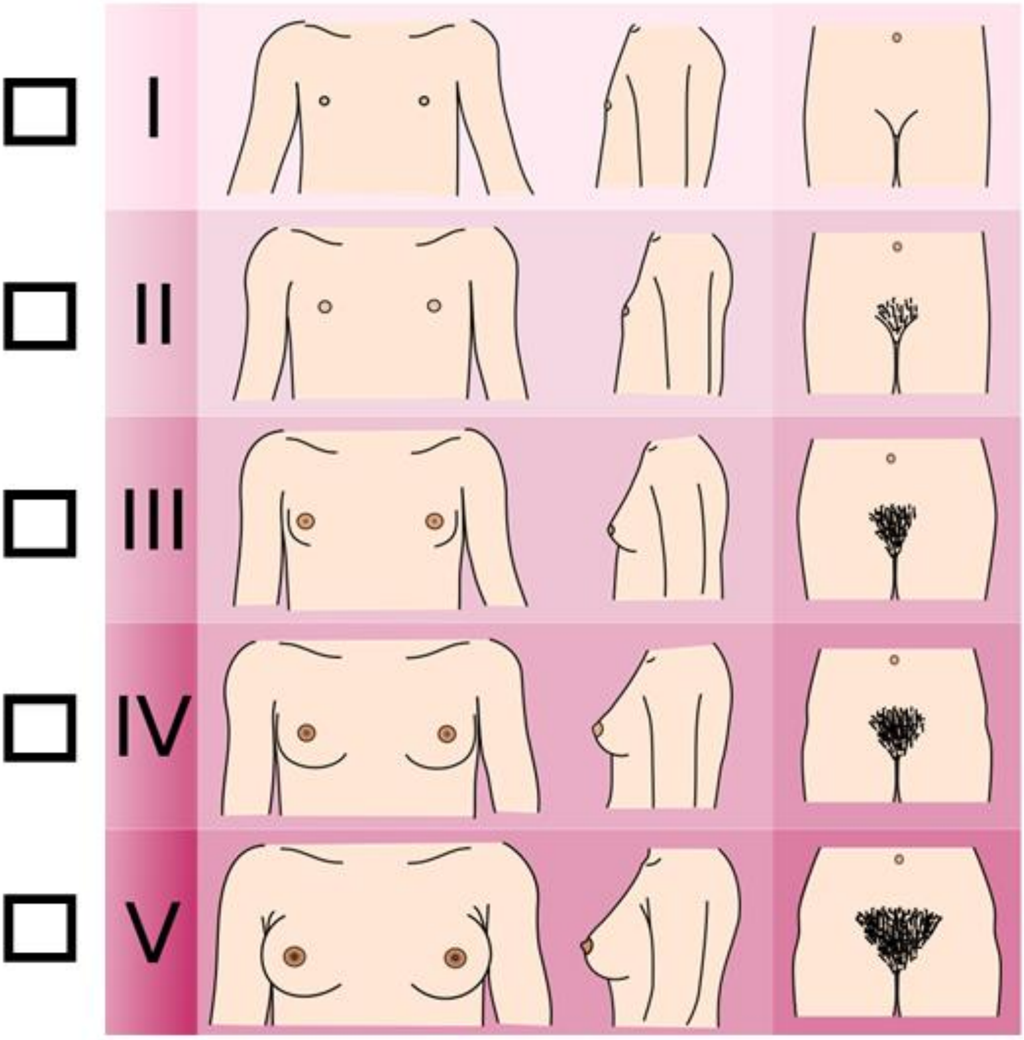
-Using the accompanied information "Tanner Scale of Sexual Maturity", please select the stage (I thru V) that you identify with the most.



Version Date: 17-MAY-2017
Adapted from the Public Health Agency of Canada: Canadian Guidelines on Sexually Transmitted Infections, Section VII, Appendix H. http://www.phac-aspc.gc.ca/std-mts/sti-its/cgsti-ldcits/app-ann-h-eng.php

Classification of Tanner Stage: Pubescent Development

-Using the accompanied information "Tanner Scale of Sexual Maturity", please select the stage (I thru V) that you identify with the most.



Version Date: 17-MAY-2017
Adapted from the Public Health Agency of Canada: Canadian Guidelines on Sexually Transmitted Infections, Section VII, Appendix H. http://www.phac-aspc.gc.ca/std-mts/sti-its/cgsti-ldcits/app-ann-h-eng.php

APPENDIX D – Supplemental Material for Chapter 5, Article 1

Appendix D1. Study characteristics.

Author	*Participant (age mean ± SD)*	*Tasks performed*	*Phase tested*	*Muscles tested*	*EMG variables*	*EMG normalization*	*Main findings*
Beaulieu (2009)	30 elite soccer players 15 females (21.1 ± 3.6), 15 males (22.9 ± 3.7)	Unanticipated side cuts	Pre- & post-landing	VM, VL, RF, BF, ST, MG, LG, TA	Onset of activity, amplitudes	MVIC	Earlier ST onset in females. Higher RF, TA, & LG amplitudes in females. No sex differences in VM, VL, BF, or MG.
Bencke (2011)	24 elite handball players 12 females (22.7 ± 3.1), 12 males (23.1 ± 3.4)	Side cuts	Pre-landing	VM, VL, BF, ST	Amplitudes	MVIC	Lower ST & BF pre-activity in females. Higher Q-H pre-activity ratio in females. No sex differences in VM & VL.
Chappell (2007)	38 recreational athletes 19 females (22.3 ± 2.2), 17 males (22.6 ± 2.2)	Bilateral vertical stop jumps	Pre-landing	VM, VL, RF, BF, SM, ST	Amplitude	MVIC	Higher quadriceps & hamstrings amplitudes during early flight phase in females.
Cowling (2001)	18 recreational athletes 11 females, 7 males (22.6 ± 2.5)	Unilateral abrupt stop	Pre- & post-landing	RF, VM, VL, SM, BF, MG	Duration of muscle burst, onset of activity, onset of peak activity	None	Delayed onset and peak activity of SM in males. No sex differences in RF, VL, VM, BF, or MG.
de Britto (2014)	30 recreational athletes 15 females (28.4 ± 6.0), 15 males (28.9 ± 4.0)	Bilateral drop jumps (20cm & 40cm)	Pre-landing	VM, RF, BF, MH	Amplitudes	Max of task	Higher pre-activity of MH in females. No sex differences in RF, VM, & BF.
Del Bel (2017)	34 soccer players 17 females (9.0 ± 1.2), 17 males (10.2 ± 1.5)	Unanticipated side cuts	Post-landing	VM, VL, MH, LH, MG, LG	Onset of activity, amplitudes	MVIC	No sex differences in any muscles.
Ebben (2010)	24 collegiate athletes 12 females (19.9 ± 0.9), 12 males (21.0 ± 1.2)	Bilateral vertical jump landings & side cuts	Pre- & post-landing	RF, VM, VL, MH, LH	Onset of activity, amplitudes	MVIC	Lower LH amplitudes during jump-landing, and both LH & MH during stance phase of cut in females. Later activations in pre-

							landing in VL & VM, and longer duration of RF & VM during stance phase of cut in females.
Fagenbaum (2003)	14 collegiate basketball players 8 females, 6 males (age not reported)	Unilateral forward jumps & drop jumps (25cm & 50cm)	Pre- & post-landing	VM, VL, MH, LH	Onset of activity, amplitudes	MVIC	Higher VL & VM, and lower MG amplitudes in females.
Garrison (2005)	16 collegiate soccer players 8 females (22.1 ± 2.4), 8 males (19.3 ± 1.5)	Unilateral drop jumps (60cm)	Post-landing	RF, VL, BF, Gmed	Amplitudes	Single-leg stance	No sex differences in any muscles.
Hamstra-Wright (2005)	36 children 19 females (9.4 ± 0.7) , 17 males (8.9 ± 0.6)	Bilateral drop vertical jumps (24cm)	Pre-landing	VM, MH	Amplitudes & Q:H co-contraction ratios	Max of task	No sex differences in any muscles/ratios.
Hanson (2008)	40 collegiate soccer players 20 females (19.8 ± 1.1), 20 males (19.4 ± 1.4)	Running & box drop side cuts	Pre- & post-landing	RF, VL, MH, LH, Gmax, Gmed	Amplitudes & Q:H co-contraction ratio	MVIC	Higher VL amplitude during both phases, and Gmed during stance phase in females. Higher Q:H ratios in females.
Hart (2007)	16 collegiate soccer players 8 females (22.0 ± 2.1), 8 males (19.1 ± 1.4)	Unilateral forward jumps	Post-landing	BF, VL, MG, Gmed	Amplitudes	Single-leg stance	Lower Gmed amplitude in females.
Hughes (2015)	20 recreational athletes 10 females (20.3 ± 1.3), 10 males (21.3 ± 0.9)	Unilateral vertical jump & side cuts	Post-landing	RF, BF, Gmax	Amplitudes	MVIC	Higher peak RF amplitude for both tasks in females. Lower peak BF activity in jump in females. No sex differences in Gmax.
Kipp (2014)	27 soccer players 16 females (11.1 ± 0.5), 11 males (11.4 ± 0.8)	Unilateral drop landing	Pre- & post-landing	RF, VM, VL, MH, LH, SO	Muscle synergies & co-contraction ratios	Max of task	Higher MH activity at preparatory and contact, and lower VM activity at landing in females.

Landry (2007)	42 adolescent elite soccer players 21 females (16.7 ± 1.0), 21 males (17.1 ± 0.6)	Unanticipated straight run & cross cuts	Post-landing	RF, VM, VL, MH, LH, MG, LG	Onset of activity, amplitudes & medial-lateral imbalance	MVIC	Higher LG amplitude during both tasks in females. Higher LG compared to MG during both tasks only in females. Lower LH & MH amplitudes during cross-cut, and just lower LH in run in females. Higher MH compared to LH during cross-cut in only females. Higher VM, VL & RF amplitudes during running, and just higher RF in cross-cut in females.
Landry (2007)	42 adolescent elite soccer players 21 females (16.7 ± 1.0), 21 males (17.1 ± 0.6)	Unanticipated side cuts	Post-landing	RF, VM, VL, MH, LH, MG, LG	Onset of activity, amplitudes & medial-lateral imbalance	MVIC	Higher overall LG & MG activity, and imbalance with higher LG compared to MG during side-cut in females. No sex differences in hamstrings or quadriceps.
Landry (2009)	42 adolescent elite soccer players 21 females (16.7 ± 1.0), 21 males (17.1 ± 0.6)	Unanticipated side cuts & cross cuts	Pre- & post-landing	RF, VM, VL, MH, LH, MG, LG	Onset of activity, amplitudes & medial-lateral imbalance	MVIC	Higher LG & MG amplitudes during pre-contact and stance of side-cut, and LG amplitudes during cross-cut in females. Earlier LH (cross-cut) and MH (side-cut) peak activity during pre-contact in females. Higher RF amplitudes during both tasks in females.
Malinzak (2001)	20 recreational athletes 9 females (24.6 ± 1.0), 11 males (24.5 ± 2.5)	Running, side cuts, & cross cuts	Post-landing	VM, VL, MH, BF	Amplitudes	MVIC	Higher quadriceps activity (running, side-cut) and lower hamstrings activity (running, cross-cut) during mid-stance in females.
Medina (2008)	57 adolescent athltes 18 female athletes (14.8 ± 1.1) 19 male athletes (14.9 ± 0.8)	Unilateral drop landing (32cm)	Post-landing	RF, MH, LH, MG	Onset of Activity	None	Later RF onset in female non-athletes compared to female & male athletes.

	20 female non-athlete (15.7 ± 1.3)						
Nagano (2007)	37 recreational athlete 19 females (19.4 ± 0.9), 18 males (19.8 ± 4.6)	Unilateral drop landing	Pre- & post-landing	RF, BF, SM	Amplitudes	MVIC	Higher RF activity during pre-landing phase in females.
Padua (2005)	21 recreational athletes 10 females (24.1 ± 3.8), 11 males (27.8 ± 4.4)	Bilateral hops	Pre- & post-landing	RF, VM, MH, LH, MG, LG, SO, TA	Amplitudes & Q:H co-contraction ratio	MVIC	Higher RF, VM, SO, & Q:H ratios in both hopping tasks in females. No sex differences in LH, MH, LG, MG, TA, or Gastroc/TA ratios
Palmieri-Smith (2009)	21 recreational athletes 11 females (24.0 ± 5.2), 10 males (23.6 ± 3.8)	Unilateral forward jumps (100cm)	Post-landing	VM, VL, MH, LH	Q:H co-contraction ratios (medial, lateral, medial-to-lateral) & amplitudes	MVIC	Lower medial-lateral and Q:H co-contraction ratios in females.
Pappas (2007)	32 recreational athletes 16 females (28.2 ± 5.4) 16 males (28.8 ± 3.9)	Unilateral & bilateral drop jump landings (40cm)	Post-landing	RF, MH, LH, MG	Amplitudes	Functional MVC (drop-jump from 20cm)	No sex differences in any muscles.
Rozzi (1999)	34 collegiate soccer/basketball players 17 females (18.9 ± 0.9), 17 males (20.4 ± 1.7)	Unilateral drop jumps (25cm)	Post-landing	VM, VL, MH, LH, MG, LG	Amplitudes & onset of activity	MVIC	Higher LH peak amplitudes in females.
Russell (2007)	55 recreational athletes 14 girls (9.3 ± 0.9) 14 boys (9.6 ± 1.0) 14 women (24.2 ± 2.3) 13 men (23.6 ± 3.4)	Bilateral maximum countermovement jumps	Pre- & post-landing	VM, MH, BF	Amplitudes & Q:H co-contraction ratio	Max of task	Lower Q:H ratios in children compared to adults. No sex differences.
Sell (2006)	35 high school basketball players	Unanticipated bilateral stop jumps	Post-landing	VM, VL, BF, SM	Amplitudes & Q:H co-contraction ratio	MVIC	Higher ST amplitude & Q:H ratios during unanticipated jumps in females.

	17 females (15.9 ± 1.1), 18 males (16.4 ± 1.4)						
Shultz (2009)	78 participants (not specified) 39 females (22.2 ± 2.9), 39 males (22.6 ± 2.6)	Bilateral drop vertical jumps (45cm)	Pre- & post-landing	VM, VL, MH, LH	Amplitudes & Q:H co-contraction ratio	MVIC	Higher quadriceps & hamstrings amplitudes during pre- and post-landing phases in females.
Sigward (2006)	30 collegiate soccer players 15 females (19.4 ± 1.5), 15 males (19.6 ± 1.9)	Side cuts	Post-landing	VL, BF, MH	Amplitudes	MVIC	Higher VL activity during deceleration phase in females. No sex differences in hamstrings.
Urabe (2005)	15 collegiate basketball players 8 females (22.0 ± 1.0), 7 males (22.2 ± 0.4)	Unilateral maximum countermovement jumps	Post-landing	VM, VL, BF, SM	Amplitudes & Q:H co-contraction ratio	MVIC	Higher VM & Q:H ratios during deceleration of drop-jump landing in females.
Zazulak (2005)	22 collegiate athletes 13 females, 8 males (age not reported)	Unilateral drop jumps (30.5cm & 45.8cm)	Pre- & post-landing	RF, Gmax, Gmed	Amplitudes	MVIC	Higher peak RF activity and lower Gmax peak activity during post-landing phase in females.

SD: standard deviation, RF: rectus femoris, VM: vastus medialis, VL: vastus lateralis, MH: medial hamstring, LH: lateral hamstring, MG: medial gastrocnemius, LG: lateral gastrocnemius, SO: soleus, TA: tibialis anterior, Gmax: gluteus maximus, Gmed: gluteus medius, Q:H: quadriceps-to-hamstrings, MVIC: maximum voluntary isometric contractions.

Appendix D2. Characteristics of EMG data collection and analysis.

Author	*Muscles tested*	*Electrode location*	*EMG amplitude processing*	*Other EMG analysis*	*Normalization*	*Main findings*
Beaulieu (2009)	VM, VL, RF, BF, ST, MG, LG, TA	Placed over the muscle belly of each muscle	Linear envelope with a critically damped filter, cut-off frequency of 6 Hz	Onset of activity: detected using a double-threshold detector	MVIC	Earlier activity in females: ST, Greater activity in females: RF, TA, & LG, No sex differences: VM, VL, BF, & MG
Bencke (2011)	VM, VL, BF, ST	Placed over the middle part of muscle bellies according to the guideline (Hermens et al., 2000)	Linear envelope using 4th order Butterworth low pass filter at 10 Hz		MVIC	Lower pre-activity in females: ST & BF, Higher Q-H pre-activity ratio in females, No sex differences: VM & VL
Chappell (2007)	VM, VL, RF, BF, SM, ST	Placed on the skin over each muscle	Linear envelope using 4th order Butterworth low pass filter at 10 Hz		MVIC	Greater quadriceps & hamstrings amplitudes during early flight phase in females
Cowling (2001)	RF, VM, VL, SM, BF, MG	Placed over the muscle bellies parallel to the line of action of the muscle fibers	Linear envelope using low pass filter at 20 Hz	Onset/offset timing: 14 consecutive samples of linear envelope exceed or passed under a threshold of 7% of max amplitude	None	Delayed onset and peak activity of SM in males, No sex differences: RF, VL, VM, BF, MG
de Britto (2014)	VM, RF, BF, MH	Placed according to the guideline (Hermens et al., 2000). Placed between the motor point and the distal tendon	Linear envelope using 2nd order Butterworth low pass filter at 8 Hz		Max of task	Greater pre-activity of MH in females, No sex differences: RF, VM, BF
Del Bel (2017)	VM, VL, MH, LH, MG, LG	Placed over the muscle bellies according to the guideline (Hermens et al., 2000)	Linear envelope with a critically damped filter, cutoff frequency of 6 Hz	Timing of peak activity: >90% of the variance within the waveforms	MVIC	No sex differences in any muscle
Ebben (2010)	RF, VM, VL, MH, LH	Placed on the longitudinal axis of the muscles. RF: halfway between the greater trochanter and medial epicondyle of the femur; VL: 1/4 of the distance from the lateral line of the knee joint to the anterior	RMS with 125 ms moving window	Onset/offset timing: 150% of baseline	MVIC	Smaller LH amplitudes during jump-landing, and both LH and MH during stance phase of cut in females; Later activations in pre-landing in VL and

		superior iliac spine; VM: 1/5 of the distance from the medial joint line to the anterior superior iliac spine; LH: halfway between the ischial tuberosity and the fibular insertion site, at least 5 cm proximal to the musculotendinous junction; MH: halfway between the ischial tuberosity and the tibial insertion point, at least 4 cm proximal to the musculotendinous junction.				VM, and longer duration of RF and VM during stance phase of cut in females
Fagenbaum (2003)	VM, VL, MH, LH, gastrocnemius	Placed approximately 1/3 of the distance from the knee joint space to the greater trochanter over the muscle bellies of VM, VL, MH and LH. Placed over the middle of the gastrocnemius muscle, 1/3 of the distance from the knee joint space to the base of the calcaneus	ARV		MVIC	Greater VL & VM, and lower gastrocnemius amplitudes in females
Garrison (2005)	RF, VL, BF, Gmed	Placed over the muscle belly of each muscle according to Fagenbaum et al. (2003)	RMS with 3 ms window		Single-leg stance	No sex differences in any muscle
Hamstra-Wright (2005)	VM, MH	Placed on each muscle	RMS	Q:H co-contraction ratios	Max of task	No sex differences in any muscle/ratios
Hanson (2008)	RF, VL, MH, LH, Gmax, Gmed	GM: halfway between the iliac crest and the greater trochanter of the femur; Gmax: 20% of the distance between the spinous process of the second sacral vertebrae and a point 10 cm distal to the greater trochanter; quadriceps & hamstrings: midsection of the muscle bellies.	RMS with 20 ms moving window	Q:H co-contraction ratios: (RF + VL)/(MH + LH)	MVIC	Greater VL activity during both phases, and Gmed during stance phase in females; Greater Q:H ratios in females
Hart (2007)	BF, VL, MG, Gmed	Placed over the muscle belly parallel to muscle fiber direction	RMS with 6 sample moving average		Single-leg stance	Lower Gmed activity in females.

Hughes (2015)	RF, BF, Gmax	Placed according to the guideline (Hermens et al., 2000). RF: half way between the anterior inferior iliac spine and the proximal border of the patella; BF: half way in between the ischial tuberosity and lateral epicondyle of the tibia; GM: diagonally half way between the sacral vertebrae and the greater trochanter	RMS with 5 ms moving window		MVIC	Greater peak RF activity for both tasks in females; Lower peak BF activity in jump in females; No sex differences for Gmax
Kipp (2014)	RF, VM, VL, MH, LH, SO	Placed longitudinally over the muscle bellies according to the guideline (Hermens et al., 2000)	Linear envelope with a critically damped filter, cutoff frequency of 6 Hz, muscle synergy analysis		Max of task	Greater MH activity at preparatory and contact, and lower VM activity at landing in females
Landry (2007)	RF, VM, VL, MH, LH, MG, LG	Placed along the direction of the muscle fibers	Linear envelope with a critically damped filter, cutoff frequency of 6 Hz	Medial-lateral imbalance (MG-LG, MH-LH)	MVIC	Greater LG amplitudes during both tasks in females; Greater LG compared to MG during both tasks only in females; Lower LH and MH amplitudes during cross-cut, and just lower LH in run in females; Greater MH compared to LH during cross-cut in only females; Greater VM, VL and RF amplitudes during running, and just the RF having greater activity for the cross-cut in females
Landry (2007)	RF, VM, VL, MH, LH, MG, LG	Placed along the direction of the muscle fibers	Linear envelope with a critically damped filter, cutoff frequency of 6 Hz	Medial-lateral imbalance (MG-LG, MH-LH)	MVIC	Greater overall LG and MG activity, and imbalance with greater LG compared to MG during side-cut in females; No sex

						differences in hamstrings or quadriceps
Landry (2009)	RF, VM, VL, MH, LH, MG, LG	Placed along the direction of the muscle fibers	Linear envelope with a critically damped filter, cutoff frequency of 6 Hz	Medial-lateral imbalance (MG-LG, MH-LH)	MVIC	Greater LG and MG amplitudes during pre-contact and stance of side-cut, and LG amplitudes during cross-cut in females; Earlier LH (cross-cut) and MH (side-cut) peak activity during pre-contact in females; Greater RF amplitudes during both tasks in females
Malinzak (2001)	VM, VL, MH, BF	Placed on the surface of each muscle	Integrated in a 4.2 ms moving window		MVIC	Greater Quads activity (running, side-cut) and lower Hamstrings activity (running, cross-cut) during mid-stance in females
Medina (2008)	RF, MH, LH, MG	Placed in line with the muscle fibers	RMS with 20 ms window	Onset of activity: two standard deviations above baseline	None	Slower RF onset of activity in female non-athletes compared to female and male athletes
Nagano (2007)	RF, BF, SM	Placed over the muscle bellies. RF: at the midpoint of the top of the patella to the posterior superior iliac spine; BF & SM: distally 1/3 of the distance from the knee joint space to the ischial tuberosity	RMS	H:Q ratio	MVIC	Greater RF activity during preparatory phase of landing in females; Lower H:Q ratio in females
Padua (2005)	RF, VM, MH, LH, MG, LG, SO, TA	Placed over the muscle belly of each muscle	Smoothed by using a Hanning integrator set to 20 points	Q:H co-contraction ratio	MVIC	Greater RF, VM, SO, and Q:H ratios in both hopping tasks in females; No sex differences in LH, MH, LG, MG, TA, or Gastroc/TA ratios

Palmieri-Smith (2009)	VM, VL, MH, LH	Placed over the muscle bellies in line with the muscle fibers according to Delagi and Perotto (1980)	RMS with 50 ms moving window	Q:H co-contraction ratios (medial, lateral, medial-to-lateral)	MVIC	Lower medial–lateral and Q:H co-contraction ratios in females
Pappas (2007)	RF, MH, LH, MG	Placed according to the guideline (Hermens et al., 2000). Placed between the motor point and the distal tendon	Linear envelope using 2nd order Butterworth low pass filter at 6 Hz		Functional MVC (drop-jump from 20 cm)	No sex differences in any muscle
Rozzi (1999)	VM, VL, MH, LH, MG, LG	Placed over the muscle belly of each muscle	Amplitude of first contraction	Onset time: time from landing until the first muscle contraction (signal exceeded the set trigger level of 10% of the maximal voluntary contraction)	MVIC	Greater LH peak amplitudes in females
Russell (2007)	VM, MH, BF	Placed parallel to the muscle fibers over the longitudinal midline between the motor point and the tendon	RMS	H:Q ratio: (MH + BF)/VM	Max of task	Lower Q:H ratios in children compared to adults, No sex differences
Sell (2006)	VM, VL, BF, ST	Placed over the muscle belly in line with the direction of the fibers according to Delagi and Perotto (1980)	Integrated EMG	Q:H co-contraction ratio: VL/ST	MVIC	Greater ST iEMG and Q:H ratios during unanticipated jumps in females
Shultz (2009)	VM, VL, MH, LH	Placed between the motor point and the distal tendon	RMS with 25 ms time constant		MVIC	Greater quadriceps and hamstrings amplitudes during pre- and post-landing phases in females
Sigward (2006)	VL, BF, MH	Placed according to Cram et al. (1998)	RMS over a 75 ms interval		MVIC	Greater VL activity during deceleration phase in females, No sex differences in hamstrings
Urabe (2005)	VM, VL, BF, SM	Placed according to Perroto (1994)	RMS with 20 ms time constant	H:Q activation ratio	MVIC	Greater VM and Q:H ratios activity during deceleration of drop-jump landing in females

Zazulak (2005)	RF, Gmax, Gmed	Placed over the muscle bellies and parallel to the line of action of the muscle fibers according to Cram et al. (1998)	RMS with 40 ms window	MVIC	Greater peak RF activity and lower Gmax peak activity post-contact phase in females

Appendix D3. Check-list for the ISEK standards for reporting EMG data.

Author	***Surface electrode***						***EMG detection***								***Sampling***		***Rectification***	***Amplitude processing***	***Normalization***			
	Material	*Shape*	*Size*	*Skin preparation*	*Interelectrode distance*	*Location*	*Type of differential*	*Input impedance*	*CMRR*	*SNR*	*Gain*	*Filter types*	*Cut-off frequencies*	*Slopes of cut-offs*	*Frequency*	*Bits*			*Training*	*Joint angle*	*Rate of rise*	*Velocity of contract-ion*
Beaulieu (2009)	+	–	–	+	–	–	–	–	–	–	+	–	–	–	+	–	±	+	–	–	–	–
Bencke (2011)	+	–	–	–	+	+	+	–	–	–	–	–	–	–	+	–	±	+	–	–	–	–
Chappell (2007)	+	–	–	+	–	–	–	–	–	–	–	+	+	–	–	–	±	+	–	+	–	–
Cowling (2001)	+	–	–	+	+	+	+	+	–	–	–	+	+	–	+	–	+	+	–	–	–	–
de Britto (2014)	+	–	–	+	+	+	+	+	+	–	+	–	+	–	+	–	+	+	–	–	–	–
Del Bel (2017)	–	–	–	–	–	+	+	+	+	–	+	–	+	–	+	–	+	+	–	+	–	–
Ebben (2010)	+	+	+	+	+	+	+	+	+	–	–	–	+	–	+	–	–	+	–	–	–	–
Fagenbaum (2003)	–	–	–	–	–	±	–	–	+	–	–	–	+	–	+	–	±	+	–	–	–	–
Garrison (2005)	–	–	–	–	–	±	+	+	+	–	–	–	+	–	+	–	+	+	–	–	–	–
Hamstra-Wright (2005)	+	–	–	+	+	–	+	+	+	–	+	+	+	–	+	–	±	+	–	–	–	–
Hanson (2008)	+	–	+	+	+	+	+	+	+	+	–	+	+	–	+	–	±	+	+	+	–	–
Hart (2007)	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+	–	–	+	–	–	–	–
Hughes (2015)	–	–	–	+	+	+	–	–	+	+	–	–	+	–	+	–	–	+	+	+	–	–
Kipp (2014)	+	–	–	+	+	+	–	–	–	–	–	–	–	–	+	–	+	+	–	–	–	–

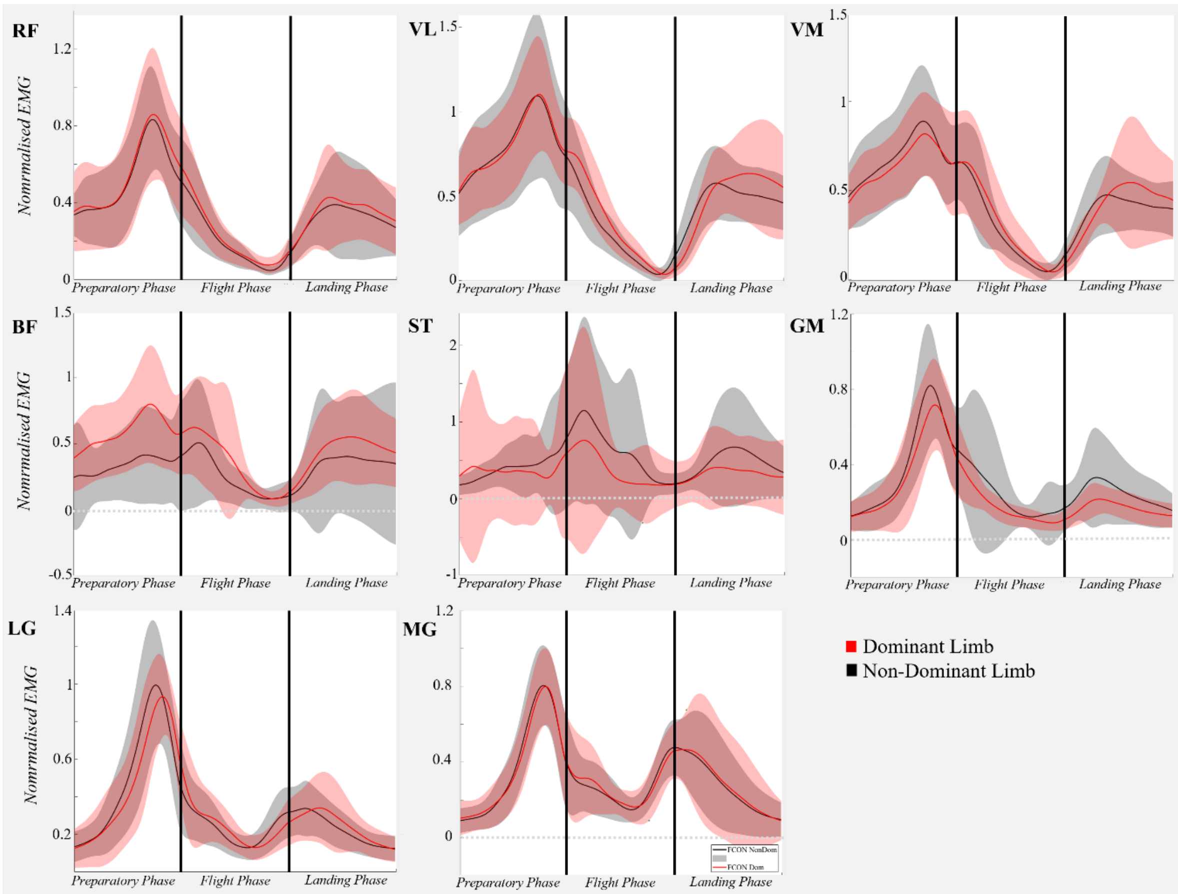
Landry (2007)	+	–	+	+	+	+	+	+	+	–	–	–	+	–	+	–	+	+	–	+	–	–
Landry (2007)	+	–	+	+	+	+	+	+	+	–	–	–	+	–	+	–	+	+	–	+	–	–
Landry (2009)	+	–	+	+	+	+	+	+	+	–	–	–	+	–	+	–	+	+	–	+	–	–
Malinzak (2001)	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	±	+	–	+	–	–
Medina (2008)	+	–	+	+	+	±	+	–	–	–	–	–	+	–	+	–	–	–	–	–	–	–
Nagano (2007)	–	–	–	–	–	±	–	–	+	–	–	–	–	–	+	–	–	+	–	–	–	–
Padua (2005)	+	–	+	+	+	+	+	+	+	–	+	–	+	–	+	–	±	+	–	+	–	–
Palmieri-Smith (2009)	+	–	+	+	+	+	+	–	–	–	–	+	+	–	+	–	–	+	–	–	–	–
Pappas (2007)	–	–	–	+	+	+	+	–	–	–	–	–	–	–	+	–	–	+	–	–	–	–
Rozzi (1999)	+	–	+	+	+	–	–	+	+	–	–	–	+	–	+	–	–	–	–	–	–	–
Russell (2007)	+	–	–	+	+	+	+	–	+	–	+	+	+	–	+	–	±	+	–	–	–	–
Sell (2006)	+	–	–	+	+	+	+	–	–	–	–	–	–	–	+	–	–	+	–	+	–	–
Shultz (2009)	+	–	–	+	+	+	+	+	+	–	–	+	+	–	+	–	–	+	–	+	–	–
Sigward (2006)	–	–	–	+	–	+	+	–	–	–	–	+	+	–	+	–	+	+	–	+	–	–
Urabe (2005)	–	–	–	+	+	+	+	–	–	–	–	–	+	–	+	–	±	+	–	–	–	–
Zazulak (2005)	+	–	+	–	–	+	+	+	+	–	–	–	+	–	+	+	–	+	–	–	–	–

Appendix D4. Methodological quality assessment.

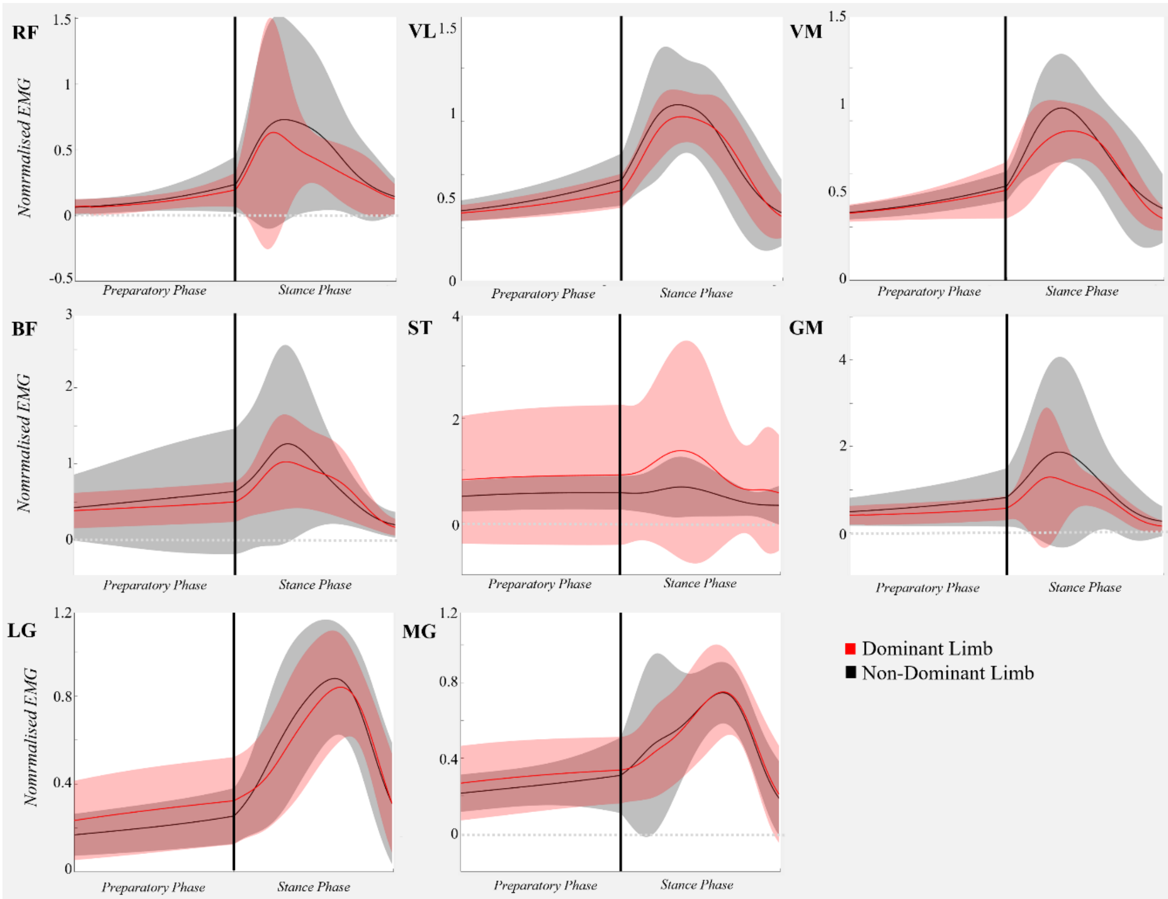
	1. Were the aims/objectives clear?	2. Was the study design appropriate for the stated aims?	3. Was the sample size justified?	4. Was the target population clearly defined?	5. Was the sample frame taken from an appropriate population base?	6. Was the selection process likely to select participants that were representative of the target population?	8. Were the outcome variables measured appropriate to the aims of the study?	9. Were the outcome variables measured correctly using instruments/measurements trialed, piloted or published previously?	10. Is it clear what was used to determined statistical significance and/or precision estimates?	11. Were the methods sufficiently described to enable them to be repeated?	12. Were the basic data adequately described?	15. Were the results internally consistent?	16. Were the results presented for all the analyses described in the methods?	17. Were the authors' discussions and conclusions justified by the results?	18. Were the limitations of the study discussed?	19. Were any funding sources or conflicts of interest declared?	20. Was ethical approval or consent of participants attained?	Total (/17)
Beaulieu (2009)	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	15
Bencke (2011)	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0	1	14
Chappell (2007)	1	1	0	1	0	0	1	0	1	1	0	1	1	1	1	0	1	11
Cowling (2001)	1	1	0	0	0	0	1	1	1	0	0	1	1	0	0	1	1	9
de Britto (2014)	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	15

Del Bel (2017)	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	15
Ebben (2010)	1	1	0	1	0	0	1	1	0	0	1	1	1	1	0	0	1	10
Fagenbaum (2003)	1	1	0	1	1	0	1	1	1	1	0	1	1	0	1	1	1	13
Garrison (2005)	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	0	1	14
Hamstra-Wright (2006)	1	1	0	1	1	0	1	1	1	1	1	1	0	1	0	0	1	12
Hanson (2008)	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	0	1	14
Hart (2007)	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	0	1	14
Hughes (2015)	1	1	0	1	1	0	1	1	1	1	1	1	1	1	0	1	1	14
Kipp (2014)	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	15
Landry (2007)	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	15
Landry (2007)	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	15
Landry (2009)	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	15
Malinzak (2001)	1	1	0	1	0	0	1	1	0	1	1	1	1	1	0	0	1	11
Medina (2008)	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	0	1	14
Nagano (2007)	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	15
Padua (2005)	1	1	0	1	0	0	1	1	1	1	1	1	1	1	1	0	1	13
Palmieri-Smith (2009)	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	0	1	14
Pappas (2007)	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	0	1	14
Rozzi (1999)	1	1	0	1	1	0	1	0	1	1	1	1	1	1	0	0	1	12
Russell (2007)	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	16
Sell (2006)	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	15
Shultz (2009)	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	15

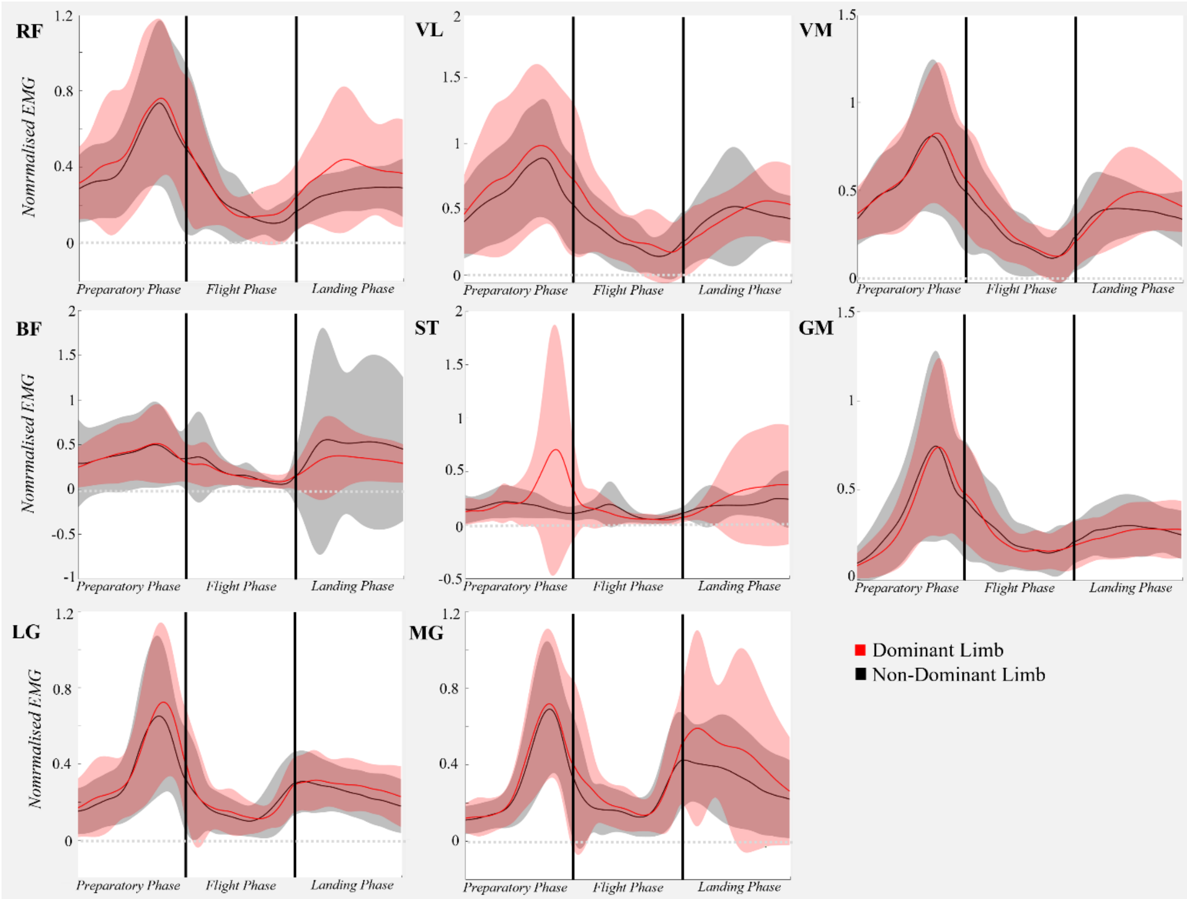
Sigward (2006)	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	15
Urabe (2005)	1	1	0	1	1	0	1	1	1	1	1	1	1	1	0	0	1	13
Zazulak (2005)	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	15
Kappa agreement	0.65	1.0	1.0	0.65	0.61	1.0	1.0	0.65	1.0	0.78	1.0	0.78	1.0	1.0	0.71	0.61	0.65	N/A



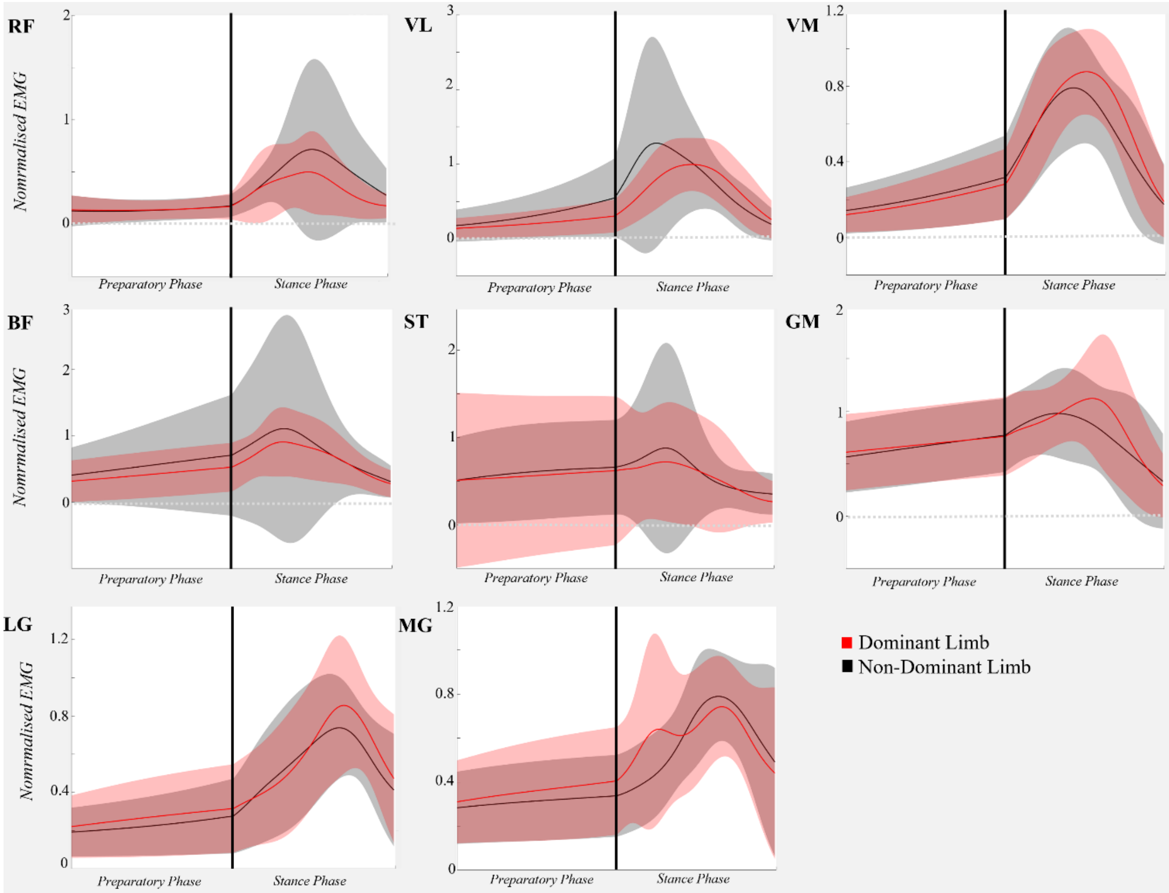
Appendix E1. Individual muscle waveforms of adolescent females throughout the three defined phases of the countermovement jump for the dominant (red) and non-dominant (black) limbs.



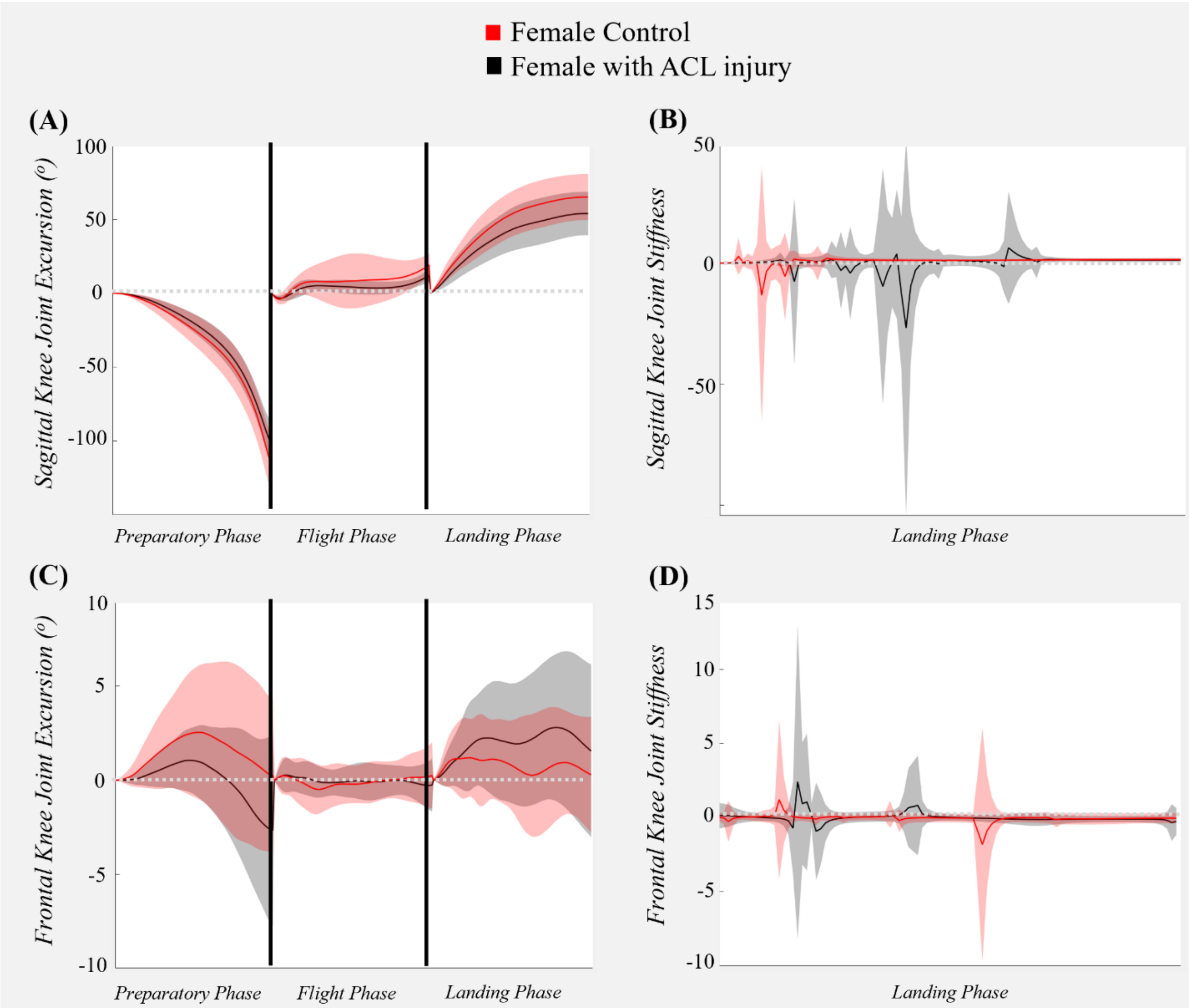
Appendix E2. Individual muscle waveforms of adolescent females throughout the two defined phases of the side-cut for the dominant (red) and non-dominant (black) limbs.



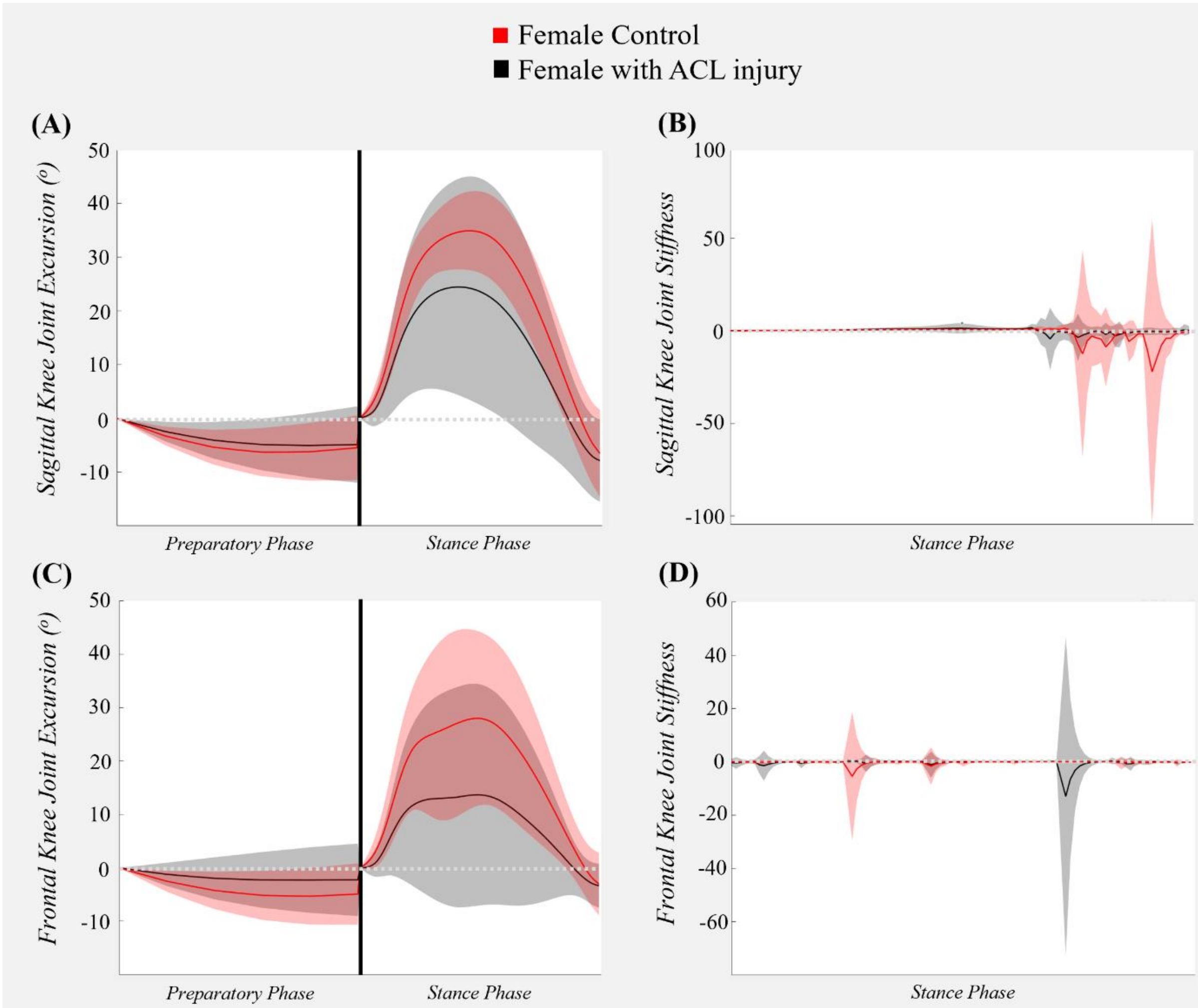
Appendix E3. Individual muscle waveforms of adolescent males throughout the three defined phases of the countermovement jump for the dominant (red) and non-dominant (black) limbs.



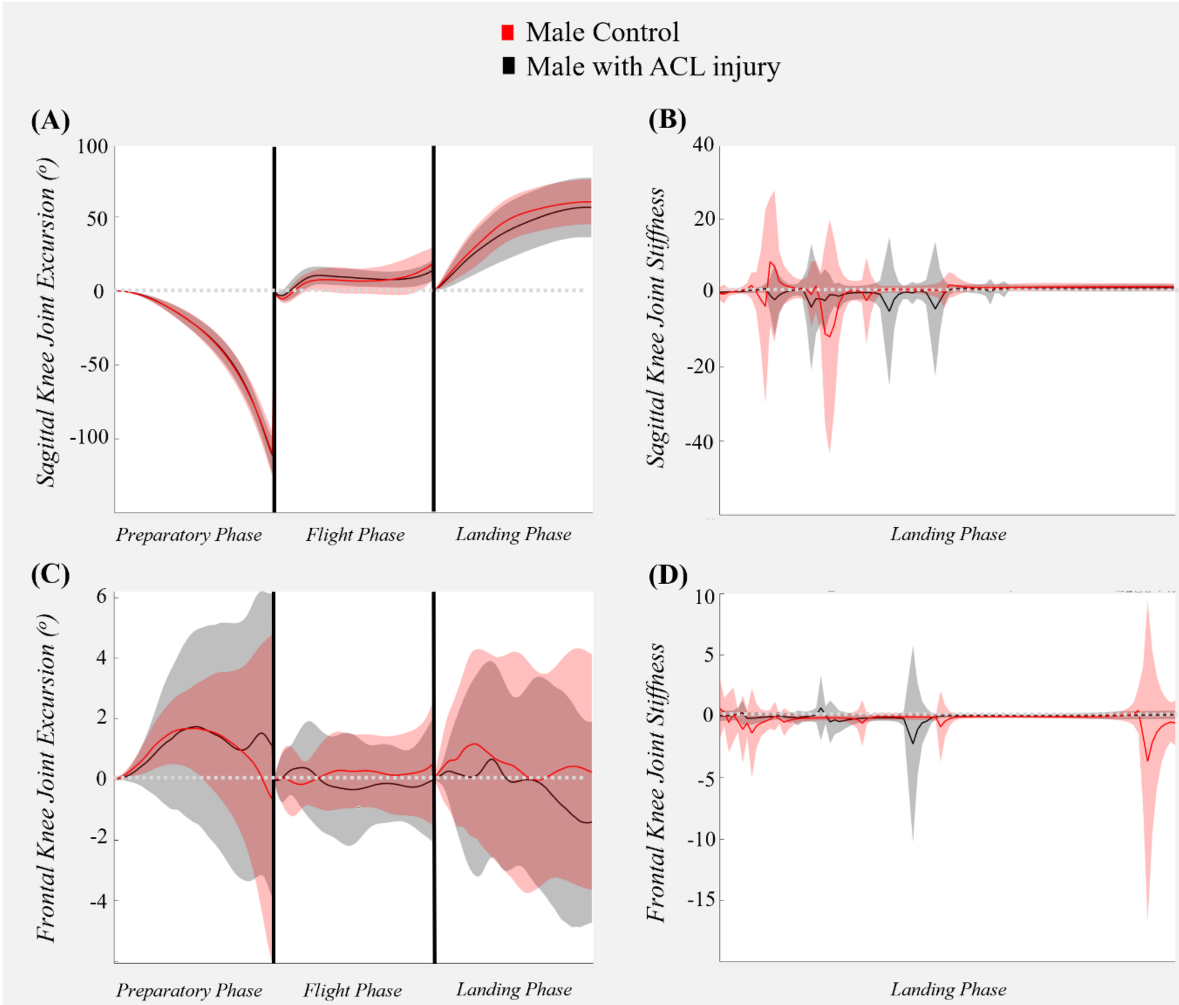
Appendix E4. Individual muscle waveforms of adolescent males throughout the two defined phases of the side-cut for the dominant (red) and non-dominant (black) limbs.



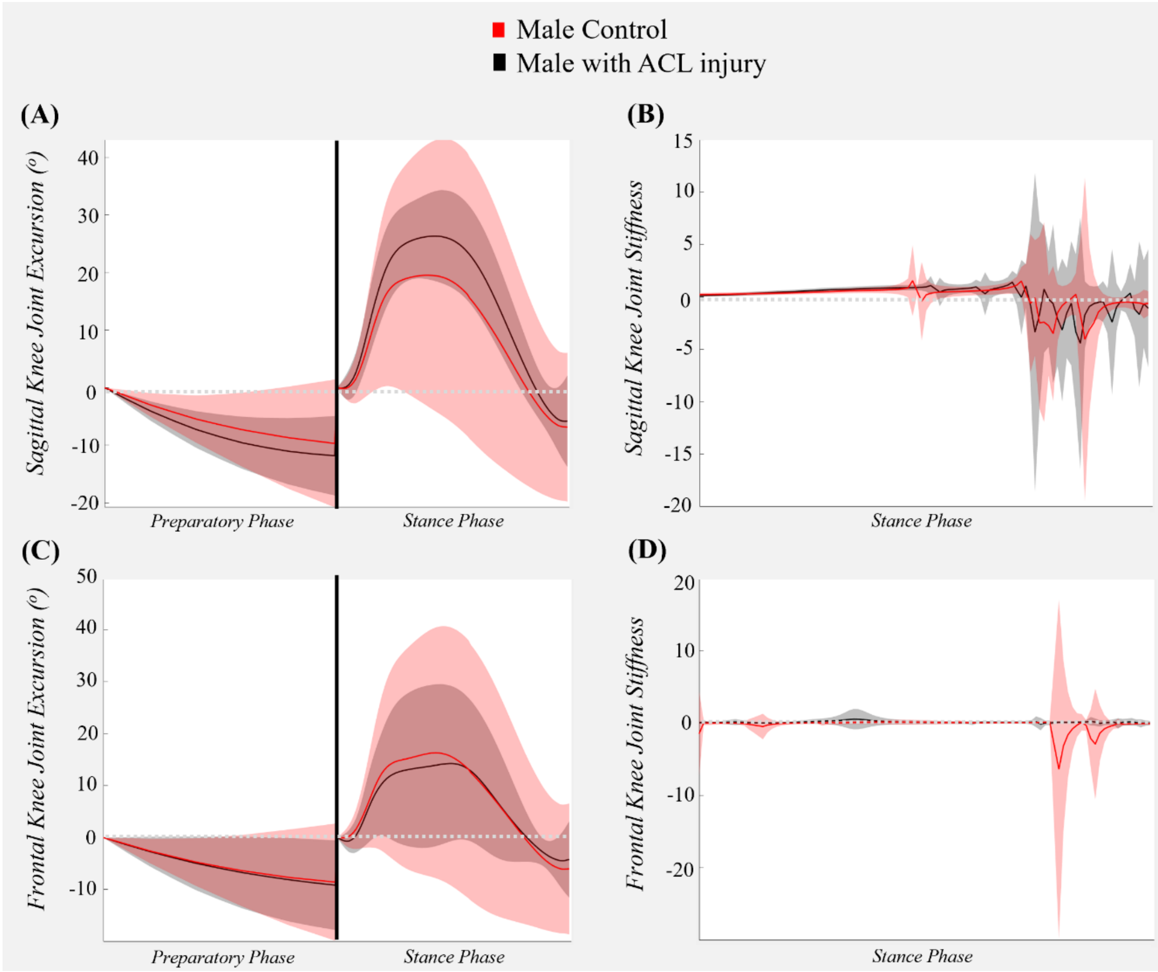
Appendix F1. SPM Mann Whitney-U test for **(A)** sagittal knee joint excursion **(B)** sagittal knee joint stiffness, **(C)** frontal knee joint excursion, and **(D)** frontal knee joint stiffness of females with ACL injuries (black) and female controls (red) during the three normalised phases of a countermovement jump.



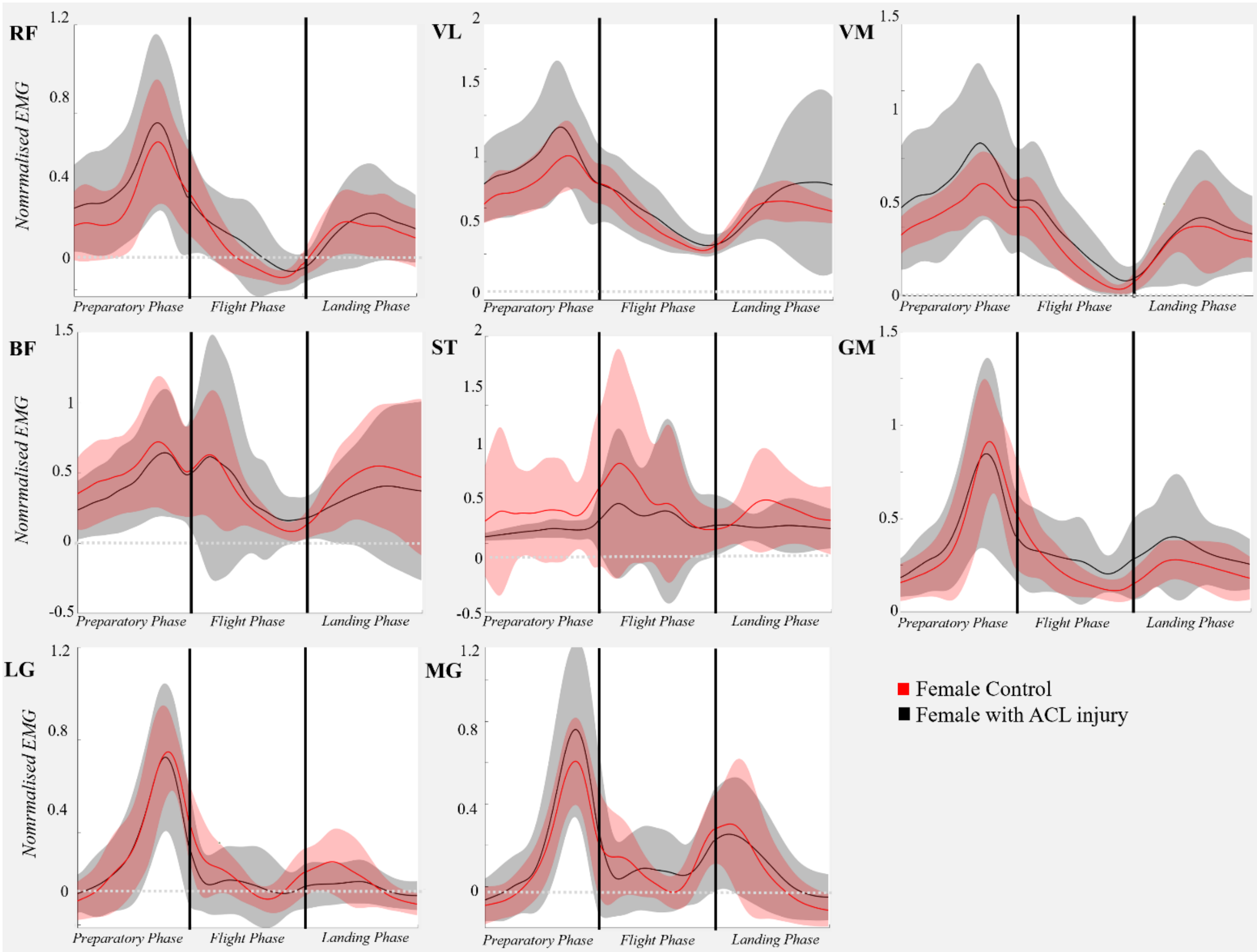
Appendix F2. SPM Mann Whitney-U test for **(A)** sagittal knee joint excursion **(B)** sagittal knee joint stiffness, **(C)** frontal knee joint excursion, and **(D)** frontal knee joint stiffness of females with ACL injuries (black) and female controls (red) during the two normalised phases of side-cuts.



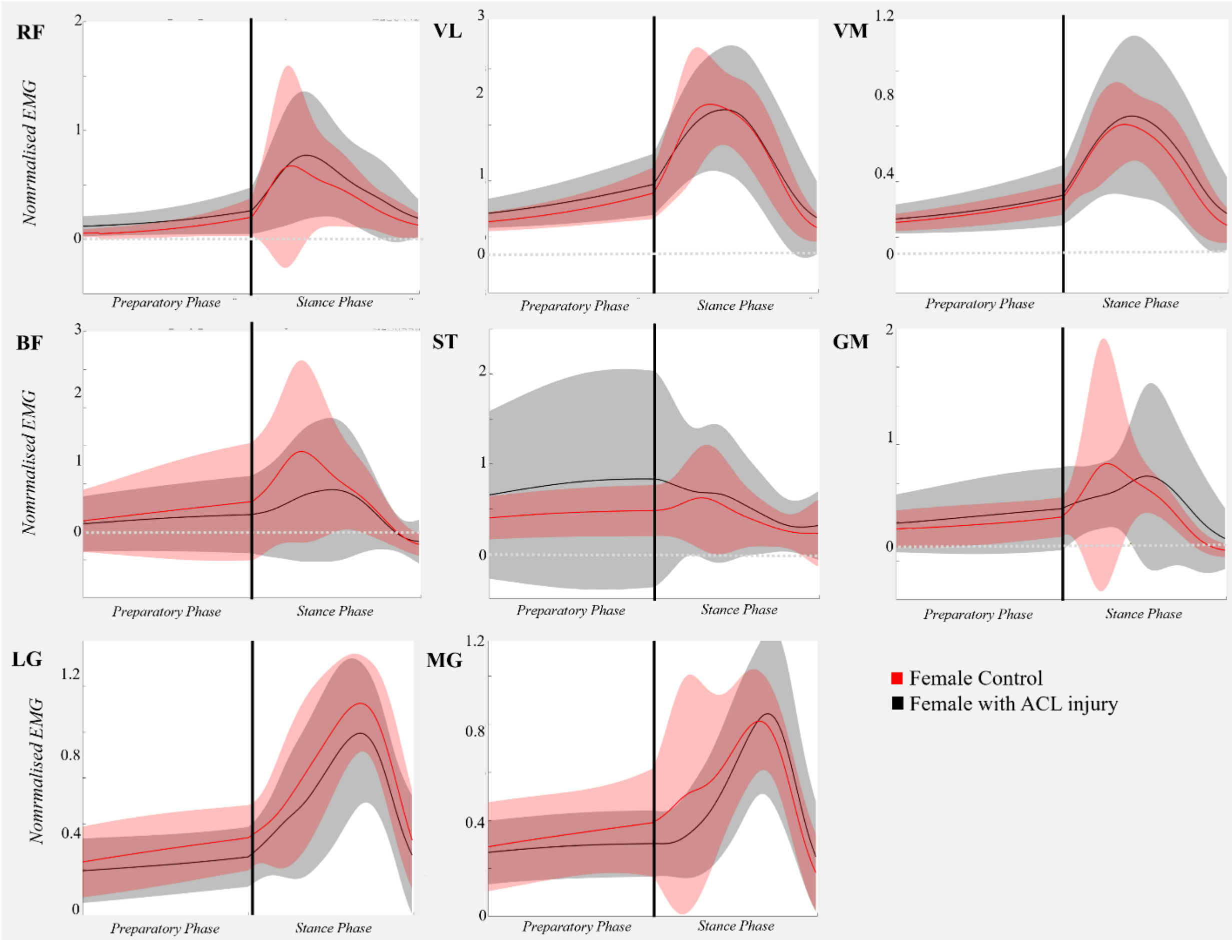
Appendix F3. SPM Mann Whitney-U test for **(A)** sagittal knee joint excursion **(B)** sagittal knee joint stiffness, **(C)** frontal knee joint excursion, and **(D)** frontal knee joint stiffness of males with ACL injuries (black) and male controls (red) during the three normalised phases of countermovement jumps.



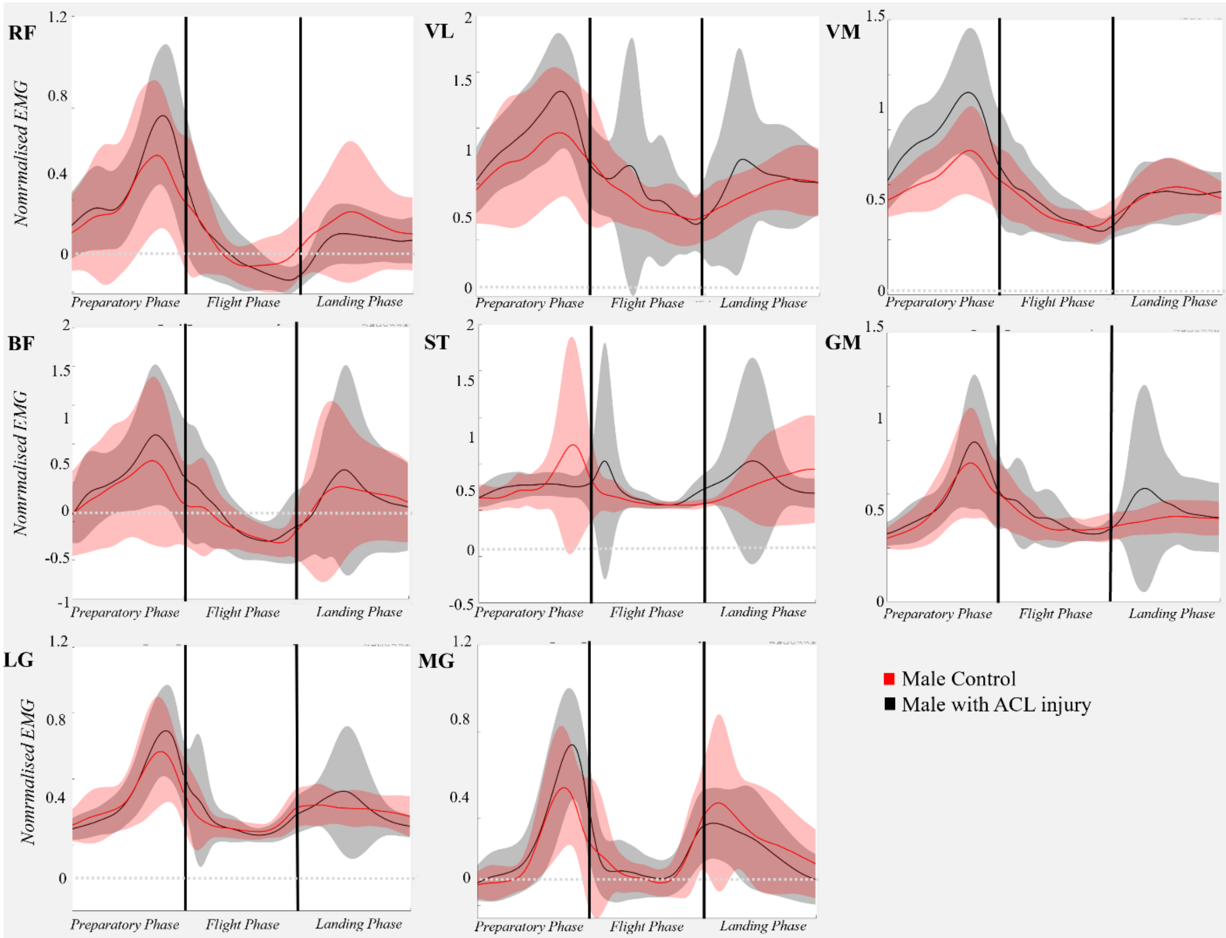
Appendix F4. SPM Mann Whitney-U test for **(A)** sagittal knee joint excursion **(B)** sagittal knee joint stiffness, **(C)** frontal knee joint excursion, and **(D)** frontal knee joint stiffness of males with ACL injuries (black) and male controls (red) during the two normalised phases of side-cuts.



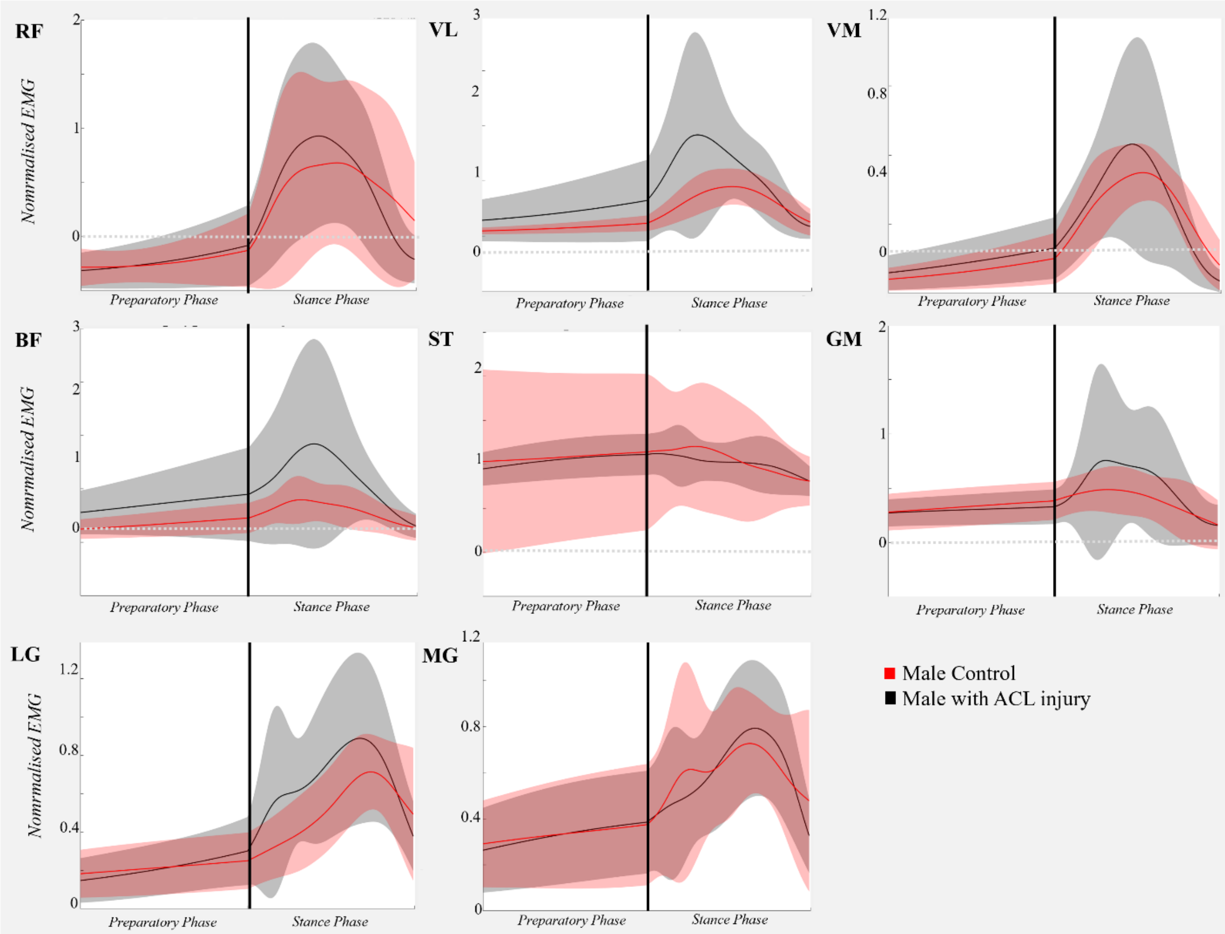
Appendix F5. Individual muscle waveforms of adolescent females with ACL injuries (black) and uninjured matched controls (red) throughout the three defined phases of the countermovement jump. Rectus femoris (RF), vastus lateralis (VL), vastus medialis (VM), biceps femoris (BF), semitendinosus (ST), gluteus medius (GM), lateral gastrocnemius (LG) and medial gastrocnemius (MG).



Appendix F6. Individual muscle waveforms of adolescent females with ACL injuries (black) and uninjured matched controls (red) throughout the two defined phases of the side-cut. Rectus femoris (RF), vastus lateralis (VL), vastus medialis (VM), biceps femoris (BF), semitendinosus (ST), gluteus medius (GM), lateral gastrocnemius (LG) and medial gastrocnemius (MG).



Appendix F7. Individual muscle waveforms of adolescent males with ACL injuries (black) and uninjured matched controls (red) throughout the three defined phases of the countermovement jump. Rectus femoris (RF), vastus lateralis (VL), vastus medialis (VM), biceps femoris (BF), semitendinosus (ST), gluteus medius (GM), lateral gastrocnemius (LG) and medial gastrocnemius (MG).



Appendix F8. Individual muscle waveforms of adolescent males with ACL injuries (black) and uninjured matched controls (red) throughout the two defined phases of the side-cut. Rectus femoris (RF), vastus lateralis (VL), vastus medialis (VM), biceps femoris (BF), semitendinosus (ST), gluteus medius (GM), lateral gastrocnemius (LG) and medial gastrocnemius (MG).